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Journal of Molecular and Cellular Cardiology 37 (2004) 161–375
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

XVIII World Congress International Society for Heart Research

7-11 August 2004 Brisbane Convention & Exhibition Centre
Brisbane · Queensland · Australia

Abstracts from the XVIII World Congress of the International Society for Heart Research Brisbane, Australia 7-11 August, 2004

doi:10.1016/j.yjmcc.2004.05.003

2004 ISHR World Congress Meeting

Abstract N° A1

Length remodeling study of sarcomeres by uniform strain via microtextured surfaces

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Our goal was to investigate remodeling of sarcomere length in response to mechanical strain. To do so, we cultured neonatal rat cardiomyocytes on microfabricated peg- and-groove, laminin-coated silicone surfaces and rapidly applied a uniaxial static strain of 10%. We found uniform lengthening of sarcomeres as determined by fast Fourier transform analysis of images. The time course peaked in length in minutes and had a half time of recovery of 2 h. Focal adhesion kinase (FAK) is located in the costamere where the cardiac myocyte is anchored to the extracellular matrix. A quick strain caused increases in FAK autophosphorylation at Y397 lasting over 30 min, as detected with phosphospecific antibody both in confocal images and in Westerns (increased 54+/–18%, $n = 3$). To assess the importance of FAK, cardiomyocytes were infected with an Adv encoding a dominant-negative inhibitor containing the focal adhesion targeting sequence, but lacking the Y-397 auto-phosphorylation site and the kinase domain (Adv-GFP-FRNK). Adv-GFP-FRNK prevented resting sarcomere length recovery, whereas a control Adv encoding only GFP did not. To assess the location of newly synthesized proteins we infected cells with adenoviruses of alpha-tropomyosin with a FLAG epitope or CapZ with a green fluorescent protein tag. Both are first detectable under 12 h. A sudden strain applied at 10, 12 or 14 h after infections timed to catch incorporation of newly synthesized and labeled Adv-proteins showed uniform labeling throughout all sarcomeres suggesting the process of new sarcomere addition or dynamic exchange occurs throughout the myocyte. In conclusion, using our novel culture system, we provide evidence indicating that the length remodeling process requires FAK and occurs throughout the myocyte. Funded by NIH HL 64956, HL 62426, HL 07692.

Abstract N° A2

Effects of myosin binding protein C on contractile efficiency and oscillatory work production

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We examined the effect of cardiac myosin binding protein-C (cMyBP-C) on contractile efficiency in isovolumically contracting left ventricle (LV) and on internal viscosity and oscillatory work production in skinned myocardial strips. Propyl-2-thiouracil (PTU) was fed to wild-type ($+/+_{PTU}$) and homozygous truncated cMyBP-C (t/t_{PTU}) mice, leading to a myosin isoform profile of ~10% α-myosin heavy chain (MHC) vs. 90% β-MHC in both groups. Western blot analysis confirmed that cMyBP-C was present in the $+/+_{PTU}$ and effectively absent in the t/t_{PTU}. Total LV mechanical energy per beat was quantified as pressure-volume area (PVA). O_2 consumption (VO_2) per beat was plotted against PVA at varying LV volumes. The reciprocal of the slope of the linear VO_2–PVA relation represents the efficiency of conversion of O_2 to mechanical energy (contractile efficiency). Contractile efficiency was significantly enhanced in t/t_{PTU} (26.1 ± 2.6%) compared to $+/+_{PTU}$ (17.1 ± 1.6%). In maximally calcium-activated skinned myocardial strips, isometric tension was similar between groups. Sinusoidal length perturbations applied from 0.125 to 250 Hz demonstrated that maximum oscillatory work occurred at higher frequencies and was more sensitive to phosphate concentration in the t/t_{PTU}. Under rigor conditions the internal elastic stiffness and viscous load was significantly reduced in the t/t_{PTU}. These results collectively suggest that contractile efficiency is most likely enhanced in t/t_{PTU} through reduced loss of mechanical energy by a viscous load normally provided by cMyBP-C and through a gain of phosphate-dependent oscillatory work normally inhibited by cMyBP-C.

Abstract N° A3

Differential roles of estrogen and insulin in cardiac myofilament activation

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Alterations in cardiac function in postmenopausal and diabetic (Diab) conditions result in the final outcome of heart failure. No additive effect of insulin and estrogen on suppressing maximum myofibrillar Ca^{2+} ATPase activity points to a similar effect of the hormones on myosin heavy chain (MHC) isoform expression. Electrophoretic analyses of cardiac MHC in ovariectomized (Ovx), Diab, and Diab–Ovx rats were then examined. With the same magnitude of suppressed myofibrillar ATPase activity among the groups, levels of α-MHC was found to be highest in Ovx and Diab–Ovx but lowest in Diab hearts. These results indicate that the mechanistic regulation of estrogen on cardiac myofilament activity is not solely dependent on MHC. The dominant effect of estrogen on changes in myofilament Ca^{2+} sensitivity and intracellular Ca^{2+} transients through suppressions of both the activity and content of SR Ca^{2+} ATPase (SERCA) proteins in Diab–Ovx and Ovx hearts, respectively, led to a proposed change in SERCA activity by estrogen but not insulin. Surprisingly, a significant decrease in maximum SERCA activity with an increase in Ca^{2+} sensitivity was also demonstrated in Diab group in the same manner as those detected in Ovx and Diab–Ovx groups. Supplementation of

the hormone in each group completely abolished the changes in SERCA activity. These results thus imply a differential role of estrogen and insulin in cardiac myofilament activation.

Abstract N° A4

Genomic structure and functional characterization of the promoters of mouse ssTnI

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Two major troponin I (TnI) genes are expressed in the mammalian heart under the control of a developmentally regulated program. Fetal TnI, which is identical to slow skeletal TnI (ssTnI), is expressed first and predominates throughout embryonic and fetal development. After birth, ssTnI is downregulated and eventually disappears in the adult heart. Meanwhile cardiac TnI (cTnI) is upregulated and becomes predominant in the adult heart. In our previous studies, we have demonstrated that ssTnI cannot re-express in adult mouse heart even in the case of hypertrophy. In this study, the up-stream part (~1800 bp) of mouse ssTnI has been identified by PCR of genomic DNA. There is a high homology in this ssTnI region between mouse and rat. Analysis of the sequence with a Genomatix software package has revealed several potential regulatory domains and binding sites such as GA-rich sequences (Sp-1 binding sites), GATA binding site, SMAD4, MEF2, AP1 NFkB, etc. The results from transfection assays have indicated that conserved GA-rich sequences, an Oct binding site and a CCAAT box within the first 300 bp upstream of the transcription start site are critical for the gene expression. An inhibitory domain has been revealed within the sequence between –1700 and –1780. The inhibitory effect seems more significant in C2C12 Myoblast cells than that in CHO cells, suggesting that some inhibitory factors existed inside of muscle type cells. In summary, the up-stream regulatory domain of mouse ssTnI has, at the first time, been identified and characterized. This provides us with a useful tool to investigate the expression regulation of this gene during heart development (Supported partly by a Grant-in-Aid from AHA-Florida and a research grant from the Center of Excellence for Biomedical and Marine Biotechnology (P200310) at FAU).

Abstract N° A5

Studies on the interactions between calpain1 and cytoskeletal proteins of the heart

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In order to study the relations between calpain1 and cytoskeletal proteins of heart, we screened the proteins interacted with calpain1 using yeast two-hybrid system, then sequencing the positive clones and BLAST analysis the DNA fragments. The sequences of clone No. 12 and 22 (pACT2-12 and pACT2-22) have the 99% and 100% similarity to cardiac muscle alpha-actin (ACTC), respectively. The sequences of clone No. 16 (pACT2-16) have the 100% similarity to cardiac myosin-binding protein C (MyBP-C3). The sequences of clone No. 37 (pACT2-37) have the 100% similarity to cardiac alpha 2 actinin (ACTN2). To further confirm which domain of calpain1 was contained during interactions, positive clones were transformed into the AH109 with the domain II, III or IV of calpain1. pACT2-12(ACTC) could interact with domain II or III of human calpain1; pACT2-16(MYBPC3) could interact with domain II, III or IV of calpain1; while pACT2-37(ACTN) could not interact with domain II, III or IV of human calpain1. Colorimetry to quantitative analysis the activities of β-galactosidase (β-Gal). The stronger of the interaction, the higher of the β-Gal. When pACT2-12, 16 or 37 interacted with calpain1, the activities of β-Gal (units/mg) are 122.28, 142.23, 110.26, respectively. Actin and actinin participate cardiomyocyte contraction. Our results showed calpain1 could interact with actin or actinin. If calpain1, as proteinase, could hydrolyze actin or actinin during physiological or pathological states, it will affect the cardiomyocytes contraction. MyBP-C3 is cardiac-specific subtypes and one of the necessary conditions forming normal thick filaments in the cardiomyocyte. Our results showed the interaction between calpain1 and MyBP-C3, and the interaction is relatively strong. It takes a clue that calpain1 is concerned with the regulation of cardiomyocyte contraction through MyBP-C3.

Abstract N° A6

Post-infarct ventricular rupture is due to matrix collagen damage in mice

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Ventricular rupture occurs in mice with acute myocardial infarct (AMI). We studied the role of extracellular matrix (ECM) collagen in the risk of rupture in mice. After coronary artery occlusion, rupture occurred during days 2-6 in mice. Collagen content in the infarcted tissue, as determined by hydroxyproline assay, was unchanged at Day-2 but increased by 60 % at Day-4 after AMI, indicating de novo collagen synthesis. We then determined in vitro tension-to-rupture (TTR) using left ventricular (LV) rings of hearts with AMI for 4 days. There was a 60 % reduction in TTR of infarcted vs. non-infarcted LV rings either from the same animal or from sham-operated mice, indicating a reduced muscle tensile strength. We previously showed that mice with cardiac overexpression of α_2-adrenoceptors had a reduced risk of rupture (J Cardiovasc Res 2002 ;40 :632). The transgenic mouse heart had a 30-40 % higher TTR than that of wildtype mice with or without AMI, in keeping with a 55 % increase in collagen content (2.63 ± 0.18 vs 1.61 ± 0.07 μg/mg d.w., $p < 0.01$, n = 6 each). To compare these findings with a non-

rupture rodent, we also similarly studied Spraque-Dawley rats. In rat hearts with AMI for 4-days, TTR was unchanged relative to non-infarcted LV rings. Collagen content in the heart was 50 % higher in rats than that in mice (2.32 ± 0.04 vs 1.38 ± 0.04 μg/mg d.w., $p < 0.01$, $n = 6$ each). We then determined levels of matrix metalloprotenase (MMP)-2 and -9 by zymography in mouse and rat hearts with AMI at Day-4. The major form of MMP that increased by AMI was MMP-9 in mice but MMP-2 in rats with a MMP-9 :MMP-2 ratio of 6 in the mouse and 0.6 in the rat. In summary, ECM collagen content correlates to the myocardial tensile strength which is significantly reduced in mice. Following AMI, MMP-9 activation likely degrades collagen and lowers mechanical strength leading to rupture. Differences in the mouse and rat in altered tensile strength, subtypes of MMPs and collagen levels, imply collagen damage in rupture pathogenesis.

Abstract N° A7

Post-infarct ventricular rupture is due to matrix collagen damage in mice

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Ventricular rupture occurs in mice with acute myocardial infarct (AMI). We studied the role of extracellular matrix (ECM) collagen in the risk of rupture in mice. After coronary artery occlusion, rupture occurred during days 2–6 in mice. Collagen content in the infarcted tissue, as determined by hydroxyproline assay, was unchanged at day-2 but increased by 60% at day 4 after AMI, indicating de novo collagen synthesis. We then determined in vitro tension-to-rupture (TTR) using left ventricular (LV) rings of hearts with AMI for 4 d. There was a 60% reduction in TTR of infarcted vs. non-infarcted LV rings either from the same animal or from sham-operated mice, indicating a reduced muscle tensile strength. We previously showed that mice with cardiac overexpression of β_2-adrenoceptors had a reduced risk of rupture (J Cardiovasc Res 2002;40:632). The transgenic mouse heart had a 30–40% higher TTR than that of wildtype mice with or without AMI, in keeping with a 55% increase in collagen content (2.63 ± 0.18 vs. 1.61 ± 0.07 mg/mg d.w., $P < 0.01$, $n = 6$ each). To compare these findings with a non-rupture rodent, we also similarly studied Spraque–Dawley rats. In rat hearts with AMI for 4 d, TTR was unchanged relative to non-infarcted LV rings. Collagen content in the heart was 50% higher in rats than that in mice (2.32 ± 0.04 vs. 1.38±0.04 mg/mg d.w., $P < 0.01$, $n = 6$ each). We then determined levels of matrix metalloprotenase (MMP)-2 and -9 by zymography in mouse and rat hearts with AMI at day-4. The major form of MMP that increased by AMI was MMP-9 in mice but MMP-2 in rats with a MMP-9:MMP-2 ratio of 6 in the mouse and 0.6 in the rat. In summary, ECM collagen content correlates to the myocardial tensile strength which is significantly reduced in mice. Following AMI, MMP-9 activation likely degrades collagen and lowers mechanical strength leading to rupture. Differences in the mouse and rat in altered tensile strength, subtypes of MMPs and collagen levels, imply collagen damage in rupture pathogenesis.

Abstract N° A8

Changes in cardiac function and beta adrenoceptors are not dependent upon cardiac remodeling

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Cardiac function and beta-adrenoceptors are either upregulated or downregulated in the failing heart. Although cardiac remodeling has been shown to be responsible for heart failure, its relation with subcellular remodeling needs to be understood. Heart dysfunction in rats was induced by myocardial infarction (MI), aortocaval shunt (AV shunt) or pressure overload (PO). Cardiac remodeling was determined by changes in heart weight whereas hemodynamic parameters were used to determine changes in left ventricle function. Since beta-adrenergic receptor signal transduction plays an important role in the regulation of cardiac function, we examined the status of beta-adrenergic receptors in this study. Our results show that cardiac remodeling was evident at both early (4 week) and later (24 week) stages of heart failure but changes in left ventricular function, isoproterenol-induced positive inotropic effect on cardiac performance and isoproterenol-induced increase in intracellular calcium were dependent on the type and stage of heart failure. Furthermore, beta-adrenergic receptor density in left ventricles was downregulated, upregulated or unchanged in crude membranes at 4 week due to MI, AV shunt or PO rats; this parameter was downregulated in all heart failure types at the later stages. These results thus support the view that changes in cardiac function and beta-adrenoceptor due to different stimuli are not dependent upon cardiac remodeling during the development of heart failure.

(Supported by CIHR Group in Experimental Cardiology)

Abstract N° A9

Norepinephrine-induced changes in the rat cardiac TGF-beta isoform expression pattern: studies in male rats

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Transforming growth factor-beta (TGF-β) is a ubiquitous growth-regulating protein with an essential role in tissue repair and formation of the extracellular matrix (ECM). To better understand the role of different isoforms of TGF-β in the remodelling process induced by norepinephrine (NE), the expression of TGF-β1, TGF-β2 and TGF-β3 as well as of

collagen I and III and of matrix metalloproteinase 2 (MMP-2) and its inhibitor TIMP-2 was studied in hearts of male rats. NE (0.1 mg/kg per h) was i.v. infused in male Sprague–Dawley rats for 1, 3 and 4 d, and freshly obtained ventricular myocardium after 1 d was dissociated into myocyte and nonmyocyte fractions. After NE infusion the three isoforms of TGF-β were differentially induced as far as the magnitude and the time course is concerned. The increased expression of TGF-β2 was more pronounced (16-fold elevation) than that of the two other isoforms, with a clear specificity for the left ventricle (LV) after 1 d of NE treatment. The increase of TGF-β1 and TGF-β2 was significant only in the myocyte fraction. TGF-β3 was decreased in non-myocytes and not changed in myocytes after 1 d of NE treatment. The expression of collagens I and III was elevated about 13-fold after 3–4 d predominantly in the LV. The expression of MMP-2 and TIMP-2 was increased about fivefold also predominantly in the LV. All three isoforms of TGF-β showed a positive correlation with the mRNA of collagen I and III as well as with MMP-2 and TIMP-2. However, the different time course and different cell specificity of the NE-induced elevation of the expression of TGF-β isoforms implicate a different role of TGF-β isoforms in the NE-induced remodelling process of the rat heart.

Abstract N° A10

Antisense oligoribonucleotide-induced exon skipping in cardiac myocytes from dystrophin deficient mice

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Duchenne muscular dystrophy (DMD) results from the absence of a functional dystrophin protein from the membrane of muscle cells. The severe skeletal muscle wasting in DMD patients has previously overshadowed cardiac abnormalities. However, these now play a more prominent role as patients are surviving beyond their 20s with improved palliative care. The administration of small synthetic RNA-like molecules into murine skeletal muscle has been shown to restore dystrophin in *mdx* mice, which carry a nonsense mutation in exon 23. The antisense oligoribonucleotides (AOs) used targeted the donor splice site of exon 23 and induced the removal of this mutation-containing exon during pre-mRNA splicing. This AO-induced exon-skipping has been shown to produce a shorter but functional, non-immunogenic protein in skeletal muscle. This investigation therefore focussed on the potential application of AOs in cardiac tissue to induce functional dystrophin. Fluorescently labelled AOs were first used to test a range of transfection reagents to visually confirm uptake by neonatal *mdx* cardiac myocytes. AOs of different lengths and chemistries, previously efficient in skeletal myocytes, were then transfected into cardiac myocytes at different doses. These were able to induce exon-skipping, detectable by RT-PCR, however, the level of exon-skipping in cardiac myocytes was less than that previously observed in skeletal myocytes. If greater levels of exon-skipping can be induced in cardiac myocytes, it is anticipated that sufficient dystrophin may be produced, to allow for systemic delivery of the treatment and thus delay the development of dilated cardiomyopathy in DMD patients.

Abstract N° A11

Abnormal cardiac wall motion and matrix metalloproteinase activity

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Activation of matrix metalloproteinases (MMPs) in the heart is known to facilitate cardiac remodeling and progression to failure. We hypothesized that regional dyskinetic wall motion of the left ventricle would stimulate activation of MMPs. Abnormal wall motion at a target site on the anterior lateral wall of the left ventricle was induced by pacing atrial and ventricular sites of five open-chest anesthetized dogs. Changes in shortening at the left ventricular (LV) pacing site and at a remote site at the anterior base of the left ventricle were monitored with piezoelectric crystals. Simultaneous atrial–ventricular pacing resulted in abnormal wall motion at the LV pacing site yielding early shortening and late systolic lengthening, while shortening at the remote site was essentially unaffected. Global myocardial MMP activity showed a sevenfold increase in substrate cleavage at the LV pacing site relative to the remote site. Gelatin zymography revealed increases of 50-fold in 92 kDa MMP-9 activity and 10-fold in 84 kDa MMP-9 at the LV pacing site relative to the remote site, whereas MMP-2 activity was unaffected. Abnormal wall motion was associated with a twofold increase in collagen degradation and with increases in plasmin activity and inflammatory infiltrate relative to the remote site. Results indicate that regional dyskinesis by epicardial activation is sufficient to stimulate significant MMP activity in the heart, suggesting that abnormal wall motion is a major stimulus for MMP activation.

Abstract N° A12

Doxycycline flanks the zinc-binding domains of matrilysin: a putative mode of inhibition

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Matrix metalloproteinases (MMPs) play an essential role in normal and pathological collagen matrix degradation. Here, we report on the effects of a broad-spectrum MMP inhibitor, doxycycline, on the structure of the MMP matrily-

sin. Deuterium exchange mass spectrometry studies on matrilysin reveal two putative doxycycline-binding sites (residues 145–155; residues 231–243) of similar affinity that flank the zinc-binding domains of the enzyme. Examination of the X-ray crystal structure of matrilysin shows that the doxycycline-binding site at residues 231–243 is positioned within the active site cleft adjacent to the catalytic zinc atom. Comparisons of drug-bound vs. drug-free forms of matrilysin show discrete changes in deuterium exchange suggesting that drug binding induces alterations in structure. In addition, fluorescence-quenching studies of matrilysin shown minor changes in conformation induced by doxycycline. In the absence of doxycycline, tryptophan fluorescence is completely accessible to quencher, whereas in the presence of doxycycline, accessibility decreases by approximately 8%. These results suggest a mode of MMP inhibition by doxycycline that could involve interactions with the catalytic zinc atom.

Abstract N° A13

Reduction of infarct size by doxycycline: a role for plasmin inhibition

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Reperfusion of ischemic myocardium leads to the upregulation of several extracellular matrix (ECM)-degrading proteases. The purpose of this study was to determine whether doxycycline (DOX), a matrix metalloproteinase (MMP) inhibitor, would reduce infarct size following ischemia–reperfusion injury and to identify potential mechanisms for its effects. Results showed that DOX treatment reduced infarct size by ~37% in the rat. DOX did not reduce acute inflammation (i.e. neutrophil infiltration) but did attenuate increases in 92 kDa MMP-9 and plasmin levels observed in the infarct region. To investigate the role of these proteases in mediating myocardial injury, we utilized cultures of neonatal rat ventricular myocytes (NRVMs). NRVMs stimulated with physiological levels of plasminogen generated the active plasmin form of the protease in a dose-dependent manner. Stimulation with plasminogen (or plasmin), but not active MMP-9, caused cell detachment and death (i.e. anoikis). Plasminogen stimulation did not activate endogenous MMPs, and co-treatment with GM6001 did not preserve cell attachment suggesting MMP activity was not responsible for cell detachment. Interestingly, co-treatment with DOX preserved NRVM attachment and viability, and DOX inhibited plasmin activity in culture with an apparent IC_{50} of ~18 μg/ml. However, inhibition of plasmin was an indirect effect, as DOX displayed no dose-dependent or time-dependent inhibition of plasmin in an in vitro test. These results suggest a novel role for DOX in preserving cardiomyocyte cell–matrix interactions by indirectly inhibiting plasmin activity.

Abstract N° A14

Cardiovascular changes in the ageing growth hormone deficient rat

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Exogenous growth hormone has been implicated as a potential novel therapy for patients with congestive heart failure. This project has characterised the Lewis Dwarf (LD) growth hormone deficient rat model from 6 to 18 months (M) of age using echocardiography, isolated Langendorff heart preparations, single-cell microelectrode electrophysiological recordings and responses from isolated thoracic aortic rings to determine if growth hormone deficiency initiates heart failure. LD rats were moderately hypertensive (6 M, 148 ± 3*; 12 M, 157 ± 4*; 15 M, 162 ± 3*; 18 M, 161 ± 4* mmHg) compared to age-matched Wistar (W) controls (6 M, 121 ± 3; 12 M, 126 ± 3; 15 M, 133 ± 2; 18 M, 129 ± 6 mmHg) with decreased left ventricular internal dimensions in diastole (LD: 6 M, 5.4 ± 0.3*; 12 M, 5.3 ± 0.1*; 15 M, 6.1 ± 0.1*; 18 M, 6.8 ± 0.2* mm; W: 6 M, 7.1 ± 1; 12 M, 8.2 ± 0.2; 15 M, 7.8 ± 0.3; 18 M, 7.9 ± 0.3 mm) and increased left ventricular posterior wall thicknesses (LD: 6M – 1.87 ± 0.08*; 12M – 1.87 ± 0.07*; 15M – 1.89 ± 0.08*; 18M – 1.76 ± 0.1* mm; W: 6M – 1.76 ± 0.08; 12M – 1.72 ± 0.07; 15M – 1.77 ± 0.08; 18M – 1.89 ± 0.08 mm) indicative of concentric cardiac hypertrophy. Fractional shortening, ejection fraction, maximum ascending aortic blood flow velocity and maximum +dP/dt were all increased in the LD rat showing improved systolic function. LD rats showed prolonged action potential duration (APD90: LD: 6M – 47.5 ± 5.9*; 12M – 52.9 ± 4.3; 15M – 60.2 ± 5.3*; 18M – 76.5 ± 7.2* ms; W: 6M – 33.1 ± 3.7; 12M – 47.8 ± 5.9; 15M – 49.9 ± 4.1; 18M – 53.7 ± 5.6 ms). Additionally, maximal responses to noradrenaline, acetylcholine and sodium nitroprusside were significantly reduced in LD rats. Diastolic stiffness was unaltered between the groups but increased with age. Thus, chronic growth hormone deficiency produces compensated concentric cardiac hypertrophy with improved left ventricular function without symptoms of heart failure but vascular function is dramatically reduced in the LD rat.

Abstract N° A15

PKCϵ-mediated phosphorylation of Cx43 of cardiac gap junction in the diabetic heart

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Cardiac arrhythmias have been extensively documented in experimental and clinical diabetes. We studied abnormalities of the gap junction function in the streptozotocin-induced diabetic rat heart, since impairment of electrical cell-to-cell

coupling is one of factors inducing arrhythmias. Impulse conductivity and the intercellular coupling were impaired. Immunoblot for Cx43 and co-immunoprecipitation of Cx43 and PKC isoforms, PKCϵ-mediated phosphorylation of Cx43 was augmented despite of reduction of Cx43 protein. It was observed in immunohistochemistry image analysis that expression of Cx43 at the intercalated disk was reduced. These alterations were ameliorated by lysosomal inhibitors. In GK/Jcl DM model rat (hereditary, NIDDM), an activation of PKC was also observed. We concluded that disturbances of intercellular communication in the diabetic heart is possibly caused by deterioration of the gap junction due to PKCϵ-mediated phosphorylation of Cx43.

Abstract N° A16

Identification of four small molecules that increase alpha myosin heavy chain protein levels in rat neonatal cardiomyocytes

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A diverse group of cardiovascular diseases, including valvular dysfunction, cardiomyopathies, hypertension, and ischemic heart disease, eventually result in chronic heart failure (CHF). During the course of disease progression, changes in fetal gene and contractile protein gene expression are frequently observed. One of these contractile proteins, alpha myosin heavy chain (α-MyHC), is severely downregulated in human heart failure. Importantly, these changes are associated with an impairment in cardiac pump function. These findings raise the possibility that one potential treatment to enhance contractile performance in CHF patients is to increase α-MyHC protein levels. In addition, improvement of cardiac contractility in CHF patients by increasing α-MyHC protein levels may also reverse cardiac remodelling and improve cardiac pump function even further. In order to identify small molecules that upregulate α-MyHC protein levels in the heart, we have developed a high throughput screening assay in rat neonatal cardiac myocytes that detects small changes in (α-MyHC) protein levels. This assay has been used to screen a 20,000 compound library of small molecules. Four compounds were identified in this screen that increase levels of α-MyHC and decrease levels of β-MyHC. The effect of these compounds on α-MyHC levels is quite dramatic, with two- to threefold increases in protein expression levels typically observed. The effect of these compounds is almost, or in some cases, of equal magnitude to the increase in α-MyHC induced by thyroid hormone, the most potent regulator of α-MyHC both in vitro and in vivo. Further studies are currently underway to examine how these compounds affect other aspects of cardiac hypertrophy in the rat neonatal cardiomyocyte model system, and to determine the mechanism of action of these small molecular regulators of α-MyHC expression.

Abstract N° A17

Atrial osteopontin expression is not elevated in patients who develop postoperative atrial fibrillation

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Osteopontin (OPN), an extracellular matrix protein, has been shown to play an important role in the development of heart failure and post-myocardial infarction remodeling by promoting collagen synthesis and accumulation. Its plasma levels were found to be associated with the presence and extent of coronary artery disease and its expression is stimulated by angiotensin II. In chronic atrial fibrillation (AF), angiotensin-converting enzyme (ACE) mRNA expression is upregulated, and it has also been shown that angiotensin II increases OPN mRNA expression in human heart. Therefore, we were now interested whether OPN expression is elevated in atria of patients with no history of AF but who develop AF early after cardiac surgery. Ten patients (68.5 ± 9.2 years; seven males/three females; ACE inhibitor treatment 40%) who did not develop postoperative AF were compared to eight patients (66.3 ± 9.9; six males/two females; ACE inhibitor treatment 62.5%) who experienced AF within 6 d postoperatively. Right atrial appendages were obtained during cardiac surgery and OPN mRNA and protein expression was assessed by RT-PCR and western blots, respectively. The medians of OPN/HPRT mRNA ratios were 1.142 (range 0.429–17.555) and 1.376 (range 0.732–5.287; postoperative AF vs. postoperative sinus rhythm). The medians of OPN/GAPDH ratios on the western blots were 0.318 (range 0.140–0.475) and 0.243 (range 0.195–0.408). OPN mRNA as well as protein expression was not significantly different between groups (*U*-test). Our data suggest that the occurrence of AF in the early period after cardiac surgery is not correlated with the amount of atrial OPN expression at the time of surgery in the affected patients. Hence, plasma OPN determination will not be adequate to estimate the risk for postoperative AF.

Abstract N° A18

Expression pattern of troponin C and troponin T isoforms in developing and pathological heart

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During heart development, contractile and regulatory protein genes show characteristic patterns of activation and subsequent modulation of expression resulting in the formation of a mature four-chambered organ. Myocardial gene expression is also altered in the adult in response to pathological stimuli and this is thought to contribute to the altered contractile characteristics of the diseased heart. We have examined the expression of different isoforms of troponin I, T and C in the developing avian heart as well as the changes in slow skeletal muscle troponin T in the human heart during

development and in disease using whole mount in situ hybridisation and real time quantitative (TaqMan) PCR analyses. Slow skeletal muscle troponin T mRNA shows transitory and regional expression in the early fetal heart, which occurs at different times in atria and ventricles. In ventricular myocardium, expression is seen in the outer epicardial layer at a time when the coronary circulation is being established. Expression was also detected at quite low levels in the adult human heart that was significantly increased in end-stage heart failure. Similarly, expression was readily detectable during early rat heart development and was up regulated in pressure overload hypertrophy in adult. These data thus show that slow skeletal muscle troponin T mRNA is readily detectable during early human heart development and further suggest that slow skeletal muscle troponin T may be responsive to myocardial stress and that elevated levels may contribute to myocardial dysfunction in adult disease. There have been virtually no reports of the changes occurring in the isoforms of troponin C in the developing heart. In this communication, we will also describe the expression patterns of troponin C isoforms in developing avian heart.

Abstract N° A19

Regulation of scaffold protein expression during ischemia/reperfusion

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The apoptotic death of cardiomyocytes due to ischemia/reperfusion is one of the major complications of heart disease. One of the molecular mechanisms leading to apoptosis is the activation of c-Jun *N*-terminal kinase (JNK). The specificity of the activation of JNK cascade may be determined partly by scaffold proteins, which bind several kinases of a signaling cascade to form a multimolecular complex. JIP-1 and JSAP-1 are two recently identified scaffold proteins that are involved in the JNK signaling pathway. The objectives of the present study were to investigate the expressions of JIP-1 and JSAP-1 in ischemia/reperfusion and the effect of magnesium tanshinoate B, a compound purified from a Chinese medicinal herb Danshen (Radix Salvia miltiorrhizae) on their expressions. Isolated rat hearts were perfused in the Langendorff mode and subjected to 30 min global ischemia followed by reperfusion for various time periods. The expression of JIP-1 mRNA was significantly reduced in the heart after ischemia/reperfusion. In contrast, the expression of JSAP-1 mRNA was elevated. The addition of magnesium tanshinoate B reversed changes in the expression of both JIP-1 and JSAP-1 induced by ischemia/reperfusion. A decrease in JIP-1 expression might reduce the retention of JNK in the cytoplasm while JSAP-1 at a high level would facilitate the activation of JNK. In conclusion, our results suggest that JIP-1 and JSAP-1 can act in concert to facilitate JNK activation during ischemia/reperfusion. Magnesium tanshinoate B can exert differential effects on the expressions of JIP-1 and JSAP-1 indicating its potential therapeutic role in cardiovascular disorders.

Abstract N° A20

Resveratrol attenuates cardiac remodelling in DOCA-salt hypertensive rats

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Chronic cardiovascular disease induces cardiac remodelling including hypertrophy and fibrosis, the excessive deposition of collagen. Reactive oxygen species such as superoxide and hydroxyl radicals are increased in hypertension and may initiate cellular damage. To test the hypothesis that an increased oxidant stress is important in cardiac remodelling, we have determined the changes in cardiac structure and function in DOCA-salt hypertensive rats, which show increased superoxide production, after treatment with the antioxidant resveratrol (R, 1 mg/kg/d p.o. starting 4 d before uninephrectomy). DOCA-salt rats were uninephrectomied, given 1% NaCl in their drinking water and deoxyxorticosterone acetate (25 mg every 4th d s.c.) for 4 weeks; uninephrectomied rats (UNX) were used as controls (n = 6–12; $^*P <$ 0.05 vs. DOCA). R attenuated the hypertension (UNX 133 ± 5, DOCA 176 ± 4, DOCA + R 154 ± 7* mmHg) and left ventricular hypertrophy (wet weight: UNX 1.97 ± 0.04, DOCA 3.17 ± 0.07, DOCA + R 2.76 ± 0.11* mg/g bwt) and prevented both the increased stiffness (UNX 22.2 ± 1.2, DOCA 30.1 ± 1,2, DOCA + R 25.5 ± 0.4*) and decreased contractility (+dP/dt: UNX 2080 ± 70, DOCA 1610 ± 130, DOCA + R 2170 ± 100* mmHg/s). Further, the increased left ventricular interstitial collagen deposition was prevented by R treatment. In isolated thoracic aortic rings, the decreased endothelial-mediated relaxation in DOCA-salt rats was prevented (maximal response to acetylcholine: UNX 5.8 ± 0.9, DOCA 1.0 ± 0.2, DOCA + R 3.9 ± 0.7* mN). In addition, maximal responses to noradrenaline and sodium nitroprusside were improved. Thus, administration of R prevented or attenuated the changes in cardiovascular structure and function in the DOCA-salt hypertensive rat, indicating that reactive oxygen species are likely to be involved in these changes.

Abstract N° A21

Covalently bound synthetic peptides of fibronectin and laminin facilitate good cellular attachment but abnormal physiology in neonatal cardiac myocytes

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Cell growth, adhesion and migration can be regulated by the extracellular matrix and play an important role in cardiac

adaptation to stress. In cell culture, a significant number of cells become detached in response to mechanical stimulation, limiting the scope of such studies. Here, we have adhered synthetic peptides, RGD (fibronection) and YIGSR (laminin) onto silicone. We examined the morphology, gene expression of neonatal cardiac myocytes cultured on these peptides and in response to mechanical stimulation. At 100 μM peptide reaction concentration, we achieved the same degree of cellular adhesion as their respective native proteins. After 48 h of culture, cells were stained with phalloidin and visualized to assess cell morphology. The number of striated cells on the peptide surfaces was significantly reduced compared to those on native proteins. Western blot analysis showed that focal adhesion kinase (FAK) was reduced by 50% in myocytes cultured on YIGSR peptide compared with laminin (laminin vs. YIGSR $P < 0.05$ $n = 3$). B_1-integin was unchanged in all groups. Connexin 43 was more phosphorylated in cells from RGD and YIGSR peptides, similar to levels found in the adult atrium. Myocytes were subjected to cyclic strain at 20% maximum strain, 1 Hz for 48 h. Cell attachment on laminin was reduced to about 50% compared to the unstretched ($P < 0.05$ $n = 6$). In cells cultured on the synthetic peptides, there was no significant cellular detachment. Following mechanical stimulation myosin protein was decreased by about 50% in cells cultured on YIGSR peptide ($P < 0.05$ $n = 3$). However, total myosin was unchanged in cells stretched on laminin. These results suggest that synthetic peptides promote the same degree of cellular adhesion has their native proteins. However, they are unable to promote the signaling required for normal FAK expression and complete cytosketetal formation in myocytes. In addition, although these covalently adhered peptides provide improved cellular adhesion in response to mechanical stimulation, they promote aberrant myocytic gene expression. HL 64956 and HL 62426.

Abstract N° A22

***N*-cadherin determined the localization of connexin 43 through Rho pathway in cardiac myocytes**

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Gap junction formation is a critical event for normal heart development. To reveal the involvement of *N*-cadherin in the distribution of connexin 43 (Cx43) at the intercalated disk, the distribution of the cell–cell adhesion molecules, *N*-cadherin and Cx43, was analyzed in the aligned cardiac myocytes induced by mechanical stretch. Neonatal rat cardiac myocytes were plated for 3 h and exposed to 20% cyclic stretch for 24 h on silicone chambers. Stretch stimulation promoted cell orientation running in parallel to tension direction. After cultivation for 5 d, the Cx43 was co-localized with *N*-cadherin at the intercalated disk-like longitudinal oriented cell termini. Next, adenoviral gene transfer of dominant negative *N*-cadherin significantly attenuated the localization of Cx43 at cell termini. Furthermore the inhibition of Rho family pathways, downstream of cadherin, significantly cancelled the accumulation of Cx43, but not *N*-cadherin. Collectively, it is suggested that *N*-cadherin determined the localization of Cx43 through Rho pathway in cardiac myocytes. These findings provide pathophysiological insights into arrhythmogenesis resulting from the disturbance of cell–cell adhesion.

Abstract N° A23

Differential expression of the extracellular matrix (ECM) components mimecan and elastin during arteriogenesis

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Arteriogenesis, the enlargement of collateral vessels, is induced by fluid shear stress to compensate for the loss of blood flow in an occluded artery. The molecular analysis of this process identified the proteoglycan mimecan/osteoglycin (M) and elastin (E), the main component of the arterial wall, as participating molecules. Northern and western blot analysis showed a significant downregulation of M after 3, 7 d and 3 weeks of occlusion. Vascular SMC of the media were the main source of M in growing collaterals. TGF-β1 in combination with MCP-1 as well as FGF-2 and Oncostatin M (OSM) attenuated M mRNA levels in in vitro studies. Unlike M the protein levels of E were reduced only at early time points of arteriogenesis, indicative of an outward remodeling of the arteries, but increased during maturation, supported by elevated mRNA levels at 7 d and 3 weeks. Similar to M vascular SMC are the main source of E. In in vitro studies mRNA levels of E could be increased by stimulation with TGF-β1, PDGF-AB and IGF-I, while FGF-2 and OSM nearly completely abolished its expression. MCP-1 had no effect on E mRNA levels in vascular SMC. Our results implicate an extensive remodeling of the ECM during collateral growth, characterized by early downregulation of M and late upregulation of E. Arteriogenic growth factors like PDGF-AB, FGF-2 or TGF-β1, alone or in combination with MCP-1, are potent modulators of the expression of these ECM proteins and potential pharmaceutical agents for modulating arteriogenesis.

Abstract N° A24

Effect of l-arginine on cardiac function in *mdx* mice

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Duchenne muscular dystrophy (DMD) is a fatal condition occurring in approximately one in 3500 live male births caused by lack of the dystrophin protein. Initially DMD was considered a skeletal myopathy, however, improved care has caused an increased incidence and acknowledgement of car-

diomyopathy as a contributor to mortality. The dystrophin deficient *mdx* mouse is used as a model of DMD and in both *mdx* and DMD biopsy samples, deficiencies of neuronal nitric oxide synthase (nNOS) are evident. Without dystrophin, nNOS is misdirected reducing nitric oxide (NO) synthesis, possibly contributing to the muscle necrosis and fibrosis. On this basis l-arginine, a NO donor, was investigated for its potential to restore muscle function and reduce fibrosis. The *mdx* mice were treated for 6 months with 10% l-arginine in their drinking water while untreated *mdx* and C57BL10ScSn (C57) mice were used as controls. Subsequently the Langendorff technique was used to measure cardiac function. Control *mdx* hearts had reduced left ventricular contractility (d*P*/d*t*), and increased end diastolic pressure (EDP) and end systolic pressure (ESP) ($P < 0.05$) and an increase in hydroxyproline (HP) content ($P < 0.001$) relative to C57 hearts. Hearts from l-arginine treated *mdx* had a decreased stiffness and increased contractility relative to control *mdx* ($P < 0.04$ and $P < 0.06$) without affecting HP content in the left ventricle. l-arginine treatment may have potential to improve cardiac function significantly in the nNOS deficient dystrophic heart, thereby possibly improving prognosis for DMD patients.

Abstract N° A25

A novel mechanism of regulation of cardiac contractility by mitochondrial functional state

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It is generally considered that mitochondria regulate cardiac cell contractility by providing ATP for cellular ATPases and by participating in Ca^{2+} homeostasis. However, besides these well-documented functions, other possible mechanisms by which mitochondria can influence contractility have been largely overlooked. The densely packed subcellular architecture of the cardiomyocyte favors direct interactions between cellular structures such as mitochondria, sarcoplasmic reticulum and myofibrils. Functional studies using fibers with selectively permeabilized sarcolemma provide an excellent means to investigate the mitochondria–myofibrils interactions while maintaining the cellular architecture and controlling the intracellular medium, which mimics the ionic composition of cytosol. Here, we demonstrate that inhibition of the mitochondrial electron-transport chain strongly increases (by 39 ± 10%) Ca^{2+}-dependent and independent isometric force in permeabilized ventricular fibers. This effect is unrelated to the ATP-generating activity of mitochondria or Ca^{2+} homeostasis. Furthermore, various conditions that increase K^+ accumulation in the mitochondrial matrix (activation of ATP- or Ca^{2+}-dependent K^+ channels as well as inhibition of the K^+ efflux pathway via the K^+/H^+ exchanger) induce a similar mechanical response. Replacement of K^+ by another cation, tetraethylammonium, considerably diminished the mechanical response to mitochondrial de-energization. All modulators of mitochondrial function that augment isometric force also cause swelling of mitochondria in the vicinity of myofibrils in situ, as shown by confocal microscopy. Osmotic compression of intracellular structures abolishes the effect of mitochondria-induced force modulation, thus confirming the mechanical nature of interaction between the organelles. These findings suggest a novel mechanism for cellular regulation of myofibrillar function by mitochondrial K^+ homeostasis. Modulation of mitochondrial volume that imposes mechanical constraints within the cell, leading to an increase in force developed by myofibrils, might be involved.

Abstract N° A26

Inotropic responses to muscarinic stimulation of ventricular myocardium from developing chick and duck

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In mammalian ventricular myocardium, acetylcholine (ACh) shows negative inotropy only when the contractile force is elevated by stimulated adenylate cyclase. In the present study inotropic effects of ACh and carbachol (CCh) were examined in isolated ventricular preparations from embryonic and hatched chick and duck hearts. ACh and CCh produced positive inotropic response in the embryonic chick and duck ventricles, while both negative and positive inotropic responses in the hatched ventricles. Thus, positive inotropic effect was observed in both species at developing stages. Negative inotropic effect was observed only in hatched hearts and was not accompanied by action potential shortening. Experiments with specific antagonists and inhibitors revealed that the positive inotropy is mediated through M1 receptors and PLC, and the negative inotropy through M4 receptors and inhibition of adenylate cyclase. In conclusion, we found that ACh produces positive and negative inotropy in developing chick and duck ventricular myocardium, which is different from mammalian ventricle.

Abstract N° A27

Diastolic dysfunction is as bad as systolic dysfunction in heart failure on long-term basis

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Patients hospitalised for heart failure (HF) with normal left ventricular ejection fraction (LVEF) are mostly due to hypertension, LVH, DM and CAD and have mostly myocardial stiffness and relaxation abnormalities with increased Left Ventricular End Diastolic Pressures. The long-term outcome of these patients after initial hospitalisation is not very well defined. Hence 106 patients admitted with decompensated HF (NYHA class II–IV), diagnosed by pulmonary congestion on chest X-ray and evidence of volume overloading on physical examination with presence of normal LVEF and evidence of dominant diastolic dysfunction with abnormalities in *E*/*A* ratio, Deceleration time and isovolumetric

relaxation time on echocardiographic evaluation were taken as the study group. The survivors of indexed admissions were followed up for a period of 1.5–3.1 years (mean of 2.3 years). Mean age was 67.3 years. M/F ratio was 61/45 (57.5/42.5%); 53% patients were hypertensives, 44% had CAD, 39% were diabetics and 4.6% cases had HCM. Endpoints were annualised re-hospitalisation rate and mortality. Thirty deaths occurred with annualised mortality rate of 15.6% and annualised re-hospitalisation rate was 67% and these rates are comparable to those reported in patients of HF with systolic LV dysfunction (NYHA class II–IV). To conclude, patients of HF with normal EF and isolated Diastolic LV dysfunction have similar outcome especially in relation to mortality and re-hospitalisations as is the case with LV systolic dysfunction.

Abstract N° A28

Heart failure as a syndrome caused by instability of higher order process control

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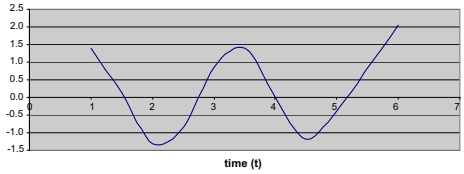
Heart failure is a complex syndrome of left ventricular systolic dysfunction, neurohormonal, inflammatory and endothelial dysfunction. Treatment focuses on neurohormonal blockade. As evidence accumulates, it is becoming increasingly apparent that the "neurohormonal theory" of heart failure is incomplete. The human cardiovascular system is under feedback control. These control loops are typically nonlinear and therefore referred to as higher order. I hypothesize that heart failure is a malfunction of process control. Specifically, heart failure is a syndrome in which feedback loops becomes unstable. The response of a control loop to a given input can be represented by the following equation: $c(t) = b_1e^{r1t} + b_2e^{r2t} + \ldots + b_ne^{rt}$ + (input terms); where $c(t)$ is the loop output or controlled variable and r_1, r_2, …, r_n are the eigenvalues or roots of the characteristic equation. Assuming that the input terms are bound as time increases, the loop's stability requires the following: for real roots $r = r$; r has to be <0 because e^{rt} approaches 0 as t approaches infinity if $r < 0$; for complex roots: $r = a + ib$; $e^{rt} = e^{at}(\cos bt + i \sin bt)$ where i is an imaginary number; a has to be <0, for $e^{at}(\cos bt + i \sin bt)$ to approach 0 as t approaches infinity. Factors affecting the eigenvalues include loop delays, changes in control gain, and structural changes of the system. Translating this to the human cardiovascular system, changes in the myocardium (MI, cardiomyopathy), vascular bed (afterload, preload) could result in an unstable control loop as shown the in example below.

Therapeutic changes including drugs and mechanical events could restore the stability of the control loop.



Abstract N° A29

Blood pressure generating capacity is a critical component in measuring overall cardiac function

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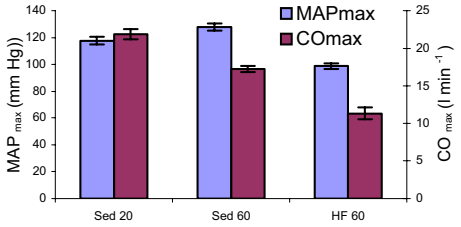
Peak exercise cardiac power (CP_{max}) incorporates both the pressure (BP) and flow (cardiac output, CO) generating capacities of the heart and is a measure of overall cardiac function (CF). Past research has tended to ignore the BP generating capacity of the heart, focusing mainly on measuring CO. CP was measured in healthy sedentary 20 and 60-year old subjects, and 60-year old heart failure (HF; NYHA III) patients. Cardiac output (CO) and mean arterial pressure (MAP) were measured non-invasively at rest and at maximal exercise, and CP (watts) calculated as (MAP × CO) × 2.2 × 10^{-3}. CO_{max} significantly ($P < 0.05$) declined (–21%) over these 40 years of healthy ageing (Fig. 1). In contrast MAP_{max} was significantly increased (+9%). Therefore, the true decline in overall function (i.e. CP_{max}) was not as great (–14%) as that predicted using CO_{max} alone (–21%). That is, the overall decline in CF was overestimated when BP was ignored. In contrast, in HF patients the decline in CF was underestimated by CO_{max}, because a significant ($P < 0.05$) decline in both CO_{max} (34%) and MAP_{max} (22%) contributed to a 61% decline in CP_{max}. These data stress the importance of measuring both BP and CO as both change in ageing and failing hearts.



Abstract N° A30

Cardiac power in the ageing, trained and failing heart

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Overall cardiac function, measured as cardiac power (CP) incorporates both the pressure and flow generating capacities of the heart. When measured in men at rest, CP_{rest} is around 1 W. However, to discriminate between the effects of age, disease or fitness levels the heart must be maximally stimulated, thereby revealing differences in CP_{max} and cardiac reserve (CR). Hence, the effects on CP of healthy ageing, with or without endurance training (Tr), and heart failure (HF) were studied. Except for the HF patients (NYHA III) all subjects were free from known diseases and medications. Cardiac output (CO) and mean arterial pressure (MAP) were measured non-invasively at rest and at maximal exercise, and

CP calculated as (MAP×CO) × 2.22 × 10^{-3}, and CR = CP_{max}–CP_{rest}. Older (60–70 years) sedentary (Sed) subjects had significantly (P < <0.05) lower (14–17%) CP_{max} than 20-year olds (Fig. 1). This decline in CP_{max} was further accentuated in HF patients (56%). In contrast, veteran athletes (Tr) demonstrated significantly higher CP_{max} (15%) and CR (17%) values than age-matched controls, with similar values to Sed 20-year olds (Fig. 1). These results indicate that CP declines with healthy ageing, and in HF. The age-related changes in CP can however be ameliorated by long-term training.

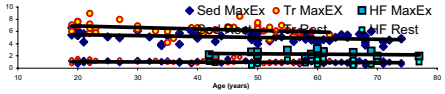

Fig. 1. Values = Mean ± SEM (n =10–15 per group).

Abstract N° A31

Age-and gender-related changes in cardiac power

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A 33% loss of cardiomyocytes has been reported between 20 and 70 years of age in men, whereas the equivalent loss in women has been much less. We tested whether these gender differences are reflected in overall cardiac function, when measured as cardiac power (CP) and incorporating both pressure and flow generating capacities of the heart. All male and female subjects were healthy and free from known diseases and medications. In all subjects at rest, CP_{rest} is around 1 Watt. However, to discriminate between the effects of age and gender the heart has to be maximally stimulated, thereby revealing differences in CP_{max} and cardiac reserve (CR). Cardiac output (CO) and mean arterial pressure (MAP) were measured non-invasively at rest and at maximal exercise, and CP was calculated as (MAP × CO) × 2.22 × 10^{-3}, with CR = CP_{max}–CP_{rest}. The rate of decline in CP_{max} and CR between 20 and 70 years for men and women were similar. However, the CR was approx 29% greater in men than women, although this was less after adjusting for body size. These data indicate that CR declines with healthy ageing in both sexes at a similar rate, despite reported greater losses of cardiomycotyes in men.

Abstract N° A32

Reduced expression of the sodium–calcium exchanger by RNAi decreases action potential duration in neonatal rat cardiomyocytes

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The sodium–calcium exchanger (NCX) is an electrogenic mechanism important for clearance of calcium that enters during the contraction cycle of the heart and is considered essential for heart function. Adenovirally delivered siRNA constructs were used to inhibit NCX expression in primary cultured neonatal rat cardiomyocytes. Following 3–6 d infection, the perforated patch clamp technique was used to record action potentials in cardiomyocytes stimulated at rates of 0.25–2 Hz. Expression of the NCX was only 5.6% in the knocked down cells relative to controls containing a scrambled sequence. Both cell types responded to electrical stimulation. Peak amplitude of the action potential was significantly lower in the NCX silenced cells compared to the scrambled controls, but there was no difference in the resting membrane potential between the two groups. Action potential durations were calculated at both 50% (APD_{50}) and 90% (APD_{90}) repolarization. No significant difference in APD_{90} was observed between the two groups at any stimulation rate tested. However, APD_{50} was significantly less in the NCX knock down cells when stimulated at 0.25–1.25 Hz compared to scrambled controls. In control cells, APD_{50} was inversely related to stimulation frequency, whereas no relation was observed in NCX silenced cells. Our data suggests the NCX is an important contributor to the shape of the cardiac action potential. This may suggest that the NCX is important in modifying L-type Ca^{2+} current. We have also shown that NCX activity is not required for beating of heart cells, raising importance of other calcium extrusion mechanisms.

Supported by the Heart and Stroke Foundation of Canada and CIHR.

Abstract N° A33

Inhibition of 4-AP sensitive transient outward potassium channel current (I_{to}) induced T-wave alternans in isolated rat heart model

Xi Wang, Weihong Liu, David A. Saint

T-wave alternans (TWA) is characterized as the beat-to-beat alternation of the morphology, amplitude and/or polarity of T-wave. TWA has been considered to be a marker of myocardial electrical instability and a predictor of lethal ventricular arrhythmias. Action potential duration alternans (APD alternans) of single myocytes is the primary basis of TWA, and block of repolarization currents such as rapid and slow delayed rectifier potassium currents are associated with TWA. However, the role of 4-AP sensitive I_{to} in TWA formation has not been determined as yet. In eight Langendorff perfused isolated rat hearts, ECG signals and monophasic action potentials (MAP) from epicardium, mid-myocardium and endocardium were simultaneously recorded. The heart was paced at basic cycle length (BCL) of 200 ms, and the effects of 4-AP at rapid pacing were investigated. Neither TWA nor alternation of MAP was induced in rat hearts without 4-AP perfusion. In the presence of 4-AP (2 mmol/l), increase in pacing rate with cycle length (CL) of 100 to 76 ms produced TWA and alternation of MAP. Mild to moderate

TWA and MAP alternans were induced at CLs ranging from 100 to 90 ms, while acceleration of CL to 86–76 ms resulted in more marked beat-to-beat alternation of T-wave and MAP. The alternation of MAP duration in epicardium was more prominent than that in mid-myocardium and endocardium. At CL of 76 ms, marked TWA and alternation of MAP were induced, followed by Torsade de pointes (Tdp) in one rat heart. Our results suggest that the blockade of I_{to} causes TWA and APD alternans in rat heart at rapid pacing and is associated with ventricular arrhythmia following acceleration of heart rate.

Abstract N° A34

Absence of mechano-electric feedback in isolated rat atrial tissue

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Mechano-electric feed back (MEF) is the process by which stretch directly affects the electrophysiology of the myocardium. Its physiological role is not clear although it has been suggested that changes in action potential morphology in stretched myocardium are important in minimising dispersion of repolarisation. Strips of isolated left ventricular and left atrial tissue from male Sprague–Dawley rats were stretched and action potentials recorded with intracellular electrodes using both repeat impalement and continuous recording. Even in the absence of stretch, action potential duration (APD), AP amplitude and resting membrane potential (RMP) were found to be surprisingly variable in closely neighbouring areas of the tissue. Repeat impalements within small regions gave more consistent results. However, even within a confined region, application of stretch produced either inconsistent changes ($n = 7$) or no discernable change in APD or RMP ($n = 10$). On a few occasions we achieved stable sustained impairments in rat left atrial tissue during stretch: in these recordings there was no change in RMP, action potential amplitude or APDs at tensions exceeding the peak of the Frank–Starling relation ($n = 4$). We conclude that MEF was not present in isolated rat atrial tissue under our experimental conditions.

Abstract N° A35

NIP-142 prolongs myocardial action potential duration through direct inhibition of GIRK1/4 channel

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NIP-142 is a novel benzopyran compound with antiarrhythmic activity against vagally induced atrial fibrillation in canine models (Circ. J. 66 (2002) 185). In anesthetized dogs, NIP-142 prolonged atrial refractory period under vagal stimulation. NIP-142 reversed carbachol- or adenosine-induced shortening of action potential. Binding assay with [^{3}H]AF-DX384 showed lack of NIP-142 binding to M_2-receptors. In isolated guinea-pig ventricular muscles, CCh showed a negative inotropic effect in the presence of forskolin, but not in its absence. NIP-142 had no effect on this negative inotropic effect of CCh indicating lack of effect on the M_2-receptor and Gi-protein. In HEK293 cells expressing hGIRK1/4 channels (I_{KACh} channel), NIP-142, as well as tertiapin, concentration-dependently inhibited hGIRK1/4 current. In conclusion, NIP-142 prolongs atrial action potential duration and refractory period through direct inhibition of the GIRK1/4 channel.

Abstract N° A36

The aging *mdx* mouse as a model of cardiomyopathy for Duchenne muscular dystrophy

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The absence of the myocyte protein dystrophin causes Duchenne muscular dystrophy (DMD), an X-linked myopathy affecting one in 3500 live male births. The incidence of cardiomyopathy has increased over the last decade as improved respiratory care has prolonged the lifespan of boys with DMD. The dystrophin deficient *mdx* mouse has been widely used for skeletal muscle research, but as a model of cardiomyopathy it has been poorly characterised. Male *mdx* mice and control C57BL10ScSn (C57) mice at 12 months of age showed increased sympathetic and decreased parasympathetic activity relative to both age and sex matched C57s and 12 week old male C57s ($P < 0.05$). In tissue bath studies, (—)-isoprenaline had a lowered efficacy and potency ($P < 0.05$) associated with a reduced affinity to the β_1-selective antagonist CGP20712A. Other manifestations of cardiac dysfunction include an increased incidence of arrhythmias in vivo as recorded by electrocardiography and in vitro in spontaneously beating right atria ($P < 0.05$). In left atria, a reduced potency and efficacy to calcium was evident ($P < 0.05$), associated with a delayed relaxation in spite of a shorter action potential at 90% repolarisation ($P < 0.05$). Similarly, ventricular contractility measured using the Langendorff technique reveals impaired contractility, and elevated end systolic and end diastolic pressure ($P < 0.05$). Finally, ventricles from *mdx* mice showed increased collagen levels evident by hydroxyproline measurements and picrosirius red staining of ventricular sections ($P < 0.05$). Thus the *mdx* mouse exhibits many features of cardiomyopathy that are similar to those evident in patients with DMD.

Abstract N° A37

Cardiac electro-mechanics: from CellML to the whole heart

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We have developed a computational modelling framework for cardiac electromechanics. The framework has been applied to the simulation of electrical activation and mechanical contraction of cardiac cells, myocardial tissue and anatomically based models of ventricular geometry. Detailed tissue microstructure is included in the tissue and ventricular models. Cellular models are specified using CellML (http://www.cellml.org), an XML language designed to store and exchange computer-based biological models. The CMISS (http://www.cmiss.org) computational package is used to integrate and describe the tissue and ventricular models. The integration of CellML into CMISS has been an important aspect of this work, resulting in the development of the open source C++ implementation of the CellML 1.0 Application Program Interface (API) available at http://cellml.sourceforge.net/. The framework we have implemented provides the ability to specify not only spatial variation of cellular material parameters (e.g. transient outward channel density through the ventricular wall), but also spatial variation of the cellular models themselves (e.g. Purkinje fibre models coupled to ventricular myocytes). By using CellML as the standard format for the cellular models, we have allowed for the models to be defined by any piece of cellular modelling software capable of exporting the model to CellML, and gained access to public model repositories. This work is one of the first steps in the development of a simulation environment for the IUPS Physiome Project (http://www.physiome.org.nz), aimed at providing a framework for the simulation of models describing organisms from proteins through to whole organ systems.

Abstract N° A38

Involvement of death receptor signaling in mechanical stretch-induced cardiomyocyte apoptosis

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Mechanical overload associated with hypertension causes cardiomyocyte apoptosis, yet the intracellular signaling mechanism leading to apoptosis has not been fully characterized. In the present study, we presented evidences suggesting the involvement of death receptor signaling in mechanical stretch-induced apoptosis. The major finding are as following: (1) the stretch-induced activation of caspases 8, 9 and 3, upregulation of Fas, FasL expression and cell surface trafficking of death ligands (FasL and TRAIL); (2) exogenous death ligand (TRAIL) enhanced while soluble death receptor (sDR5) neutralized stretch-induced apoptosis; (3) adenovirus delivered dominant negative FADD significantly decreased apoptosis and caspases 8, 9, and 3 activation; (4) moreover, it also attenuated stretch-induced cytochrome *c* release from mitochondria. These data demonstrated the involvement of death receptor signaling in cardiomyocyte apoptosis-induced by mechanical stretch and further suggested the crosstalk between the death receptor signaling and mitochondria-dependent apoptotic signaling. Our data provide novel clues for further understanding the mechanism of mechanical overload-induced cardiomyocyte apoptosis.

Abstract N° A39

Improvement of vasorelaxation by endothelin receptor antagonist CPU 0213 is mediated by suppression on preproET-1 of the vascular wall in diabetic rats

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Aim. – The endothelial lesion in diabetes could be related to an excess of endothelin (ET)-1 which causes dysfunction of vasorelaxation. A novel ET receptor antagonist CPU 0213 a dual blockader is tested to recover the impairment of vasorelaxation in the STZ-induced diabetes.

Methods. – Rats were injected with STZ 60 mg/kg i.p. developed sustained hyperglycemia in 4 weeks and treated with CPU0213 80 mg/kg p.o. and aminoguanidine (AGD) 100 mg/kg p.o. for 28 d, separately. The vasorelaxation to acetylcholine, measurement of ET-1, mRNA of preproET-1 in myocardium and thoracic aorta, iNOS and NO in serum were conducted.

Results. – ET-1 raised significantly in serum in diabetic rat by 39.5% and a reduction in ET-1 level was significant by CPU 0213 (–40.4%) and AGD (–30.0%). In the diabetic model the mRNA abundance of preproET-1 was increased dramatically in the myocardium (104%) and aortic wall (581%), respectively. CPU 0213 and AGD reduced preproET-1mRNA in myocardium (–42% and –28%, respectively) and thoracic aorta (–83% and –80%). The iNOS mRNA increased in diabetic aorta (305%) and was reduced by 0213 and AGD (–70% and –38%). The iNOS activity in serum was elevated in the model (865%), and reduced by 0213 and AGD (–81% and –51%). The NO in serum was increased in model significantly (96%) and reduced by 0213 and AGD (–16% and –18%). The maximal vasorelaxation was reduced in the model (–69%), and improved by 0213 and AGD(151% and 125%).

Conclusion. – The novel endothelin receptor antagonist CPU 0213 which improves significantly vasorelaxation of the diabetic thoracic aorta is more effective to suppress overexpression of preproET-1 mRNA in thoracic wall.

Supported by a key project of National Science Foundation of China No. 30230170.

Abstract N° A40

Alterations in cardiac dihydropyridine receptors and calcium channel function in *mdx* mice

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Duchenne muscular dystrophy (DMD) is a fatal neuromuscular condition affecting approximately one in 3500 live male births resulting from the lack of the myocyte protein dystrophin. The absence of dystrophin in cardiac myocytes is associated with calcium overload which in turn activates calcium-dependent proteolytic enzymes contributing to congestive heart failure, muscle necrosis and fibrosis. To date, the basis for the calcium overload has not been determined. Since L-type calcium channels are a major mediator of calcium influx we determined their potential contribution to the calcium overload. Male muscular dystrophy (*mdx*) mice and control C57BL10ScSn (C57) mice aged 12–16 weeks were used in all experiments. In tissue bath studies, isolated contracting left atria from *mdx* revealed a reduced potency to the dihydropyridine (DHP) agonist BayK8644 and antagonist nifedipine ($P < 0.05$). Similarly, radioligand binding studies using the DHP antagonist [^{3}H]-PN 200-110 showed a reduced potency ($P < 0.05$) in isolated membranes, associated with an increased receptor density ($P < 0.05$). The increased receptor density was supported by RT-PCR experiments revealing increased RNA for the DHP receptor. Patch clamp studies revealed the presence of a diltiazem sensitive calcium current that showed delayed inactivation in isolated *mdx* myocytes ($P < 0.01$). In conclusion, the increased number of DHP binding sites and the delay in L-type current inactivation may both contribute to increased calcium influx and hence calcium overload in the dystrophin deficient *mdx* cardiac myocytes.

Abstract N° A41

Testing AKT modulation of I_{CAL} by gene transfection in HEK293 cells

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The kinase AKT mediates antiapoptotic/hypertrophic signaling downstream to phosphatidilinositol kinase PI3K, a pathway activated by numerous growth factors and involved in "compensatory" cardiac hypertrophy. This study tests whether the DHP-sensitive Ca^{2+} channel α-subunit (DHPR) is a target of AKT-mediated modulation.

Methods. – HEK293 cells were transfected with the relevant plasmid vectors and the current/voltage relation of DHP-sensitive Ca^{2+} current (I_{CaL}) was evaluated by patch-clamp; Ba^{2+} was substituted to Ca^{2+} as charge carrier.

Results. – Transfection with a vector encoding DHPR and the reporter GFP under the same promoter (DHPR/GFP) led to expression of I_{CaL} in all GFP + cells (peak density at 25.6 ± 3 mV = 29.3 ± 2.8 pA/pF; $n = 17$). Co-transfection of these cells with constitutively active AKT isoform (AKT+) led to complete suppression of I_{CaL} in 83% (10/12) of cells. A similar result was obtained with inactive AKT isoform (AKT–), which suppressed I_{CaL} in 83% (5/6) of cells. In both cases, co-transfection of AKT isoforms markedly enhanced GFP fluorescence signal. In cells with stable expression of AKT+, DHPR/GFP transfection induced I_{CaL} in 43% of GFP+ cells only (3/7, peak density = 8.0 pA/pF). A similar result was obtained in cells with stable AKT–.

Summary. – Co-transfection with AKT inhibited DHPR functional expression irrespective of whether its kinase activity was enhanced or inhibited. Since DHPR-bound GFP signal was enhanced, DHPR/GFP gene transcription was probably stimulated, rather than inhibited by AKT co-transfection. Thus, a mechanism downstream to regulation of gene transcription and independent of AKT kinase activity is likely to account for AKT-induced inhibition of DHPR functional expression in HEK293 cells. While biochemical assays are required to confirm this view, our preliminary data suggest that this experimental model may be unsuitable to test DHPR modulation by AKT-mediated phosphorylation.

Abstract N° A42

Role of the L-type Ca current in the dissociation between systolic Ca and sarcoplasmic reticulum Ca content observed at physiological stimulation frequencies in the rat

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Previously, we have investigated systolic Ca and sarcoplasmic reticulum (SR) Ca content under conditions of physiological temperature and stimulation rates using current clamp control. Here on increasing stimulation frequency between 4 and 6 Hz SR Ca content and Ca transient amplitude increased in parallel. However at 8 Hz SR content was increased yet Ca transient amplitude decreased compared to 6 Hz data. This was not due to changes in action potential duration. Thus, we have investigated the role of the L-type Ca current. Changes in $[Ca]_i$ were measured using Fluo-3 AM. Data are presented as mean ± SEM from n experiments. Peak L-type Ca current amplitude decreased with stimulation frequency at 1, 4, 6 and 8 Hz (7.2 ± 1.3, 5.1 ± 1.0, 3.6 ± 0.7 and 2.3 ± 0.5 pA pF^{-1}; $P < 0.05$, $n = 5$). The time constant of decay of the L-type Ca current increased with stimulation frequency (1 Hz, 8.1 ± 0.7; 4 Hz, 8.7 ± 0.4; 6 Hz, 9.7 ± 0.3 and 8 Hz, 11.3 ± 0.5 ms, $n = 5$). Under these conditions, Ca transient amplitude decreased with increasing stimulation frequency (285 ± 45, 243 ± 41, 181 ± 31 and 116 ± 24 nmol l^{-1}; $P < 0.05$ between 4, 6 and 8 Hz, $n = 5$–6).

This was partly due to a rise in diastolic Ca ($P < 0.05$ between all frequencies). We suggest that a decrease in trigger Ca for Ca-induced Ca release, brought about by a reduction in peak L-type Ca current at high stimulation frequencies, may contribute to the dissociation between systolic Ca and SR Ca content observed under current clamp conditions. The associated decrease in Ca transient amplitude may be responsible for the increasing time constant of inactivation of the L-type channel due to a reduction in Ca-dependent inactivation.

Abstract N° A43

Reverse engineering the L-type Ca channel α_{1c} subunit in adult cardiac myocytes using novel adenoviral vectors

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The α_{1c} subunit is the main component of the cardiac L-type Ca^{2+} channel, containing the channel pore, voltage- and Ca^{2+}-dependent gating structures, and drug binding sites. Although well-studied in heterologous expression systems, many questions regarding the biophysical properties of cardiac L-type channels in intact cardiomyocytes remain unanswered, including the physiological sites of channel phosphorylation, and which structures influence cardiac excitation–contraction coupling. The latter has been impossible to study due to the inability to reconstitute the spatial organization of the dyad. To overcome these problems, we developed adenoviral constructs with E1, E3 and fiber gene deletions, to permit incorporation of full length, α_{1c} gene cassettes into the adenovirus backbone. Full-length wild type ($\alpha_{1c\text{-}wt}$) and mutant ($\alpha_{1c\text{-}qm}$), as well as a C-terminal truncated α_{1c} subunit ($\alpha_{1c\text{-}dm}$) adenoviruses were constructed; the two latter viruses were mutated at residues critical for dihydropyridine binding. $\alpha_{1c\text{-}wt}$, $\alpha_{1c\text{-}qm}$ and α4.19 pA/pF $n = 5$; $\alpha_{1c\text{-}qm}$ 3.2 pA/pF $n = 30$; $\alpha_{1c\text{-}dm}$ 19.36 pA/pF, $n = 5$), and, as expected, I_{Ca} carried by $\alpha_{1c\text{-}dm}$ (K_i 10.8 μM) was markedly less sensitive to nitrendipine than $\alpha_{1c\text{-}wt}$ (K_i 80 nM); a feature exploited to discriminate between engineered- and native-channel currents in transduced guinea-pig myocytes. Ten micromolar nitrendipine blocked only 51 ± 5% ($n = 9$) of I_{Ca} in $\alpha_{1c\text{-}dm}$-transduced myocytes, in comparison to 86 ± 8% ($n = 9$) of I_{Ca} in control myocytes. Further, evoked calcium transients, measured with indo-1, were observed in $\alpha_{1c\text{-}dm}$ -transduced cells, but were largely blocked in control myocytes, in the presence of 10 μM nitrendipine, indicating that the engineered channels were coupled to sarcoplasmic reticular Ca^{2+} release. These novel α_{1c} adenoviruses provide an unprecedented method for structure-function studies of cardiac excitation–contraction coupling and L-type channel regulation in the native myocyte background.

Abstract N° A44

Age associated alterations in the cardiac L-type calcium current: role in excitation–contraction coupling

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Ageing is the greatest risk factor for the development of heart failure. We have investigated whether ageing is associated with alterations in cardiac excitation–contraction coupling that may predispose the aged myocardium to heart failure. Experiments were performed on left ventricular mid-myocardial myocytes from young (<1.5 years) and aged (>8 years) sheep. Cells were voltage clamped and Fluo-3AM used to measure changes in intracellular calcium concentration. Cells from aged animals exhibited a 26% increase in membrane capacitance, 21% increase in width and 4% increase in length ($P < 0.005$) indicating cellular hypertrophy with ageing. The systolic calcium (Ca^{2+}) transient amplitude increased in aged myocytes (115 ± 16 vs. 178 ± 17, $P < 0.05$). This was not due to a difference in sarcoplasmic reticulum Ca^{2+} content (43 ± 4 vs. 45 ± 3 μmol/l, $P > 0.6$). In aged myocytes peak L-type Ca^{2+} current increased by 175% (–40 to 8 mV, $P < 0.001$) and integrated Ca^{2+} entry during the voltage clamp pulse increased by 55% ($P < 0.01$). In addition, there were also slight changes in the properties of activation and voltage-dependent inactivation of the L-type Ca^{2+} current such that the window current increased by 20% in the ageing myocytes. We conclude that together, the increase in peak Ca^{2+} current (that which triggers release of Ca^{2+} from the sarcoplasmic reticulum) and the increased total Ca^{2+} entry via the L-type Ca^{2+} current, will serve to produce the observed increase in the systolic Ca^{2+} transient in aged hearts without the need to change sarcoplasmic reticulum calcium content. Furthermore the greater window current will facilitate a larger Ca^{2+} entry to support contraction under normal physiological conditions where the aged cells have a longer action potential duration.

Supported by the British Heart Foundation.

Abstract N° A45

Differences in L-type calcium current between infant and adult human atrial myocytes

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Developmental differences in the amplitude of L-type calcium current (I_{Ca}) and its regulation by sympathetic tone have been demonstrated in several animal studies. We investigated differences in β-adrenergic regulation of I_{Ca} between infant (INF, 1–12 months), young adult (YAD, 14–18 years),

and older adult (AD) human atrial myocytes. Atrial myocytes were dissociated from biopsies of right atrial appendages of patients undergoing open-heart surgery. Basal I_{Ca} in INF atrial myocytes (1.2 ± 0.1 pA/pF) was significantly smaller than YAD (2.5 ± 0.2 pA/pF) or AD (2.6 ± 0.3 pA/pF) myocytes. The maximal I_{Ca} produced by isoproterenol was similar for INF (8.4 ± 1.1 pA/pF), YAD (9.6 ± 1.0 pA/pF), and AD (9.2 ± 1.3 pA/pF) cells. The efficacy of isoproterenol was larger for INF (E_{max} 607 ± 50%), than for YAD (E_{max} 371 ± 29%) or AD (E_{max} 455 ± 12%) myocytes. The potency was higher for AD (EC_{50} 0.82 ± 0.09 nM) or YAD (EC_{50} 0.41 ± 0.14 nM) than for INF myocytes (EC_{50} 7.6 ± 3.5 nM). Western blotting showed similar levels of $G_{i\alpha2}$ but much greater levels of $G_{i\alpha3}$ for INF vs. AD or YAD atrial tissue. When we inhibited the activity of $G_{i\alpha3}$, by including the C-terminal peptide of $G_{i\alpha3}$ in the pipette, both basal I_{Ca} and the response to 10 nM isoproterenol were increased for INF cells but not for YAD cells. In summary, there are significant differences in the properties of I_{Ca} between INF and either YAD or AD human atrial myocytes. We propose that basal I_{Ca} and response to low-dose isoproterenol are inhibited in INF (but not YAD or AD) cells by constitutive inhibitory effects of $G_{i\alpha3}$.

Abstract N° A46

NADP$^+$/NADPH plays a role in regulating K$^+$- and Ca^{2+} channels in vascular smooth muscle and cardiac myocytes

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Recent studies from our laboratory have demonstrated that inhibition of pentose phosphate pathway (PPP) by 6-aminonicotinamide, epiandrosterone and dihydroepiandrosterone dilates aorta, coronary (CA) and pulmonary (PA) artery, and inhibits myocardial contractility. In the CA, PPP inhibitors have been found to suppress intracellular Ca^{2+} release and influx, and in the PA inhibition of PPP decreases Kv channel currents. Evidences in the smooth muscle indicate that neither cAMP nor cGMP mediates relaxation induced by the PPP inhibition. In the isolated rat hearts, inhibition of PPP decreases $\pm dp/dt$ and suppresses myocardial contractility. Electrophysiological evidences further suggested that PPP inhibitor epiandrosterone decreases L-type Ca^{2+} channel activity, since PPP inhibition decreases activation and accelerates decay of the Ca^{2+} currents. Furthermore, steady-state inactivation curve is shifted to more negative potentials by PPP antagonist, this indicates that PPP inhibition stabilized channels in inactivated state as well as inactivated open channels. Therefore, these results suggest that PPP-derived NADPH and/or $NADP^+$ may be involved in directly regulating ion channel function.

Abstract N° A48

Proteomics approach to the study of cardiovascular disease

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Heart failure is not a uniform disease entity, but a syndrome with various causes, including hypertension, ischemia and congenital heart disease, cardiomyopathy, myocarditis and intoxication. The characterization of biological processes on the basis of alterations in the cellular proteins, or "proteomic" analysis, is a powerful approach that may be adopted to decipher the signaling mechanisms that underlie various pathophysiological conditions, such as ischemic heart disease. The application of proteomics provides major opportunities to elucidate disease mechanisms and to identify new diagnostic markers and therapeutic targets. The purpose of the present study was to evaluate difference of the cardiac marker protein expression in three types of heart tissue (control, ischemic preconditioning (IPC), and ischemia–reperfusion) using two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). We have compared rabbit control heart 2DE gel with ischemia preconditioning and ischemia 2DE gels, respectively. Appearances in expression of some proteins were observed in ischemia–reperfusion heart gel, but this spots were not detected or decreased in control and IPC heart gels. For further examination of molecular characteristics, this spots in 2DE were isolated and subjected to trypsin digestion followed by MALDI-MS analysis. We were able to identify this spots, and which was confirmed by western immunoblot. Our result was consistent with previous studies of cardiac marker in ischemic heart disease and showed that proteins are suitable cardiac marker as measure of myocardial injury. In addition, through the multiple techniques, including 2DE, MALDI-MS, and western immunoblot, proteomic analysis is appropriate means of the identification of cardiac marker in study of IPC and ischemia–reperfusion.

Abstract N° A49

Pivotal role of gp91^{phox}-containing NADPH oxidase in early ischemic preconditioning

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Reactive oxygen species (ROS) signaling is implicated in early ischemic preconditioning (PC). A gp91^{phox}-containing NADPH oxidase is a recognised source of ROS in cardiac myocytes, whose activity is augmented by preconditioning-

mimetics such as angiotensin II. We hypothesised that this oxidase is an essential source of ROS in PC. Hearts from wild-type (WT) and gp91^{phox} knockout (KO) mice were Langendorff perfused and subjected to 35 min ischemia/reperfusion with or without preceding PC or drug treatment. Infarct size was measured by triphenyl tetrazolium chloride staining and NADPH oxidase activity by lucigenin chemoluminescence. PC significantly attenuated infarct size in WT (26 ± 2% vs. WT control, 38 ± 2%, $P < 0.05$), yet was ineffective in KO hearts (33 ± 3% vs. KO control, 34 ± 3%). Concomitantly, PC significantly increased NADPH oxidase activity in WT (+41 ± 13%; $P < 0.05$), but not in KO (+1 ± 28%, P = NS). The ROS scavenger *N*-2-mercaptopropionyl glycine (MPG, 300 nmol/l) abrogated PC in WT (39 ± 2% vs. 33 ± 1%). 2-Chloro-*N*6-cyclopentyl adenosine (CCPA 200 nmol/l), a putative ROS-independent PC trigger, significantly attenuated infarct size in WT, MPG-treated WT and KO hearts (24 ± 2%, 23 ± 1% and 20 ± 3%, respectively, $P <$ 0.05). Furthermore, CCPA did not augment NADPH oxidase activity over control (+22 ± 11%, P = NS). Inhibition of protein kinase C (PKC) with chelerythrine (CHE, 2 nmol/l), completely abrogated both PC (36 ± 2% vs. CHE alone, 33 ± 3%) and associated increases in oxidase activity (+3 ± 10%, P = NS). Thus, we have demonstrated PKC-dependent activation of a gp91^{phox}-containing NADPH oxidase is pivotally involved in early ischemic PC. However, adenosine receptor activation is able to trigger a ROS and gp91^{phox} independent PC pathway.

Abstract N° A50

Ischemic postconditioning reduced infarct size in normal and hypercholesterolemic rabbit hearts

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Brief episodes of ischemia/reperfusion performed immediately after a prolonged ischemia episode (ischemic postconditioning) reduce infarct size in dogs. The objective was to determine if this mechanism was present not only in normal rabbit hearts, but in hypercholesterolemic animal hearts as well. Isolated and isovolumic rabbit hearts were perfused according to Langendorff technique and subjected to 30 min of global ischemia followed by a 30 min reperfusion (G1, n = 9). In G2 (n = 8) the protocol of G1 was repeated, but immediately after ischemia an ischemic postconditioning protocol was performed that consisted of two episodes of ischemia/reperfusion of 30 s each one. In G3 (n = 6) and G4 (n = 6) protocols of G1 and G2 were, respectively, repeated but the animals were previously fed with 1% cholesterol enriched diet, during 4 weeks. The left ventricular developed pressure (LVDP, mmHg), end diastolic pressure (LVEDP), and infarct size were measured using TTC after 30 min of reperfusion. Plasma cholesterol levels were determined as 47.3 ± 9.3 mg/dl in normal animals and 340 ± 128.8* mg/dl in animals fed with cholesterol enriched diet.

	LVDP	LVEDP	Infarct size
G1	39.8 ± 4.1	48.2 ± 4.5	16.6 ± 2.1
G2	37.6 ± 4.4	66.6 ± 12.5	4.2 ± 1.5 *
G3	29.2 ± 5.3	56.1 ± 6.1	30.6 ± 2.6 *
G4	35.1 ± 8.1	61.5 ± 8.8	5.5 ± 1.1**

X ± SEM.
*$P < 0.05$ vs. G1.
**$P < 0.05$ vs. G3.

Ischemic postconditioning reduced infarct size in normal and hypercholesterolemic rabbit hearts without modifying postischemic ventricular function. Although infarct was greater in hypercholesterolemic animals, the protection reached was similar in both groups.

Abstract N° A51

Ischemic preconditioning induced by coronary angioplasty in patients: effect of hyperlipidemia

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Although ischemic preconditioning provides a remarkable cardioprotection in animals and humans, its effectiveness is limited in some disease states, such as hyperlipidemia. We studied ischemic preconditioning induced by coronary angioplasty in hyper- and normocholesterolemic patients by means of beat-to-beat analysis of intracoronary ST-segment elevation. Thirty patients of significant single-vessel coronary disease were divided into normocholesterolemic and hypercholesterolemic (n = 15 each) groups. Intracoronary ECG and intra-aortic pressure were continuously recorded during three 2 min balloon inflations with 5 min intervals. In normocholesterolemic patients, ST-segment was continuously increasing during occlusions. Repeated occlusions significantly attenuated ST-elevation from 1.28 ± 0.67 to 0.88 ± 0.51 mV ($P <$ 0.001) when measured at the end of the last occlusion, showing the effect of preconditioning. In hypercholesterolemic patients, ST-segment rapidly increased in the initial 30 s of the first occlusion. However, in these patients, repeated occlusions did not attenuate ST-segment elevation at the end of the last occlusion (1.24 ± 1.11 mV vs. 1.21 ± 1.09 mV, non-significant). We conclude that (i) beat-to-beat analysis of ST-segment shift is a suitable method for the fine assessment of the evolution of the severity of ischemia; (ii) hypercholesterolemia attenuates the anti-ischemic effect of preconditioning in patients, and leads to an early, rapid increase in ST-segment elevation during the first ischemic challenge. These results show that the ability of the heart to adapt to ischemic stress is diminished in hypercholesterolemic patients.

Abstract N° A52

Hypoxia-inducible factor-1α mediates anti-apoptosis of hypoxic preconditioning in cardiomyocytes from neonatal rats

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Ischemic preconditioning (IPC) is a term that is used to describe the phenomenon that brief ischemic episodes can attenuate tissue injury caused by subsequent sustained ischemia/reperfusion. The cardioprotection of IPC including anti-apoptosis has been demonstrated in hypoxic preconditioning model of cardiomyocytes. To investigate whether hypoxia-inducible factor-1α (HIF-1α) mediates anti-apoptosis of hypoxic preconditioning in cardiomyocyte, we measured the apoptosis rate of cardiomyocytes at 6 or 12 h after hypoxia/reoxygenation, activities of extracellular signal-regulated protein kinases (ERKs), and expression of HIF-1α in cultured cardiomyocyte from neonatal S.D. rats. We found that the apoptosis rate of cardiomyocytes in hypoxic preconditioning group decreased 10.92% and 14.34% at 6 and 12 h after hypoxia/reoxygenation ($n = 6$, $P < 0.05$), respectively. We also found that hypoxic preconditioning increased the abundance of phospho-ERK1/2 by 3-folds and expression of HIF-1α by onefold in whole cell extracts from hypoxic preconditioned cardiomyocytes. PD98059, an inhibitor of the upstream kinase of ERKs, abolished the anti-apoptosis effect, ERKs activation, and expression of HIF-1α induced by hypoxic preconditioning. We concluded that hypoxic preconditioning protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis and upregulation of HIF-1α through ERKs pathway mediates the cardioprotection of hypoxic preconditioning.

Keywords: Hypoxia-inducible factor–1α; Hypoxic preconditioning; Apoptosis

Abstract N° A53

ERKS-mediated phosphorylation of HIF-1α is associated with cardioprotection of hypoxic preconditioning

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Hypoxia-inducible factor-1α (HIF-1α) is a heterodimer composed by HIF-1α and HIF-1β. The phosphorylation of HIF-1α is upregulated upon hypoxia and regulates transcription of the hypoxia-inducible genes and cellular survival following hypoxia. To determine whether the cardioprotection of hypoxic preconditioning (HPC) is mediated by phosphorylation of HIF-1α through extracellular signal-regulated protein kinases (ERKs) pathway, neonatal cardiomyocytes from Sprague–Dawley rats were divided into HPC, hypoxia/reoxygenation (H/R), PD98059 + HPC, BDM + HPC, and control groups. We measured viability of cardiomyocytes, lactate dehydrogenase (LDH) release, ERKs activity, and phosphorylation of HIF-1α in HPC neonatal cardiomyocytes. We found that the HPC cardiomyocytes showed an increase by 22% in survival, a decrease by 52% in LDH release from cardiomyocytes subjected to lethal H/R 24 h after HPC. The protective effects of HPC were abolished either by PD98059 which inhibits ERKs, or by BDM (an activator of protein phosphatase). Western blot analysis showed activated ERKs in whole cell extracts from HPC cardiomyocytes. SDS-PAGE mobility shift experiments showed increased phosphorylation level of HIF-1α in HPC group, and the phosphorylation was blocked by PD98059 or BDM. We conclude that ERKs-induced phosphorylation of HIF-1α is associated with cardioprotection of HPC.

Keywords: Hypoxic preconditioning; Cardiomyocyte; Hypoxia-inducible factor-1

Abstract N° A54

Different mechanisms contribute to preconditioning vs. d-myo-inositol trisphosphate-induced cardioprotection

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Recent studies from our group revealed that: (1) concentrations of the second messenger inositol trisphosphate (IP3) are increased in hearts 'preconditioned' (PC) with brief antecedent ischemia; and (2) pretreatment with a synthetic analog of IP3 (d-myo-IP3) elicits a significant reduction of infarct size (IS). The mechanisms by which d-myo-IP3 evokes cardioprotection are unknown; but may involve cardiac gap junctions (a site at which external IP3 receptors are purportedly located), and protein kinase C (PKC: a mediator of PC, also reportedly co-localized and involved with gap junction phosphorylation). To assess these potential candidates, isolated buffer-perfused rabbit hearts underwent 30 min of coronary artery occlusion and 3 h of reflow, with IS delineated by tetrazolium staining and expressed as a percentage of the risk region (RR). In Protocol 1, hearts were randomized to receive d-myo-IP3 (6 μM), 5 min brief PC ischemia or a matched control period. Protocols 2, 3, and 4 were identical, except all hearts were treated with the PKC inhibitor chelerythrine, the classic but non-specific gap junction blocker heptanol, and the novel and selective peptide-based gap junction inhibitor, Gap 27, respectively. Protocol 1 confirmed that IS/RR was reduced in both PC and d-myo-IP3-treated groups vs. controls (29* + 4% and 31* + 7% vs. 52 + 6%; *$P < 0.05$). Chelerythrine, at a dose known to block translocation of PKC, had no effect on IS in controls (49 + 6%), abrogated the benefits of PC (IS/RR: 48 + 6%), but failed to block d-myo-IP3-induced protection (IS/RR: 29 + 4%; $P < 0.05$ vs. matched controls). Conversely, heptanol and Gap 27, at doses confirmed to block gap junctions, abrogated d-myo-IP3-induced protection (IS/RR: 45–54%; P = ns vs. matched controls), but did not block PC (IS/RR: 14–21%; $P < 0.05$ vs. matched controls). Thus, while both d-myo-IP3 and PC limit IS, this comparable protection is achieved via different cellular mechanisms.

Abstract N° A55

Bcl-2 levels are increased in endothelial cells by antioxidant supplementation but not exercise training

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Atherosclerotic plaque contains apoptotic cells of endothelial origin with oxidative stress implicated in this process. The dietary supplements vitamin E and α-lipoic acid have been shown to be a potent antioxidant combination with the potential to prevent endothelial apoptosis. Furthermore, regular exercise is known to increase myocardial protection however little research has investigated the effects on the endothelium. Therefore, the purpose of this study was to examine the effect of antioxidant supplementation and/or exercise training on Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic) proteins in coronary endothelial cells. Young male rats received either a control or antioxidant-supplemented diet (vitamin E and α-lipoic acid) and were assigned to sedentary or exercise-trained groups for 14 weeks. Left ventricular endothelial cells were isolated and Bcl-2 and Bax protein levels were measured. Antioxidant supplementation alone caused a fourfold increase in Bcl-2 ($P < 0.05$) with no significant change in Bax ($P > 0.05$). Consequently, Bcl-2:Bax, a marker of cellular protection, was increased sixfold with antioxidant supplementation compared to non-supplemented animals ($P < 0.05$). Exercise training had no significant effect on Bcl-2, Bax or Bcl-2:Bax either alone or combined with antioxidant supplementation ($P > 0.05$). Antioxidant supplementation did not affect myocardial Bcl-2 compared to non-supplemented animals ($P > 0.05$). We conclude that dietary supplementation of vitamin E and α-lipoic acid increases endothelial cell Bcl-2 whereas exercise training has no such effect.

Abstract N° A56

Intermittent hypoxic training protects canine myocardium from infarction

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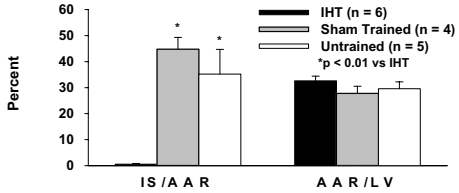
This investigation examined cardiac protective effects of intermittent hypoxia training (IHT). Six dogs underwent IHT for 20 d in a normobaric chamber ventilated intermittently with N_2 to reduce O_2 to 9.5–10%. To assess resistance of ventricular myocardium to infarction, after anesthesia the left anterior descending coronary artery (LAD) was occluded for 60 min and then reperfused for 5 h. Radioactive microspheres were injected systemically to assess coronary collateral flow into the ischemic region. After 5 h reperfusion, the heart was dyed to delineate the area at risk (AAR), and stained to identify infarcted myocardium (IS). During LAD occlusion and reperfusion, systemic hemodynamics and global left ventricular function were stable. Average collateral flow in the inner 2/3 of the center of the AAR was 0.20 ± 0.09 ml/min/g (four hearts had collateral flow <0.12 ml/min/g). In four hearts no infarcted tissue was detected, and in two hearts, infarction equaled 0.5 g (1.4% of AAR). In contrast, four sham trained dogs and five untrained dogs subjected to similar periods of LAD occlusion and reperfusion had large infarctions (see Fig. 1). In conclusion, IHT protects canine myocardium from infarction.



Support: NIH grant HL64785 and Hypoxia Medical Academy, Moscow, Russia.

Abstract N° A57

BAX ablation protects against myocardial infarction

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Isolated hearts lacking the *bax* gene demonstrated more cardioprotection than wild type, following myocardial ischemia/reperfusion injury. To explore the effect of the *bax* gene in vivo following myocardial infarction (MI), two groups were studied: homozygotic *bax* gene deficient (–/–) knockout and matched wild type mice, which underwent left anterior descending coronary artery (LAD) ligation. Echocardiography was performed before surgery and at 1 or 4 weeks after MI. Left ventricular end diastolic diameter (LVEDd), end systolic diameter (LVESd), and fractional shortening (FS), infarct size and caspase 3 activity were measured. Post-infarct mortality was 25% in both groups. The progressive increase in LVEDd and LVESd in Bax (–/–) was significantly smaller compared to the wild type, 28 d following MI ($P < 0.03$). Concomitantly, FS was higher in Bax (–/–), (35 ± 4.1% and 27 ± 2.5%, $P < 0.001$). Infarct size was smaller in the Bax (–/–) than wild type (24 ± 3.7% and 37 ± 3.3%, $P < 0.001$). Caspase 3 activity was elevated at 2 h after MI only in the wild type, but reduced to baseline values at 1 and at 28 d post-MI. Hearts of Bax knockout mice demonstrated reduced infarct size and superior myocardial function. Further investigation is required concerning the *bax* gene and its intracellular mechanisms that appear to play a significant role in the post-MI response.

Abstract N° A58

Role of Erk-mediated suppression of gap junction permeability in cardioprotection afforded by mitoK_{ATP} channel activation

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Our recent study showed that suppression of gap junction (GJ) permeability during myocardial ischemia significantly limited infarct size (IS) (Am. J. Physiol. 286 (2004) H214). In the present study, we examined the hypothesis that cardioprotection afforded by activation of the mitochondrial ATP-sensitive K^+ channel (mitoK_{ATP}) is mediated by ERK-induced suppression of GJ permeability. To assess GJ permeability in the ischemic myocardium, rabbit ventricular myocardium was incubated in anoxic buffer containing Lucifer yellow (2.5 μg/ml). A mitoK_{ATP} opener, diazoxide (100 μM), induced phosphorylation of ERK1/2 and reduced transport of Lucifer yellow during 20-min ischemia by 70%. PD98059 (10 μM), a MEK1/2 inhibitor, abolished suppression of GJ permeability and ERK phosphorylation by diazoxide. In isolated perfused rabbit hearts, IS after 30-min global ischemia was 57.1 ± 3.7% of the left ventricle. Pretreatments with 100 and 10 μM diazoxide reduced IS to 5.0 ± 1.3% and 21.5 ± 10.5% of the left ventricle, respectively. PD98059 inhibited cardioprotection by 10 μM diazoxide (IS = 55.8 ± 7.3%) but not by 100 μM (IS = 6.8 ± 0.3%). These results suggest that ERK-mediated suppression of GJ permeability is crucial for cardioprotection by a low level of mitoK_{ATP} activation and that additional mechanisms play roles in protection by a high level of mitoK_{ATP} activation.

Abstract N° A59

Detection of Ca^{2+}-activated and ATP-sensitive potassium channels in the rat cardiac mitochondria

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Ion channels on the mitochondrial membrane influence cell function in specific ways that can be detrimental or beneficial to cell survival. At least one type of potassium channel, mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channels play a pivotal role in early and late ischemic preconditioning. And another channel with properties similar to the surface membrane calcium-activated K^+ channel was found on the mitochondrial inner membrane (mitoK_{Ca}) of rat ventricular cells. In this study, we investigated the subunit composition of the rat heart mitoK_{ATP} channels and K_{ca} channel proteins were present in mitochondrial membrane. Mitochondria were isolated and purified by differential centrifugation and visualized by confocal microscopy and visualized mitoK_{ATP} channels by means of green fluorescence probe BODIPY-glibenclamide labeling. Enriched mitochondrial protein was estimated by Bradford method. Western blotting was performed with antibodies against the known K_{ATP} channel subunits (the sulfonylurea receptor, SUR1, -2 and the inwardly rectifying potassium channel Kir6.1 or 6.2) and K_{Ca} channel (the surface membrane BK_{Ca} channel). The results showed that two known bands of ~44 and ~46 kDa Kir6.2, and ~46 kDa Kir6.1 proteins was enriched in the mitochondria. And we observed that heart mitochondria appeared to be significantly enriched in SUR2-specific bands found at ~140 kDa. We also observed that two major bands of about 55 and 140 kDa, similar to the predicted size of the α-subunit and β-subunit of K_{Ca}. Our results indicate that mitoK_{ATP} channels compose of Kir6.1, Kir6.2 subunits and a SUR2-related sulfonylurea binding protein in rat heart mitochondria. Also our results findings identify mitoK_{ca} in rat cardiac mitochondria.

Abstract N° A61

Changes in myocardial protein expression in hypoxic preconditioned rat heart

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Two-dimensional gel electrophoresis (2-DE) is a powerful tool for proteomic analysis, providing us with valuable information on differential protein expression. The aim of the present study is to detect the changes in myocardial protein expression in hypoxic-preconditioned (HPC) rat heart. Male Sprague–Dawley rats (200–220 g) were randomly divided into either HPC or control group. Rats in HPC group were subjected to systemic hypoxic exposure (10 ± 0.4 O_2) for 4 h followed by a 24-h period of normoxic reoxygenation. Rats in control group were time matched with the preconditioned group and maintained under normoxic conditions for 28 h. Rats were euthanized and the hearts were removed. Whole cell extracts were prepared from myocardium. 2-DE was carried out with IPG-DALT systems and the protein spots were stained and detected by ImageMaster 2D Elite software. The results were that 1271 ± 93 protein spots were resolved in HPC myocardium and the match rate is 82.2 ± 7.5%. The results also showed that 45 protein spots displayed quantitative changes in HPC myocardium, among which 33 showed higher expression and 22 decreased in abundance. Therefore, HPC induces differential expression of myocardial proteins in rats. We suggest that the alteration of protein expression may attribute to the cardioprotection of HPC.

Keywords: Hypoxic preconditioning; Two-dimensional polyacrylamide gel electrophoresis; Myocardium

Abstract N° A62

Preconditioning improves post-ischemic mitochondrial function and diminishes oxidation of mitochondrial proteins

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This study examined the hypothesis that preconditioning improves post-ischemic mitochondrial function by attenuating oxidation of mitochondrial proteins. Isolated rat hearts were exposed to 25 min global ischemia (37 °C), and 60 min reperfusion. Hearts were preconditioned by short ischemia and reperfusion (IP); or by 50 μM nicorandil (Nic). IP and Nic significantly ($P < 0.05$) improved post-ischemic hemodynamic function. The respiratory control ratio (RCR) in isolated mitochondria was depressed by 61% in control hearts at the end of reperfusion. IP and Nic significantly ($P < 0.05$) improved post-ischemic RCR. Improvement was partially abolished by 200 μM 5-hydroxydecoanate (5HD) or 300 μM *N*-(2-mercaptopropionyl)-glycine (MPG). Furthermore, mitochondria from ischemic hearts had significantly ($P < 0.05$) less ability to resist swelling upon Ca^{2+} loading, which was improved by both IP and Nic. Carbonyl content of multiple mitochondrial proteins was elevated after ischemia; and still elevated by the end of reperfusion. IP and Nic attenuated the increased protein oxidation observed at the end of ischemia. The protective effects of IP and Nic were partially abolished by 5HD. These studies support the conclusion that one mechanism for enhanced post-ischemic function in the preconditioned heart is improved mitochondrial function as a result of decreased oxidation of mitochondrial proteins.

Abstract N° A63

Effects of nuclear factor-κB on inhibition of cardiomyocyte apoptosis induced by hypoxic preconditioning

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Apoptosis is one of the characteristics of ischemia/reperfusion injury. Hypoxic preconditioning (HPC) attenuates cell apoptosis. In the present study, we investigated whether protein kinase C (PKC)-mediated upregulation of nuclear factor-κB (NF-κB) attributed to inhibition of apoptosis by HPC in neonatal cardiomyocytes subjected to hypoxia/reoxygenation. Cultured cardiomyocytes from neonatal Sprague–Dawley rats were divided into four groups: Hypoxia/reoxygenation, hypoxia preconditioning, hypoxia preconditioning + PKC inhibitor H7, and control. Morphological changes in apoptotic cardiomyocytes were detected by the DNA specific fluorochrome Hoechst 33258 under the invert microscope. The apoptotic rates of cardiomyocytes were measured. Whole cell extracts were prepared for western blotting analysis to measure the expression of PKC and NF-κB. It was found that the HPC reduced apoptosis ratio of cardiomyocytes subjected to sustained hypoxia/reoxygenation by 28.7% and upregulated nPKC ϵ expression to 2.5-fold and NF-κB expression to 1.3-fold. The effects of HPC on nPKC ϵ, NF-κB expression, and cardioprotection were abolished by H7, an inhibitor of PKC.We conclude that HPC protects cardiomyocytes from apoptosis induced by hypoxia/reoxygenation and PKC-mediated upregulation of NF-κB is involve in the cardioprotection.

Keywords: NF-κB; Hypoxic preconditioning; Apoptosis

Abstract N° A64

Roles of microtubules in ischemic preconditioning against myocardial infarction

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The aim of this study was to determine roles of microtubules in ischemic preconditioning (PC). Myocardial infarction was induced in isolated rabbit hearts by 30-min global ischemia and 2-h reperfusion, and infarct size was expressed as a percentage of the left ventricle (% IS/LV). Using separate groups of rabbits, ventricular biopsies were taken before and after PC for determination of protein kinase C (PKC) translocation and p38-mitogen activated protein kinase (p38MAPK) activation. To depolymerise microtubules, we used two structurally different agents, colchicine (50 μM) and nocodazole (1 μM). PC with two cycles of 5-min ischemia/5-min reperfusion significantly reduced IS from 60.1 ± 5.0% to 20.0 ± 5.0%. Although neither colchicine nor nocodazole modified IS in non-preconditioned hearts, these agents abolished the IS-limiting effects of PC (% IS/LV = 56.8 ± 2.2% and 53.4 ± 2.5%, respectively). Colchicine prevented PKC-ϵ translocation and p38MAPK activation by PC. Furthermore, PKC translocation by infusion of 1-oleyl-2-acetyl-sn-glycerol was also prevented by colchicine. However, colchicines did not interfere with negative inotropic and chronotropic responses of the myocardium to adenosine. These results suggest that microtubules play a crucial role in transmission of signals from G proteins to PKC in the mechanism of anti-infarct tolerance afforded by PC.

Abstract N° A66

Biochemical changes in cardiac myocytes obtained at different stages of ischemic preconditioning from rabbit model

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Various biochemical markers are associated with ischemic preconditioning (IP) of cardiac myocytes. We studied the role of various markers like superoxide dismutase (SOD), glutathione peroxidase (GSH), malondialdehyde (MAD), intracellular calcium (Ca^{2+}), protein kinase C (PKC), norepinephrine (NEp), and nitric oxide byproducts–(citrulline) and reactive nitrogen intermediates (RNI) in IP of rabbit heart using isolated cardiac myocyte preparation. Each of Sham group (A) and five preconditioned groups (B–G) had eight rabbits. Preconditioned groups were–as follows (B) 5 min coronary ligation, (C) 5 min ligation → 10 min recovery, (D) 30 min ligation, (E) 5 min ligation → 10 min recovery → 30 min ligation, (F) 5 min ligation → 10 min recovery → 30 min ligation → 30 min recovery, and (G) adenosine infusion → 30 min ligation → 30 min reperfusion. Following anaesthesia, left anterior descending (LAD) was ligated. Thereafter, myocytes were isolated from LAD, left circumflex (LCx) and right coronary artery (RCA) territory to estimate various markers. SOD and GSH were significantly increased in all three territories in Groups F and G. MAD level in membranous fraction of myocytes showed significant increase in Groups C and E in all three territories. Intracellular Ca^{2+} was significantly increased in Groups F and G in LAD territory only. In Groups F and G of LAD territory, the translocation of PKC from cytosol to membrane was significantly increased. Citrulline and RNI levels were significantly increased in E, F and G Groups in all three territories. Groups F and G did not show significant increase in NEp level in any territory. Various biochemical markers like SOD and GSH, membranous MAD, intracellular Ca^{2+}, citrulline, RNI, NEp, and PKC are contributing to IP in Group F (preconditioned group) and adenosine treated group (Group G).

Abstract N° A67

Effect of mechanical stress on myocardial anti-oxidant enzymes and ischemic preconditioning in rabbit heart

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We studied the contribution of mechanical stress in raising anti-oxidant enzymes levels in cardiac myocytes during ischemic preconditioning in rabbit hearts. Eight rabbits in each sham group and pulmonary artery ligation group were studied. Following anaesthesia, pulmonary artery (PA) was ligated partially for 2 min (till right ventricular dilatation was visualized), followed by 30 min of reperfusion. In sham group, the heart was directly removed without PA ligation. Thereafter, cardiac myocytes were isolated from left anterior descending (LAD), left circumflex (LCx), and right coronary artery (RCA) territory to estimate enzymes namely superoxide dismutase (SOD) and glutathione peroxidase (GSH). Values of levels of SOD and GSH in two groups are presented in the table as mean ± SEM. Both SOD and GSH levels in cardiac myocytes were significantly increased in PA ligation group compared to sham group in RCA territory. The levels were higher but not statistically significant in other territories compared to sham group. The table denotes * $P < 0.05$ as compared to sham. Mechanical stress can produce a rise in the anti-oxidant enzyme levels and can play an important role in ischemic preconditioning. Groerritory RCA territory

Groups	LAD territory	LCx territory	RCA territory
SOD (Enzyme U/mg protein)			
Sham group	0.56 ± 0.09	0.52 ± 0.11	0.57 ± 0.06
PA Group	0.61 ± 0.02	0.70 ± 0.04	1.06 ± 0.02 *
GSH (nmol/mg protein)			
Sham group	3.07 ± 0.26	2.93 ± 0.40	3.03 ± 0.40
PA group	3.72 ± 0.26	3.10 ± 0.13	6.71 ± 0.20 *

Abstract N° A68

Heat shock protein 70 mediates effects of metabolic inhibition preconditioning or kappa opioid receptor stimulation on calcium homeostasis in the rat ventricular myocytes subjected to ischaemic insults

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Heat shock protein 70 (HSP70) plays an important role in delayed cardioprotection of preconditioning. Attenuation of cytosolic calcium ($[Ca^{2+}]_i$) overload is believed to be responsible for cardioprotection. There is evidence suggesting a link between HSP70 and $[Ca^{2+}]_i$ homeostasis. We hypothesize that activation of HSP70 by preconditioning may restore $[Ca^{2+}]_i$ homeostasis altered by ischaemic insults. To test the hypothesis, we determined the effects of preconditioning with metabolic inhibition or U50,488H (a κ-opioid receptor agonist) on viability and injury, HSP70 expression, and $[Ca^{2+}]_i$ alterations in ventricular myocytes subjected to ischaemic insults (MI and anoxia, MI/A), with blockade of the synthesis of HSP70. In myocytes with vehicle pretreatment, the percentage of dead cells detected by trypan blue exclusion, the injury reflected by release of lactate dehydrogenase (LDH) and the resting $[Ca^{2+}]_i$ significantly increased, while the amplitude of electrically induced $[Ca^{2+}]_i$ transient decreased, after 10 min incubation with 10 mM 2-deoxy-d-glucose (2-DOG) and 10 mM sodium dithionite, known to cause MI/A. However, when the myocytes were subjected to 30 min pretreatment with either 20 mM lactate and 10 mM 2-DOG (MIP) or 30 μM U50,488H (UP) 20 h before MI/A, the changes in viability and injury, and $[Ca^{2+}]_i$ responses were significantly attenuated. These were accompanied by a

significant increase in HSP70 expression. Furthermore, blockade of the synthesis of HSP70 with a selective antisense oligonucleotides abolished the beneficial effects of MIP or UP on cellular viability and $[Ca^{2+}]_i$ responses. This study provides evidence that activation of HSP70 induced by preconditioning, which conferred delayed cardioprotection, restored, at least partially, the $[Ca^{2+}]_i$ homeostasis altered by ischaemic insults. The observations also suggest that activation of HSP70 may confer cardioprotection by restoring $[Ca^{2+}]_i$ homeostasis.

Supported by HKRGC.

Abstract N° A69

Cardioprotective actions of melatonin: receptor-mediated or due to its reactive oxygen species scavenging?

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Melatonin (mel), a hormonal product of the pineal gland, is a powerful cardioprotectant, probably due to its reactive oxygen species (ROS) scavenging properties. Mel receptors have been identified in the myocardium, but it is unknown whether they participate in mel-induced cardioprotection. In contrast, mel may counteract the protective effects of ischaemic preconditioning (IP), a process dependent on ROS release. The aims of this study were to investigate the effect of (a) mel on IP as well as NO-induced preconditioning and (b) mel antagonists on mel-induced cardioprotection. Isolated perfused rat hearts were subjected to either 20 min global ischaemia or 35 min regional ischaemia preceded by (i) no interventions (non-IP), (ii) 3 × 5 min IP in the presence or absence of mel 50 μM, (iii) 3 × 5 min NO preconditioning (induced by sodium nitroprusside (SNP) 100 μM), in the presence or absence of mel 50 μM. The effect of mel 50 μM on infarct size was studied after 35 min regional ischaemia in the presence or absence of the mel-receptor blockers, Luzindole 5 μM or *N*-acetyl tryptamine (NAT) 5 μM.

Aortic output (ml/min) during reperfusion

Non-IP	IP	IP + Mel	SNP	SNP + Mel
6.25 ± 1.70	19.1 ± 1.7 *	7.1 ± 2.88	14.10 ± 1.31 *	16.6 ± 3.06 *

P < 0.05 vs. non-IP

Effect of melatonin on infarct size

Non-IP	Mel	Mel+Luzindole	Mel+NAT
34.8 ± 1.8	16.8 ± 2.6 *	27.2 ± 2.1 **	21.4 ± 2.8

* Non-IP vs. Mel $P < 0.001$.
** Luzindole vs. Mel $P < 0.001$.

Conclusions. – Mel-induced cardioprotection is partially receptor mediated. Due its ROS scavenging properties it attenuates IP, but has no effect on NO preconditioning.

Abstract N° A70

Possible involvement of changes in mitochondrial calcium uptake in cardio-protection by ischemic preconditioning

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Intensive studies have demonstrated that the mechanism of cardioprotection by ischemic preconditioning (IPC) is closely related to mitochondria. It was reported that IPC can reduce mitochondrial calcium content in ischemic myocardium. However, whether mitochondrial calcium uniporter participates in the cardiac effect of IPC has not been elucidated. The aim of this study is to determine whether mitochondrial calcium uniporter plays a role in the cardioprotection by IPC and its relationship to mitochondrial permeability transition pore (MPTP). Isolated rat hearts were subjected to 30 min regional ischemia (ligation of left anterior descending artery) followed by 120 min reperfusion. We found that IPC (5 min global ischemia + 5 min reperfusion, two cycles) reduced the infarct size (IPC vs. control, 13.09 ± 3.65% vs. 44 ± 3.6%, $P < 0.01$), which was associated with improved recovery of left ventricular developed pressure (LVDP) and left ventricular end-diastolic pressure (LVEDP) at 30 min of reperfusion ($P < 0.01$). Spermine, an activator of mitochondrial calcium uniporter, which was administrated at 20 μM during first 10 min of reperfusion, attenuated IPC-induced decrease of infarct size (IPC + spermine vs. IPC, 35.6 ± 4.0% vs. 13.09 ± 3.65%, $P < 0.01$), and preserved recovery of LVDP and LVEDP ($P < 0.01$). Release of lactate dehydrogenase from coronary effluent in spermine-treated IPC heart was more than that in the IPC heart. During first 10 min of reperfusion, application of ruthenium red (5 μM), an inhibitor of mitochondrial calcium uniporter, had similar effect with IPC. Treatment with cyclosporin A (CsA, 0.2 μM, an inhibitor of MPTP opening) during last 5 min of ischemia and first 15 min of reperfusion also showed similar cardiac effect with IPC. Co-administration of spermine (20 μM) with CsA (0.2 μM) during reperfusion abolished the effect of spermine. The results indicate that inhibition of mitochondrial calcium uptake is involved in the cardioprotection of IPC via inhibiting MPTP.

Abstract N° A71

University of Wisconsin vs. Celsior for donor heart preservation with cyclosporin A

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In previous studies, 12 h of heart preservation using University of Wisconsin solution (UW) at 4 °C and micro perfusion (1 ml/min) in a canine heart model resulted in 100% functional recovery. Eighteen hours of preservation resulted in 50–60% functional recovery with no necrosis but significant amounts of apoptotic cells (TUNEL). Blocking apopto-

sis with cyclosporin A (CsA) by treating the donor animal (10 mg/Kg) and adding CsA to preservation solution (10^{-5} mol/l), resulted in functional recoveries of 100% after 18 and 24 h of preservation. The viscosity of UW at 4 °C may prevent adequate perfusion during preservation. In the present experiments, Celsior (Cs) was used, (less viscous) to determine the efficacy of heart preservation with and without CsA. After preservation for 24 h, hearts were heterotopically transplanted and studied biochemically, morphologically and functionally during 6 h of reperfusion. When combined with CsA, both preservation solutions were equally effective with 100% functional recovery (Cs hearts reached this level faster-between 2 and 3 h while 5–6 h for UW). Apoptosis was less with Cs and CsA preservation. In conclusion, Cs was a better solution for extended periods of heart preservation. Although the destructive processes are not blocked or eliminated by CsA, their delay through mitochondrial mechanisms (permeability transition pore blockade) provides a viable heart graft after 18–24 h of preservation.

Abstract N° A72

Anti-infarct effect of magnesium is not mediated by adenosine A1 receptor in the globally ischemic isolated rat heart

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Attention is growing for a potential role of magnesium (Mg) in the pathophysiology of cardiovascular disease. Clinical impact of Mg therapy remains controversial in acute myocardial infarction. The present study investigated the infarct size. Isolated rat hearts ($n = 28$) were used for Langendorff perfusion. Control hearts were perfused at 37 °C for 180 min, global ischemia (GI) hearts received 30 min of global ischemia and 120 min reperfusion, Mg hearts received a bolus injection of magnesium sulfate ($MgSO_4$) 15 min before the global ischemia, Mg/DPCPX, a selective antagonist adenosine A1 receptor, hearts received DPCPX (200 nM) 5 min before the $MgSO_4$. Infarct size was measured by triphenyltetrazolium chloride method. The rate pressure product (RPP) was calculated as an index of ventricular contractile function and RPP 120 min after reperfusion was taken as an endpoint of functional protection. Infarct size was significantly smaller in Mg group (0.2 ± 0.13%) compared to GI group (43.41 ± 1.63%) $P < 0.001$. The infarct size limiting effects of Mg was not abolished by DPCPX. Mg enhanced post-ischemic functional recovery. RPP was significantly enhanced to 88.34 ± 3.1% ($P < 0.001$ vs. GI). The present findings raise the possibility that reduction of infarct size with Mg dose not involve enhanced adenosine-mediated cardioprotection but adenosine mediate protection action of Mg on post-ischemic function recovery. Another important finding of this study is that Mg administration has an infarct size limiting effect independently its effect on post-ischemic functional recovery in rats.

Abstract N° A73

Adenosine A1 and opioid receptor cross-talk in the isolated murine heart

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We have previously shown cross-talk between adenosine and opioid receptors in vivo. As there is evidence supporting an interaction between opioids and adenosine in the CNS, we sought to examine if the cross-talk observed in vivo was present in a model devoid of CNS innervation. To this end, we employed an isolated mouse heart model where hearts were subjected to 25 min global ischemia followed by 45 min reperfusion. Following reperfusion, untreated hearts exhibited an elevated end-diastolic pressure (EDP) of 29 ± 3 mmHg, while rate–pressure product (%RPP) returned to 40 ± 5% of baseline. Treatment with CHA (1 μM) or morphine (30 μM) significantly reduced EDP (17 ± 1 and 15 ± X mmHg, respectively, $P < 0.05$) and improved contractile function (62 ± 2.2% and 66 ± 2% RPP, respectively, $P < 0.05$). Lactate dehydrogenase (LDH) release was significantly reduced in both agonist-treated groups. Pretreatment with opioid antagonist naloxone (100 μM) effectively reduced the recovery of %RPP in CHA-treated hearts (50 ± 3%) implying a role for the adenosine A1 receptor. Furthermore, 200 nM of the A1 adenosine receptor antagonist, DPCPX, abrogated the cardioprotective effects of morphine. Phosphporylation of GSK-beta and ERK1/2 was also significantly increased in morphine-treated hearts. This increase in phosphorylation was completely blocked by DPCPX. Thus, cross-talk between adenosine A1 and opioid receptors appears to exist in a model lacking intact CNS innervation, and may provide novel approaches to cardioprotective therapeutics.

Abstract N° A74

The delta opioid agonist fit is protective 48–120 h after administration via PI3K

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Chronically administered morphine achieves an enhanced cardioprotective effect, however, the mechanism of protection, including the opioid receptor subtype and protein kinases involved, are unknown. Recent evidence suggests that the phosphatidylinositol-3 kinase (PI3k) pathway is involved in acute opioid-induced cardioprotection (OIC), however, it is unknown whether PI3k is involved in chronic OIC. Hence, we analyzed the efficacy and window of cardioprotection provided by FIT, a novel irreversible δ opioid agonist, and whether PI3k mediates chronic OIC. Male Sprague–Dawley rats were administered FIT either acutely or chronically and then subjected to 30 min ischemia and 2 h of reperfusion followed by infarct size (IS) assessment (Mean ± SEM%, significance, $^{*}P < 0.01$ or $^{**}P < 0.001$). Acute doses of FIT showed greatest IS reduction at 0.01 mg/kg given either

10 min before ischemia or 5 min before reperfusion compared to control (41.7 ± 1.0*, 45.9 ± 1.8* vs. 60.0 ± 1.1%, respectively). Chronic IS reduction was also present with FIT (0.01 mg/kg) 48, 72, 96, and 120 h vs. control (45.4 ± 1.3*, 42.4 ± 3.1**, 30.0 ± 3.3**, 46.2 ± 0.7*, vs. 60.0 ± 1.1%, respectively). Wortmannin (WORT, 15 μg/kg), an irreversible PI3k inhibitor, given 1 h prior to FIT, abolished IS reduction at 96 h (55.3 ± 4.9%). Data obtained from H9C2 cells showed that FIT (1 μM) phosphorylates GSKβ, a kinase downstream of PI3k, 10 min after stimulation. This effect was abolished by pretreatment with WORT (100 nM). These data suggest that chronic OIC via the δ opioid receptor occurs via PI3k.

Abstract N° A75

Cardioprotection of k-opioid receptor stimulation with interleukin-2 or U50,488H involves inhibition of MPTP opening

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Previous study demonstrated that kappa opioid receptor stimulation with a kappa opioid receptor (κ-OR) agonist, U50,488H (U50), or interleukin-2 (IL-2) confers cardioprotection. Inhibiting mitochondrial permeability transition pore (MPTP) opening at reperfusion has also been shown to confer cardioprotection of ischaemic preconditioning. We tested the hypothesis that stimulation with U50 or IL-2 also confers cardioprotection by inhibiting MPTP opening. Cardiac injury was evaluated by measuring infarct size and lactate dehydrogenase (LDH) release in response to 30 min ischemia and 120 min reperfusion in the isolated rat heart. The MPTP opening was observed in calcein-loaded isolated myocytes and isolated mitochondria. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) also was determined in the isolated ventricular myocytes suffered from 5 min anoxia and 10 min reoxygenation by spectrofluorometry. Pretreatment of the isolated perfused heart with IL-2 (50 U/ml) or U50 (10 μM) decreased the infarct size and LDH release, effects blocked by blockade of κ-OR with a selective κ-OR antagonist, nor-BNI. Moreover, blockade of protein kinase C (PKC) with its inhibitor, GF109203X (GF, 10 μM), also attenuated the protective effect of both IL-2 and U50. The infarct and LDH reducing effects of IL-2 or U50 or PMA were blocked by atractyloside (Atr, 20 μM), a MPTP opener. Inhibition of the MPTP opening with cyclosporin A (CsA, 0.2 μM) reduced both infarct size and LDH release, which could not be blocked by GF. In isolated ventricular myocytes subject to anoxia and reperfusion, the end diastolic $[Ca^{2+}]_i$ increased while the amplitude of $[Ca^{2+}]_i$ transient decreased. These changes were attenuated by either IL-2 or U50. The effects of IL-2 and U50 were abolished with opening of the MPTP with Atr. Finally we showed that IL-2 and U50 decreased MPTP opening in calcein-loaded myocytes, and reduce calcium-induced mitochondrial swelling. The present study provides the first evidence that κ-OR stimulation with IL-2 and U50 protects the myocardium by inhibiting of MPTP opening. PKC may be one of the upstream components of signal transduction pathway.

Supported by The Research Grants Council, Hong Kong.

Abstract N° A76

The delta opioid agonist fit is protective 48-120 hours after administration via PI3k

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Chronically administered morphine achieves an enhanced cardioprotective effect, however, the mechanism of protection, including the opioid receptor subtype and protein kinases involved, are unknown. Recent evidence suggests that the phosphatidylinositol-3 kinase (PI3k) pathway is involved in acute opioid-induced cardioprotection(OIC), however, it is unknown whether PI3k is involved in chronic OIC. Hence, we analyzed the efficacy and window of cardioprotection provided by FIT, a novel irreversible d opioid agonist, and whether PI3k mediates chronic OIC. Male Sprague Dawley rats were administered FIT either acutely or chronically and then subjected to 30 minutes ischemia and 2 hours of reperfusion followed by infarct size (IS) assessment (Mean ± SEM %, significance = $*P < 0.01$ or $**P < 0.001$). Acute doses of FIT showed greatest IS reduction at 0.01mg/kg given either 10 minutes before ischemia or 5 minutes before reperfusion compared to control (41.7 ± 1.0*, 45.9 ± 1.8* vs. 60.0 ± 1.1 %, respectively). Chronic IS reduction was also present with FIT (0.01mg/kg) 48, 72, 96 and 120 hours versus control (45.4 ± 1.3*, 42.4 ± 3.1**, 30.0 ± 3.3**, 46.2 ± 0.7*, vs.60.0 ± 1.1 %,respectively). Wortmannin (WORT, 15μg/kg), an irreversible PI3k inhibitor, given 1 hour prior to FIT, abolished IS reduction at 96 hours (55.3 ± 4.9 %). Data obtained from H9C2 cells showed that FIT(1μM) phosphorylates GSKβ, a kinase downstream of PI3k, 10 minutes after stimulation. This effect was abolished by pretreatment with WORT(100nM). These data suggest that chronic OIC via the δ opioid receptor occurs via PI3k.

Abstract N° A77

Sarcolemmal Na/Ca exchange and mitochondria are both involved in Ca paradox induced cell injury: effects on $[Ca]_i$ and $[Ca]_m$

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The Ca paradox results in marked Ca overload and cell damage. Recently, it has been reported that mitochondrial K_{ATP} channel (mitoK_{ATP}) and mitochondrial permeability transition pore (mPTP) could be involved in the cardioprotective effects against Ca overload. We investigated the un-

derlying mechanisms of protective effects by monitoring $[Ca]_i$ (by fura-2 ratio:R) and the cell death during Ca paradox protocol in intact rat myocytes, and $[Ca]_m$ (by rhod-2) in saponin-permeabilized myocytes. (1) On Ca repletion following 15 min Ca depletion, 90 of 210 cells (43%) died, and R increased in these cells (ΔR; 1.51 ± 0.06, means ± S.E., $P < 0.01$). (2) KB-R7943 (10 μM; an inhibitor of Na/Ca exchange) completely inhibited both the cell death and increase in R (0%, 0.14 ± 0.02, $n = 30$, $P < 0.01$). (3) Diazoxide (Dz; 100 μM, a mitoK_{ATP} opener), ruthenium red (RR; 10 μM, an inhibitor of Ca uniporter) and cyclosporin A (CsA; 1 μM, an inhibitor of mPTP) prevented cell death (26%, 15%, 17%, $n = 81–125$, $P < 0.05$), and the protective effects of Dz were abolished by 5-hydroxydecanoate (100 μM; a mitoK_{ATP} inhibitor). (4) Dz and RR decreased $[Ca]_m$, but CsA did not. (5) Dz decreased NADH fluorescence whereas RR or CsA did not alter it. In conclusion, (1) a rapid increase in cytosolic Ca concentration via Na/Ca exchange causes cell damage in Ca paradox, (2) the protective effects of Dz could be ascribed to altered Ca regulation by decreasing $[Ca]_m$ and by modifying oxidative phosphorylation, and (3) the protective effects of CsA could be directly associated with mPTP.

Abstract N° A78

Cardioprotection with diazoxide and pinacidil: protonophoric mechanism of action

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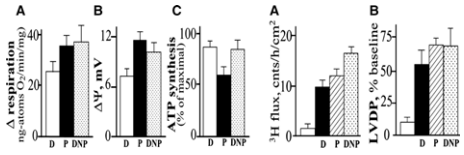
Excessive build-up of mitochondrial protonic potential is harmful to cellular homeostasis, and modulation of inner membrane permeability a proposed countermeasure. Here, we demonstrate that structurally distinct potassium channel openers, diazoxide (D) and pinacidil (P), facilitated transmembrane proton translocation generating H^+-selective current. Both openers depolarized mitochondria, activated state 4 respiration and reduced oxidative phosphorylation, recapitulating the signature of mitochondrial uncoupling (Fig. 1). This effect was maintained in K^+-free conditions, and shared with the prototypic protonophore 2,4-dinitrophenol (DNP). D, P, and 2,4-DNP (Fig. 2), but not 2,4-dinitrotoluene lacking protonophoric properties, preserved functional recovery of ischemic heart. The identified protonophoric property of potassium channel openers, thus, implicates a previously unrecognized component in their mechanism of cardioprotection.



Abstract N° A79

Antimycin A (AA) a mitochondrial free radical generator mediates cardioprotection via p38 mapkinase (p38)

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Low concentration free radicals may have a role in cell signalling. Antimycin A (AA) is a mitochondrial free radical generator thought to work at site III of the electron transport chain. The p38 has a controversial role in cardioprotection. We examined the effect of AA on p38 activation. We also examined whether AA is cardioprotective and if so whether SB203580 (SB), a p38 inhibitor has any effect on cardioprotection. C57Blk/6 or MKK3 knockout (KO) mice were treated in a Langendorff model with 3 min AA (0.1 μg/ml) followed by 5 min perfusion then freeze clamped for protein analysis by western blotting. Also C57Blk/six hearts were perfused with AA for 3 min and 10 min washout followed by 30 min global ischaemia and 2 h reperfusion. A similar protocol was performed but with the AA bracketed with 1 μM SB. Controls of SB alone and DMSO were used. Infarct size (IS) was analysed in all groups. AA caused activation of p38 and HSP27. This was similar in the MKK3 KO mice. AA caused a significant reduction in infarct size and this reduction was lost when AA was bracketed with SB. The p38 appears to be activated by AA independently of MKK3 and inhibition of p38 blocks AA cardioprotective effect. Infarct size/left ventricle size (%IS/LV) $P < 0.05$:

Group ($n = 6$ *in each group*)	% IS/LV ± SEM
DMSO	48.3 ± 5.2
SB	48 ± 4.5
AA	22.8 ± 6.1
SB + AA	60.7 ± 6.5

Abstract N° A80

LPS treatment of neonatal rat cardiomyocytes alters mitochondrial membrane potential and increases uncoupling protein expression

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Cardiac myocyte cell death is a clinically important consequence of endotoxic shock. Our studies have identified an innate protective phenotype of the neonatal rat heart against LPS-induced cell death. Using deconvolution laser microscopy we have demonstrated that LPS treatment of neonatal rat ventricular myocytes (NRVM) leads to a transient change in the mitochondrial membrane potential (ΔΩ). This loss of ΔΩ is temporally associated with an increase in the expression of mitochondrial uncoupling proteins, UCP2 and UCP3, and a decrease in cellular ATP levels. Thus, implying that LPS treatment of NRVM's can reversibly un-

couple oxidative phosphorylation, facilitating a proton leak across the mitochondrial membrane, decreasing $\Delta\Omega$, and thereby reducing the production of cell-damaging reactive oxygen species. LPS treatment has been reported to reduce fatty acid oxidation and decrease the levels of fatty acid binding protein. In our LPS treated cell model we observed a rapid decrease in the level of phosphorylated AMP-activated protein kinase (AMPK). Decreasing active AMPK leads to increases in cardiac malonyl CoA levels and a concomitant decrease in fatty acid oxidation. In this situation the intramitochondrial levels of long chain fatty acid anions, which cannot be metabolised, may increase. UCP3 may act as an anion transporter, protecting the mitochondrion by removing long chain fatty acids, which are not esterified to acetyl-CoA, from the mitochondrial matrix.

Supported by the US Army (TATRC) DREAMS Project.

Abstract N° A81

Cardioprotection by pretreatment of tumor necrosis factor-alpha: role of mitochondrial permeability transition pore

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It is known that brief exposure to tumor necrosis factor-alpha (TNF-alpha) triggers subsequent cardioprotection. TNF-alpha can activate multiple downstream signaling cascades related to mitochondria. However, it is not clear if mitochondrial permeability transition pore is involved in TNF-alpha-induced cardioprotection. In the present study, we examine whether TNF-alpha can inhibit mitochondrial permeability transition pore opening. In isolated rat hearts subjected to 30 min regional ischemia (occlusion of left anterior descending artery) and 120 min reperfusion, pretreatment with TNF-alpha at 10 U/ml for 7 min followed by 10 min washout reduced the infarct size (TNF vs. control, $15.9 \pm 4.5\%$ vs. $44.86 \pm 4.4\%$, $P < 0.01$) and lactate dehydrogenase (LDH) release ($P < 0.01$). The pretreatment of TNF-alpha also improved the recovery of the left ventricular performance (left ventricular developed pressure and $+dP/dt_{max}$) during reperfusion as well as the left ventricular end-diastolic pressure (LVEDP). Administration of atractyloside (Atr), an opener of mitochondrial permeability transition pore, at 20 μmol/l for 20 min (last 5 min of ischemia and first 15 min of reperfusion) attenuated the reduction of infarct size (Atr + TNF vs. TNF, $51.56 \pm 1.03\%$ vs. $15.9 \pm 4.5\%$, $P < 0.01$), LDH release and LVEDP induced by TNF-alpha ($P < 0.01$). In addition, treatment of paxilline (Pax, 1 μmol/l), a calcium-activated potassium channel antagonist, for 5 min before ischemia, diminished the decreasing effect of TNF-alpha on infarct size (Pax + TNF vs. TNF, $44.69 \pm 3.08\%$ vs. $15.9 \pm 4.5\%$, $P < 0.01$) and LDH release ($P < 0.01$). The findings suggest that in the isolated heart model, TNF-alpha protect myocardium against ischemia and reperfusion injury via inhibiting mitochondrial permeability transition pore opening as well as activating calcium-activated potassium channel (*Department of Neurology, Harbin Medical University First Clinical College, China).

Abstract N° A82

Attenuated mitochondrial calcium uptake as mechanism of protection on heart reperfusion injury. Study with an oxygen-bridged dinuclear amine ruthenium complex (Rth_{360})

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A growing body of evidence has demonstrated that reperfusion injury is mediated by mitochondria. Mitochondrial oxidative damage is enhanced during reperfusion, probably due to an increased mitochondrial calcium uptake. Mitochondrial calcium overload occurs via the calcium-uniporter; high $[Ca^{2+}]_m$ induces the onset of a highly permeable state of the mitochondrial inner membrane, called the mitochondrial permeability transition. Oxygen-bridged dinuclear amine ruthenium complex (Rth360) is the most potent and specific inhibitor of the mitochondrial calcium uniporter. This study reports the effect of Rth360 in isolated and in vivo rat hearts after ischemic–reperfusion injury. Rth360 completely abolished post-reperfusion arrhythmias in the whole animal model. Also, it was observed a decrease in tissue injury marker enzymes and an intact mitochondrial phosphorylating capacity was observed. In isolated hearts subjected to global ischemia a threefold recovery of mechanical performance at 20 min of reperfusion was found. Isolated mitochondria obtained from reperfused hearts previously treated with Rth360 showed 18% inhibition in calcium transport. Possible Ruth360 interactions with another calcium transport systems were evaluated. We measured Rth360 effect on calcium release and uptake of sarcoplasmic reticulum vesicles (SRV). SRV showed a very low affinity for the inhibitor. These data demonstrated that the main protection mechanism of Rth360 on reperfusion injury is by direct attenuation of mitocondrial calcium transport.

Abstract N° A83

Blocking electron transport during cardiac ischemia protects mitochondria

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Mitochondria are sources and targets of injury during cardiac ischemia (isch). In the isolated rabbit heart, 45 min isch decreases the content of the oxidatively sensitive phospholipid cardiolipin (CL) in subsarcolemmal mitochondria (SSM) located beneath the plasma membrane. The decrease in CL is accompanied by a decrease in the content of cytochrome *c* (cyt *c*) and decreased respiration through cytochrome oxidase (complex IV, CIV). Isch did not alter CIV, CL, or cyt *c* in interfibrillar mitochondria located between the myofibrils. The contents of CL and cyt *c*, and oxidation

through CIV, did not further decrease further during reperfusion in SSM. We thus focused on isch, and asked if electron leak from sites in the electron transport chain (ETC) proximal to CIV depletes CL, predisposing to loss of cyt *c* and the defect in CIV. Isolated rabbit hearts were treated immediately before isch with rotenone (ROT), an irreversible inhibitor of complex I to block electron flow in the ETC during isch. ROT treatment preserved CL (nmol/mg pr) and cyt *c* (nmol/mg pr). CIV, measured as TMPD-ascorbate respiration (nAO/min/mg), was also preserved. Inhibition of the proximal ETC during isch preserves CL, cyt *c*, and improves respiration through CIV in SSM. During isch, the ETC mediates damage to mitochondria, probably via electron leak that produces reactive oxygen species. Limitation of electron flow during isch is a novel approach to limit mitochondrial damage and possibly limit subsequent mitochondrial-derived cardiomyocyte injury during ischemia and reperfusion.

SSM	Pre-isch (*n = 6)*	45 min isch (*n = 10)*	45 min isch + ROT (*n = 6)*
CIV	484 ± 32	324 ± 20 *	436 ± 37 **
CL	41 ± 2	34 ± 1 *	43 ± 2 **
Cyt *c*	0.17 ± 0.01	0.10 ± 0.01 *	0.15 ± 0.02 **

±SEM.
* $P < 0.05$ vs. pre-isch.
** $P < 0.05$ vs. 45 min isch.

Abstract N° A84

Blockade of electron transport before cardiac ischemia with the reversible inhibitor amytal protects rat heart mitochondria

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Ischemia (ISCH) damages the electron transport chain (ETC). Irreversible blockade of the ETC at complex I by rotenone blunts ischemic damage to mitochondria at cytochrome oxidase (complex IV, CIV) in the distal ETC. However, eventual therapeutic intervention to protect myocardium during ISCH and reperfusion (R) requires the use of a reversible inhibitor that allows resumption of oxidative metabolism during R. Interestingly, R does not worsen oxidative function through CIV. Thus, we propose that if protection of oxidative function during ISCH can be achieved with reversible inhibitors of ETC, then R can occur in the setting of preserved mitochondrial oxidative function. Amytal is a reversible inhibitor at the rotenone site of complex I. We asked if amytal administered immediately before ISCH protects mitochondria. Isolated rat hearts were perfused for 15 min followed by 25 min global ISCH at 37 °C. Non-ISCH hearts were only perfused for 15 min. Amytal-treated hearts received drug for 1 min before ISCH. Subsarcolemmal (SSM) and interfibrillar (IFM) populations of mitochondria were isolated after ISCH and oxidation through CIV measured. ISCH decreased respiration through CIV. Amytal decreased damage at CIV in both SSM and IFM in a dose-dependent manner. Peak protection occurred at doses of 2 or 2.5 mM (*n* = 3 each, combined below). Lower or higher doses provided suboptimal protection. Thus, blockade of the ETC during ISCH with a reversible agent protects oxidative function. Thus, R with relatively preserved mitochondrial function is a feasible goal.

Respiration through CIV (nAO/min/mg protein (mean ± S.D.)

	SSM	IFM
Non-ischemia (*n = 7)*	550 ± 76	829 ± 103
Ischemia alone (*n = 3)*	354 ± 34 *	583 ± 101 *
2 or 2.5 mM amytal (*n = 6)*	536 ± 91 **	817 ± 99 **

* $P < 0.05$ vs. non-ischemia.
** $P < 0.05$ vs. ischemia.

Abstract N° A85

Impact of imidapril on cardiac mitochondrial function in an ex vivo animal model of global myocardial ischemia

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Myocardial ischemia has a negative impact on both cardiac mitochondria structure and function; it is thought that the main cause of this deleterious affect is mediated by the induction of the mitochondrial permeability transition. This phenomenon is believed to be triggered by the oxidative stress that occurs within the mitochondria as a result of calcium overload. It is, therefore, clinically relevant to identify drugs capable of reverting or preventing these alterations. Angiotensin converting enzyme (ACE) inhibitors, due to its modulation of the renin-angiotensin-aldosterone system, are a pharmacological class that already showed in several clinical trials to have an important role in the treatment of heart failure and coronary artery disease patients. However, their impact on the cardiac mitochondrial function during ischemia is not fully understood. Our aim was to assess the impact of imidapril, an ACE inhibitor, on the calcium-induced mitochondrial damage during myocardial ischemia. We used an ex vivo animal model of global myocardial ischemia, with a Langendorff perfusion system. Thirty Wistar rat hearts were divided in to three groups: A (75 min of perfusion with a Krebs-modified solution), B (15 min of perfusion, followed by 60 min of ischemia), and C (as in B, but in the presence of imidapril 4 μM). Several parameters of mitochondrial function were assessed: velocity of the respiratory state 3 (using a Clark-type electrode), membrane electrical potential (TPP^+-electrode) and rate and amplitude of mitochondrial swelling (by turbidimetry). Regarding the velocity of the respiratory state 3, no significant differences were observed between ischemic and imidapril groups. However, imidapril significantly increased membrane electrical potential and prevented, in cardiac mitochondria submitted to ischemia, the calcium-induced increase on the rate and amplitude of mitochondrial swelling ($P < 0.05$, see Fig. 1). These results demonstrate that imidapril has a positive impact on the calcium-induced mitocondrial-damage, and can help us to understand the good results that

ACE inhibitors have already demonstrated in the treatment of heart failure and coronary artery disease patients.

Abstract N° A86

Carvedilol protects ischemic cardiac mitochondria from oxidative stress-induced injuries

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During ischemia, there is an increase in production of reactive oxygen species (ROS), due to the decrease of antioxidant defenses and to the reduced state of several respiratory chain components, thus increasing electron escape and ROS production, leading to higher oxidative damage. Oxidative stress can cause mitochondrial dysfunction and, ultimately, lead to cell death. The use of drugs capable of reversing this mitochondrial dysfunction can be clinically relevant. This work aimed to assess the impact of carvedilol, a beta adrenoreceptor blocker with antioxidant properties, on the activation level of the apoptotic cascade induced by oxidative stress during global myocardial ischemia. Twenty-one Wistar rat hearts were divided in three groups: A, 75 min of perfusion with a Krebs-modified solution; B, 15 min de perfusion, followed by 60 min of global ischemia; C, as in B, but in the presence of carvedilol 40 μM. Oxidative stress was measured by the thiobarbituric acid reactive substances test (TBARS) and the activities of caspases 1, 3, 8, and 9 was determined by using their respective colorimetric assays. In cardiac mitochondria from ischemic hearts, carvedilol decreased oxidative stress levels ($P < 0.05$, see Table 1). Ischemia increased the activity of caspase 8, without significant differences on the activities of caspases 1, 3, and 9 (see Table 1). Regarding this parameter, carvedilol did not significantly change the activity of the caspases evaluated. Our results show that carvedilol decreases oxidative stress. However, under these experimental conditions, carvedilol showed no impact on the apoptotic cascade.

Abstract N° A87

Carvedilol protects cardiac mitochondria submitted to ischemia–reperfusion from oxidative stress-induced injuries

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In an ischemia–reperfusion setting, there is a marked increase in reactive oxygen species (ROS) production in mitochondria, as a result of the sudden availability of oxygen in the cardiac tissue. This phenomenon can cause oxidative damage in the inner mitochondrial membrane, thus resulting in mitochondrial dysfunction and, ultimately, cell death (oxygen paradox). Our aim was to assess the impact of carvedilol, a beta adrenoreceptor blocker with antioxidant properties, on the activation level of the apoptotic cascade induced by oxidative stress during global myocardial ischemia–reperfusion. Twenty-one Wistar rat hearts were divided in three groups: A, 90 min of perfusion with a Krebs-modified solution; B, 15 min de perfusion, followed by 60 min of global ischemia and 15 min of reperfusion; C, as in B, but in the presence of carvedilol 40 μM. Oxidative stress was measured by the thiobarbituric acid reactive substances test (TBARS) and the activities of caspases 1, 3, 8, and 9 was determined by using their respective colorimetric assays. Carvedilol decreased TBARS levels (an indicator of oxidative stress) in cardiac mitochondria submitted to ischemia–reperfusion ($P < 0.001$, see Fig. 1). Ischemia followed by reperfusion induced an increase in the activity of caspase 1, without altering the activity of caspases 3, 8, and 9. Carvedilol significantly decreased the activity of caspases 1 and 8 (see Fig. 1). In this ischemia–reperfusion model, carvedilol significantly decreased the oxidative stress and caspase cascade activity. These findings may explain some of the previously reported cytoprotective effects of carvedilol under ischemia–reperfusion conditions.

Parameters/groups	Ischemic	Carvedilol	*P*
TBARS (% control)	90 ± 5	45 ± 12	<0.05
Caspase 1 activity (% control)	127 ± 7	102 ± 10	<0.05
Caspase 3 activity (% control)	121 ± 3	103 ± 12	n.s.
Caspase 8 activity (% control)	110 ± 6	76 ± 11	n.s.
Caspase 9 activity (% control)	102 ± 20	101 ± 18	n.s.

Abstract N° A88

Auranofin increases apoptosis and ischemia–reperfusion injury in the isolated rat heart

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Auranofin, an anti-rheumatic gold compound is an inhibitor of selenocysteine enzymes such as thioredoxin reductase and glutathione peroxidase. These antioxidant enzymes play an important role in protecting cardiac tissue from oxidative stress generated during ischemia–reperfusion. Auranofin was administered to rats by suspending in corn oil and gavaging with an oral dosing needle 24 h prior to sacrifice. We chose to use 100 mg auranofin/kg bodyweight to selectively downregulate thioredoxin reductase activity without altering glutathione peroxidase. Sham controls were gavaged with corn oil only. Hearts were then isolated and perfused using the Langendorff model where they were subjected to 22.5 min ischemia and 45 min reperfusion. The activity of thioredoxin reductase and glutathione peroxidase was determined in heart extracts subjected to normoxic perfusion and ischemia–reperfusion. Caspase-3, a marker of apoptosis, was also measured in heart extracts post-ischemia–reperfusion. There was significantly less thioredoxin reductase activity in nor-

moxic auranofin treated hearts whilst the level of glutathione peroxidase remain unchanged, demonstrating that the dose of auranofin was able to selectively inhibit one of these enzymes. Rats treated with auranofin displayed significantly impaired recovery from ischemia–reperfusion with elevated end diastolic pressures and decreased contractile function. The level of caspase-3 activity was also significantly increased in the auranofin treated hearts suggesting auranofin is pro-apoptotic. Given that auranofin is prescribed as an anti-rheumatic drug, often to older patients at risk of cardiovascular disease, this data would suggest that these patients would recover poorly from an ischemic insult such as myocardial infaction or related clinical procedures.

Abstract N° A89

Age-related changes in purine catabolism in normoxic and ischemic–reperfused hearts

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Impaired tolerance to ischemia–reperfusion with age may stem in part from altered purine catabolism. This study characterized potential changes in adenosine/purine catabolism in young (2–4 months) and aged (18 months) C57/B16 mice. To interrogate purine metabolism we employed 10 μM EHNA to block adenosine deaminase (ADA), and 5 μM iodotubercidin to block adenosine kinase (AK), individually or in combination. Datya revealed that absolute adenosine formation (with deamination and rephosphorylation blocked) under normoxic conditions was 12 nmol/min/g in young vs. 9 nmol/min/g in aged hearts. Of this, 4.5 nmol/min/g was rephosphorylated and 5.2 nmol/min/g deaminated in young hearts vs. 1.5 nmol/min/g rephophorylated and 3.5 nmol/min/g deaminated in aged hearts. This was consistent with 50% reductions in measured adenosine deaminase and AMP-specific 5′nucleotidase activities with age. Effects of ADA/AK inhibition reveal 45% of normoxic purine efflux occurs via 5′-AMP catabolism and 55% via IMP catabolism in young hearts vs. 50% for each in aged hearts. Young and aged hearts exhibited similar purine efflux following 20 min ischemia and 30 min reperfusion, approximating 1800 nmol/g. Data indicate ~1100 nmol/g of 5′-AMP is hydrolysed in both age groups, with 200 nmol/g of the adenosine formed being rephosphorylated, giving a net washout of 800 nmol/g 5′-AMP-derived metabolites in both ages. An equal quantity (800 nmol/g) of purine washout was derived from IMP. Thus, 50% of purines lost during ischemia–reperfusion are derived from 5′-AMP hydrolysis and 50% from IMP hydrolysis. In summary, our data reveal IMP and 5′-AMP are equally important sources of extracellular purines (and potentially damaging xanthine-oxidase derived radicals) during normoxic and ischemic conditions, and that minor differences in purine handling are unlikely to account for substantial differences in ischemic tolerance with age.

Abstract N° A90

Age-related changes in ischemic tolerance and adenosinergic cardioprotection in mouse hearts

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The genesis of ischemia intolerance with age is poorly understood. We examined effects of aging on ischemic tolerance and adenosinergic protection with adenosine, adenosine deaminase blockade (10 μM EHNA), adenosine kinase blockade (5 μM iodotubercidin) in C57/Bl6 mouse eharts subjected to 20 min ischemia and 45 min reperfusion. Post-ischemic contractility was impaired by aging with elevated ventricular diastolic contracture (32 ± 3 mmHg vs. 19 ± 2 mmHg in young) and reduced ventricular developed pressure (39 ± 4 mmHg vs. 83 ± 5 mmHg in young). Lactate dehydrogenase (LDH) loss, indicative of necrotic damage, was also enhanced (27 ± 2 IU/g vs. 16 ± 2 IU/g). Treatment with 50 μM adenosine improved diastolic (8 ± 2 mmHg) and developed pressures (132 ± 6 mmHg), and LDH loss (8 ± 2 IU/g) in young hearts, yet failed to modify outcomes in aged hearts. This was despite the presence of functional A_1 and A_2 receptor mediated responses to 50 μM adenosine in both age groups. Similarly, while enhanced endogenous adenosine with EHNA and iodotubercidin exerted protective effects comparable to adenosine in young hearts, both agents failed to modify outcome in aged hearts (despite 3- to 4-fold elevations in endogenous extracellular adenosine levels). In contrast to adenosinergic treatment, direct mitochondrial K_{ATP} activation with 50 μM diazoxide reduced ischemic injury in both age groups. Effects of PKC activation with PMA were also assessed. Reduced protection is not the result of impaired A_1 receptor transcription or expression (comparable in both ages), but may involve reduced A_3 receptor transcription with both aging and ischemia–reperfusion (potentially limiting adenosinergic protection in older hearts). Data collectively reveal failure of adenosinergic cardioprotection, potentially involving a "lesion" in signaling proximal to mitochondrial K_{ATP} channels, together with changes in A_3 receptor expression.

Abstract N° A91

The effects of dietary hempseed on cardiac ischemic-reperfusion injury

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Polyunsaturated fatty acids (FA) are known to possess significant cardioprotective effects. For example, n-3 FAs are known to be anti-arrhythmic. However, n-6 FAs are suggested to have detrimental effects on the heart. Hempseed is a unique food enriched in both linoleic acid (LA), an n-6 FA, and alpha linolenic acid (ALA), an n-3 FA. The purpose of

our study was to determine if chronic dietary supplementation with hempseed would provide beneficial cardioprotection vs. ischemic challenge. Sprague–Dawley rats were administered a regular diet or one supplemented with 5% hempseed, or 10% hempseed, or 10% hempseed cake (lipid depleted) or 1% palm oil for 6 or 12 weeks. Global ischemia was induced in Langendorff-perfused hearts for 10 min followed by normal reperfusion. Plasma LA and ALA levels were significantly increased at 6 and 12 weeks in the two hempseed supplemented groups. After 6 weeks of dietary intervention, there were no differences in the recovery of any parameters of cardiac contractile performance in any of the groups. The incidence of extra systoles was not statistically different amongst the groups. After 12 weeks of dietary intervention, hempseed supplementation induced a significantly better recovery of post-ischemic cardiac contractile tension in comparison to the seedcake supplemented group. The dietary interventions did not influence the incidence of extra systoles. In summary, our data would argue against a detrimental effect of elevated n-6 fatty acids in ischemic/reperfusion injury to the heart. Conversely, extended dietary supplementation with hempseed provided a better recovery of cardiac contractile performance after global ischemia. This is likely due to the unique n-6/n-3 FA content.

This study was supported by CIHR, the Heart and Stroke Foundation of Canada and Hemp Oil Canada.

Abstract N° A93

Significance of serum calcineurin activity and the protective role of eugenol in isoproterenol-induced cardiac hypertrophy

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The role of calcineurin (CaN) in cardiac hypertrophy is rapidly gaining importance since CaN inhibitors can reverse this disease. We thus evaluated the significance of serum calcineurin in heart disease patients. Oxidative stress in all groups of patients was evidenced by (a) increase in lipid peroxidation and activities of antioxidant enzymes and (b) decreased glutathione contents. CaN activity was enhanced in patients diagnosed for cardiac hypertrophy but not in other cardiac disorders. Since we found that eugenol could inhibit CaN activity in vitro, its in vivo effects on isoproterenol-induced cardiac hypertrophy were evaluated in Wistar rats. Intraperitonial (i.p.) administration of isoproterenol resulted in tachycardia, increase in heart weight, accumulation of reactive oxygen species, decreased total glutathione contents, increased activities of CaN and protein kinase C and enhanced percentage of apoptotic cells. Administering eugenol alone for 3 d followed by simultaneous administration of isoproterenol with eugenol, resulted in reversal of cardiac hypertrophy and normalization of the above changes, even though it could only decrease the number of apoptotic ventricular cells to a limited extent. These results suggest that eugenol, a natural antioxidant, may offer potential benefits in the management of cardiac hypertrophy.

Abstract N° A94

Activation of calcineurin in human failing heart ventricle by endothelin-1, angiotensin II and urotensin II

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The calcineurin (CaN) enzyme-transcriptional pathway is critically involved in the hypertrophy of heart muscle in some animal models. Currently, there is no information concerning the regulation of calcineurin (CaN) enzyme activation by endogenous agonists in human heart. Therefore, we investigated whether endothelin-1 (ET-1), angiotensin II (Ang II) and human urotensin II (hUII) can directly activate calcineurin in human heart in vitro.

Methods. – Human right ventricular trabeculae were obtained from explanted human (14 males/one female) failing hearts, set up in a tissue bath and electrically paced at 60 beats/min. Tissues were incubated with or without 100 nM ET-1, 10 μM Ang II or 20 nM hUII for 30 min. Tissues from three patients were incubated with 200 nM FK506 for 60 min and then incubated in the presence or absence of ET-1 for a further 30 min. Tissues were then snap frozen and used to determine calcineurin enzymatic activity with detection of free phosphate released from a specific phospho-substrate. SDS-PAGE was used to quantify calcineurin A protein level.

Results. – ET-1 (100 nM) increased contractile force in all 12 patients with an overall $P < 0.001$. Ang II (10 μM) and hUII (20 nM) also increased contractile force in three of seven and four of nine patients separately with overall insignificant P-value. FK506 (200 nM) had no effect on contractile force. ET-1, Ang II and hUII increased calcineurin activity by 26%, 92%, and 10%, respectively ($P = 0.001$, $n = 12$; $P = 0.03$, $n = 7$; $P = 0.04$, $n = 10$). FK506 caused reduction in basal calcineurin activity (basal 0.019 ± 0.003, FK506 0.013 ± 0.003 nmol/μg protein/30 min, $n = 3$ patients, $P = 0.001$). ET-1 stimulation in the presence of FK506 did not restore calcineurin activity (ET-1 + FK506 0.014 ± 0.004, $P = 0.1$ FK506 vs. ET-1 + FK506). Despite the increase in calcineurin activity there was no difference in calcineurin A protein level in the right ventricle in different patients.

Conclusion. – Endogenous cardiostimulants which couple to Gαq-proteins can directly activate calcineurin in human heart. There is not correlation between calcineurin activity and muscle contractile force.

Abstract N° A95

Failure of the calcineurin enzymatic assay to detect calcineurin activation in left ventricular hypertrophy

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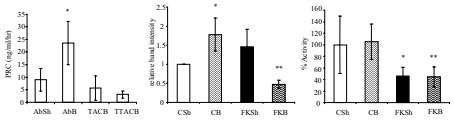
Translocation of NFAT to the nucleus by the enzyme calcineurin is thought to mediate induction of left ventricular hypertrophy (LVH), based on published animal models that mimic renal artery stenosis by tightly banding the abdominal aorta, resulting in large pressure gradients. Whether calcineurin activity in the heart is increased by such models is controversial. We measured LV calcineurin activity in a selection of in vivo models where pressure-overload LVH was induced by abdominal aortic banding (Ab) or thoracic aortic banding–mild (TAC) and severe (TTAC). NFAT3 nuclear content was also measured and used as an alternative index of calcineurin activation. Male Wistar rats were either banded (B) or sham-operated (Sh). These two groups were subdivided into treatment with vehicle (C) or FK506, a calcineurin inhibitor (FK). Whilst the conventional enzymatic assay could only detect significant inhibition of calcineurin activity due to blockade, NFAT3 nuclear content was shown to be increased over twofold at 1 d post-abdominal banding, but not by thoracic banding. Plasma renin concentration was also measured for these models, and was also only increased in the abdominal CB group. A significant limitation of the calcineurin assay is the absence of cardiac intracellular calcium data. Measurement of NFAT3 provides information on the downstream effect of calcineurin which circumvents this issue.



Abstract N° A96

Selectivity of bisindolylmaleimides as inhibitors of protein kinase C vs. p90 ribosomal S6 kinase ($p90^{RSK}$) isoforms in vitro and in cardiac myocytes

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Bisindolylmaleimide inhibitors of protein kinase C (PKC), such as GF109203X (GF) and Ro318220 (Ro), have been used to investigate the roles of PKC isozymes in the regulation of many cellular processes, including sarcolemmal Na^+/H^+ exchanger activity in cardiac myocytes. However, data from in vitro kinase assays suggest that GF and Ro also inhibit the $p90^{RSK}$ family of enzymes (RSK1, 2 and 3). We therefore investigated the selectivity of GF and Ro for PKCα and PKCϵ vs. RSK2, the predominant $p90^{RSK}$ isoform in the heart. GF and Ro inhibited the activities of recombinant PKCα, PKCϵ and RSK2 in vitro (at 5 mM [ATP], IC_{50} values for inhibition of PKCα, PKCϵ and RSK2, respectively, were 0.31, 0.17 and 7.4 μM for GF, and 0.15, 0.14 and 0.93 μM for Ro). To determine the effects of GF and Ro on in situ $p90^{RSK}$ activity in cultured adult rat ventricular myocytes (ARVM), phosphorylation of the eukaryotic elongation factor 2 kinase (eEF2K) at Ser366, a known $p90^{RSK}$ target, was used as the index of such activity. Adenoviral expression of a constitutively active form of mitogen activated protein kinase kinase 1 (MEK1) induced $p90^{RSK}$ activation and eEF2K phosphorylation 42 h post-infection. eEF2K phosphorylation was abolished by UO126 (1 μM), a selective inhibitor of MEK1, and significantly reduced by GF (at ≥3 μM) and Ro (at ≥ 1 μM). Both agents significantly inhibited PMA-induced PKC activity in ARVM (as indexed by PKC substrate phosphorylation) at 1 μM. Thus, PKC inhibitory concentrations of GF and Ro additionally inhibit $p90^{RSK}$, which may contribute to their cellular effects in ARVM.

Abstract N° A97

Angiotensin II-induced phosphorylation of the SSXS motif of R-SMAD 2 in cardiac myofibroblasts is mediated by PKC and PI3K

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Cardiac myofibroblasts contribute to remodeling of the cardiac collagen matrix in failing hearts. Transforming growth factor-β1 (TGF-β1) and angiotensin II (angiotensin) both influence myofibroblast function. TGF-β1 signals through Smads and other signaling pathways, including kinases. We have previously determined that angiotensin type I receptor activation led to rapid specific phosphorylation of R-Smad 2 at Ser 465/467, and that this event is linked to enhanced collagen deposition; however, the precise kinase pathways mediating this phenomenon are unresolved. We investigated angiotensin and TGF-β1-signaling in primary cardiac myofibroblasts in vitro, using inhibitors of protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K). We examined (i) phosphorylation of R-Smad 2 at Ser 465/467; (ii) the effects of chelerythrine (PKC inhibitor), and LY 294002 (PI3K inhibitor) on such phosphorylation; (iii) the nuclear translocation of phosphorylated R-Smad 2; and (iv) the effects of TGF-β1 neutralizing and anti-TGF-β type II receptor (TβRII) antibodies on the phosphorylation of R-Smad 2. Angiotensin-mediated phosphorylation of R-Smad 2 at Ser 465/467 is associated with nuclear translocation in myofibroblasts and increased collagen type I synthesis. Chelerythrine (1 μM) or LY294002 (10 μM) treatment ablated angiotensin-induced phosphorylation of R-Smad 2 protein. Pretreatment of cells with either chelerythrine or LY 294002 decreased nuclear translocation of phosphorylated R-Smad 2 in both TGF-β1 and angiotensin treatments. Ablation of TGF-β1 signaling with either TGF-β1 neutralizing antibody (TGF-β1 NA) or anti-TGF-β type II receptor neutralizing antibody (TβR-II NA) did not alter angiotensin-

specific phosphorylation of R-Smad 2. We suggest that the PKC and PI3K pathways in cardiac myofibroblasts participate in non-classic (i.e., non-TGF-β1 induced) phosphorylation of R-Smad 2 to enhance collagen deposition by myofibroblasts.

Abstract N° A98

Inhibition of phenylephrine-induced cardiomyocyte hypertrophy by activation of multiple adenosine receptor subtypes

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Plasma adenosine levels are elevated in cardiovascular disease including hypertension and heart failure and the nucleoside has been proposed to serve as an endogenous anti-myocardial remodelling factor. We studied the modulation of phenylephrine-induced hypertrophy by adenosine receptor activation in isolated neonatal cultured ventricular myocytes. Phenylephrine (10 μM) increased cell size by 35% and significantly increased expression of atrial natriuretic peptide. These effects were completely prevented by the stable adenosine analogue 2-chloro adenosine as well as the adenosine A_1 receptor agonist CPA (1 μM), the A_{2a} receptor agonist CGS21680 (100 nM) and the A_3 receptor agonist IB-MECA (100 nM). The antihypertrophic effects of all three agonists were completely reversed by their respective antagonists. Phenylephrine significantly upregulated expression of the immediate early gene c-fos especially within the first 30 min of phenylephrine treatment. These effects were almost completely inhibited by all adenosine receptor agonists. Although phenylephrine also induced early stimulation of both p38 and ERK, these responses were unaffected by adenosine agonists. The expression of the G-protein regulatory factor RGS2 was increased fivefold by phenylephrine treatment although this was completely prevented by adenosine receptor agonists. These agents also blocked the ability of phenylephrine to upregulate Na-H exchange isoform 1 (NHE1) expression in hypertrophied myocytes. Thus, our results demonstrate an antihypertrophic effect of adenosine acting via multiple receptor subtypes through a mechanism involving downregulation of NHE1 expression. The ability to prevent RGS2 upregulation further suggests that adenosine receptor activation minimizes signalling which leads to hypertrophic responses.

Abstract N° A99

Protective effects of A_3 adenosine receptor activation against doxorubicin induced cardiotoxicity in rats

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Background. – Anthracycline antibiotic doxorubicin (DOX) induced cardiotoxicity. We have previously shown that activation of the A_3 adenosine receptor (A_3R) in newborn cultured cardiomyocytes by highly selective agonist Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide) induced protection after treatment with DOX.

Objectives. – To explore the effect of Cl-IB-MECA against DOX cardiac injury in an in vivo model.

Methods. – Four groups of rats: control, DOX, Cl-IB-MECA and DOX + Cl-IB-MECA were tested. DOX 2.5 mg/kg (i.p.) and Cl-IB-MECA 33 μg\kg (i.v.) were injected six times every alternate day. Left ventricular end diastolic diameter (LVEDd), end systolic diameter (LVESd) and fractional shortening (FS) were assessed by echocardiography. A stress test with dobutamine was conducted. To determine whether DOX changed SR Ca^{2+} content and uptake, we exposed cultured rat cardiomyocytes to 10 mM caffeine to release SR calcium.

Results. – LVEDd, LVESd were higher and FS was smaller in DOX treated animals than controls ($P < 0.01$). The stress test showed superior contractility dP/dt_{max} at each dose point in DOX and Cl-IB-MECA treated hearts vs. DOX. Twenty micromolar DOX increased $Ca^{2+}{}_i$ diastolic level, decreased amplitude and $Ca^{2+}{}_i$ transients following caffeine treatment. Cl-IB-MECA significantly improved SERCA2a efficiency.

Conclusions. – DOX+Cl-IB-MECA improved cardiac hemodynamic function, partially caused through a decrease in calcium overload and activation of SR Ca^{2+} uptake.

Abstract N° A100

The effect of chronic l-arginine on cardiac adenosine receptor expression and function

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l-Arginine increases myocardial nitric oxide (NO) production. NO mediates many cardiovascular actions of adenosine and modulates adenosine metabolism. In this study we examined the effect of chronic l-arginase (5%) intake on adenosine receptor expression and β-adrenergic induced stimulation of cardiac function and adenosine production. Our results indicate that 4 week chronic ingestion decreases the expression of all the cardiac adenosine receptors, with reductions in adenosine A_1 (20-fold), A_{2A} (7.7-fold), A_{2B} (25.6-fold) and A_3 (76-fold) mRNA. Stimulation of β-adrenoceptors with noradrenaline induced an increase in purine release in hearts from control and l-arginine treated rats ($P < 0.05$). Chronic l-arginine treatment did not alter catecholamine induced purine efflux ($P > 0.05$), it did however reduce contractile responses to noradrenaline with a significant decrease in left ventricular developed pressure (LVDP) and dP/dt_{max} and myocardial oxygen consumption. At basal and low noradrenaline concentrations a significant increase in coronary resistance was observed in l-Arginine treated hearts, however with an increase in contractility observed at higher levels of noradrenaline, coronary perfusion

pressure decreased to control values ($P < 0.05$). These results indicate that chronic l-arginine treatment reduced the expression of all cardiac adenosine receptor and although it attenuates β-adrenoceptor induced stimulation of the heart it does not alter adenosine efflux. As a number of cardiac actions of adenosine receptor work via NO-cGMP mediated mechanisms, elevated NO production with chronic l-Arginine intake may result in down regulation of upstream stimulators of nitric oxide synthase activity.

Abstract N° A101

A novel Ca^{2+}-dependent Akt phosphorylation in endothelial cells

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The protein kinase Akt participates in such important functions of endothelial cells (ECs) as nitric oxide (NO) production and angiogenesis, activities that involve changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). However, it is not known if Akt activity is linked to Ca^{2+} signaling in these cells. Here we have investigated possible mutual interaction between phosphatidylinositol 3-kinase (PI3-kinase)/Akt activation and Ca^{2+} signaling during agonist stimulation in cultured porcine aortic ECs. Intracellular Ca^{2+} concentration was measured using fura-2/AM. Phosphorylation of Akt and eNOS, known as a downstream substrate of Akt, was assessed by western blotting. Thapsigargin (TG, 1 μM) and bradykinin (BK, 10 nM) stimulated biphasic Ca^{2+} response in ECs, which were not influenced by inhibition of PI3-kinase/Akt using wortmannin or by expression of a dominant-negative form of Akt. TG and A23187 (5 μM), a Ca^{2+} ionophore, induced phosphorylation of both Akt and eNOS. Wortmannin prevented phosphorylation of Akt but not of eNOS. On the other hand, removal of extracellular Ca^{2+} prevented both phosphorylation of Akt and eNOS. In conclusion, PI3-kinase/Akt is not involved in the regulation of agonist-stimulated Ca^{2+} signals in ECs. However, Akt phosphorylation appears to require both Ca^{2+} entry and PI3-kinase activity, while eNOS phosphorylation requires extracellular Ca^{2+} but does not involve the PI3-kinase/Akt-linked signaling pathway.

Abstract N° A102

Intracellular nitric oxide detection in adult cardiomyocytes: assessment with a fluorescent probe

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The role of nitric oxide (NO) as a biological mediator of cardioprotection during ischaemia–reperfusion injury and ischaemic preconditioning is a fast growing field of interest in heart research. Despite this, understanding NO's exact cellular mechanism remains elusive. Although measurement of intracellular NO levels is essential for gaining more insight, many studies depend on indirect methods to assess NO release. Diaminofluorescein-2/diacetate (DAF-2/DA), a NO-specific fluorescent probe, provides an affordable method to detect intracellular NO generation. DAF-2/DA has been shown to detect NO production in endothelial cells by flow cytometry, but this method has not yet been tested in adult cardiomyocytes. We assessed whether intracellular NO release in these cells could be detected with DAF-2/DA by flow cytometric analysis. Adult rat cardiomyocytes were isolated by collagenase perfusion and incubated with 10 μM DAF-2/DA for 180 min with or without (i) the NO-donor SNP (100 μM), (ii) eNOS activator cyclosporine A (CsA, 10 μM), and (iii) hypoxia (120 min). The NOS inhibitor l-NAME (50 μM) was administered to groups (ii) and (iii). Incubation was followed by FACS analysis. SNP increased mean fluorescence by 36.4% ($P < 0.05$ vs. control), CsA by 42.4% ($P < 0.05$), and hypoxia by 16.6% ($P < 0.05$). l-NAME resulted in a 16% attenuation of mean fluorescence in control cells ($P < 0.05$), 35% in CsA-treated cells ($P < 0.05$), and 13% in hypoxic myocytes ($P < 0.05$). This study demonstrates a method for the direct detection of intracellular NO in adult cardiomyocytes by flow cytometry, using DAF-2/DA. CsA and hypoxia both increase NO-production in cardiomyocytes secondary to NOS activation. This method, not previously described in isolated adult cardiomyocytes, could be used in future studies to help elucidate intracellular NO mechanisms in the myocardium.

Abstract N° A103

Nitric oxide does not significantly contribute to altered pulse pressure amplification during aerobic exercise

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Nitric oxide modulates blood pressure and arterial stiffness at rest. Normally there is amplification of pulse pressure from central (aorta) to peripheral (brachial) arteries, which is increased during exercise. However, amplification is significantly attenuated during exercise in people with hypercholesterolemia, who also have reduced nitric oxide bioavailability and increased arterial stiffness. The aim of the present study was to determine the contribution of nitric oxide to pulse pressure amplification and markers of arterial stiffness during exercise in people free from cardiovascular risk factors that may confound results. Twelve healthy males aged 29 ± 1 years (mean ± SEM) exercised at 60% of their

maximal heart rate on a bicycle ergometer. Non invasive measures of central blood pressure, estimated aortic pulse wave velocity (Tr) and systemic arterial stiffness (AIx) were obtained by pulse wave analysis during iv infusion of saline (control), NG-monomethyl-l-arginine (l-NMMA; a nitric oxide-synthase inhibitor) at 6 mg/kg/h or noradrenaline (NE; control vasoconstrictor) at 50 ng/kg/h. Pulse pressure amplification was defined as the ratio of peripheral to central pulse pressure (PPP:CPP). Cardiac output and stroke volume was determined by electrical bioimpedence. Both l-NMMA and NE caused a significant increase in mean arterial pressure (7 and 8 mmHg, respectively; $P < 0.01$) and AIx (5% and 11%, respectively; $P < 0.01$), a decline in PPP:CPP (–4% and –8%, respectively; $P<0.05$) and a trend towards reducing Tr (–4 and –7 ms; $P = 0.35$ and 0.07, respectively) at baseline. Exercise caused significant elevation of PPP:CPP ($P < 0.001$), whereas AIx and Tr declined ($P < 0.05$) during saline and drug infusions. However, during exercise no significant differences were observed in AIx, PPP:CPP or Tr between infusion procedures ($P > 0.50$). Heart rate, peripheral vascular resistance and cardiac output did not differ during exercise between saline, l-NMMA or NE. Nitric oxide contributes to systemic arterial stiffness and pressure amplification at rest, but has no significant effect on these variables during aerobic exercise.

Abstract N° A104

Central pressure during exercise is significantly increased with age and hypercholesterolaemia

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Due to amplification of pulse pressure, central (aortic) blood pressure may differ significantly from recordings at peripheral (e.g. brachial artery) sites. Although, the brachial blood pressure response to exercise and resting central blood pressures are more powerful predictors of cardiovascular outcome compared to resting peripheral measures alone, the effect of exercise on central blood pressure in people with risk factors for cardiovascular disease is unknown. This study aimed to assess the central and peripheral haemodynamic response to exercise with increasing age and hypercholesterolaemia. Twenty healthy young subjects (aged 29 ± 5 years; mean ± S.D.), 20 healthy older (aged 57 ± 5 years) and 10 matched hypercholesterolaemic (aged 59 ± 7 years) men exercised at 60% of their predicted maximal heart rate on a bicycle ergometer. Central blood pressure and augmentation index (AIx), a marker of systemic arterial stiffness, were obtained non-invasively using pulse wave analysis. Amplification was calculated as the ratio of peripheral to central pulse pressure. Resting haemodynamic measures were not different between the hypercholesterolaemics and controls. During exercise there was no difference in brachial systolic blood pressure (SBP) ($P = 0.7$), but hypercholesterolaemics had significantly higher mean arterial pressure, brachial diastolic blood pressure (DBP), central SBP and AIx ($P < 0.05$ for all). Young subjects had significantly lower AIx and central SBP during exercise ($P < 0.001$) compared to older subjects. For the whole population, total cholesterol and amplification during exercise were negatively correlated (r=–0.60; $P < 0.001$). With increasing age, wave reflection is augmented during exercise, resulting in elevated central pressure. These effects are exacerbated by hypercholesterolaemia and would contribute to cardiovascular risk by mechanisms associated with central hypertension.

Abstract N° A105

Role of nitric oxide and protein kinase C in the tachyphylaxis to vasopressin in rat aortic rings

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The purpose of the present study was to assess the contribution of nitric oxide (NO) and protein kinase C (PKC) in the tachyphylaxis to arginine vasopressin (AVP) in the rat aorta. Endothelium-intact (E+) and -denuded (E–) rings obtained from the rat thoracic aorta were exposed to three repetitive administrations of a supramaximal concentration of AVP (100 nM) lasting 20 and 45 min apart. The first contraction to AVP was 17 ± 2 mN in E+ and 27 ± 1 mN in E– rings. Compared with the first, the third contraction to AVP was equal to 15 ± 2% and 46 ± 8% in E+ and E– rings, respectively. NG-nitro-l-arginine (NNLA), a non-selective inhibitor of all isoforms of NO synthase, led to a significant diminution of AVP tachyphylaxis in both E+ (67 ± 12%) and E– rings (86 ± 6%). Inhibition of PKC by either bisindolylmaleimide I-HCl (Bis I) or chelerythrine diminished significantly the tachyphylaxis to AVP in E– rings only (125 ± 20% and 95 ± 5%, respectively). Furthermore, activation of PKC with phorbol-12-myristate-13-acetate (PMA) simulated tachyphylaxis to AVP in E– rings only (12 ± 3% compared to 120 ± 17% in E+). This effect of PMA on AVP tachyphylaxis in E– rings was antagonized by the NO donor, sodium nitroprusside. Results obtained suggest that NO modulates the implication of PKC in the tachyphylaxis to AVP. Thus, PKC plays a role in AVP tachyphylaxis only in E– rings, the presence of NO probably diminishing the effects of this kinase.

Abstract N° A106

Endothelial CB1 receptors confer cardiac protection through nitric oxide production

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Both cannabinoid receptor subtypes CB1 and CB2 have been identified in the rat ventricles and selective agonists can produce a significant cardiac protection against ischemia. The aim of the study was therefore to identify differences in

the localization of these receptors as well as their signaling pathways and specific effectors. Isolated rat hearts were perfused with Krebs–Henseleit buffer on a Langendorff setup and submitted to severe ischemia and reperfusion. Some hearts were formalin fixed and paraffin embedded for immunohistochemical experiments, some were stained with 1% TTC after reperfusion to measure infarct size and some hearts were snap frozen at different time to extract protein and RNA to perform biochemical assays. ACEA and JWH133 (respectively, CB1 and CB2 agonists) as well as SR141716A and SR144528 (respectively, CB1 and CB2 antagonists) were perfused before and throughout ischemia. CB1 receptor was identified on capillary and arterial endothelial cells only in control hearts but appeared as well on myocytes in ischemic hearts. CB2 receptor was identified on myocytes and arterial endothelial cells but ischemia did not alter its expression. CB1 and CB2 agonists caused an independent cardioprotection activating different signaling pathway (respectively, ERK1/2 and p38 MAPK) as assessed by phosphorylation and activity experiments. *N*-Ω-nitro-l-arginine selectively inhibited CB1 mediated cardioprotection, and CB1 agonist caused a selective induction of iNOS protein, thus suggesting a strong implication of nitric oxide in the cardiac protection conferred by CB1 receptor.

Abstract N° A107
Practical and fundamental considerations in quantification of nitrite as an index of nitric oxide formation in vivo

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Nitrite is now assigned to be an alternative candidate for an index of nitric oxide (NO) formation in vivo, as nitrate or NO*x* (nitrite and nitrate) in plasma or in whole blood (including red blood cells) are not considered to be reliable index of NO formation in vivo anymore. However, there is no practical information available in handling for quantification of the ion, though nitrite contamination from laboratory ware or through whole procedure is anticipated. Therefore, we explored nature of the contamination and way to avoid that for accurate evaluation of nitrite. Nitrite was measured by ENO10 or 20 (Eicom, Kyoto, Japan) with sensitivity of ~3 nM by loading volume of 100 μl. Every solution contained small amount of nitrite. It was not possible to obtain nitrite-free solution even by the usage of an ion exchange column (Milli Q, Millipre). Degrees of nitrite contamination from laboratory ware were variable. However, they were lower than those of nitrate and were attenuated by pre-wash with water containing lower nitrite. For pre-treatment of plasma, use of some kinds of ultrafiltration units may be a cause of severe nitrite contamination, however, a unit with negligible contamination is available. As nitrite contamination is suspected in methanol, attention has to be paid to the use of the agent for deproteinization of plasma. A solution kept in room air without seal absorbed nitrite and the concentration increased time dependently, while solution sealed within a gas-tight ware did not, regardless whether air of the dead space was replaced with argon gas or not. These results indicated that we should avoid nitrite contamination through whole procedure in any way possible for accurate evaluation of nitrite in biological samples.

Abstract N° A108
Identification of the nitroxyl anion as a coronary vasodilator

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Nitric oxide (NO) is a well-known endogenous vasodilator molecule that can exist in several different redox forms, including the free-radical form ($NO^{\bullet}$) and the nitroxyl anion (NO^{-}). Like $NO^{\bullet}$, NO^{-} causes vasorelaxation and can be produced endogenously. The role of NO^{-} in the coronary vasculature is currently unknown, so this study aimed to examine the ability of NO^{-} to cause vasodilation in the intact rat coronary circulation. Hearts from male Sprague–Dawley rats (350–550 gm) were excised and perfused by the Langendorff method with Krebs' solution containing EDTA (26 μM). Perfusion pressure was monitored throughout the experiments. Following a 15 min equilibration period, perfusion pressure was raised to approximately 100 mmHg by infusing phenylephrine (100 mM, 0.5–2 ml/min) and giving bolus additions of the thromboxane mimetic U46619 (0.1–10 nmol) as required. Concentration response curves to the NO^{-} donor, Angeli's salt (1 pmol–100 nmol) were constructed in the presence and absence of inhibitors that were added to the perfusion solution. Angeli's salt caused a concentration-dependent vasodilation (EC_{50}: 1.12 ± 3.4 nmol, maximum vasodilation: 36.7 ± 12.5 mmHg, $n = 4$) that was reproducible over time. This response was unaffected by the NO scavenger, hydroxocobalamin (100 μM) but significantly inhibited by the NO^{-} scavenger l-cysteine (l-cys) (3 mM) (Max dilation (mmHg) control 27.9 ± 4.8; l-cys 11.4 ± 4.7, $P < 0.05$, $n = 6$) indicating the response is mediated via NO^{-}. Vasodilator responses to Angeli's salt were significantly attenuated by ODQ (10 μM), and 30 mM K^{+} Krebs. The response was unaffected by the Kv channel blocker 4-aminopyridine (1 mM), but attenuated by the K_{ATP} channel blocker glibenclamide (10 μM). This is the first demonstration of vasodilation by NO^{-} in the coronary circulation. NO^{-} mediates coronary vasodilation independently of $NO^{\bullet}$ and via the activation of sGC and K^{+} channels. These findings raise the possibility that NO^{-} donors may be useful therapeutic agents for treating coronary vascular disease.

Abstract N° A109

A decrease in endothelium-derived hyperpolarizing factor in femoral resistance arterioles of spontaneously hypertensive rats

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The aim of our study is to clarify the mechanism of impairment of endothelium-dependent relaxation (EDR) in the resistance arterioles of hypertensive rats. Femoral arterioles (75) of male spontaneously hypertensive rat (SHR) and age matched Wistar-Kyoto rat (WKY) were isolated and examined acetylcholine (ACh)-induced EDR with microvideoscopy. ACh-induced dilatation in prehypertensive rat (4 weeks) was not significantly decreased without any inhibitor (control). Also there was no difference in nitric oxide (NO)-mediated dilatation (difference between control and dilatation with NG-monomethyl-l-arginine (l-NMMA) and indomethacin (INDO)) and EDHF-mediated dilatation (difference between dilatation with l-NMMA, INDO and dilatation with l-NMMA, INDO, CTX, apamin and 40 mM KCl) between SHR and WKY. In the resistance arterioles endothelium-derived hyperpolarizing factor (EDHF)-mediated dilatation was more than NO-mediated dilatation (45 ± 4% vs. 23 ± 3%, percentage of maximal dilatation by nitroprusside). Blood pressure of 16 weeks SHR was significantly higher than WKY. Control dilatation in 16 weeks SHR was attenuated than that in WKY (64.5% vs. 100%). EDHF-mediated dilatation is significantly decreased in SHR (16 weeks) as compared to age matched WKY (46.5 ± 5% vs. 14.5 ± 3%). There were no differences in NO-mediated dilatation between SHR and WKY. EDHF-mediated dilatation was decreased in the smaller arterioles of established hypertensive rats.

Abstract N° A110

Prostaglandin E1 induces vascular endothelial growth factor-1 in human adult cardiac myocytes but not in human adult cardiac fibroblasts via a cAMP-dependent mechanism

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Prostaglandin E1 (PGE1) has been used to treat pulmonary hypertension and peripheral artery occlusive disease and has been successfully employed for pharmacological bridging to transplantation in patients with chronic end stage heart failure. In addition to its vasoactive effects PGE1 was shown to stimulate angiogenesis in animal models. Recently, we showed that PGE1-induced angiogenesis in hearts of patients with ischaemic heart disease. We proposed that the angiogenic action of PGE1 is mediated by vascular endothelial growth factor (VEGF). The purpose of this study is to show a possible effect of PGE1 on the expression of VEGF-1 in cultured human adult cardiac myocytes (HACM) and cultured human adult cardiac fibroblasts (HACFB), respectively, to identify a cellular source of VEGF-1 in patients treated with PGE1. We also aimed to delineate mechanisms involved in a possible regulation of VEGF-1 by PGE1 in these cells. When HACM, isolated from human myocardial tissue, were treated with PGE1, a significant up to threefold increase in VEGF-1 production could be observed. These results could be confirmed on the level of specific mRNA expression as determined by real-time PCR. The effect of PGE1 on VEGF-1 expression could be blocked by H089, an inhibitor of cAMP-dependent protein kinase A. In HACFB, also isolated from human myocardial tissue, no effect of PGE1 on VEGF-1 production was seen. If this effect of PGE1 is also operative in the in vivo situation, one could speculate that cardiac myocytes could be a cellular source of PGE1-induced VEGF-1 expression in patients treated with this drug. This mechanism might lead to angiogenesis and subsequently to better collateral blood flow in the hearts of these patients and thus might offer an explanation of beneficial effects of PGE1 treatment not attributable solely to its vasodilative activity.

Abstract N° A111

Expression equilibrium between matrix metalloproteinases and their inhibitors (TIMPs) in human compensated left ventricular hypertrophy

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The development of left ventricular hypertrophy (LVH) is an adaptive response to pressure overload following aortic stenosis, but chronic alterations may lead to heart failure. Remodeling of myocardial extracellular matrix caused by a disequilibrium between matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) activities is likely to play a crucial role in the progression of LVH and in the transition to heart failure. During aortic valve replacement tissue samples from the interventricular septum were obtained from patients with aortic stenosis and consecutive LVH (n = 13). The hypertrophy was verified by echocardiography, while all patients were clinically in a compensated stage of the disease. Equivalent autopsy samples from traffic accident victims who had no heart disease, served as controls (n = 12). mRNA and protein expression for MMP-2, MMP-9, TIMP-1, TIMP-2, TIMP-3 and TIMP-4 was assessed by semiquantitative RT-PCR and western blots. Additionally, MMP activity was determined by gelatin zymography. We did not find any significant differences in MMP and TIMP expression between LVH patients and controls, neither on the mRNA nor on the protein level. Correspondingly, MMP-2 and MMP-9 activity on zymogram gels were similar between groups. Our data suggest, that in human compensated LVH,

the expression and activity of MMPs and TIMPs is still balanced, confirming recent results in a rat model. The response to chronic pressure overload concerning MMPs and their endogenous inhibitors might be more pronounced and important during the transition from a compensated stage of the disease to heart failure. It will be of great interest to investigate whether the time period of transition could be marked by plasma MMP and TIMP levels.

Abstract N° A112

Oncostatin M upregulates tissue inhibitors of metalloproteinase-1 and 2 in human adult cardiac myocytes

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Myocardial infarction (MI), a leading cause of death, results in pathological remodelling, thinning of ventricular wall, dilation and heart failure. Following MI, however, a reorganization of the extracellular matrix (ECM) and, consequently, the myocytes occurs which contributes to loss of heart function. Dynamic expression and activation of matrix metalloproteinases (MMPs) may mediate many of the morphological changes that occur after MI. Tissue inhibitors of MMPs (TIMPs) function as an important regulatory control on the activity of MMPs by stabilizing the proenzymes and by inhibiting the active enzymes. Downregulation of TIMPs, as shown in the failing heart, alters the ECM equilibrium towards matrix degradation and leads to an increase in tissue turnover and accelerated remodelling. In a recent publication, by showing that human adult cardiac myocytes (HACM) in vitro express plasminogen activator inhibitor-1 (PAI-1) and that this expression is significantly upregulated by the inflammatory mediator oncostatin M (OSM), we speculate on a protective role of PAI-1 against excessive matrix degradation by proteases during inflammation and cardiac repair processes. To expand on these observations we studied expression patterns and possible regulatory mechanisms of MMPs and TIMPs in HACM. We could show that HACM express MMP-1, -2, -3 and -9, and TIMP-1 and -2 constitutively. Whereas TIMP-1 and -2 were upregulated threefold and twofold, respectively, after treatment with OSM for 4 h as determined by quantitative real-time PCR, no significant changes were observed on the expression of MMPs studied. Thus HACM might not only be indirectly involved in ECM degradation—by production and dynamic expression of components of the plasminogen system—but also directly affect ECM equilibrium and consequently ventricular geography and pump function by differential regulatory patterns of MMPs and TIMPs.

Abstract N° A113

Matrix metalloproteinases-3 and -7 have differing effects on atherosclerotic plaque stability

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Matrix metalloproteinases (MMPs) are thought to be involved in the destabilization and rupture of atherosclerotic lesions. We have previously demonstrated that MMP-9 and MMP-12 have divergent effects on atherosclerotic plaque stability in the fat-fed apolipoprotein E (apoE) knockout mouse model of plaque rupture: the former is protective, whilst the latter is destructive. We aimed to evaluate the roles of two further MMPs in the same model, focussing on apoE/MMP-3 (stromelysin-1) and apoE/MMP-7 (matrilysin-1) double knockouts. Plaques in the proximal 150 μm of the brachiocephalic artery were substantially larger in apoE/MMP-3 double knockouts (n = 26) than in age-, strain- and sex-matched apoE knockout controls (n = 26) (31150 ± 7380 μm^2 vs. 7060 ± 2860 μm^2; $P < 0.01$). The frequency of previous, now healed, plaque rupture (seen as buried fibrous layers within the plaque) was sevenfold higher in apoE/MMP-3 double knockouts than in controls (0.31 ± 0.11 vs. 0.04 ± 0.04; $P < 0.05$). Additionally, an 80% reduction in smooth muscle density was observed in plaques from apoE/MMP-3 double knockouts (4.5 ± 1.6 vs. 0.9 ± 0.1 cell/mm^2; $P < 0.001$). The effects of knocking out MMP-7 were quite different. There was no significant difference in plaque size (68660 ± 11210 μm^2 vs. 63690 ± 8680 μm^2 in apoE/MMP-7 double knockouts (n = 25) and controls (n = 25), respectively). Also, there were no significant differences in the frequency of healed plaque rupture or smooth muscle cell density. These data indicate that, like MMP-9, MMP-3 plays a protective role, limiting plaque growth and promoting plaque stability. However, absence of MMP-7 has no effect on plaque stability. This challenges the concept that MMPs simply degrade matrix and thus destabilize plaques, and suggests that members of the MMP family have diverse effects on plaque stability. Selective MMP inhibitors would allow further dissection and understanding of the roles of MMPs in plaque rupture, and selective targeting of MMPs will be essential if such drugs are to be developed as plaque stabilizing agents.

Abstract N° A114

Changes in hearts of spontaneously hypertensive rats induced by salt drinking

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Elevated blood pressure in spontaneously hypertensive rat (SHR) leads to pathological changes in the myocardium that ultimately may result in heart failure (HF) after 18 months of age. To investigate the mechanisms contributing to the transition from cardiac hypertrophy to HF, adult SHR were given

1% NaCl solution (SHR-Na) to drink for the period of 3 months. Control group of SHR (SHR-C) was given tap water for drinking. At the age of 6 months hemodynamic parameters were measured with Millar tip catheters, hearts were then excised, and the changes in the mRNA for cytokines and extracellular matrix were investigated using ribonuclease protection assay (RPA). The hemodynamic measurements showed high left ventricular systolic pressure and high peripheral resistance in both groups. The hemodynamic parameters were not significantly different between the water-drinking (SHR-C) and NaCl-drinking (SHR-Na) rats. Also the mRNA for cytokines showed no significant differences between hearts of SHR-C and SHR-Na rats. SHR-Na rats, however, had significant hypertrophy of the right ventricle (RV) as compared to SHR-C (RV weight/body weight ratio 0.56 ± 0.01 vs. 0.49 ± 0.01, $P < 0.05$). The changes in RV were also prominent at the level of mRNA for extracellular matrix proteins. There was significant increase in the mRNA for atrial natriuretic peptide (ANP) (360% of SHR-C, $P < 0.001$), collagen I (248% of SHR-C, $P < 0.001$), collagen III (190% of SHR-C, $P < 0.002$), and colligin (150% of SHR-C, $P < 0.05$) in the RV. This was accompanied by changes in LV in mRNA for ANP (296% of SHR-C, $P < 0.001$), collagen III (144% of SHR-C, $P < 0.002$), and tissue inhibitors of matrix metalloproteinases-2 (TIMP-2, 122% of SHR-C, $P < 0.01$). These results suggest, that the 6-month-old SHR rats compensate functionally the additional stress. At the subcellular level there are changes that may contribute to the increasing stiffness of the myocardium (increased collagen I/collagen III ratio) possibly contributing to the development of HF.

Abstract N° A115

Studies of the effects of IFN-γ on proliferation and apoptosis of smooth muscle cells and matrix metalloproteinase activity

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The aim is to study the effects of interferon-γ (IFN-γ) on proliferation and apoptosis of smooth muscle cells (SMCs) and matrix metalloproteinases (MMPs) activity and its effect on plaque stability. The SMCs from male Sprague–Dawley rat thoracic aorta were primarily cultured in vitro. Different IFN-γ concentrations (10, 100, and 200 u/ml) were added into plates to be co-incubated with the cells, respectively. MTT method was used to detect the cell proliferation at 24, 48 and 72 h. After different IFN-γ concentrations were incubated with SMCs for 24 h, the cell cycle was detected by flow cytometer to observe the effect of IFN-γ on cell apoptosis. Macrophages were isolated from rat abdomen and allowed to grow in cell culture with different IFN-γ concentrations (10, 100, and 200 u/ml). The supernatant of the macrophages cultures was collected 24 h later. Zymography was performed by SDS-PAGE in 10% gels containing 0.1% (wt/vol) gelatin. After destained gelatinolytic activity was established by a clear white band against the blue background of stained gelatin. The activities of MMPs could be detected by the grey level of the band. IFN-γ inhibited the proliferation and accelerated apoptosis of SMC with dose depend. IFN-γ increased the activity of MMPs significantly with dose depend. IFN-γ in plaque is produced by active T-cells. IFN-γ can increase plaque instability by decreasing the amount of SMCs in plaques and increasing the degradation of extracellular matrix in fibrous cap.

Abstract N° A116

Transition from hypertrophy to heart failure in patients with aortic stenosis is accompanied by changes of MMP-2 level in plasma and pericardial fluid

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The transition from hypertrophy to heart failure (HF) in human hearts is characterized by myocardial remodeling where matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) take an active part. We studied the expression of these enzymes in different stages of hypertrophy progression. We investigated three groups of patients with aortic stenosis (AS) (I group: ejection fraction (EF) > 50%, II: 50–30%, III: <30%). Intraoperative biopsies (28 patients), pericardial fluid (12 patients) and serum (11 patients) were analyzed. Biopsies from five patients with mitral stenosis but normal EF and two donor hearts; pericardial fluid of three patients without valve pathology and serum of five healthy volunteers were used as controls. Quantitative analysis by confocal microscopy and western blotting showed upregulation of MMP1, 2, 3, 9, 13, 14 already in group I and further increase in later stages. TIMP1, 2 were enhanced, TIMP4 was decreased in comparison to control. Zymography demonstrated that MMP-2 significantly ($P < 0.05$) increased 1.2- (group I), 1.5- (group II) and 1.6-fold (group III) over control. We found that gelatinolytic activity of MMP2 in serum and pericardial fluid was increased in patients with AS in comparison to control, but the content of MMPs in the serum was lower than in pericardial fluid. Increased MMP2 levels in both serum and pericardial fluid correlated with hypertrophy progression.

Conclusions. – (1) Changes in MMPs and TIMPs levels indicate the ongoing left ventricular (LV) remodeling process during the transition from hypertrophy to HF and finally lead to an increased rate of fibrosis and a reduction of cardiac function. (2) MMPs profiles in myocardial tissue are comparable with serum and pericardial fluid levels. These measurements may have a diagnostic and prognostic significance in patients with AS.

Abstract N° A117

Combined guanethidine and capsaicin administration: effect on sensory and sympathetic innervation of the rat heart

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It has been well documented that lasting chemical sympathetic and sensory denervation can be achieved by administration of guanethidine (G) and capsaicin (C), respectively. The aim of our study was to investigate the effect of combined G and C treatment on norepinephrine (NE), dopamine (DA) and calcitonin gene-related peptide (CGRP) levels in the rat heart compartments. Newborn rats were randomly divided into five groups: G: rats treated with G 50 mg/kg daily for 3 weeks after birth, C: animals given C 50 mg/kg on postnatal days 2 and 3, CG: rats treated with both drugs in the above mentioned scheme, C+G: animals given C as above and G from postnatal day 10 for 3 weeks, Cont: rats injected with saline only. The following table shows NE, DA and CGRP concentrations in the right atria of 60-d-old rats as determined by RIA:

Group	NE (ng/g)	DA (ng/g)	CGRP (ng/g)
Cont	826 ± 67	8.7 ± 1.7	9.5 ± 0.6
G	24 ± 7 *	5.5 ± 1.4	20.2 ± 0.8 *
C	1278 ± 106 *	57 ± 9 *	1.4 ± 0.2 *
CG	130 ± 11 *	19 ± 2.3 *	23.8 ± 3.1 *
C+G	41 ± 4 *	14 ± 1.2 *	24.5 ± 1.1 *

Separate G and C administrations led to the expected shifts in NE and CGRP levels in all heart compartments. Interestingly, combined simultaneous administration of C and G (CG) resulted in 84% decrease in NE level and in significant increase in DA and CGRP concentrations (220% and 250% of controls, respectively). Delayed administration of G (C+G) led to more effective depletion of NE (5% Cont). Nevertheless, CGRP levels were still increased to the same extent as in G and CG protocols. In conclusion, the plasticity of the cardiac sensory innervation seemed to exceed that of the sympathetic neurons.

Supported by GACR, Grant No. 305/04/0144.

Abstract N° A119

Developmental changes in the action of moderate hypoxia on intrinsic membrane properties of intracardiac neurones

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The action of moderate hypoxia on the electrical properties of intracardiac neurones (ICNs) was investigated in an intact ganglion preparation from adult (≥6 weeks), juvenile (2–3 weeks) and neonatal (2–8 d) Wistar rats, maintained at 37 °C. Measurements were taken in normoxic Krebs (95% O_2/5% CO_2, pO_2 ~200 mmHg), after 20 min hypoxia (95% argon/5% CO_2, pO_2 ~80 mmHg) and upon reversion to normoxic Krebs (45 min). At each stage of development, hypoxia hyperpolarized the E_m by ~2 mV, and increased input resistance by ~15% in neurones from neonates and ~40% in both juveniles and adult neurones. There were no changes in action potential overshoot or after hyperpolarization (AHP) amplitude. Hypoxia increased the AHP_{50} (50% of AHP maximum duration) from 19.2 ± 6.2 to 23.5 ± 7.5 ms (n.s., $n = 3$) in adults; 11.4 ± 0.8 to 15.1 ± 1.7 mV $P = 0.02$, $n = 6$) in juveniles and 12.4 ± 3.3 to 17.9 ± 4.5 ms ($P = 0.01$, $n = 4$) in neonates. The reversal of these actions was dependent on postnatal age. Differences in ion channel expression have been reported for dissociated neonatal and adult ICN (Auton. Neurosci. 98 (2002) 75). These alterations may contribute to developmental changes in sensitivity to hypoxia in ICN.

Abstract N° A120

Developmental regulation of GABA-ergic responses in intrinsic cardiac neurons of the rat heart

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The amino acids, γ-aminobutyric acid (GABA) and taurine, have been proposed to be neuromodulators in the mammalian heart. Intravenous administration of GABA has been shown to lower blood pressure and to induce bradycardia in several species. Furthermore, GABA has been shown to be released from nerve endings terminating in the sinus node of the guinea pig heart and directly modulate its function by enhancing the release of acetylcholine via activation of $GABA_A$ receptors. We investigated the effects of GABA and taurine on acutely dissociated intracardiac ganglion neurons from neonatal and adult rat hearts using whole-cell patch-clamp recording technique. Focal application of GABA and taurine evoke a depolarizing, excitatory response in neonatal but not adult rat intracardiac neurons. Under voltage clamp, both GABA and muscimol elicit inward currents in a concentration-dependent manner with half-maximal activation (EC_{50}) of 28.1 ± 1.2 μM ($n = 8$). The fast, desensitizing currents could be inhibited by micromolar concentrations of the GABA antagonists, bicuculline and picrotoxin. The $GABA_C$ antagonist, (1,2,5,6-tetrahydropyridin-4-yl)methyl phosphonic acid (TPMPA), had no effect on GABA-induced currents, suggesting that $GABA_A$ receptor channels mediate the response. The GABA-evoked current was age dependent whereby the current density measured at a holding potential of –80 mV was ~20 times higher in intracardiac neurons obtained from neonatal rats (P5: –164.4 ± 7.3 pA/pF) compared to adult rats (P49: –8.4 ± 1.8 pA/pF). The decrease in GABA sensitivity occurs during the first 2 weeks post-natally as maturation of the sympathetic innervation of the rat heart occurs. Antibodies against GABA and taurine demonstrate the presence of GABA-ergic innervation of the intracardiac nerve plexus in the rat heart. These results suggest that GABA and taurine may act as modulators of neurotransmission and cardiac function in the developing mammalian intrinsic cardiac nervous system.

Abstract N° A122

CP-154,526, A CRF1 receptor antagonist, reduces cardio-vascular responses during acute psychological stress in rabbits

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CP-154,526 is a selective non-peptide antagonist of CRF1 receptors, with anxiolytic properties (Proc. Natl. Acad. Sci. USA 93 (1996) 10477; Psychopharmacol 138 (1998) 55). We examined the effect of CP-154,526 on changes in heart rate (HR) and mean arterial pressure (MAP) induced by salient stimuli in conscious unrestrained rabbits with pre-implanted telemetric probes. Stimuli were presented after administration, on different days, of CP-154,526 (30 mg/kg i.v.), propranolol (1.5 mg/kg i.v.) or vehicle. Neither treatment affected basal level of HR or MAP. CP-154,526 substantially reduced pressor and HR responses (both brady- and tachycardic) elicited by stressful stimuli. Propranolol inverted tachycardia induced by a pinprick and reduced airjet-elicited tachycardic and pinprick-elicited pressor responses.

	Sound	Cage drop	Pinprick	Airjet
Effects on HR responses (BPM)				
Vehicle	–29 ± 8	–6 ± 9	62 ± 9	30 ± 3
CP154526	–9 ± 3 *	3 ± 3	21 ± 3 *	14 ± 4 *
Propranolol	–26 ± 4	–20 ± 6	–16 ± 12 *	15 ± 3 *
Effects on MAP responses (mmHg)				
Vehicle	4 ± 1	8 ± 1	16 ± 2	17 ± 1
CP154526	2 ± 1	2 ± 1 *	3 ± 1 *	6 ± 2 *
Propranolol	2 ± 1	7 ± 1	7 ± 2 *	14 ± 2

* Significantly different from control ($P < 0.01$, $n = 6$).

Thus, CP-154526 reduced both vagally and sympathetically mediated cardiac effects. Blocking CRF1 receptors attenuates cardiovascular responses to environmental stimuli, presumably by reducing the probability that a particular stimulus will prove salient.

Abstract N° A123

Disinhibition of the medullary raphe/parapyramidal region increases cardiac contractility in anaesthetized rabbits

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Recent studies (Brain Res. 980 (2003) 1; J. Physiol. 546 (2003) 243) suggest that medullary raphe may contain cardiac presympathetic neurons. We further investigated this possibility in anaesthetized, paralyzed and mechanically ventilated rabbits ($n = 6$). We recorded changes in ECG, mean arterial pressure (MAP), heart rate (HR) and left ventricular pressure (LVP) elicited by pharmacological disinhibition of raphe region by bicuculline. Local microinjection of bicuculline (1 nmol/100 nl) increased MAP (from 99 ± 6 to 112 ± 7 mmHg, $P < 0.01$), arterial pulse pressure (from 59 ± 7 to 66 ± 8 mmHg, $P < 0.05$), LVdP/dT (from 3727 ± 348 to 4110 ± 398 mmHg/s, $P < 0.01$), and slightly decreased HR (from 305 ± 5 to 295 ± 6 BPM, $P < 0.05$). The onset of bradycardia was delayed compared to the increase in MAP and ventricular contractility. Electrical stimulation (50 Hz, 50–200 μA, 5 s) of the same area produced similar haemodynamic changes accompanied by ventricular ectopic beats. Propranolol (1 mg/kg i.v.) caused sustained reduction in MAP (92 ± 7 to 73 ± 9 mmHg, $P < 0.05$), pulse pressure (54 ± 5 to 46 ± 4 mmHg, $P < 0.05$), LVdP/dT (3549 ± 330 to 1694 ± 395 mmHg/s, $P < 0.01$) and HR (298 ± 3 to 206 ± 8 BPM, $P < 0.01$). After propranolol, bicuculline injection evoked only minor pressor responses, without affecting other haemodynamic parameters. Our data indicate that raphe area participate in the sympathetic control of cardiac contractility.

Abstract N° A124

Evidence for the involvement of the opioidergic system in the development of heart failure

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Opioid peptides and their receptors have been shown to be present in the heart, with the κ-opioid receptor the most highly expressed, and to modulate normal cardiac function by inducing bradycardia and a negative inotropic state, as well as inhibiting β-adrenergic receptor signaling. It has also been shown that levels of leu-enkephalin and dynorphin, two endogenous opioid peptides, are altered in the spontaneously hypertensive rat, a model of heart failure. However, the role of the opioidergic system in the development and progression towards heart failure has yet to be elucidated. To investigate the functional capacity of the opioidergic system in heart failure, a hamster model of spontaneous hypertension (H) was employed. Previous characterization of these animals found that hypertension is first observed at 8 weeks resulting in a mean atrial pressure of 162 ± 3 mmHg for H and 94 ± 4 mmHg for control hamsters (C). Utilizing the isolated work-performing heart, 1 months C and H hamsters, those without a hypertensive phenotype, and 10 months C and H hamsters, non-hypertensive controls and chronic hypertensive hamsters, were employed to investigate the effect of κ-opioid receptor stimulation on cardiac function via a cummulative concentration–response curve. Also, an immunoassay was utilized to investigate changes in endogenous opioid peptide levels (β-endorphin, dynorphin-A, and leu-enkephalin) as the animals progressed towards heart failure. Administration of a κ-selective opioid agonist led to a concentration dependent decrease in systolic and diastolic functional parameters that was similar in both 1 months C and H hearts. However, the negative inotropic and lusitropic response to opioid administration was markedly attenuated in 10 months H hearts compared to age-matched C hearts ($P < 0.05$). Cardiac opioid peptide levels also differed between the normotensive and hypertensive lines. These observations suggest that the opioidergic system may be a pivotal modu-

lator in the etiology of heart failure as well as present a possible mechanism for therapeutic intervention.

Abstract N° A125

Cardiac methionine-enkephalin levels are decreased in a mouse model of primary hypertrophy

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The opioid peptide methionine-enkephalin (ME) is produced by heart muscle cells and by neural afferents terminating in the heart. Apart from countering the effects of noradrenalin-mediated stimulation, ME can suppress DNA synthesis and act as a 'negative' growth factor in cardiac tissue. The transgenic AOGN mouse overproduces angiotensin II in the heart, which leads to primary cardiac hypertrophy. In this study we investigated whether alterations in ME concentrations are altered during the development of hypertrophy in AOGN.

Methods. – Hearts were isolated from 14- and 28-week-old AOGN and wild-type (WT) mice. Cardiac tissues were immediately homogenised in acid mixture and boiled for 15 min to inactivate enkephalinases. Enkephalins were extracted and ME content was measured by radioimmunoassay.

Results. – An increase in atrial ME content with age was seen in the WT group (from 3968 ± 627 to 9291 ± 2656 pg/g, $n = 7$, $P = 0.0748$). In the AOGN group the increase in atrial ME content was significantly attenuated compared to WT (from 3745 ± 539 to 5833 ± 1071 pg/g, $n = 7$, $P = 0.02$). No differences in ME were detected between ventricular and atrial tissues.

Conclusion. – The growth promoting effects of elevated cardiac angiotensin II levels in this model of hypertrophy are linked with suppression of ME levels. The coincident regulatory shift in these mediators requires further exploration as to their molecular interaction and contribution to the development of primary cardiac hypertrophy.

Abstract N° A126

Met-enkephalin gradient across the heart decreases with increased sympathetic activation in heart failure

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Within the myocardium, two cellular production sources of Met-enkephalin (ME) have been identified, these being cardiomyocytes and sympathetic nerves. In the context of neuronal release of ME, it appears that the opioid peptide is co-released with noradrenaline (NA) and can oppose the tachycardic, vasoconstrictive and positive inotropic effects of NA. Our group has characterised the presence of cardiac sympathetic nervous system overactivity in heart failure (HF) patients, as indicated by heightened cardiac NA release using radiotracer "spill-over" methodology. In the present study we hypothesised that cardiac ME release correlates with the NA "spill-over" and may be subject to similar baroreceptor control.

Methods. – 3H NA was infused through a peripheral vein in 10 HF patients undergoing cardiac catheterisation for transplantation assessment. Baseline haemodynamic parameters, coronary sinus (CS) and arterial blood samples (9 ml) were collected. Subjects were then tilted on a 20° angle for 10 min and measurements were repeated. ME levels were measured via I^{125} radioimmunoassay, NA was determined using high performance liquid chromatography, 3H NA was measured by liquid scintillation spectroscopy.

Results. – Before upright tilt the CS-arterial gradient in ME correlated with the high NA CS-arterial gradient ($P = 0.052$). The NA CS-arterial gradient increased during upright tilt (427 ± 110 to 585 ± 117 pg/ml, $P = 0.004$). In contrast, after upright tilt ME gradient across the heart decreased significantly (8.8 ± 6.8 to –17.4 ± 9.3 pg/ml, $P = 0.04$), suggesting significant cardiac uptake of ME. Upright tilt invoked a reduction of ME in CS (44.8 ± 26.3 to 14.2 ± 6.4 pg/ml, $P = 0.246$) and arterial samples (36.0 ± 26.5 to 31.6 ± 11.5 pg/ml, $P = 0.433$).

Conclusion. – In HF, ME release appears to be subject to similar baroreceptor control as NA. However, sympathetic stimulation provoked by upright tilt increases NA CS-arterial gradients but decreases ME gradients across the heart.

Abstract N° A127

Effects of peptide molecular mass and PEG chain length on vasoactivity of VIP and $PACAP_{1–38}$ in PEGylated phospholipid micelles

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We have previously shown that vasoactive properties of certain amphipathic peptides are amplified when self-associated with sterically stabilized micelles (SSMs) composed of polyethylene glycol (PEG)-conjugated phospholipids. The purpose of this study was to determine the effects of amphipathic peptide molecular mass and PEG chain length on vasoreactivity evoked by vasoactive intestinal peptide (VIP), a 28-amino acid neuropeptide, and pituitary adenylate cyclase-activating $peptide_{1–38}$ ($PACAP_{1–38}$) associated with PEGylated phospholipid micelles in vivo. Both peptides were incubated for 2 h with SSM composed of PEG with molecular mass of 2000 or 5000 grafted onto distearoylphosphatidylethanolamine (DSPE-PEG2000 or DSPE-PEG5000) before use. We found that regardless of peptide

molecular mass, PEG chain length had no significant effects on peptide–SSM interactions. Using intravital microscopy, VIP associated with DSPE-PEG5000 SSM incubated at 25 °C evoked similar vasodilation as VIP associated with DSPE-PEG2000 SSM in the intact hamster cheek pouch microcirculation. Likewise, $PACAP_{1–38}$-induced vasodilation was PEG chain length independent. However, SSM-associated $PACAP_{1–38}$ evoked significantly smaller vasodilation than that evoked by SSM-associated VIP ($P < 0.05$). When the incubation temperature was increased to 37 °C, SSM-associated $PACAP_{1–38}$-induced vasodilation that was now similar to that of SSM-associated VIP. This response was associated with a corresponding increase in α-helix content of both peptides in the presence of phospholipids. Collectively, these data indicate that for a larger amphipathic peptide, such as $PACAP_{1–38}$, greater kinetic energy and/or longer incubation period is required to optimize peptide–SSM interactions which, in turn, amplify its vasoactivity in vivo.

Abstract N° A128

VIP and its receptors in diabetic heart

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Vasoactive intestinal peptide (VIP) is a vasorelaxant peptide. Its actions are mediated through G-protein-coupled receptors that also recognize pituitary adenylate cyclase-activating peptide and are denoted VPAC1 and VPAC2 receptors. It stimulates insulin secretion and mediates anti-inflammatory effects, and has been proposed for treatment of type 2 and autoimmune diabetes. In the heart, VIP is produced and released primarily by intrinsic neurons and improves cardiac perfusion and function. Here, we investigated the involvement of this system in the events underlying development of diabetic cardiomyopathy in the rat model of streptozotocin-induced diabetes by real-time RT-PCR, and VPAC1- and VPAC2-immunohistochemistry. Cardiac neuropathy of VIP containing neurons manifests progressively during the first 4 months of diabetes at both mRNA and peptide level, and is accompanied by initial downregulation of VPAC2 at one prime target of VIP containing axons, i.e. smooth muscle cells of coronary arterioles. After initial changes that are specific for atria and ventricles, respectively, both VPAC1 and VPAC2 expression return to control or slightly elevated levels at 16 weeks, despite ongoing loss of VIP. Given the cardioprotective role of the VIP signaling system, this persistence of receptors has therapeutic implications, as it can be expected that treatment with VPAC2 agonists will also exert a beneficial effect on diabetic cardiomyopathy.

Granted by: GACR 305/03/D180, GACR 305/04/0144, and by german-czech cooperation grant (WTZ CZE 01/014).

Abstract N° A129

Effect of atropine on vasoactive intestinal peptide concentrations in the rat heart atria

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The effects of acute and chronic atropine (ATR) administration on vasoactive intestinal peptide (VIP) levels in the separated rat heart atria were investigated. Young adult rats (n = 30) received ATR 10 mg/kg daily for 10 d (A group) or saline in corresponding volume (n = 30; C group). One day after the last injection, all animals were anesthetized, pretreated with metipranolol and artificially ventilated. Ten animals from each group were subjected to bilateral vagus nerve stimulation lasting 30 min. Ten rats from each group were pretreated with ATR and then subjected to bilateral vagus nerve stimulation lasting 30 min. Remaining animals from both C and A groups were ventilated for 30 min without any other intervention and served as controls. ECG was recorded throughout each experiment. After the end of experiment, atria were excised and extracted for VIP determinations. The following table shows VIP levels in the right atria (RA) and left atria (LA) of experimental rats:

Group	Intervention	VIP (ng/g)—RA	VIP (ng/g)—LA	n
C	0	3.44 ± 0.3	4.33 ± 0.3	10
A	0	2.09 ± 0.2 *	2.67 ± 0.3 *	10
C	Stimulation	2.16 ± 0.2*	3.48 ± 0.2	10
A	Stimulation	1.94 ± 0.2*	2.72 ± 0.2 *	10
C	Stimulation + ATR	1.88 ± 0.1 *	3.61 ± 0.3	10
A	Stimulation + ATR	2.01 ± 0.2 *	2.45 ± 0.2 *	10

Chronic ATR administration led to a significant decrease in VIP levels in both atria. Vagus nerve stimulation alone decreased VIP concentrations in RA only. ATR administration before the stimulation had no further effect on the tissue VIP levels. The present results suggest that chronic ATR treatment might interfere rather with VIP synthesis than its release in the rat heart atria. VIP releasable by the vagus nerve stimulation seemed to be located preferentially in RA.

Supported by Grant Agency of CU, Grant No 75/2001.

Abstract N° A130

Contractile effects of neurotensin in normal and hypertrophic rat left ventricles

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Although neurotensin (NT)-induced positive inotropic responses on isolated rat atria have been reported in some studies, its inotropic potency in ventricular myocardium has not been determined. In the present study, we assessed inotropic responses to NT (10^{-15}–10^{-7} M) in isolated, isovolumically contracting hearts from normal and isoproterenol (ISO)-treated (0.04 mg/kg/d, intraperitoneally, 5 weeks) Sprague–Dawley rats. The hearts were retrogradely perfused with physiological saline solution (10 ml min^{-1} g of heart weight) and paced at a frequency of 330 beats min^{-1}. In normal rats NT produced marked positive inotropic respon-

ses developing within 30 s after the beginning of peptide infusion. The magnitude of NT-induced responses was equivalent to those of norepinephrine and histamine, but greater than those for serotonin and angiotensin II. Comparisons of the EC_{50} values revealed NT to be as potent as serotonin and angiotensin II, but more potent than norepinephrine and histamine at eliciting contractile responses. A comparison of the inotropic activity of the NT (1–6) and (8–13) fragments, and the NT analogue, [D-Trp^{11}]-NT, showed that the contractile effects of NT were largely attributed to the C-terminal part of this peptide. ISO-induced left ventricular hypertrophy was accompanied by a reduced contractile response both to NT and norepinephrine. Indeed, the maximal inotropic response achieved at a dose of 10^{-7} M of NT was approximately halved in ISO-treated rats, although the EC_{50} value was not altered. Taken together, these findings suggest that NT promotes ventricular contractility, an effect that is downregulated in myocardial hypertrophy.

Abstract N° A131

Factors determining NT-proBNP levels in patients with end-stage renal failure

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Introduction. – Serum N-terminal pro brain natriutretic peptide (NT-proBNP) determination in the general population can help identify patients with left ventricular systolic dysfunction. NT-proBNP levels are, however, often elevated in patients with end-stage renal failure (ESRF), the reason for which is largely unknown. The present study aimed at (i) identifying the factors that affect the concentrations of circulating NT-proBNP in patients undergoing regular hemodialysis and (ii) determining the effect of dialysis on NT-proBNP levels.

Methods. – One hundred and nine patients (82 males, mean age 62 years) on chronic hemodialysis underwent physical examination, ECG, echocardiography, cardiac MRI, and measurement of 24-h ambulatory blood pressure. NT-proBNP was measured before and after hemodialysis using Elecsys 2010 (Roche Diagnostics, Germany).

Results. – Eighty percentage of the cohort had hypertension treated to a mean systolic blood pressure of 144 ± 22 mmHg, 50% had left ventricular hypertrophy and 27% had documented CAD. The mean left ventricular ejection fraction (LVEF) was 50 ± 13%. NT-proBNP was remarkably elevated in these patients (predialysis 4079 pg/ml (median; interquartile range 1893–14549); postdialysis 2773 pg/ml (1073–10773), $P < 0.001$; normal value <334 pg/ml). Logistic regression analysis demonstrated a strong correlation between predialysis NT-proBNP and LVEF ($P < 0.001$), systolic blood pressure ($P = 0.008$) and 24-h urine production ($P = 0.016$). As opposed to the general population there was no correlation between levels of NT-proBNP and age, sex, BMI or volume overload (maximum weight above estimated dry weight) in our cohort.

Conclusion. – Serum concentrations of NT-proBNP are greatly elevated in patients with ESRF undergoing dialysis. Hence they cannot be used as a marker of heart failure in this population. Dialysis induces a significant decrease of NT-proBNP levels, most likely due to loss during dialysis, however, reduced synthesis may also contribute considering the abrupt fall in intravascular volume occuring during dialysis. Further, the correlation between 24-h urine production and NT-proBNP levels can be explained by both increased renal excretion of NT-proBNP and reduced synthesis.

Abstract N° A132

Thyroid hormone enhances calcification of vascular smooth muscle cells in vitro

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Thyroid hormones have marked effects on various tissues and organs, including the cardiovascular system. Although nuclear receptors for thyroid hormones are expressed in vascular smooth muscle cells, their functional roles have been unclear. As thyroid hormones play critical roles in bone remodelling, and both smooth muscle cells and chondrogenic/osteogenic cells are derived from mesenchymal stem cells, we hypothesized that thyroid hormones are also associated with vascular smooth muscle calcification. To test this hypothesis, we examined the effects of 3′,3,5-triiodo-l-thyronine (T3) on rat aortic smooth muscle cells, which were cultured in the presence of 10% thyroid hormone-depleted serum. As assessed by the colorimetric method based on *o*-cresolphthalein complexion, the treatment with a physiological concentration of T3 (15 pM free T3, 5 d) significantly increased the calcium deposition in the smooth muscle cells by 38.7%. The T3 treatment also increased the alkaline phosphatase activity, a phenotypic marker of osteogenesis, by 14.2%. In contrast, aortic smooth muscle tissues isolated from methimazole-induced hypothyroid rats (400 mg/l drinking water, 28 d) showed an increase in the calcium content by 32.9%, compared with that of the control euthyroid rats. Our findings suggest that hypothyroidism directly attenuates vascular smooth muscle cell calcification, but this effect is apparently masked in vivo, presumably due to atherosclerosis by abnormal cholesterol metabolism in the hypothyroid liver. This is the first evidence for the direct effect of thyroid hormone on the calcification of vascular smooth muscle cells.

Abstract N° A133

Overexpression of diacylglycerol kinase zeta inhibits endotheline-1-induced cardiomyocyte hypertrophy

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Diacylglycerol kinase (DGK) is an enzyme that is responsible for controlling the cellular level of diacylglycerol (DAG) by converting it to phosphatidic acid, thus acting as a regulator of protein kinase C (PKC). However, the functional role of DGK has not been rigorously examined in cardiomyocytes. Since DGK inactivates DAG, a strong activator of PKC, we hypothesized that overexpression of DGK inhibited endothelin-1 (ET-1)-induced activation of downstream signaling cascade and subsequent cardiomyocyte hypertrophy. Since it was reported that DGK zeta (DGKζ was predominant isoform in the heart, we overexpressed DGKζ in neonatal rat cardiomyocytes using recombinant adenovirus that encoded rat DGKζ. The translocation of both PKCα and ϵ by ET-1 was inhibited by overexpression of DGKζ. We next examined the activator protein-1 (AP-1) DNA-binding activity by dual-luciferase assay. The increased AP-1 DNA-binding activity by ET-1 was inhibited by overexpression of DGKζ (4.54 ± 0.23 vs. 1.36 ± 0.71-folds over control, $P < 0.01$) Furthermore, ET-1-induced increases in cell surface area were attenuated by DGKζ. We demonstrated for the first time that overexpression of DGKζ inhibited ET-1-induced activation of subcellular signaling pathway and resultant cardiomyocyte hypertrophy.

	PKCa (M/C ratio)	PKCϵ (M/C ratio)	Cell surface area (fold increase)
Control + LacZ	0.17 ± 0.04	0.52 ± 0.03	1.00 ± 0.07
Control + DGKζ	0.24 ± 0.06	0.72 ± 0.20	0.95 ± 0.05
ET-1 + LacZ	0.54 ± 0.11 *	4.93 ± 0.35 *	1.42 ± 0.07 *
ET-1 + DGKζ	0.31 ± 0.04 **	1.31 ± 0.14 **	1.23 ± 0.05 **

M/C ratio: membrane/cytosol ratio as indices for the translocation of PKC.
* $P < 0.01$ vs. control + LacZ.
** $P < 0.01$ vs. ET-1 + LacZ.

Abstract N° A134

S100B expression modulates left ventricular hypertrophy and apoptosis following pressure overload in mice

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Myocardial hypertrophy is an adaptive response of the heart to increased workload, but it is also associated with a high risk of cardiac mortality. S100B, a 20 kDa, Ca^{2+}-binding dimer is an intrinsic negative regulator of myocardial hypertrophy expressed following cardiac disease, suggesting that S100B overexpressing transgenic (TG) and S100B knock-out (KO) mice could be used to relate varying degrees of hypertrophy to cardiac structure and function. We compared 15 wild-type (WT) and 15 TG mice over 28 d following aortic banding (AB) with similar matched sham-operated controls. Of those, 6/15 TG-, 2/15 WT-banded mice and 0/30 sham-operated mice died during the observation period. KO mice did not survive surgery. In both strains, AB increased arterial pressure by approximately 40 mmHg. Echocardiography and postmortem examination indicated that the WT group of banded mice mounted a hypertrophic response (30% increase) accompanied by a program of fetal gene re-expression including β-myosin heavy chain, skeletal α-actin and atrial natriuretic factor, while the S100B overexpressing TG-banded group showed no detectable hypertrophy or the associated fetal gene expression, but marked myocyte apoptosis, as evidenced by DNA fragmentation and increased mRNA expression of the apoptotic related genes Caspase-8, Fas-1. Our results suggest that the absence of adaptive hypertrophy, and the marked apoptosis associated with S100B expression in TG mice may contribute to the poor survival in response to pressure-overload induced by AB.

Abstract N° A135

Expression and functional consequences of RAGE (receptor for advanced glycation end products)/S100B expression in rat myocardium

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RAGE and its ligands including the calcium-binding protein S100B have been implicated in a range of disorders leading to diverse cellular responses varying from cytokine expression to cell survival. We show here that RAGE mRNA and protein is enhanced (approximately 10-fold) in ventricular tissue in a manner overlapping that of its ligand S100B in a rat model of myocardial infarction, coronary artery ligation. In addition, using a co-immunoprecipitation strategy we demonstrated an interaction between S100B and RAGE in ventricular myocardium following coronary artery ligation. To determine the functional role of the RAGE/S100B interaction, rat neonatal cardiac myocyte cultures co-transfected with a full-length cDNA of the RAGE gene or a cytoplasmic deletion mutant of RAGE plus a green fluorescent protein (GFP) expression plasmid were stimulated with nanomolar (1–100) concentrations of recombinant bovine brain S100B. In RAGE/GFP expressing myocytes, S100B-induced myocyte apoptosis in a concentration-dependent manner as evidenced by DNA fragmentation. This effect seems to be RAGE dependent as myocytes expressing the cytoplasmic deletion mutant of RAGE were not apoptotic. S100B also induced RAGE-dependent increased mRNA expression of the apoptotic related genes Caspase-8 (fivefold), and Fas-1 (threefold). Thus, the expression and engagement of RAGE with its ligand S100B post myocardial infarction may play a role in myocyte apoptosis.

Abstract N° A136

Regulation of the human S100A6 gene in myocardial cells by trophic stimuli

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The calcium-binding protein S100A6 has been implicated in the general regulation of distinct genetic programs known to be activated in terminally differentiated non-dividing adult myocytes by trophic stimuli both under physiological conditions and during hypertrophy. To begin to define the regulation of S100A6 expression under basal conditions, and in the setting of trophic stimulation with the α_1-adrenergic agonist phenylephrine (PE), angiotensin II (AII), triiodothyronine (T3), PDGF, TGFβ, prostaglandin $F_2\alpha$, phorbol myristate acetate (PMA) or the activated transcription factors, transcription enhancer factor 1 (TEF-1) and activator protein-1 (AP-1), we characterized the human S100A6 promoter and mapped its upstream regulatory elements in rat neonatal cardiac myocytes in culture, using a luciferase reporter system. The functional S100A6 promoter was localized to a region –167/+134 containing 167 bp upstream of the transcription initiation site of the gene. The human S100A6 promoter is regulated by both positive and negative regulatory elements located upstream in the 5′ flanking DNA regions. The regions –361/–167 and –588/–361 contain strong positive regulatory elements. Negative regulatory elements were mapped to the regions –1371/–1194 and –3000/–1371 of the gene. PE, PMA, $F_2\alpha$, AII, T3, PDGF, TGFβ, all induce the basic S100A6 promoter approximately 1.25 to 3-fold. α_1-Adrenergic stimulation had no effect on promoter activity. The transcription factors, TEF-1 and AP-1 influenced transcription from the basic promoter, implicating active MCAT and AP-1-binding sites, respectively. TEF-1 transrepressed (0.35-fold) and AP-1 transactivated (fivefold) the basic S100A6 promoter. Our results suggest that multiple signaling pathways induce the S100A6 gene and that this induction is modulated by TEF-1 and AP-1.

Abstract N° A137

The signaling pathway of macrophage migration inhibitory factor induces MMPs expression

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The aim of this study is to investigate the potential role of macrophage migration inhibitory factor (MIF) in the destabilization of atherosclerotic plaques and the signal transduction pathways of MIF inducing matrix metalloproteinases (MMPs) expression in macrophages in vitro. Real-time PCR showed that constitutive expression of MMP-2, MMP-9 and MT-MMP-1 mRNA was low in normal macrophages. Addition of MIF strongly induced MMP-2, MMP-9 and MT-MMP-1 mRNA expression by macrophages in a time- and a dose-dependent manner, being significant twofold increase at 3 h after MIF stimulation. Similarly, western blot analysis demonstrated MIF-induced MMP-2, MMP-9 and MT-MMP-1 protein synthesis by macrophages in a time- and a dose-dependent fashion, being significant at 12 h after MIF stimulation. The specificity of MIF to induce MMPs mRNA or protein expression in macrophages was further demonstrated by the addition of a neutralizing MIF mAb. In addition, the upregulation of MMPs by MIF stimulation can be blocked completely by MEK-1 inhibitor PD98059, partly by JNK inhibitor SP600125, but not blocked by p38 inhibitor SB203580. Induction of dominan-negative MEK1 by retrovirus blocked MMPs expression significantly, but induction of dominan-negative ERK by a replication-deficient adenovirus blocked MMPs synthesis slightly. Similarly, dominan-negative MEK1, not dominan-negative ERK, blocked the phosphorylation of transcription activators c-jun. This process can be confirmed by c-jun *trans*-reporting system. Thus, MIF may play a critical role in the destabilization of human atherosclerotic plaques by upregulation of MMP-2, MMP-9 and MT-MMP-1 expressions by MER1–ERK–c-jun-dependent pathways.

This work was supported by grants from National Natural Science Foundation of China (30271287, 30300421) and Guangdong Provincial Natural Science Foundation for Research Team (015015).

Abstract N° A138

Using antisense RNA to inhibit the macrophage migration inhibitory factor expression in human umbilical vein endothelial cell

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Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine involved in atherosclerosis. By blocking new mRNA synthesis with antisense RNA, post-transcriptional gene silencing phenomenon in human umbilical vein endothelial cells (HUVECs) is reported in this study. A vector (pcDNA3) was used that transcribed antisense RNA and hairpin-double-stranded RNA (dsRNA) of MIF gene separately to transfect HUVECs by using lipofectamine 2000. By using G-418 sulfate, two HUVECs which were transfected by antisense RNA or hairpin-dsRNA of MIF separately were obtained and identified by PCR. The apoptotic cells were visualized with the fluorescent DNA-

binding dyes Hoechest 33258 and propidium iodide under fluorescent microscope. The real-time PCR and western blot analysis showed that MIF mRNA and MIF protein was upregulated significantly by the stimulation of lipopolysaccharide (LPS) at 10 ng/ml for 30 h. In the present of antisense RNA or hairpin-dsRNA, the MIF expressions were both significantly inhibited ($P < 0.01$), but the apoptosis was observed only in the HUVECs which were transfected by hairpin-dsRNA. The results indicate that antisense RNA can specifically inhibit the MIF express, but the hairpin-dsRNA (348 bp) can reduce the genes expression in HUVECs by unspecific apoptosis. Thus, antisense RNA not full hairpin-dsRNA (348 bp) is suitable for gene therapy, small interfering RNAs (siRNAs) may be more potential for RNA interference which will be performed in our further study.

This work was supported by grants from National Natural Science Foundation of China (30271287, 30300421) and Guangdong Provincial Natural Science Foundation for Research Team (015015), and Guangdong Provincial Natural Science Foundation (033189).

Abstract N° A139

PKC reduces sarcoplasmic reticulum calcium and induces cardioprotection

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Protein kinase C (PKC) activation has been shown to be cardioprotective. We previously showed that activation of PKC-epsilon reduced that rate of decline in ATP during ischemia and improved postischemic contractile function. We also found that reducing sarcoplasmic reticulum (SR) calcium cycling during ischemia is cardioprotective. To examine the role of SR calcium in the cardioprotection associated with activation of PKC, we used freshly isolated single myocytes from adult rat ventricular myocardium. To confirm that PKC activation is protective in the myocyte model, myocytes were exposed to 3 μM DOG for 10 min to activate PKC. Myocytes were gently pelleted and covered with oil in 1.5 ml tubes to simulate ischemia and incubated at 37 degrees. Trypan blue was applied to assess myocyte viability. Untreated (control) myocytes had increased cell death compared to myocytes treated with DOG after simulated ischemia. Cell death was 57.9 % in controls vs 47.3 % in DOG treated myocytes after 1 h of simulated ischemia, 65.5 % vs 48.3 % at 2 h, 68.0 % vs 50.2 % at 3 h, and 70.7 % vs 53.3 % at 4 h. Once we confirmed that DOG was cardioprotective, we examined the effect of DOG on SR calcium using fura-2 fluorescence to monitor calcium transients and caffeine releasable calcium as a measure of SR calcium content. At 10 min after addition of DOG, there was a decrease in the amplitude of the $[Ca^{2+}]_i$ transient, to 82 ± 22 % of the pretreatment level. Caffeine-releasable SR calcium measured after 10 min of DOG treatment was reduced by 12 %. We also analyzed the $[Ca^{2+}]_i$ transient curve as an indicator of SERCA and Ryanodine Receptor function. The relaxation time after 2 min of DOG exposure was prolonged compared with control, suggesting decreased SERCA activity. These data suggest that activated PKC reduces SR Ca^{2+} content, and that reduced SR Ca^{2+} may be important in cardioprotection.

Abstract N° A140

Vardenafil : a novel type 5 phosphodiesterase inhibitor reduces myocardial infarct size following ischemia/reperfusion injury via opening of mitochondrial K_{ATP} channels in rabbit

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We previously demonstrated that sildenafil (Viagra), a potent inhibitor of phosphodiesterase-5 (PDE-5) that enhances erectile function in men through upregulation of cGMP, induces powerful preconditioning like cardioprotective effect following ischemia/ reperfusion (I/R) injury. In the present study, we further investigated the effect of vardenafil (Levitra), a more selective and biochemically potent inhibitor of PDE5 in protection against I/R injury. Rabbits were treated with vardenafil (0.014 mg/kg, iv) or equivalent volume of saline, 30 min prior to 30 min of sustained regional ischemia followed by 3 hrs of reperfusion. 5-hydroxydecanoate (5-HD, 5 mg/kg, iv) or HMR 1098 (HMR, 3 mg/kg, IV), the respective specific blockers of mitochondrial or sarcolemmal K_{ATP} channel were administered 5 min before I/R. Infarct size was measured by computer morphometry of tetrazolium stained sections. Mean arterial blood pressure decreased from 93.5 ± 2.6 to 82.2 ± 1.5 mmHg and heart rate increased from baseline value of 151 ± 20 to 196 ± 4.6 (mean ± SEM, $p < 0.05$) within 5 minutes after treatment with vardenafil. The infarct size (% of risk area) was reduced from 33.8±1.3 in control rabbits to 14.3±2.2 (58 % reduction, $p < 0.05$). 5-HD abolished vardenafil-induced protection with increase in infarct size to 34.5 ± 2.3 ($p < 0.05$, n-6/groups). In contrast, HMR failed to block the protective effect of vardenafil (infarct size, 14.3 ± 2.2 versus 16.3 ± 1.0 in vardenafil + HMR, $p > 0.05$). Both inhibitors of the K_{ATP} channel had no influence on infarct size in the control rabbits, as shown by infarct size of 34.9 ± 1.1 and 33.3 ± 1.4 in animals treated with 5HD and HMR, respectively. For the first time, we demonstrate that vardenafil induces protective effect against I/R injury via opening of mitochondrial K_{ATP} channel. These results further support our hypothesis that novel class PDE-5 inhibitors, in addition to their well known clinical effects in the treatment of erectile dysfunction in men, also induce protective effects in the ischemic heart.

Abstract N° B1

Hypoxia/reoxygenation studies on mouse embryonic stem cells

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It is important to understand the cellular responses to hypoxia since hypoxia conditions lead to serious human disease such as ischemia and tumorigenesis. Hypoxia recently has been shown as a factor that promotes the self-renewal of murine and human normal hematopoietic stem cells. We have recently cultured murine embryonic stem cells (CCE27) in LIF maintained culture medium. The pluripotency of these embryonic stem cells has been proven by successfully differentiating into embryoid bodies (EBs) with distinctive germ layers. About 50% of the induced EBs show rhythmic beating after 12 d of culture in differentiation medium. Two weeks cultures of EBs on gelatin-coated dishes show abundant expression of cardiac troponin-T and organized myofibrils. The hypoxia/anoxia effects are being tested by treating the cells with an anoxic environment in anoxic chambers. Our results show that extensive exposure to anoxia induces apoptosis in the embryonic stem cells. Western blotting has shown clearly that BNIP3, a hypoxia-induced pro-apoptosis protein in the stem cell, is expressed in the hypoxia/reoxygenization-treated stem cells. In our differentiated stem cell cultures, we have observed the translocation of protein kinase C (PKC)-epsilon to myofibril structures after phorbol ester treatment, indicating the involvement of PKC-epsilon in the hypoxia response in stem cell-derived cardiomyocytes. Hypoxia/reoxygenization treatment of stem cells on potential differentiation ability into specific cell types is being studied with emphasis on cardiomyocyte formation. Our preliminary data suggest that hydrogen peroxide is an inducing factor for cardiomyocyte differentiation from embryonic stem cells; 10 nM H_2O_2 treatment on the EBs has significantly increased the numbers of beating centers to twice that of untreated or 1 μM H_2O2 overtreated undifferentiated cells. Microarray assays are used to elucidate the genome level responses of embryonic stem cells to hypoxia/reoxygenization during differentiation, apoptosis and self-renewal processes.

Supported by NIH HL58435 and HL61246 and AHA Grant-in-aid to LFL.

Abstract N° B2

Histone deacetylase inhibitors reverse hypertrophic growth and gene expression in cultured neonatal cardiomyocytes

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Cardiac hypertrophy is an independent risk factor for the development of heart failure. Although it was originally assumed that hypertrophy was a necessary compensatory response of the heart to increased load, more recent evidence suggests that contractile function can be maintained in the absence of cardiomyocyte hypertrophy. Thus, inhibiting the onset of hypertrophy, or reversing established hypertrophic growth, may be of therapeutic value. Histone deacetylase inhibitors (HDAC-I) are a novel class of antineoplastic agents currently in clinical trials for various types of cancer. It has been shown previously that hypertrophy of cultured neonatal cardiomyoctyes is inhibited by HDAC-I. This was correlated to increased levels of histone acetylation. We have now extended this investigation to demonstrate that the hypertrophic phenotype of cardiomyocytes can be at least partially reversed by the administration of HDAC-I. Stimulation of cardiomyocytes with phenylephrine for 48 h induced the typical expression of fetal genes and led to increases in cell size. An additional 48 h culture in the presence of phenylephrine plus HDAC-I resulted in the repression of fetal gene expression, reduction of protein accumulation, and decrease of cell size. This phenotypic change was accompanied by a reduction in global histone acetylation. Similar results were obtained with different chemical classes of HDAC-I. These results suggest that HDAC-I may be effective at modulating cardiac hypertrophy both in a prophylactic as well as a therapeutic setting.

Abstract N° B3

Occurrence of bone-marrow-derived cardiomyocytes in transgenic mice with cardiac overexpression of monocyte chemoattractant protein-1

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Only recently, it became apparent that in cardiac diseases characterized by myocyte loss, cardiomyocytes die by multiple mechanisms and probably proliferate. We have studied transgenic (TG) mice with prolonged cardiac overexpression of monocyte chemoattractant protein-1 (MCP-1). These slowly develop an autoimmune inflammatory cardiomyopathy characterized by cardiomyocyte loss by autophagy, antigen presentation, necrosis but not apoptosis, and low rate of proliferation. In order to study whether myocyte loss might be counteracted by myocyte replacement from bone-marrow-derived cells (BMDCs), MCP-1 TG mice were lethally irradiated and subsequently transplanted with BMDCs from enhanced green fluorescent protein (eGFP) ubiquitously expressing mice. At 4 months after transplantation the hearts were analyzed for detection of GFP cells using confocal laser scanning microscopy, myocytes were identified by sarcomeric actin labeling, and nuclei by TOTO 3. MCP-1 transplanted mice showed significant levels of haematopoietic reconstitution (81–91% of nucleated blood cells expressed eGFP), which was measured by flow cytometry. At 4 months after transplantation, GFP-BMDCs were found

incorporated into the ventricular wall. The vast majority of green cells were identified as interstitial cells. However, several GFP-positive cells exhibited cross-striated actin labeling and had a morphology and alignment that was indistinguishable from the surrounding cardiomyocytes, following the orientation of the other fibres. These data indicate that in MCP-1 TG mice myocyte loss is partially counteracted by myocyte replacement from BMDCs.

Abstract N° B4

The study on gene transfer into different passages of bone marrow mesenchymal stem cell

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The efficiency and stability of adenovirus-medicated gene transfer into different passages of bone marrow mesenchymal stem cells (BMSCs) were investigated. BMSCs were obtained from bone marrow of Sprague–Dawley rat and cultured. Then P3 and P8 BMSCs were transfected with Ad-CMV-green fluorescent protein (GFP), respectively. The transfection ratio was evaluated by flow cytometer at 2, 4, 7 and 10 d after transfection. At the same time Coxsackie and Ad receptor (CAR) of different passages of BMSCs was estimated by RT-PCR and western blot. The green fluorescence could be observed 24 h after transfection, while the strength of fluorescence were rising with time and the peak was at 7 d. It was seen that the transfection ratio was over 80% and there was no difference between P3 and P8 BMSCs; $P > 0.05$. Flow cytometer analysis by different gates showed the transfection ratio was high in BMSCs in period of productive metabolism. The gene expression of CAR in P3, P6, and P8 was similar, and the same change was in the protein expression of CAR in P3 and P8 BMSCs. Ad-CMV-GFP could be transferred to BMSC effectively and sustained about 28 d. It was suspected that BMSC in mitotic phase were easy to be transferred by Ad-CMV-GFP and different passages of BMSCs from P3 to P8 BMSCs could be as high-effectively gene vehicle.

Abstract N° B5

Induction of controlled therapeutic angiogenesis by L-Lysine hydrochloride in ischaemic myocardium

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Acute and chronic ischemia in myocardium produce higher yield of angiogenic agents in situ as a normal response, which is inadequate on demand. Simpler mode of therapy for induction of controllable therapeutic angiogenesis, especially for myocardial salvage in ischemic heart disease are under search. Based on the profound cellular expansion abilities of L-Lysine hydrochloride, the molecule has been examined in the current experimental work, for its possible role in reperfusing ischemic myocardium, in an acute mode of experimentation. Two phasic sheep experiments were conducted with Madras Red breed sheep of similar age group and body weight. Four sheep hearts were accessed through left anterolateral thoracotomy in the first phase and an upper diagonal branch tied in each to induce myocardial infarction, whereas in the second phase through median sternotomy the apical diagonal branches were tied in 6 sheep. Injectable L-Lysine (250mg), was used for various routes of injection, such as locally at the infarcted area, and/or aortic root or intravenously in a dose of 4gm per day for 10 days. One control in each group had 1 ml normal saline injected locally. Preoperative and post operative 2D echocardiography assessment of the sheep hearts were done till the day of sacrifice ; phase 1 sheep were sacrificed after 6 weeks and phase 2, after 4 weeks. Macroscopic findings and histopathological examinations of the infarcted area by various stains are noted. Quantitative increase of neoangiogenesis is noticed in the experimental animals having L-Lysine than the control. Quantitation done by averaging the observation of five fields of 200 magnification and confirmation done by immunohistochemistry. L-Lysine creates considerable neoangiogenesis in ischemic myocardium, even through intravenous administration in sheep model ; being an essential amino acid it will be easy to use clinically for human trial in ischemic heart disease.

Abstract N° B6

The kelch family protein Nd1 and actin depolymerising proteins are differentially expressed in growing collateral arteries

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Fluid shear stress (FSS) induces collateral growth (arteriogenesis). To identify genes involved in this process, two models of chronic high FSS were used. The femoral arteries of rabbit or pig were ligated (LC) bilaterally and a unilateral arteriovenous shunt (SC) was created. Genes differentially expressed between LC and SC were identified by a combination of 2D-PAGE, array analysis and suppression subtractive hybridisation. Northern blot analysis was used to verify the results. Results showed differential expression of genes involved in actin turnover and stabilisation. In particular, the actin depolymerising proteins Cofilin1 (36% up-regulated) and Cofilin2 (72% down-regulated on SC) are expressed reciprocally ($P < 0.05$) in the porcine model. Additionally, the kelch family protein Nd1, known to stabilise actin filaments, is 69% downregulated on SC in the rabbit model ($P < 0.009$). Similar results were obtained in the porcine model. Our results indicate an important role of proteins involved in actin regulation during arteriogenesis. The reciprocal expression of the Cofilins and the reduced expression of Nd1 seem to be responsible for the “switch” from the contractile to the

synthetic phenotype and the restart of proliferation during collateral growth. We hypothesise that actin depolymerisation and polymerisation are important events during arteriogenesis, necessary for the migration of vascular smooth muscle cells.

Abstract N° B 7

Hypoxic preconditioning induces myocardial angiogenesis through ERKs-mediated HIF-1α expression

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Angiogenesis plays an important role in cardioprotection of ischemic preconditioning. Hypoxia-inducible factor-1 (HIF-1) is involved in transcription of the hypoxia-inducible genes including vascular endothelial growth factor (VEGF), which is involved in angiogenesis. To determine whether hypoxic preconditioning (HPC) induces myocardial angiogenesis through extracellular signal-regulated protein kinases (ERKs)-mediated HIF-1α expression, 12 male Sprague–Dawley rats were divided into either HPC or control group. Animals in HPC group were subjected to systemic hypoxic exposure (10 ± 0.4 O_2) for 4 h. Animals in control group were time matched with the preconditioned group and maintained under normoxic conditions for 4 h. Rats were euthanized and the hearts were removed at 1, 7, and 21 d after HPC. The myocardial samples were submitted for immunohistochemical analysis to detect microvascular density with the antibody against factor-VIII-related antigen. Remaining samples were submitted for western blot analysis to measure activities of ERKs with phospho-specific antibody against ERK1/2 and expression of HIF-1α with HIF-1α antibody. We found that microvascular density appeared increased by 36.99% and 37.76% in myocardium 7 and 21 d after HPC, respectively. Western blot analysis showed activated ERKs and expression of HIF-1α in whole cell extracts from hypoxic preconditioned cardiomyocytes. We conclude that upregulation of HIF-1α by ERKs mediates angiogenesis of hypoxic preconditioning.

Keywords: Angiogenesis; Hypoxic preconditioning; Hypoxia-inducible factor-1

Abstract N° B 8

Gene transfer of stromal cell-derived factor-1α enhances ischemic vasculogenesis and angiogenesis via VEGF/eNOS-related pathway

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Background. – Stromal cell-derived factor-1α (SDF-1α) is implicated as a chemokine for endothelial progenitor cells (EPCs). We, therefore, hypothesized that SDF-1α gene transfer would induce therapeutic neovascularization in vivo by functioning as a chemokine of EPC.

Methods and results. – To examine SDF-1α-induced mobilization of EPC, we used bone marrow-transplanted mice whose blood cells ubiquitously express β-galactosidase (LacZ). We produced unilateral hindlimb ischemia in the mice and transfected them with plasmid DNA encoding SDF-1α or empty plasmids into the ischemic muscles. SDF-1α gene transfer mobilized EPCs into the peripheral blood, augmented recovery of blood perfusion to the ischemic limb, and increased capillary density associated with partial incorporation of LacZ-positive cells into the capillaries of the ischemic limb, suggesting that SDF-1α-induced vasculogenesis and angiogenesis. SDF-1α gene transfer did not affect ischemia-induced expression of vascular endothelial growth factor (VEGF), but did enhance Akt and endothelial-type nitric oxide synthase (eNOS) activity. Blockade of VEGF or nitric oxide synthesis prevented all such SDF-1α-induced effects.

Conclusion. – SDF-1α gene transfer enhanced ischemia-induced vasculogenesis as well as angiogenesis in vivo through a VEGF/eNOS-related pathway. This strategy might become a novel chemokine therapy for next generation therapeutic neovascularization.

Abstract N° B 9

Augmentation of arteriogenesis via cell therapy

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The treatment of vascular occlusive disease is of tremendous clinical importance for alleviation of morbidity and mortality in the western world. Although some patients have developed a collateral circulation, the net effect was rarely adequate to return to normal blood flow. Instead of invasive bypassing or catheter intervention, cell therapy via monocytes (Mo) transplantation could be used for promotion of collateral vessel growth. Mo play a key role for the remodelling of a pre-existing arteriole into a collateral artery (ca) by various arteriogenic cytokines. In this study, we test the hypothesis that Mo transplantation is able to increase arteriogenesis and demonstrate the homing of vehicle Mo for a gene therapeutic approach histochemically. Therefore, Mo were isolated and intravenously (i.v.) re-injected into rabbits 24 h after ligation of their femoral artery ($n = 6$ for each condition). One week later collateral conductance (CC) was determined in vivo (CC = flow/mean central pressure–mean peripheral pressure, ml/min/100 mmHg). Additionally angiographic evaluation of ca within the rabbit hind limbs was done. Transplantation of allogenic Mo (same species) resulted in a 83% rise of CC from 105 ± 20, ca = 11 ± 2 (ligation without transplantation) to CC = 192 ± 18, ca = 16 ± 1, $P < 0.001$ vs. autologous Mo (same animal) (CC = 130 ± 29,

ca= 11±1). The homing of re-injected Mo was demonstrated by visualization of LacZ-transduced Mo close to collateral vessels. Their transplantation after ex vivo adenoviral transduction with GM-CSF was able to efficiently augment arteriogenesis (CC = 211 ± 46, ca = 19 ± 2, $P < 0.001$) in comparison to control-transduced cells (CC = 122 ± 7, ca = 14 ± 2) being close to values determined in unligated control vessels (CC = 262 ± 31, ca = 5 ± 1). The observations derived from our study have the potential to offer a method for systemic cell administration for transplantation into areas of arteriogenesis. Obtaining Mo is currently widely available, all of which makes the ultimate goal of applying this strategy to humans for therapy of vascular disease eminently attractive.

Abstract N° B10

Effects of hydrogen peroxide on proliferation and messenger RNA of MMP-2 and TIMP-2 in vascular smooth muscle cells

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To determine whether hydrogen peroxide alters the activation of transcription of matrix metalloproteinases-2 (MMP-2) and tissue inhibitors of metalloproteinase-2 (TIMP-2) in rat vascular smooth muscle cells (VSMCs), we measured the proliferation and messenger RNA (mRNA) levels of MMP-2 and TIMP-2 by using MTT method and RT-PCR, respectively. We found that hydrogen peroxide at range of 0.01–200 μM did not, but at more than 300 μM decreased viability of VSMCs. The OD value in MTT test was increased by treatment of hydrogen peroxide at 10 μM, which reached a peak at 24 h and maintained similar level to 48 h. Hydrogen peroxide induced transcription of mRNA of MMP-2 in dose-dependent and time-dependent manner, and the peak of MMP-2/beta-actin ratio occurred at 24 h of incubation, but TIMP-2 mRNA levels were similar during 12–48 h incubation with hydrogen peroxide. The results indicate that hydrogen peroxide acts as a stimulator for proliferation and transcription of MMP-2 of VSMCs, suggesting that it may play a role in the vascular remodelling.

Abstract N° B11

Estrogen modulates cardiac mast cell-mediated extracellular matrix degradation

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There are fundamental differences between males and females with regard to susceptibility to heart disease. Although numerous animal models of heart failure have demonstrated that premenopausal females are afforded cardioprotection and therefore fare better in the face of cardiac disease than their male counterparts, many questions as to how this occurs still exist. We have shown that increased mast cell density is associated with adverse ventricular remodeling. Also, we have demonstrated that chemically induced mast cell degranulation with compound 48/80 caused marked changes in matrix metalloproteinase (MMP) activity, cardiac collagen structure, and cardiac diastolic function in male rats. With the known gender differences in cardiac disease in mind, we sought to examine the effects of chemically induced cardiac mast cell degranulation in isolated, blood perfused, functioning hearts of intact females, ovariectomized females (OX), and OX treated with 17β-estradiol (OX + EST). In response to mast cell degranulation, no change in cardiac function, MMP-2 activity, or collagen volume fraction (CVF) was observed in intact females or OX + EST. However, a significant rightward shift in the pressure–volume relationship occurred post-48/80 in OX hearts, accompanied by a significant reduction below control in CVF (0.455 + 0.23 vs. 0.730 + 0.072, $P < 0.05$), and a significant 132.6% increase in active MMP-2 values over that seen in intact female hearts post-48/80 ($P < 0.05$). These findings indicate that estrogen's cardioprotective role could be partially mediated by its effects on cardiac mast cells, MMPs, and the extracellular matrix.

Abstract N° B12

Development and evaluation of matrix-type transdermal patch for controlled delivery of carvedilol

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Matrix-type transdermal patches were fabricated using Chitosan. Patches were fabricated by casting method using lactic acid. The drug load was adjusted to 0.3%. Patches thus fabricated were evaluated for drug release using modified Franz diffusion cell using dorsal part of rattus skin and Cadaver skin as rate limiting membrane. Patches were also evaluated for their physico-chemical properties (i.e. thickness, tensile strength, drug load, content uniformity and physical appearance). The rate of drug release was calculated as cumulated percentage of drug release and analysed for its release kinetics. The rate of drug release was found to be 92–99% in 48 h. The release kinetics when statistically analysed, showed that drug releases from these patches following zero-order kinetics ($R = 0.98$). Therefore, it may be possible that optimized patch containing Carvedilol can be used to deliver Carvedilol in systemic circulation at a controlled rate up to 48 h. The in vivo studies in rats and rabbits to evaluate its pharmaco-kinetics parameters are in progress.

Acknowledgements: Government of NCT of Delhi, India, AIIMS, New Delhi, Cipla Ltd, Mumbai.

Abstract N° B13

Cardioprotection in mast cell deficient rats with chronic volume overload

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We have previously reported that cardiac mast cell-mediated myocardial remodelling contributes to the development of ventricular dilatation associated with heart failure. We have also demonstrated that elevations in tumour necrosis factor (TNF)-alpha contribute to the adverse myocardial remodelling in chronic volume overload. In order to further characterize the role mast cells play in the initial myocardial remodelling and subsequent development of heart failure, 8-week-old male mast cell deficient (Ws/Ws) and normal wildtype (WT) rats were divided into three groups as follows—Sham-operated control, 5 d aortocaval fistula and 56 d fistula groups. In contrast to the significant increase of 120% in left ventricular (LV) matrix metalloproteinase (MMP) activity in the WT hearts at 5 d post-fistula, MMP-2 activity in the Ws/Ws hearts remained at control levels. LV levels of TNF were significantly increased sixfold in the WT hearts, while TNF levels in the Ws/Ws hearts were similar to control. Consistent with these differences in MMP-2 activity and TNF levels, serial echocardiography demonstrated significant differences in the extent of ventricular dilatation induced by chronic volume overload. While the LV end diastolic dimension (LVEDD) was significantly increased by 82% above baseline in WT hearts at 8 weeks post-fistula, the LVEDD in the mast cell deficient Ws/Ws hearts was only 37% larger than baseline measurements. These findings are consistent with our previous observations that cardiac mast cell degranulation is responsible for the MMP activation, extracellular matrix degradation and ventricular dilatation induced by chronic biventricular volume overload. These findings also suggest that cardiac mast cells regulate activation of latent TNF in the myocardium and demonstrate that the mast cells play a crucial role in regulating the myocardial remodelling responsible for heart failure.

Abstract N° B14

Synthesis and evaluation of new chelators for use as MMP inhibitors using cardiac cell cultures

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Matrix metalloproteinases (MMPs) are hydrolytic enzymes involved in the breakdown of connective tissue. MMP activity is associated with a number of illnesses including arthritis, cancer, and cardiovascular disease. Compounds that can inhibit MMP activity may prove to be useful therapeutic agents. The MMP active site contains a Zn(II) ion bound to three histidine ligands with open coordination sites for substrate binding. Our research program is focused on developing novel MMP inhibitors and exploring the binding modes of known inhibitors. Using hydrotris(pyrazolyl)borate (Tp) model complexes the interaction of MMP inhibitors with the enzyme has been reproduced. This strategy has revealed several chelators that may be promising compounds for MMP inhibition. These lead compounds were evaluated in an MMP activity assay that utilizes a fluorescent peptide substrate. The results of these assays indicate that the newly identified ligands have activities ranging from 3- to 600-fold more potent than the hydroxamic acid ligands used in most clinically tested MMP inhibitors. These activities and lack of evidence for cytotoxicity were validated using cultures of rat cardiac fibroblasts. We propose that these new chelators represent a significant step toward producing improved, next-generation MMP inhibitors.

Abstract N° B15

Recellularisation of photo-oxidised pericardium using immature foreign body reaction in the construction of a tissue engineered heart valve

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Foreign body reaction (FBR), a mechanism encapsulating non-degradable implants, deposits a layered structure of macrophages, fibroblasts and mesothelium. We hypothesise that the immature stage of FBR can be used to repopulate durable biological matrix material for construction of a tissue engineered heart valve. Eight sheep received an intraperitoneal implant of an acellular photo-oxidised bovine pericardium patch suspended in a stainless steel cage. After 3 d, the retrieved patch showed the presence of both macrophages and a blast-like vimentin positive cell population. From this material, a valve was constructed and immediately implanted in the pulmonary artery. Additional sheep served as control, receiving an identical valve construct without prior intraperitoneal cell seeding. Weekly echocardiography showed normal functioning valves. The control and four sheep were sacrificed at day 8, the remaining four at 1 month. The explants were fixed for immunohistochemistry (IHC) and matrix stability (sirius red) study. IHC shows a clear in- and overgrowth of cells in/on the photo-oxidised pericardium with a two- to fourfold increase in cell number with time in situ. Positive for both vimentin and α-smooth muscle actin, but negative for heavy chain myosin; we conclude that the cells are of the (myo)fibroblast phenotype. Additionally IHC showed a limited but progressive endothelialisation of the valve surface. Specific IHC indicated a trivial presence of macrophages in the overgrowth and complete absence in the original matrix. The original matrix showed remodelling over time: at 8 d the total collagen markedly increased in both the wall and leaflet, but normalised at 1 month. The amount of organised collagen diminished gradually in parallel with the increased recellularisation. We conclude that by this method we have repopulated the valve with (myo)fibroblast, i.e. its original interstitial cells, and induced spontaneous endothelialisation without causing chronic macrophage infiltration or matrix degeneration.

Abstract N° B16

An attempt to develop an experimental model of rheumatic heart valve in vitro using tissue engineering techniques

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Tissue engineering of heart valve represents a new experimental concept to improve current mode of therapy in rheumatic valvular heart disease and to develop an experimental model of rheumatic heart disease (RHD) in vitro. For the treatment of heart valve disease, mechanical or bioprostheses are currently in use. But the drawbacks of glutaraldehyde-fixed tissue valves or mechanical valves include the short durability or the need for life-long anticoagulation, respectively. Both have in common the inability to grow, which makes valvular heart disease problematic especially in children. The aim of the present study is to develop a new methodology for the tissue engineered heart valve combining human cells and xenogenic acellularized matrix. Porcine aortic as well as pulmonary valves were acellularized by deterging cell extraction using Triton. Endothelial cells as well as myofibroblast cells were isolated in parallel from human saphenous veins and expanded in vitro. Specimens of the surface of the acellular matrix will be seeded with myofibroblast cells and endothelial cells. Analysis of acellularity was performed by light microscopy and scanning electron microscopy. Cell viability following seeding will be assayed by fluorescence staining of viable cells. The tissue-engineered valve then will be cultured with sensitized serum and lymphocytes of RF/RHD patients to see the pathological lesion. The acellularization procedure resulted in almost complete removal of the original cells while the 3D arrangement of the matrix fibers was grossly maintained. The porcine matrix could be seeded with in vitro expanded human myofibroblast and endothelial cells and maintained in culture for up to 3 d to document the formation of confluent cultures. The xenogenic matrix will be reseeded with human endothelial and myofibroblast cells. This approach may eventually lead to the engineering of tissue heart valves repopulated with the patient's own autologous cells.

Abstract N° B17

Different responses to inflammation of left- and right-sided heart valves

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Infective endocarditis (IE) rarely involves right-sided heart valves as compared to left-sided valves. One reason may be lower haemodynamic stress; however, we hypothesized that there may be a difference in immune response. In our established rabbit model of IE, surgically-induced valve insufficiency and bacterial inoculation (*Staphylococcus aureus*) were performed. Untreated animals, animals with bacteraemia only and with surgery only served as controls. Using semi-quantitative RT-PCR, mRNA expression of the macrophage colony-stimulating factor (MCSF-1), the MCSF-1 receptor c-fms and of β_1-integrin on valve tissues was analysed for comparison of left- and right-sided heart valves. MCSF-1 increased on mitral and tricuspid valves after the surgical procedure alone ($P < 0.05$). Bacterial inoculation resulted in a further increase of MCSF-1 on the mitral ($P < 0.05$), but not on the tricuspid valve. Corresponding data were obtained for the aortic and pulmonary valves (analyses performed on pooled valves). Comparison of β_1-integrin-expression (pooled tissue) gave evidence of downregulation in right-heart valves, while left-heart valves again remained unaffected. c-fms was equally decreased on both sides of the heart after the surgical trauma; bacteraemia did not induce additional changes. Our results suggest a more pronounced inflammatory response to endothelial trauma and bacteraemia on left-sided heart valves. The lack of β_1-integrin downregulation on left-sided heart valves may facilitate fibronectin binding and bacterial growth, thus promoting the formation of vegetations. We conclude, that there is a different immune response to bacterial infection between right and left heart.

Abstract N° B18

Impacts of hemodynamic factors on the myocardium after intra-abdominal heart transplantation in mice

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Mouse intra-abdominal heart transplantation (IAHT) model is increasingly being used to understand the immunologic bases of allograft rejection. However, little is reported on myocardial adaptation to altered hemodynamic factors, particularly over a long period of time. IAHT was performed in mice. Graft function was confirmed by palpation and periodic echocardiography. At various time points, grafts were removed for routine histological evaluation. After 3 months of transplantation, some of the grafts had prominent concentrating hypertrophy of both right and left ventricles. Occasionally, the left ventricle cavity contained fibrous tissue most likely as a result of a thrombotic mass organization, which often was complicated with calcification. In some cases, fibrotic tissue was moderately spread into the myocardium. Some hearts had dilatation of the right ventricle cavity with or without thrombus formation and local aneurism. Accumulation and infiltration of inflammatory cells were observed to a different extent primarily at the level of pericardium, sometimes spreading into the myocardium and perivascular regions. No advanced atherosclerotic lesion was observed in the major coronary arteries. Altered hemodynamic parameters after intra-abdominal heart transplantation may result in myocardial alterations. More studies are needed to explore the mechanisms of these tissue modifications.

Supported by the Heart and Stroke Foundation of Canada.

Abstract N° B19

Successful repair of acute myocardial infarction by skeletal muscle myoblasts

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The adult heart injured by an ischemic episode has a limited capacity to regenerate. We administered skeletal myoblasts to assess their suitability for repair of acute myocardial infarction. Using confocal fluorescent microscopy and a variety of specific immunomarkers and echocardiography we have provided anatomical evidence for the viability and the long-term survival of these cells and the possible functional benefits of such implants. All cells were transfected with adenovirus containing β-galactosidase gene such that their migration from the site of injections could be traced. Skeletal myoblasts (Mb) isolated from adult guinea-pigs and inbred rats (*M. soleus*) were expanded in vitro. Most (95%) of the transfected Mb were identified 7 d post-transplantation mainly in the infarcted area. During this time and thereafter they proliferated and differentiated into myotubes forming new regularly striated myofibers which occupied most (50–70%) of the infarcted area by the 2–3 week. These newly formed myofibers maintained their skeletal muscle origin evidenced by their expression of myogenin and fast skeletal myosin. With time, skeletal phenotype appeared to downregulate, while the Mb partially transdifferentiated into cardiac phenotype indicated by labeling for cardiac-specific troponin T and cardiac myosin heavy chain (MHC). At the third week post-transplantation, the new myofibres formed apparent contacts with the native cardiomyocytes via aputative gap-juntions@ expressing connexin 43. The myocardial performance of the animals successfully transplanted with myoblasts was significantly improved. They remained anatomically and functionally unchanged for 12 weeks post-transplantation without any sign of atrophy.

Abstract N° B20

Fibre death and regeneration in skeletal muscle following exposure to elevated catecholamines

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High levels of catecholamines are found in patients with heart failure and cachexia and may be causally linked to the muscle wasting. We have recently shown that a single injection (s.c.) of adrenaline or isoprenaline (0.01–20 mmol/kg) in vivo causes myocyte apoptosis and necrosis in the soleus muscle of male Wistar rats (205 ± 7 g). Animals were killed at various times over the next 28 d, the soleus muscle isolated, cryosectioned and the total number of normal, dying and regenerating fibres quantified using image analysis (Fig. 1). Data were analysed using one-way ANOVA. Seventeen percent of the total number of fibres were injured and lost 5 d after exposure to the catecholamine. However, newly regenerating fibres (identified using an antibody against embryonic/neonatal myosin) were soon evident (solid symbols), with these restoring the full complement of fibres within 28 d. The time course of these events, are shown below.

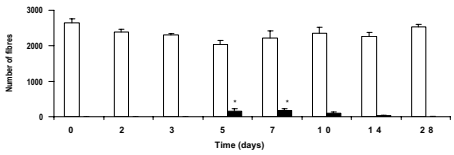

Fig. 1. Fibre loss and regeneration with time. Viable (□) and regenerating (■) fibres. Mean ± SEM, $n = 5–6$, $^*P < 0.05$

Abstract N° B21

Obstruction of aortic valve using a left ventricular catheter in a mouse model

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Pressure volume loops generated using a conductance catheter are increasingly used to measure cardiac contractility in mice. Catheters are introduced in the left ventricle (LV) either by LV puncture or following carotid (ICA) cannulation by crossing the aortic valve (AV). We questioned whether the catheter, although small (0.47 mm diameter, 0.17 mm^2 cross-section area), might cause obstruction when placed across the murine AV (0.88 + 0.05 mm diameter, 0.62 ± 0.07 mm^2 area in C57Bl/6 mice 25–33 g), using the ICA route. We simultaneously measured aortic pressure (AP) using left ICA cannulation and LV pressure (LVP) using either direct LV puncture (uncrossed group, $n = 6$) or using right ICA cannulation (crossed group, $n = 6$).

Results obtained:

Parameters	Uncrossed	Crossed	*P-value, t-test*
Body weight (g)	28.8 ± 1.0	29.3 ± 1.0	NS
Heart rate (per min)	369 ± 16	373 ± 6	NS
Stroke volume (μl)	5.87 ± 0.86	4.14 ± 0.60	NS
Cardiac output (ml/min)	2.21 ± 0.40	1.54 ± 0.21	NS
LVP–AP (mmHg)	5.30 ± 1.53	11.19 ± 3.77	NS

NS: Not significant.

Thus, no statistically significant differences were seen between the groups. Furthermore, the trend toward an increase AV gradient in the crossed group is of no functional significance. We conclude that this technique does not compromise cardiac function or physiology.

Abstract N° B22

Sheng Mei for mild to moderate cardiorenal syndrome in heart failure

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Cardiorenal syndrome in chronic heart failure is recently recognized as the most common reason that symptoms cannot be relieved despite aggressive management. Our previous observation found that 1 month Sheng Mei therapy for cardiorenal syndrome is safe (Circulation (2002) 106(19) II-352). We hypothesised that short-time Sheng Mei treatment for cardiorenal syndrome may show both efficacy and safety.

Methods and results. – The study in 127 patients with mild to moderate cardiorenal syndrome and class III or VI and LVEF <25 was performed. They were randomly divided into Sheng Mei group (60 ml/d $n = 64$) and placebo ($n = 63$). Sex, age, serum creatinine, LVEF, 6 min walking distance and MLWHF scores between the two groups were similar. Six minute walk test, LVEF, MLWHF score and serum creatinine were assessed at beginning and after 6 months of treatment. Serum creatinine level in Sheng Mei group decreased slightly but increased in placebo ($P < 0.005$). LVEF, 6 min walking distance and QOL in Sheng Mei group were more remarkable than those in placebo (all $P < 0.01$). There was a significant difference in mortality between two groups (four patients in Sheng Mei died vs. nine died in placebo).

Conclusions. – Sheng Mei may improve cardiac function, exercise capacity and QOL without increasing serum creatinine and decrease mortality in patients with mild to moderate cardiorenal syndrome during 6 month treatment.

Abstract N° B23

Structural differences in stable and unstable coronary artery atheromatous plaque

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The aim of our study was the establishment of structural differences between stable and unstable atheromatous plaque (AP). Coronary segments ($n = 146$) were harvested from the hearts of 94 patients who died of coronary artery disease. All segments were studied by morphological and morphometrical methods. Presence of erosion, dystrophic and necrotic signs with fissure or rupture of fibrous cap (FC), intraplaque hemorrhage, and parietal or obstructive thrombosis was considered as morphological features of AP instability. We estimated the intensity of inflammtory cell infiltration (CI) of FC using ocular net with 280th magnification in 5–10 fields of vision. Intensity of CI was divided into four types—mild, moderate, intensive and severe. Thickness of FC was established by ocular micrometer. It was considered that thick FC formed >30% of the diameter of AP, and thin FC >20%. Destruction of FC of instable AP with fissure from the direction of vessel lumen occurred in 36.5% of cases, with rupture—in 50.0%, with fissure from the direction of lipid core—in 8.5%. Our data suggest that severity of destruction depends on FC thickness ($P < 0.025$), rupture was more frequent in AP with thin FC (61.4%), and fissure was more frequent for thick FC (56.0%). There was no correlation between level of inflammatory CI and thickness of FC, both for stable and unstable AP. Instable AP with thin FC revealed mild and moderate CI in 51% of cases, intensive and severe—in 49%, those with thick AP—in 48% and 52% correspondently. Instability of AP was concerned with disorganization of connective tissue and presence of necrotic focuses in FC ($P < 0.001$). Disorganization of connective tissue was not related to intensity of CI ($P > 0.1$). Reliable difference in quality characteristic of CI (ratio monocyte/lymphocyte) of FC for stable and instable AP was discovered ($P < 0.001$). In FC of stable AP prevalence of lymphocytes was observed in 51.4% of cases, monocytes—in 5.7%. For instable AP monocyte prevalence in 30.6%, and lymphocyte prevalence—in 26.1% of cases was established.

Abstract N° B25

Total circulation time in heart failure patients measured using an acetylene technique

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While exercise testing represents an important prognostic tool in patients with chronic heart failure (CHF) it provides only a broad index of the cardiovascular response. Patients with CHF have traditionally been thought to have a blunted cardiovascular response to exercise. Circulation (circ) time may provide a further index of the cardiovascular impairment to exercise in CHF. Traditionally circ time has required arterial blood sampling and the introduction of a labeled dye. We hypothesized that a mean circ time could be estimated from the reappearance of end tidal acetylene ($PetC_2H_2$) in the expired air following a single inhalation. Moreover, we hypothesized that there would be an attenuated fall in circ time with progressive exercise in patients with CHF, relative to healthy subjects. Four healthy subjects (39 ± 11 years,) and four gender-matched CHF patients (46 ± 14 years) completed two exercise tests during which circ time (test 1) and cardiac output (CO, test 2) were measured. The circ time and CO were measured at four exercise intensities 25%, 50%, 75% and 100% of peak. CO was measured using the open circuit C_2H_2 washing technique. In the healthy subjects, circ time decreased and CO increased from rest (circ time = 42 ± 3 s, CO = 4.5 ± 0.5 l min^{-1}) to peak exercise (circ time = 19 ± 1 s, CO = 19.2 ± 0.7 l min^{-1}). Similarly, in CHF patients circ time decreased and CO increased from rest (circ time = 48 ± 3 s, CO = 3.6 ± 0.7 l min^{-1}) to peak exercise (circ time = 19 ± 1 s, CO = 8.8 ± 1.6 l min^{-1}). At a matched CO, circ time was longer in the healthy subjects ($P < 0.05$). This study demonstrated that circ time can be measured using the reappearance

of $PetC_2H_2$ and at a similar CO during exercise, CHF patients had a faster circ time, possibly suggesting an enhanced peripheral vasoconstriction.

Supported by HFRC, Griffith University and HL71478.

Abstract N° B26

Vascular smooth muscle role on right ventricular-arterial coupling

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We analyzed the effects of the vascular smooth muscle (VSM) activation to right ventricular (RV) afterload and ventricular–arterial coupling. Pulmonary flow, pressure and diameter and biventricular pressures were measured in six anesthetized sheep. Acute pulmonary hypertension was induced by phenylephrine (AH) and a high-pressure (PH) mechanical occlusion. The pulmonary artery (PA) viscous/elastic indexes ratio (η/E) was obtained to quantify the arterial wall buffering function. Total vascular resistance (Zo) characteristic impedance (Zc), and RV hydraulic power (total, W_T and oscillatory, W_O) were quantified (flow-pressure curves). RV–arterial coupling was assessed by the efficiency transmission ratio (ETR) and transpulmonary efficiency (TPE). Total pulmonary compliance (C_T) was obtained by the diastolic time constant.

	CTL	PH	AH
PAmean pressure (mmHg)	14.5 ± 2	21.9 ± 1.5 [a]	19.9 ± 2.7 [a]
PAmean diameter (mm)	23.0 ± 2.3	23.9 ± 2.2 [a]	22.3 ± 2.4 [a,b]
E, (mmHg/mm)	5.5 ± 1.2	9.3 ± 3.5 [a]	6.4 ± 1.2 [b]
η, 10^{-2} (mmHg s/mm)	4.9 ± 0.6	4.7 ± 0.5	7.6 ± 0.7 [a,b]
η/E, 10^{-3} s	9.2 ± 1.7	5.4 ± 1.8 [a]	12 ± 1.4 [a,b]
Zo, (dyn s/cm^5)	595 ± 137	934 ± 275 [a]	965 ± 230 [a]
Zc, (dyn s/cm^5)	82 ± 21	231 ± 90 [a]	80 ± 26 [b]
C_T, (ml/mmHg)	2.0 ± 0.2	1.0 ± 0.3 [a]	1.5 ± 0.1 [a,b]
W_O/W_T, (%)	11 ± 2.6	21 ± 2.2 [a]	13 ± 3.6 [b]
ETR, (%)	45 ± 8.9	69 ± 12 [a]	35 ± 16
TPE, (ml mW/min)	15.2 ± 1.2	10 ± 3 [a]	10 ± 2.7 [a]

Mean ± S.D., $n = 6$.
[a] $P < 0.05$ vs. CTL.
[b] $P < 0.05$ vs. PH, ANOVA.

During acute pulmonary hypertension the VSM activation enhanced both, local and global buffering function of pulmonary circulation and decreased the pulsatile component of the RV hydraulic load, resulting in an improved coupling between the RV and the hypertensive pulmonary circulation.

Abstract N° B27

Differences of coronary microvascular lesion in coronary heart disease and hypertension alone

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The purpose of this study is to observe the characteristics and difference of coronary microvascular lesion (CML) in the autopsied elderly cases with essential hypertension (EHT) and coronary heart disease (CHD) with the same degree of left ventricular wall thickness (LVWT). A retrospective study was performed in 246 cases over 60 year old of EHT, CHD, EHT with CHD and 26 normal cases as control (CTRL) out of 3195 consecutive autopsied cases. The arteriole with the diameter of 10–60μm and the capillary in muscular layer were shown by the methods of HE, Elastic fiber + VG staining and immunohistochemistry of CD31. Quantitative measurements on the arteriole density (AD), the ratio of arteriole wall and lumen (RWL), and the capillary density (CD) were performed by light microscope observation and image analysis by computer. According to LVWT, the cases were divided into four degrees from I to IV, which were I–IV degrees in EHT, CHD and EHT with CHD groups and I degree in CTRL group, respectively. SAS system was used for the statistic analysis. The AD and RWL increased while CD decreased significantly with the aggravation of LVWT in EHT group (*$P < 0.05$–0.0001); there was a similar but more severe change in the group of EHT with CHD ($P < 0.001$–0.0001); the AD increased ($P < 0.001$), while RWL and CD did not change significantly in CHD group. In EHT and CHD groups, the similar change of CML appeared in AD and different changes appeared in RWL and CD. The CML was much more severe in the group of EHT with CHD. It was concluded that the CML might be one of the main causes for decreased coronary flow reserve and myocardial damage in EHT and EHT with CHD.*

Abstract N° B28

Expression of α_1-adrenoceptor mRNA in the human cystic artery: correlation with functional responses

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The α_1-adrenoceptor (α_1-AR) subtype (α_{1A},α_{1B},α_{1D}), responsible for vasoconstriction is unclear since expression varies between species and vascular bed. This study aims to identify the α_1-AR subtypes responsible for vasoconstriction of the human cystic artery and look for correlation at the mRNA expression level. Vessels obtained from cholecystectomy operations were snap-frozen or used immediately. Isolated tissue bath experiments were used to generate subtype α_1-AR selective antagonist affinities (pK_B values), which were determined from shifts in individual concentration–response curves (CRCs) to phenylephrine (PE). RNA was extracted from the vessels and mRNA expression levels were obtained using quantitative real-time polymerase chain reaction (qRT-PCR). The results are presented as mean ± SEM, with $n \geq 3$. α_1-AR antagonists produced parallel rightward shifts of PE CRCs without affecting maximum responses, and with Schild slopes not differing from unity. The α_{1A}-AR antagonists RS100329 and 5-methylurapidil had high affinity values of 9.88 ± 0.13, and 8.21 ± 0.26, respectively. The

α_{1D}-AR antagonist BMY7378 had a low affinity (5.90 ± 0.19). The α_1-AR antagonist prazosin had a high affinity (9.52 ± 0.12). The artery affinity values correlated best with the values reported for the cloned α_{1A}-AR. From the qRT-PCR total mRNA copy number for the three α_1-AR subtypes was 1.734, 383 ± 561, 807 with the α_{1A} mRNA making up 69.7 ± 19.7%, α1B 30.22 ± 18.7% and α_1D 0.06 ± 0.02% of the total α_1-AR mRNA copy number. In conclusion the cystic artery has high α_1-AR mRNA levels with a predominance of the α_{1A}-AR subtype. The α_{1A}-AR subtype was also found to mediate vasoconstriction of this artery in vitro.

Abstract N° B30

Interstitial effects account for the ability of aldosterone receptor blockade to prevent β-adrenoreceptor-mediated cardiac dilatation

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Cardiac dilatation is an important determinant of pump dysfunction in cardiac disease. Although myocardial interstitial changes are suggested to be an important mechanism by which aldosterone receptor blockade reduces cardiac cavity dimensions in cardiac disease, there is no direct evidence to support this hypothesis. We therefore evaluated the impact of spironolactone (SPIRO), an aldosterone receptor antagonist, on chamber remodelling and the potential mechanisms thereof in a rat model of cardiac dilatation (β-adrenoreceptor mediated). The effect of SPIRO (80 mg kg^{-1} d^{-1}) on left ventricular (LV) cavity dimensions, myocardial function and interstitial changes was assessed in 14 month old spontaneously hypertensive rats (SHR) receiving isoproterenol (ISO) at 0.04 mg kg^{-1} d^{-1} for 4.5 months. ISO administration resulted in an increase in 24 h urinary aldosterone excretion, enlarged cavity dimensions, produced a right shift in LV end-diastolic (LVED) pressure–internal diameter and LVEDP–LVED volume relations, decreased relative wall thickness despite augmenting LV hypertrophy, reduced collagen cross-linking and increased total and non-cross-linked myocardial collagen concentrations. ISO altered neither cross-linked collagen concentrations nor intrinsic myocardial systolic function in SHR. SPIRO prevented ISO-induced increases in total and non-cross-linked myocardial collagen concentrations and chamber remodelling, but failed to modify blood pressure, cross-linked collagen concentrations, or intrinsic myocardial systolic function. These results suggest that myocardial interstitial changes are important in mediating the beneficial effects of aldosterone receptor blockade on LV dilatation.

Abstract N° B31

Interstitial changes are a critical mechanism mediating β-adrenoreceptor-induced cardiac dilatation

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β-Adrenoreceptor (β-AR) activation is an important determinant of adverse chamber remodelling. The critical mechanisms responsible for β-AR-mediated cardiac dilatation are however unclear. We evaluated the mechanisms by which chronic β-AR activation induces left ventricular (LV) dilatation. The effect of isoproterenol (ISO, 0.04 mg kg^{-1} d^{-1} from 14 to 18.5 months of age) on LV cavity dimensions, systolic chamber and intrinsic myocardial function, interstitial properties, diastolic pressure–volume relations, necrosis, apoptosis and myocardial noradrenaline (NA) release was assessed in spontaneously hypertensive rats (SHR). At 18.5 months of age SHR had LV end-diastolic (LVED) diameters, LVED pressure–internal diameter relations, LVEDP–LVED volume relations, systolic chamber and myocardial function similar to that noted in Wistar Kyoto controls (WKY). However, myocardial stiffness, relative wall thickness, myocardial NA release, collagen cross-linking, total and non-cross-linked myocardial collagen concentrations, pathological score and the percentage of myocytes with apoptosis was increased in SHR at 18.5 months. ISO administration to SHR, but not to WKY resulted in enlarged cavity dimensions, a right shift in LVED pressure–internal diameter and LVEDP–LVED volume relations, decreased relative wall thickness despite augmenting LV hypertrophy, reduced collagen cross-linking and increased total and non-cross-linked myocardial collagen concentrations. However, ISO administration to SHR failed to alter blood pressure, heart rate, myocardial NA release, cross-linked myocardial collagen concentration, pathological score, percentage of myocytes with apoptosis, or intrinsic myocardial systolic function in SHR. These results suggest that myocardial interstitial changes are a critical mechanism mediating β-AR-induced LV dilatation.

Abstract N° B32

Effect of histamine on mesenteric microcirulation, measured by intravital microscopy

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Using in vivo intravital microscopy, we examined the role of histamine on mesenteric microcirculation. The present experiments were carried out on 25 male Sprague–Dawley rats anaesthetized by subcutaneous administration of 1.25 ml of 20% solution of Urethane per 100 g body weight of animal (1) and prepared for in vivo intravital microscopy of mesenteric microcirculation. The internal diameter (i.d.) of the terminal arterioles (TA) as measured through a precalibrated micrometer eye-piece fitted to the binocular microscope (Hertell and Reuss) was found to be 18–23 μm (microns), these TA branch out to the capillaries of 4–9 μm. One millimolar solution of histamine in modified tyrode solution pH 7.4, was topically applied on the preparation and the i.d. of the vessels was measured to know the latency and magnitude of the response, any change in i.d. of the vessels was ex-

pressed as the percentage of control, the vascular flow was visually recorded. Soon after application (10–20 s) the arterioles were found to be in dilated state, and the i.d. became almost twice the control diameter between 80 and 100 s. This gradually approached the control diameter in 5 min. In most instances the number of dormant capillaries increased by 50–60%, the flow rate in the active capillaries also substantially increased. The effect presumably appears to be either a direct effect on histamine receptors or on the specific ion-channels, thus bringing about the relaxation of the smooth muscles of the terminal arterioles. It seems that the local regulatory mechanisms like the hormones and transmitters are of greater value than the enteric neural regulation alone. The possibility that the specific histaminergic receptors are present on intestinal microcirculation has not been explored in the current work, as it requires rather more comprehensive experimental work.

Abstract N° B33

Increases in leukocyte CD antigen expression during cardiopulmonary bypass in patients undergoing heart transplantation

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Cardiopulmonary bypass (CPB) is essential in heart transplantation surgery and is also associated with a post-operative inflammation (increases in IL-8, TNFα and cluster of differentiation (CD) antigens) in coronary artery bypass procedures. We have developed a novel antibody array that can simultaneously measure the expression of 72 CD antigens on isolated leukocytes. The array compares the changes in leukocyte CDs isolated from a 5 ml blood samples from 12 heart transplant patients. A preoperative blood sample acted as a baseline and was compared to a second sample taken about 2 h at the end of CPB. Two samples from lung transplantation patients where CPB was not used acted as controls. No previous studies have examined changes in the expression so many CD antigen during heart transplantation. In effect, the array data comprise the immunophenotype of the patient. We find increased expression of 10 CD antigens in leukocytes from all patients between these two samples. Detailed clinical data were obtained to evaluate the effects of epigenetic factors (smoking, diet, drinking, and medications) on the CD expression. This is the largest CD expression profiling of patients with end-stage heart failure undertaken so far. It provided us insights into the nature of inflammation associated with CPB.

Abstract N° B34

Changes in CD antigen expression in on-pump and off-pump coronary artery grafting surgery

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Coronary artery grafting surgery may be undertaken with (on-pump) or without (off-pump) cardiopulmonary bypass. Following on-pump surgery a systemic post-operative inflammatory response is often observed. This manifests as an increase in pro-inflammatory proteins like interleukin-8 and tumour necrosis factor accompanied by increases in neutrophil populations and increased expression of cluster of differentiation (CD) antigens The cause of this inflammation has been attributed to the activation of complement and leukocyte mediated inflammatory pathways via the extracorporeal circuit. However, following off-pump surgery a similar but less intense systemic inflammatory response is also observed despite the absence of cardiopulmonary bypass. Clearly there is contention as to the cause of this inflammatory response observed in both forms of cardiac surgery. Previous studies have measured pro-inflammatory proteins in serum as markers of inflammation following cardiopulmonary bypass. Others have measured the expression of CD antigens on leukocytes as possible inflammatory indicators, though these studies were limited to examining three–four CD antigens simultaneously. We have developed a novel antibody array that is able to measure changes in the expression of 72 different CD antigens simultaneously. The present study represents the first large-scale analysis of changes in CD antigen expression in on-pump and off-pump coronary artery grafting surgery.

Abstract N° B35

Improved vein graft patency using a 'no-touch' technique is due to preserved endothelial nitric oxide synthase

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The saphenous vein (SV) is the most commonly used conduit for coronary artery bypass surgery (CABG) but over 50% of grafts fail within 10 years. In conventional CABG the SV is exposed to surgical trauma during harvesting. A marked improvement in graft patency has recently been described using a no-touch technique (NT) of CABG where the SV is removed with minimal damage. Here, under local ethics committee approval and patients' informed consent we have used segments of conventional and NT SV for the localisation and assessment of endothelial nitric oxide synthase (eNOS) using immunohistochemistry and western blotting. The ability of SV to release NO was also studied using the citrulline assay. Western blot analysis revealed that eNOS

expression in conventional SV was reduced compared to NT vein segments ($P < 0.05$). Immunohistochemical studies showed that this was due to denudation of the luminal endothelium as well as damage to the tunica adventitia, both structures that are unaffected in NT CABG. Using the citrulline assay the ability of conventional SV to release NO was reduced by approximately 80% compared with NT SV. These results show that eNOS associated with the luminal endothelium and endothelial cells of microvessels and nerves within the adventitia of SVs are removed or damaged during conventional CABG. Since the NT technique is atraumatic the SV retains its normal architecture. The preservation of eNOS in such veins used for CABG maintains the vessel's ability to generate NO and plays a central role in the increased graft patency described using NT surgery.

Abstract N° B36

Telomerase in end-stage heart failure

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Heart failure can result from various cardiovascular diseases of ischemic, hypertensive, toxic or inflammatory origin. Animal studies have shown the potential role of telomerase, a ribonucleoprotein complex that stabilizes and extends the chromosome telomeric ends and thereby regulates cell replicative capacity and lifespan, in cardiac regeneration and proliferation. However, the regulation of telomerase in the failing human heart and associated coronary circulation remains unclear. The study was aimed to uncover the pathological regulation of telomerase in chronic heart failure by molecular and morphological approaches, with emphasis on epicardial coronary arteries and myocardium of the end-stage failing human heart. Forty discrete epicardial coronary arterial segments and 12 explanted failing hearts were obtained from patients who underwent heart transplantation. Functional bioactivity of the telomerase holoenzyme and cellular expression of telomerase catalytic protein, human telomerase reverse transcriptase (hTERT) were examined by sensitive PCR-ELISA-based telomeric repeat amplification protocol (TRAP) and immunocytochemical analyses, respectively. In epicardial coronary arteries, we compared with the telomerase expression in vascular tissues with or without atherosclerotic lesions form ischemic or dilated cardiomyopathy. The incidence of telomerase re-activation reached 70% in atherosclerotic coronary arteries, fourfold higher than that of non-atherosclerotic controls ($P = 0{\cdot}007$). hTERT-immunoreactivities frequently distributed in the vascular smooth muscle cells (VSMCs) with coronary atherosclerotic lesions, coupled with the mitotic index of proliferating VSMCs proven by positive Ki67-staining. The level of hTERT expression significantly correlated with telomerase bioactivity ($P = 0{\cdot}017$) and atherosclerotic severity ($P < 0{\cdot}001$). In the failing myocardial tissues, apparent telomerase bioactivity was shown in all endomyocardial biopsies, whereas most of the transverse myocardial slices of left ventricle exhibited weak enzyme activity. Histopathologically, strong hTERT-immunolabeling presented predominantly in endocardium and epicardium, including the endothelial cells, smooth muscle cells, connective tissues cells, mesothelial cells and intramural vascular cells. Some damaged myocytes or adjacent connective tissues cells showed moderate hTERT-immunoreactivities in the injury zone of myocardium. Our results indicate the unique active telomerase and hTERT expression in the coronary atherosclerosis and myocardial tissues may play a role in the cardiovascular events leading to the development of chronic heart failure.

Abstract N° B37

No-touch saphenous veingraft for coronary artery bypass grafting provides a high graft patency rate

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The aim of this study is to evaluate a new "no-touch" harvesting technique where the vein is harvested with a cushion of surrounding tissue, which protects the vein from spasm and therefore obviating the need for distension. This is a prospective and randomised study comparing the patency of veingrafts harvested with three techniques: (I) Conventional technique: Adventitial stripping, manual distension and storage in saline at room temperature. (II) Intermediate technique: Adventitial stripping, locally papaverine was used instead of distension and storage in heparinized blood. (III) "No-touch" technique: The vein was harvested with a cushion of surrounding tissue. It was not dilated and stored in heparinized blood. The patients demographics and the distribution of the grafts to the target coronary arteries were similar in the three groups. The patency rate for grafts harvested by the conventional technique was 113/127 (88.9%), for grafts in the intermediate technique was 100/116 (86.2%) and for grafts in the "no-touch" technique was 118/124 (95.4%). The statistical analysis showed a *P*-value of 0.035 for the "no-touch" technique. Futhermore, the preliminary results of the long-term angiographic follow up has also shown a high patency rate for the SV grafts that were prepared with the "no-touch" technique. The use of refined technique of harvesting SV for CABG is a prerequisite to achieve a high early veingraft patency and this can be accomplished when using our "no-touch" technique. The success of "no-touch" graft might be due to preservation of endothelial integrity and nitric oxide synthase (NOS) activity as we demonstrated in previous studies.

Abstract N° B38

C-Jun N-terminal kinase regulates pathological extracellular matrix metabolism in abdominal aortic aneurysm in vivo

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We investigated the role of c-Jun N-terminal kinase (JNK) in human abdominal aortic aneurysm (AAA) that is characterized by proteolytic degradation of extracellular matrix (ECM) by matrix metalloproteinases (MMPs). We observed a significant increase in JNK activity showing a linear correlation (r = 0.82) with MMP-9 expression in human AAA. Active JNK and MMP-9 co-localized in macrophages and vascular smooth muscle cells (VSMCs) in AAA. DNA microarray analysis in rat aortic VSMC culture revealed that MMP-9 and five of its positive regulators were ranked within top 20 JNK-induced genes, which was confirmed at protein levels. In contrast, tissue inhibitor of metalloproteinase-3 (TIMP-3), a negative regulator of MMP activity, was suppressed by JNK. In addition, collagen type III and biosynthetic enzymes for collagen and elastin fibers were suppressed by JNK, suggesting an important role of JNK in turnover of ECM in the aortic wall. In fact, specific activation of JNK by adenoviral expression of active MKK7 markedly suppressed collagen biosynthesis as measured by [3H] proline incorporation in VSMCs. SP600125, a specific JNK inhibitor, eliminated expression and secretion of MMP-9, recovered TIMP-3 expression and prevented collagen degradation in ex vivo culture of human AAA walls. Finally, specific inhibition of JNK in vivo by SP600125 completely suppressed the development of murine AAA that was induced by abluminal application of calcium chloride. These findings demonstrate the central role of JNK in the pathological ECM metabolism in AAA. JNK represents a novel therapeutic target for abdominal aortic aneurysm.

Abstract N° B39

Regression of pressure-overload induced left ventricular hypertrophy in mice

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The aims of this study are to establish left ventricular hypertrophy (LVH) and regression model in mice by surgical interventions and to characterize the time-course for the development of LVH and a subsequent regression of established LVH. Alterations in histology and certain genes expression were studied as well. Mice with C57BL/6 genetic background subjected to aortic banding, some of them followed by debanding (release of aortic narrowing), and sham operation were investigated for 10 or 14 weeks. Using echcardiography, we observed a progressive increase in LV mass, gradual LV dilatation and dysfunction during the development of LVH following aortic banding. LVH was also associated with myocyte enlargement, interstitial fibrosis, enhanced ANP and suppressed a-MHC and SERCA2a expressions. Surgical unloading induced a reversal in myocardial hypertrophy, LV chamber dilatation and dysfunction, which was accompanied with restorations of histological changes and gene expressions. Regression of LVH induced by pressure overload was very sensitive to the alteration of arterial pressure. However, the extent of LVH regression was not consistent in mice with different duration of pressure overload. A complete restoration in LV structure and function was observed in animals undergone 4 weeks aortic banding followed by 6 weeks debanding, but not in those undergone 8 weeks banding followed by 6 weeks debanding. Hypertrophied myocytes and increased interstitial collagen content could not return to control level even given a longer recovery period than pressure overload. These findings demonstrated that the degree of LVH regression was dependent on the duration and extent of LVH and histological reversal was slower and lagged behind the restoration in LV structure and function. The time courses for the development and regression of LVH induced by pressure overload and subsequent surgical unloading were clearly delineated from different levels.

Abstract N° B40

The low oxygen-carrying capacity of Krebs buffer causes a doubling in wall thickness in the isolated heart

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The coronary flow of buffer-perfused hearts is known to be higher than in vivo, due to the low oxygen-carrying capacity of Krebs buffer. We postulated that this may result in changes in cardiac dimensions that may impact on contractile efficiency. Rats (n = 4/group) were anaesthetised and their heart dimensions measured in vivo (closed chest) using a modified ultrasound system. The hearts were then excised and buffer-perfused in Langendorff mode at 100, 80, 60 or 40 cm H_2O. Two further groups of hearts were perfused at 100 cm H_2O and either given a bolus of endothelin 1 (ET1, 1×10^{-9}M) or infused with FC-43 (the perfluorochemical oxygen carrier); another group was perfused in the "working" mode.

	LVWT (mm)	LV lumen diameter (mm)	Coronary flow (ml/min)
In vivo	2.1 ± 0.3	5.7 ± 0.4	–
Langendorff 100 cm	5.6 ± 0.5 *	2.0 ± 0.1 *	16.6 ± 0.5
Langendorff 40 cm	7.1 ± 0.7 *	1.3 ± 0.3 *	6.7 ± 0.8 †
Langendorff + ET1	3.8 ± 0.9 *,†	2.2 ± 0.4 *	7.9 ± 1.2 †
Langendorff + FC-43	4.1 ± 0.4 *, †	1.8 ± 0.7 *	8.5 ± 0.6 †
Working heart	4.9 ± 0.5 *	3.9 ± 0.6 †	18.2 ± 0.7

* $P < 0.05$ vs. in vivo.

† $P < 0.05$ vs. Langendorff 100 cm.

Left ventricular wall thickness (LVWT) in Langendorff hearts (100 cm) was twice that of the same hearts in vivo, and

increased further with lower perfusion pressures. The increased LVWT was attenuated by ET1 or FC-43. The collapse of the LV lumen in Langendorff hearts was reversed by switching to the "working" mode, but was unaffected by ET1 or FC-43. We conclude that the increased wall thickness in buffer-perfused hearts is due to vasodilation induced by the low oxygen carrying capacity of Krebs buffer.

Abstract N° B41

Ventricular function and remodelling in hypertensive cardiomyopathy

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Myocardial remodelling as a result of chronic hypertension includes progressive changes in cellular architecture and extracellular matrix collagen organisation. These changes have the potential to interfere with the mechanical and metabolic properties of cardiac myocytes and affect left ventricular (LV) function. The objective of this study was to characterise the time-course of changes in LV structure and function through the progression of hypertensive cardiomyopathy. Hearts were collected from spontaneously hypertensive rats (SHRs) at different stages of hypertensive cardiomyopathy development (early hypertension (3 months), established hypertension (12 months), compensated heart failure (18 months) and de-compensated heart failure (22–24 months)) as well as from aged-matched controls, Wistar-Kyoto rats (WKYs). Standard indices of LV geometry and systolic function were determined using echocardiography prior to excision and plasma samples stored for subsequent brain natriuretic peptide assay. Passive LV pressure/volume relations were then measured in the isolated hearts (eight per group). Two further hearts from each group were fixed in Bouin's solution, stained for collagen with picrosirius red and imaged using an automated confocal microscopy system to produce detailed extended 3D images of myocardial structure spanning the LV free wall. All SHR hearts, including the dilated failing hearts, demonstrated increased passive stiffness compared to age-matched controls. Structural analysis revealed increased collagen volume fraction and density in the myocardial intralaminar spaces, the increases being systematically associated with age and disease state. Long-term hypertension causes loss of intralaminar spaces and thickening of collagen around individual myocytes, these effects being exaggerated in failing hearts.

Abstract N° B42

Phosphodiesterase inhibition promotes the transition from compensated hypertrophy to cardiac dilatation

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The cellular mechanisms responsible for the transition from compensated left ventricular (LV) hypertrophy (LVH) to LV dilatation (chamber remodeling) and heart failure are unclear. As chronic administration of a β-adrenoceptor (β-AR) agonist mediates the premature onset of cardiac remodeling without myocyte necrosis or myocardial dysfunction in LVH, we suggest that the β-AR-cyclic adenosine monophosphate (cAMP) system is critical in promoting cardiac dilatation and hence the transition from compensated LVH to heart failure. To determine the role of cAMP in promoting adverse cardiac chamber remodeling, we evaluated whether phosphodiesterase inhibition (PDEI) promotes LV dilatation in rats with compensated LVH. The impact of chronic administration of the PDEI, pentoxifylline (75 mg kg^{-1} d^{-1}), on LV remodeling and function was assessed in spontaneously hypertensive rats (SHR) with compensated LVH. Inotropic doses of the PDEI administered for 4 months to SHR failed to modify LV weight or influence intrinsic myocardial systolic function, but mediated the development of a right shift in LV end-diastolic (LVED) pressure–internal diameter and LVED pressure–volume relations, produced LV wall thinning, reduced systolic chamber function and increased myocardial soluble (non-cross-linked) collagen concentrations. In conclusion, chronic PDEI administration induces adverse geometric and interstitial cardiac remodeling in SHR, a finding that supports the notion that the β-AR-cAMP system is important in mediating the progression to heart failure by promoting interstitial remodeling and LV dilatation in LVH.

Abstract N° B43

Lymphocyte adhesion to the artery wall is increased in atherosclerosis

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Lymphocytes accumulate in atherosclerotic plaques and may have a role in plaque instability and breakdown. The aim of this study was to compare basal and thrombin-stimulated adhesion of lymphocytes to vessel segments from 16 week-old C57BL/6 and Apo-E–/– mice. Apo-E–/– received a high fat diet from 4 weeks. Lymphocytes were isolated from mouse spleen, labelled with ^{51}Cr, and added in a droplet to mouse aortic arch, thoracic aorta and abdominal aorta. After 30 min non-adherent lymphocytes were washed and the adherent lymphocytes were counted. Basal lymphocyte adhesion in Apo-E–/– mice (19.3 ± 2.4%) was significantly increased compared to C57BL/6 mice (10.8 ± 1.4%). Thrombin pre-treatment of the artery segments increased lymphocyte adhesion, however Apo-E–/– mice were more sensitive to thrombin (thrombin 1 U/ml 27.0 ± 1.7% adhesion) compared to C57BL/6 mice (thrombin 1 U/ml 12.7 ± 1.9% adhesion, thrombin 10 U/ml 27.0 ± 3.6% adhesion). Adhe-

sion of C57BL/6 lymphocytes to Apo-E–/– arteries was increased (40.0 ± 4.0% adhesion) compared to adhesion of autologous lymphocytes to C57BL/6 arteries (10.8 ± 1.8% adhesion). Adhesion of Apo-E–/– lymphocytes to C57BL/6 arteries was increased (25.0 ± 4.6% adhesion) compared to adhesion of autologous lymphocytes to Apo-E–/– arteries (19.3 ± 2.4% adhesion). These results suggest that in this model of atherosclerosis there is enhanced adherence of both the lymphocytes and the aortic wall. The greater responsiveness to thrombin suggests upregulation of thrombin receptor signalling in atherosclerosis.

Abstract N° B44

Effect of OX-LDL on proliferation of vascular smooth muscles and the promoter activities of human α2(I) procollagen gene

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To comprehend the role of OX-LDL in the formation and progression of atherosclerosis. The smooth muscle cells obtained from male Sprague–Dawley rat's thoracic aorta were primarily cultured in vitro. Different concentrations of OX-LDL50, 100, 150 and 200 μg/ml were added into the plate to co-incubated with the cells, respectively. MTT method was used to detect the cell proliferation after 24, 48 and 72 h. Smooth muscle cells were transiently transfected with pCOLH22.4 and pCOLH21.6 containing –2.4 kb and –1.6 kb of 5′flank sequence of human α2(I) procollagen gene, fused to CAT reporter gene by FuGene transfectant reagent. The effect of OX-LDL 150 μg/ml on the plasmid were determined by CAT-ELISA. OX-LDL could accelerate the proliferation of VSMC in dose-dependent manner. OX-LDL could enhance the promoter activities of human α2(I) procollagen gene significantly. OX-LDL can accelerate the proliferation of VSMC and enhance production of typeαcollagen and accelerate the formation and progression of atherosclerosis.

Abstract N° B45

SR Ca release at transverse tubule and cell surface dyads in cardiac myocytes

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Introduction. – In cardiac ventricular myocytes, it is unknown whether local Ca release from the sarcoplasmic reticulum (SR) is the same at the transverse (t-) tubule and cell surface dyads. We have addressed this question.

Methods. – Experiments were performed at room temperature on enzymatically isolated rat ventricular myocytes. The perforated patch clamp technique and fura-2 were used to record the Ca current (I_{Ca}) and whole cell Ca transient (CaTr) simultaneously. CaTr was also recorded in field-stimulated myocytes using fluo3 imaged by confocal microscopy. We compared control myocytes, in which Ca signalling is dominated by the t-tubules, with detubulated myocytes, in which Ca signalling occurs predominantly at the cell surface.

Results. – I_{Ca} and CaTr amplitude elicited at 0 mV were significantly smaller in detubulated cells than in control cells (n = 14 and 16, respectively). The gain of the Ca release process (CaTr amplitude/I_{Ca} amplitude) was not significantly different between control and detubulated cells (0.133 ± 0.015 vs. 0.122 ± 0.016, $P > 0.05$). This suggests that the effectiveness of a given I_{Ca} in causing SR Ca release is the same in control and detubulated myocytes, and therefore at the t-tubules and surface membrane. To investigate this further, we compared localised rates of rise of Ca (dF/dT) in control and detubulated myocytes using confocal microscopy. dF/dT was the same at the cell surface and center in control myocytes (3.4 ± 0.6 vs. 3.6 ± 0.7, $P > 0.05$, n = 11) and at the cell surface in detubulated myocytes (3.6 ± 0.4, n = 15).

Discussion. – Structural studies using electron microscopy have shown no difference in the size of the dyadic space at the cell surface and t-tubules (Am. J. Physiol. (1978) 235: C147), and immunocytochemistry has shown similar co-localization of I_{Ca} and RyR (J. Cell. Biol. (1995) 129 672) at the two sites. In addition, computer simulation showed that differences in the dyadic cleft would be expected to alter the gain and the rate of rise of Ca (Biophys. J. (1997) 73 112). Our data provide the first functional evidence that the geometry and the structure of the dyadic cleft, and hence coupling of Ca entry and release, is the same at the cell surface and the t-tubules.

Supported by the Wellcome Trust.

A table may be set in eight points text, as follows.

Abstract N° B46

Changes of spontaneous Ca^{2+} transients during cardiomyocyte development: the role of type 2 ryanodine receptor

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In adult cardiomyocytes, type 2 ryanodine receptor (RyR2), the cardiac sarcoplasmic reticulum Ca^{2+} release channel, plays a critical role in the regulation of cytosolic Ca^{2+} transients (CaTs), but the developmental changes of CaTs and the contribution of RyR2 during cardiomyogenesis are currently obscure. We have therefore systematically analyzed spontaneous CaTs in cardiomyocytes derived from wild-type ($RyR2^{+/+}$) and RyR2 deficient ($RyR2^{-/-}$) embryonic stem cells at early (EDS, 7 + 2–4 d), intermediate (IDS, 7 + 6–8 d) and late developmental (LDS, 7+ 10–12 d) stages. In $RyR2^{+/+}$ cardiomyocytes, the frequency and amplitude of spontaneous CaTs were increased significantly from EDS to LDS, while the duration of CaTs was decreased gradually upon development. Unexpectedly, $RyR2^{-/-}$ cardiomyocytes showed similar amplitude of CaTs to these in $RyR2^{+/+}$ cells at three developmental stages, however, the duration of CaTs

was significantly prolonged with slow contraction. The prolonged duration of CaTs was mimicked in $RyR2^{+/+}$ cells by 10 μM ryanodine in a development-dependent manner. As β-adrenergic stimulation is known to regulate CaTs, we further analysed the role of RyR2 on CaTs with isoproterenol (Iso, 3×10^{-8} M). Iso significantly increased in spontaneous frequency and amplitude of CaTs in $RyR2^{+/+}$ cardiomyocytes, whereas these responses to Iso in $RyR2^{-/-}$ cardiomyocytes were much weaker. We conclude that: (1) more rapid and higher CaTs are developed gradually during cardiomyocyte development; (2) RyR2 is essential for this rapid CaT via acting as a functional Ca^{2+} release channel in spontaneous beating and contracting cardiomyocytes and in β-adrenergic-stimulated response throughout all developmental stages, especially in terminally differentiated stage.

Acknowledgements: The study was supported in part by grant of General Program of National Natural Science Foundation (No. 30270656) and by grants of Major and Key Programs (03DJ14019 and 01JC14048) from Science and Technology Committee of Shanghai Municipality.

Abstract N° B47

PKA and CaMKII phosphorylation of ryanodine receptors in canine heart failure

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Phosphorylation/dephosphorylation of ion channels and transporters is an effective mechanism by which neurotransmitters and hormones increase cardiac performance during stress or exercise. This response is apparently altered in heart failure (HF), as failing ventricular cells appear to be insensitive to β-adrenergic stimulation. Perfusion of hearts with isoproterenol results in activation of PKA and subsequent phosphorylation of ryanodine receptors (RyR), the Ca^{2+} release channels of the sarcoplasmic reticulum (SR). However, RyRs display a unique phosphorylation site for PKA (S2809), and multiple Ca^{2+}-, CaM-dependent protein kinase II (CaMKII) sites. In principle, then, RyRs are better substrates for CaMKII than for PKA, but the exact stoichiometry of PKA/CaMKII phosphorylation of RyRs, and the extent of RyR phosphorylation in HF remain unclear. We used [^{32}Pγ]-ATP incorporation and an antibody against phospho-S2809 (P-S2809) to investigate the total level of RyR phosphorylation by CaMKII and PKA and the relative efficacy of these kinases to phosphorylate S2809, in control and HF dogs. Autoradiograms of SR proteins show that CaMKII is three- to fivefold more efficient than PKA to phosphorylate RyRs in vitro. The higher CaMKII stoichiometry cannot be ascribed to lower PKA activity, as both kinases phosphorylated phospholamban to similar levels. Phospho-S2809 signals were low in native SR, suggesting low basal levels of S2809 phosphorylation, but increased with PKA or CaMKII phosphorylation. A dephospho-S2809 antibody (deP-S2809) signal is diminished by PKA or CaMKII, indicating equal access of both kinases to this site. Neither autoradiogram nor P-S2809 signals in untreated, PKA-, or CaMKII-phosphorylated RyRs were significantly different in control and HF dogs. These results suggest that CaMKII is potentially a more effective modulator of RyRs than PKA, but that phosphorylation of RyRs does not appear to play a central role in the blunted response of cardiac myocytes to β-adrenergic stimulation.

Abstract N° B49

Vasodilation via Cl^- Channel blockade-induced Ca^{2+} flux reduction

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Cl^- channels are expressed in vascular smooth muscles (VSM), but their role in VSM contraction remains poorly understood. In order to investigate these roles, isometric tension of isolated rat aortic ring was measured with an organ bath perfusion technique. The data were reported as mean ± S.D. and were analyzed by Student's unpaired *t*-test. The Cl^- channel blockers niflumic acid (NFA) and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) completely inhibited high K^+(60 mM)-induced aorta contraction. Glt^- substitution for extracellualar Cl^- mimicked the inhibitory effects of Cl^- channel blockers (81.3 ± 2.9%, $n = 8$, $P < 0.01$), but depletion of internal Ca^{2+} stores with thapsigargin (10 μM) did not affect this inhibitory action ($n = 5$). Blockade of L-type Ca^{2+} channels ($I_{Ca,L}$) with nifedipine(1 μM) prevented the contraction. In contrast, high K^+ induced a transient contraction pretreated with NFA and NPPB, and the half inhibition time ($T_{1/2}$) for NFA and NPPB were16.3 ± 3.9 ($n = 7$) and 16 ± 4.3 min ($n = 6$) respectively, which were significantly longer than that of nifedipine (2 ± 0.8 min, $n = 10$). In addition, in Ca^{2+}-free Krebs solution, NFA and NPPB completely suppressed norepinephrine-induced contractions ($n = 5$). Taken together, these data indicated that the inhibitory effect of Cl^- channel blockers on VSM contraction was due to Cl^- channel inhibition and the resulting decreased Ca^{2+} entry carried by $I_{Ca,L}$, and Cl^- channel blockade also prevented sarcoplasmic reticulum Ca^{2+} release in VSM.

Abstract N° B50

Dispersion of RYR2 and $I_{CA.L}$ associated with exacerbated arrhythmias and cardiac failure is regressed by propranolol and CPU86017, a multi-channel blocker

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It was intended to explore if the downregulated ryanodine 2 receptor and upregulated $I_{Ca.L}$ could be dispersed in an infarcted heart associated with arrhythmogenesis and cardiac failure by over phosphorylation and in response to propranolol and CPU86017, a multi-channel blocker.

Methods. – The coronary artery in rats was ligated to develop myocardial infarction, then, isoproterenol (Isop) was medicated during d 16–20 for 5 d in parallel with interven-

tions with propranolol and three doses of CPU 86017. Cardiac arrhythmias were assessed by reperfusion mounting on Langendorff's apparatus and hemodynamics was evaluated under pentobarbital anesthesia. The severity of arrhythmias and cardiac failure was exaggerated in the MI rat with isoproterenol together with significant cardiac remodeling. The mRNA abundance of RyR2, SERCA2, NCX and $I_{Ca.L}$ current were not changed markedly in the MI group vs. normal. Downregulation of RyR2 and SERCA2 was significant in infarcted hearts stimulated with isoproterenol ($P < 0.01$) and much more depressed RyR2 and SERCA2 were presented in the LV against the right ($P < 0.01$). An upregulation of the $I_{Ca.L}$ current and mRNA of NCX were evident and a dispersion was presented by showing a more upregulated in the LV in the infarcted plus isoproterenol against the MI. The changes in channels were associated with exacerbation of arrhythmias and hemodynamics in MI plus isoproterenol group. Propranolol was effective to regress these changes. The regression was also significant in responses to CPU 86017 2 and 4 mg/kg i.p., however, no effect with 1 mg/kg. This is the first time to show dispersion of depressed RyR2, SERCA2 and upregulated ion channels NCX and $I_{Ca.L}$ in an affected heart associated with enhancement of arrhythmias and cardiac failure, resulting from the hyperphosphorylation by isoproterenol in MI heart. These were regressed completed by propranolol. Regression of exacerbation of arrhythmias and cardiac failure by hyperphosphorylation can also be achieved by CPU86017 which possessing a blockade on $I_{Ca.L}$, IKR, IKS α-receptors and a potent antioxidant effect is derived from berberine.

Abstract N° B51

Dissociation of $[Ca^{2+}]_i$ and force in trabeculae from an animal model of hypertensive failure

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We have used an animal model of hypertensive heart failure to examine the relationship between intracellular calcium ($[Ca^{2+}]_i$) and contractile force for a range of different stimulus frequencies. Simultaneous measurements were made of isometric force and $[Ca^{2+}]_i$ (measured as the ratio of fura-2/AM fluorescence), in left-ventricular (LV) trabeculae from spontaneously hypertensive rats (SHR, mean age 21.8 ± 0.5 months) in heart failure, and their age-matched, normotensive, Wistar-Kyoto controls (WKY, mean age 23.6 ± 0.5 months). In addition, the force–frequency relationship was also examined in right ventricular (RV) trabeculae from healthy Wistar rats (mean age 3.0 ± 0.4 months) to allow comparison with other studies. At 37 °C, and with an extracellular calcium concentration of 2 mM, peak stress was lower for SHR trabeculae in comparison to WKY for all stimulus frequencies examined, whereas, paradoxically, peak $[Ca^{2+}]_i$ was higher. At low frequencies, peak $[Ca^{2+}]_i$ and peak stress both decreased with increasing frequency for all rat strains. However, for frequencies above 2 Hz, although peak $[Ca^{2+}]_i$ increased, there was no parallel frequency-dependent increase in peak stress for either SHR or WKY. A bi-phasic force–frequency response was obtained for Wistar trabeculae, with peak stress increasing at higher frequencies in a similar manner to peak $[Ca^{2+}]_i$. The dissociation of stress and $[Ca^{2+}]_i$ suggest that altered excitation–contraction coupling does not explain the apparent contractile dysfunction, but that differences in the extracellular matrix might be a contributing factor.

Abstract N° B52

Suppression in sarcoplasmic reticulum calcium uptake activity underlies changes in intracellular calcium mobilization in ovariectomized rat hearts

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The hypothesis that ovarian sex hormone deficiency may suppress cardiac contraction through modification of intracellular calcium mobilization was tested here. The sarcoplasmic reticulum (SR) calcium uptake activity was determined using both left ventricular homogenate and SR membrane vesicular preparations from 10-week ovariectomized rat hearts. A significant decrease in the maximum SR Ca^{2+} uptake rate but an increase in Ca^{2+} sensitivity was demonstrated in ovariectomized hearts. Measurements of SR Ca^{2+}-ATPase (SERCA) activity also substantially revealed suppression in maximum activity but an increase in SERCA sensitivity to Ca^{2+} in ovariectomized hearts. Reductions in the SR Ca^{2+} uptake and SR Ca^{2+}-ATPase activity were correlated well with a significant downregulation of SERCA proteins detected by immunoblot analyses. Either estrogen (5 μg/rat) or progesterone (1 mg/rat) supplementation after ovariectomy completely reversed changes in the uptake activity as well as the SERCA protein expression. On the other hand, there was no effect of ovarian sex hormone deficiency on the expression of phospholamban protein. These results thus indicate a significant cardioregulatory effect of ovarian sex hormones on intracellular Ca^{2+} homeostasis through the function of SR Ca^{2+} uptake especially the SERCA activity and protein expression.

Abstract N° B53

Calmodulin triggers Ca^{2+} Pump function in native cardiac sarcoplasmic reticulum by disrupting Ca^{2+}-ATPase–phospholamban interaction

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A pivotal role for calmodulin (CaM) in supporting the Ca^{2+}-ATPase function in cardiac sarcoplasmic reticulum (CSR) was suggested by our recent observation that a high affinity CaM-binding peptide (CaM BP) strongly inhibits CSR Ca^{2+} pump function by scavenging membrane-associated CaM (Xu, A., Narayanan, N., 2000. J Biol Chem 275, 4407–4416). The present study investigated the mecha-

nism of CaM regulation of CSR Ca^{2+} pump function by determining the influence of CaM BP and CaM on the intermediate steps in Ca^{2+}-ATPase catalytic cycle and Ca^{2+}-ATPase–phospholamban (PLN) interaction in rabbit CSR. Parallel studies were also performed using rabbit fast skeletal muscle SR (FMSR) lacking PLN. Under identical assay conditions, CaM BP (5 μM) strongly inhibited decomposition of the Ca^{2+}-induced phosphoenzyme (EP) intermediate in the catalytic cycle of Ca^{2+}-ATPase in CSR but not FMSR. CaM (3 μM) reversed the CaM BP-induced blockade EP decomposition in CSR. CaM BP and CaM did not influence EP formation in CSR or FMSR. Co-immunoprecipitation studies revealed that CaM BP promoted association of Ca^{2+}-ATPase with PLN in native CSR whereas as CaM promoted dissociation of the Ca^{2+}-ATPase-PLN complex. This action of CaM was Ca^{2+}-dependent and was accompanied by CaM–Ca^{2+}-ATPase interaction. CaM BP (2.5 μM) caused relaxation failure in the intact beating rabbit heart. These findings imply that (a) PLN-bound Ca^{2+}-ATPase is catalytically inert, and (b) endogenous CaM serves to trigger CSR Ca^{2+} pump function and cardiac relaxation by dissociating Ca^{2+}-ATPase from PLN and by accelerating the rate limiting step of EP decomposition in the enzyme's catalytic cycle.

Supported by CIHR and HSFO.

Abstract N° B54

Target-dedicated function of calcium/calmodulin-dependent protein kinase in cardiac sarcoplasmic reticulum

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In the cardiac sarcoplasmic reticulum (SR), a membrane-associated CaMK is known to phosphorylate the ryanodine receptor-Ca^{2+} release channel (RyR), Ca^{2+}-ATPase and phospholamban (PLN). RyR phosphorylation results in enhanced Ca^{2+} release whereas phosphorylation of the Ca^{2+}-ATPase and PLN results in enhanced Ca^{2+} uptake. To be physiologically meaningful, the Ca^{2+} release and uptake events must occur sequentially and in concert with the contraction–relaxation cycle of the heart. The mechanism that governs the sequential activation and temporal segregation of these events is unknown. This study investigated our hypothesis that, spatial segregation of SR-CaMK as target-dedicated molecules provides a mechanistic framework for the temporal segregation of Ca^{2+} release and uptake events. As a first step, the target specificity of SR-CaMK was determined from its ability to discriminate between endogenous and exogenous substrates. SR vesicles were isolated from rabbit cardiac muscle and the phosphorylation reaction by endogenous CaMK was performed in the absence and presence of Ca^{2+}/CaM and the exogenous substrates troponin, myosin light chain (MLC), and myelin basic protein (MBP). The results showed that SR-CaMK phosphorylated the endogenous substrates RyR, Ca^{2+}-ATPase and PLN but not the exogenous substrates troponin and MLC. MBP was phosphorylated in the absence and presence of Ca^{2+}/CaM, suggesting that its phosphorylation is CaMK-independent. Ca^{2+}-ATPase in the junctional SR, but not in the longitudinal SR, underwent phosphorylation by endogenous CaMK. These findings suggest target-dedicated spatial segregation of CaMK in cardiac SR.

Supported by CIHR and HSFO.

Abstract N° B55

The frequency-induced relaxant effect is mediated by a camkii independent pathway

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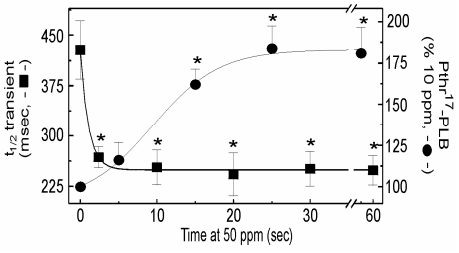
An increase in stimulation frequency (ISF) causes an acceleration of relaxation (FDAR). Several mechanisms have been postulated to explain this effect: a CaMKII-phosphorylation of Thr^{17} site of PLB ($PThr^{17}$) and/or of Ser^{38} site of SERCA, or a CaMKII-independent mechanism, whereby ISF-induced Ca^{2+} elevation would disrupt the SERCA-PLB interaction. We examined these mechanisms in Indo-1-loaded isolated cat myocytes. The ISF significantly reduced time to half Ca^{2+} transient decay, ($t_{1/2}Ca_iT$), and increased $PThr^{17}$. Comparison of the time course of FDAR with the increase in $PThr^{17}$, produced by ISF from 10 to 50 ppm, showed that the $t_{1/2}Ca_iT$ was maximally decreased after 2.5 s of ISF, whereas the increase in $PThr^{17}$ became significant only after 15 s of ISF (Fig. 1). Additionally, the CaMKII-inhibitor KN-93, significantly decreased the $PThr^{17}$ evoked by ISF, but failed to affect FDAR. The results would indicate that CaMKII-phosphorylation pathways are not involved in FDAR in cat myocytes. More likely, a direct effect of the increase in Ca^{2+} on SERCA-PLB interaction, promoting its dissociation, would lead to an increase in SR Ca^{2+} uptake and myocardial relaxation.



Abstract N° B56

Overexpression of sarcoplasmic reticulum calcium atpase in isolated rat myocytes mediated by recombinant adenovirus

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To assess value of SERCA2a in the gene therapy of congestive heart failure, furthermore to analysis the effect of transfection to pathophysiology. A recombinant adenovirus,

named rAd-trSERCA2a, was constructed with AdEasy system, in which the SERCA2a cDNA was inserted under the control of CMV promoter. The cardiac myocytes of newborn rat were cultured, and were transfected with rAd-trSERCA2a. RTPCR and western blotting were performed to detect the expression of SERCA2a in cardiac myocytes.

Results. – The sequence of SERCA2a was stably existed in rAd-trSERCA2a, which was tested by PCR and Southern blot. The quantity of mRNA of SERCA2a in cardiac myocytes increased 3.6 times compared with the control, and the protein level is 1.5 times of the control, 72 h after the transfection.

Conclusion. – This recombinant virus, rAd-trSERCA2a, can effectively deliver the SERCA2a gene into cultured cardiac myocytes and may become a feasible tool to modulate myocyte Ca^{2+} homeostasis in the failure heart.

Keywords: Heart failure; Recombinant adenovirus; SERCA2a; Gene therapy

Abstract N° B57

Inhibition of phosphoamban expression in cultured rat myocardial cells by adeno-associated virus plasmid mediated RNA interference

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Construct three plasmids containing the shRNA of phospholamban to suppress the expression of phospholamban in rat myocardial cell. A 334-bp human U6snRNA promoter was amplified from human genomic DNA by PCR and ligated to a 21 or 20 nt reverse repeated motif of phospholamban target sequence with 9 nt spacer and AAV plasmid pSNAV, pSNAV-phRis. We transfected those three plasmids into cultured rat myocardial cells to detect the effect of ϵ phospholamban expression separately. pSNAV-phR is suppresses the phospholamban mRNA expression 15, 25 and 30 by RT-PCR. pSNAV-phRis suppresses the phospholamban protein expression 10%, 14.5% and 23.6% by western blot, whereas the control GFP express efficency was 32.4%. The results showed that the short hairpin RNA of PLN can suppress it's expression in cultured rat myocardial cell.

Abstract N° B58

Knock down phospholamban to improve heart function by AAV- and adenovirus-mediated RNAi

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We developed serials of methods to introduce RNAi into cultured mammal cell by adenoassistant virus (AAV) and adenovirus for improving heart function by AAV- and adenovirus-mediated RNAi.

Methods. – (1) We constructed three AAV plasmids, named pSNAVpRi1, 2 and 3, in which short 21nt stem hairpin RNA duplexes under the control of the U6 snRNA promoter were cloned into pSNAV-1. After generation of AAV cell lines by G418 chosen, the AAV-pRi vectors were saved out by HSV1-rc/DUL2. (2) The long hairpin duplexes were constructed by linkage two 657 and 541 bp segments cloned from phospholamban mRNA with 5′-5′ mode, which transcriptional RNA would form to a 541 bp stem and 116 bp loop structure. Then the duplexes were inserted into the pTract-CMV plasimd under CMV promoter to product recombination adenovirus AV-lpRi. (3) Cultured rat cardiac myocytes were transfected with these siRNA plasmids or infected with AAV-pRis and AV-lpRi. The cells were collected in 72 h after infections. Western blot were performed to compare the difference between the expression of phospholamban. RT-PCR were used to quantified the mRNA levels.

Results and conclusion. – Western blot and RT-PCR were shown that two virus could inhibit phospholamban expression no more than 50%. And three AAV plasmids pSNAV-pRi1, pSNAV-pRi2 and pSNAV-pRi3, could inhibit phospholamban expression about 10%, 14.5% and 23.6%. Considering the low transfections efficiency of control plasmids (about 20–30%) into cultured rat cardiac myocytes, we will finish the other two AAV-pRis virus vectors in the further study. On the other hand, it has shown that the cardiac myocytes infected with AV-lpRi virus did not show the nonspecific mRNA degradation and translation inhibition. We thought this long hairpin RNA duplexes with long-short mode introduce RNAi weakly. Next we would construct the long duplexes adenovirus with an additional loop.

Abstract N° B59

Endoplasmic reticulum Ca^{2+} depletion induces apoptosis independent of caspase-12 signaling pathway

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Apoptosis of endothelial cells (ECs) is now regarded to be an initial step inducing atherosclerosis. Recent study reported that the depletion of endoplasmic reticulum (ER) Ca^{2+} stores plays an important role of apoptosis. Caspase-12 is a key signal to lead ER stress-induced apoptosis. However, it is not known if the depletion of ER Ca^{2+} is linked to caspase-12 signaling in ECs. Here, we have investigated the interaction of Ca^{2+} signaling and caspase-12 cleavage in apoptosis of cultured porcine aortic ECs. Cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) was measured using fura-2/AM. Apoptosis was assessed by DNA ladder formation and cleavage of caspase-12 by western blotting. Thapsigargin (2 μM), an inhibitor of the ER-associated Ca^{2+}-ATPase, increased $[Ca^{2+}]i$ and induced persistent ER calcium depletion, cleavage of caspase-12 and apoptosis. Bradykinin (10 nM) increased $[Ca^{2+}]i$ but induced neither cleavage of caspase-12 nor apoptosis. However, when ECs were treated with

BAPTA/AM, BK caused the Ca^{2+} depletion of ER and apoptosis without the cleavage of caspase-12. These results suggested that the increase of $[Ca^{2+}]i$ did not play an important role to cause apoptosis in ECs and ER Ca^{2+} depletion-induced apoptosis independent from caspase-12 linked signaling pathway.

Abstract N° B60

Regulatory myosin light chain signaling pathways in urotensin-II-mediated positive inotropic effect in human right atrium

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Urotensin-II (UII) is the most potent endogenous cardiostimulant yet identified, however the signaling mechanism is not known. Phosphorylation of regulatory myosin light chain (MLC-2) increases force by sensitisation of myofilaments to Ca^{2+}, and this may be modulated by protein kinase C (PKC) and myosin light chain kinase (MLCK). RhoA/Rho kinase may prevent dephosphorylation of MLC-2 by inhibition of myosin light chain phosphatase. The role of these pathways on the UII response was investigated using human right atrial trabeculae in a tissue bath preparation. UII increased phosphorylation of MLC-2 (1.76 ± 0.40-fold, $n = 6$, $P < 0.05$), and PKCαβII (1.42 ± 0.18-fold, $n = 7$, $P < 0.05$). UII increased force of contraction and this was attenuated by desensitisation of PKC with phorbol 12-myristate 13-acetate (PMA) but not 4α-phorbol, which does not desensitise PKC (Table 1). The cardiostimulatory effect was inhibited by the PKC inhibitor chelerythrine, but not the MLCK inhibitor, wortmannin.

Table 1
Effect of inhibitors on inotropic response of UII

Treatment	Control (mN)	Treated (mN)	*n, P values*
10 μM PMA	1.81 ± 0.46	0.21 ± 0.15	6, *P = 0.008*
10 μM 4α-phorbol	1.22 ± 0.19	1.24 ± 0.36	5, *P = 0.968*
10 μM chelerythrine	1.56 ± 0.31	0.61 ± 0.13	7, *P = 0.016*
10 μM Wortmannin	1.93 ± 0.75	3.90 ± 1.03	5, *P = 0.159*

Developed force (mean ± SEM). $P < 0.05$, significance (Student's *t*-test).

Although the Rho kinase inhibitor Y-27632 (10 μM) inhibited the UII response, basal force of contraction was also reduced. No detectable activation of RhoA was observed by affinity immunoprecipitation. The findings indicated that UII increased phosphorylation of MLC-2 and that the positive inotropic effect involved activation of PKC, and was independent of MLCK and RhoA-Rho kinase signaling pathways.

Abstract N° B61

Both β_1- and β_2-adrenoceptors mediate increases in contractile force and hastening of relaxation in human atrium

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Right atrial emptying initially occurs passively when the tricuspid valve opens and then by contraction of atrial muscle. Ensuing relaxation allows venous filling of the right atrium. Right atrial muscle contraction and relaxation is modulated by activation of β_1- and β_2-ARs. We therefore investigated the effects of stimulation of β_1- and β_2-ARs and phosphorylation of proteins that affect contractile function in right atrium from non-failing and failing hearts. Both (–)-noradrenaline in the presence of 50 nM ICI 118,551 (NA, selective β_1-AR activation) and (–)-adrenaline in the presence of 300 nM CGP 20712A (ADR, selective β_2-AR activation) caused increases in contractile force and hastening of relaxation associated with increases in cyclic AMP, phosphorylation of phospholamban, troponin I and C-protein in trabeculae taken from right atrial appendages of patients undergoing coronary artery bypass surgery, not taking β-blockers (non BB) prior to surgery. Further studies also investigated trabeculae dissected from human right atrial appendages obtained from coronary artery bypass surgery from patients with non-failing hearts (NF) receiving BB (atenolol/metoprolol) and trabeculae from right atrium of explanted hearts from patients with terminal heart failure (HF). There was no difference in basal contractile force between trabeculae from the three groups, however, the time to reach peak force (TPF) was longer in heart failure patients (non BB 102.3 ± 1.4 ms; BB 104.8 ± 1.6 ms; HF 113.0 ± 3.7 ms, $P = 0.0021$, $n = 37$—115 trabeculae) with a similar trend for time to reach 50% relaxation. The difference was not related to endogenous cyclic AMP, phospho-serine16-phospholamban (P-Ser16PLB) or phospho-threonine17-PLB (P-Thr17PLB) content. NA and ADR caused concentration-dependent increases in contractile force (NA $-\log EC_{50}$ NF BB 7.4 > NF non BB 7.0 > HF 6.4 $P < 0.0001$; ADR NF BB 7.95 > NF non BB 7.24 ≅ HF 6.95, $P < 0.0001$) and hastening of relaxation in right atrium associated with increases in phosphorylation of PLB. These studies emphasize the importance of β_2-AR in addition to β_1-AR coupling to Gsα-protein-cyclic AMP and phosphorylation of proteins implicated in hastening of relaxation.

Abstract N° B62

Different types of heart failure induced by overexpression of cardiac NCX1.1 or its mutant in transgenic mice

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Na^+/Ca^{2+} exchange is the primary mechanism of Ca^{2+} extrusion from cardiac myocytes during diastole. There are many reports indicating that NCX1 expression levels are elevated in heart failure; however, the role of NCX1 in the pathophysiology of cardiac disease is not well understood. To determine the in vivo cardiac function of NCX1, we generated transgenic mice with cardiac-specific overexpression of NCX1.1, and also mice where the exchanger had a mutated XIP region (Y224W/Y226W/Y228W/Y231W), devoid of Na^+-dependent inactivation. Cardiac-specific overexpression of exchangers in these transgenic mice was confirmed by western blotting and immunohistochemistry with an anti-NCX1 antibody. Increased Na^+/Ca^{2+} exchange activities were also demonstrated by measuring $^{45}Ca^{2+}$ uptake into membrane vesicles and exchange currents in whole cell patch-clamp myocytes. We found that homozygous NCX1.1-transgenic mice, but not heterozygous mice, develop cardiac hypertrophy, and heterozygous NCX1.1 mutant-transgenic mice produce dilated cardiomyopathy. These models will be useful for understanding the role of NCX1 in cardiac disease.

Abstract N° B63

The reverse mode of vascular Na^+/Ca^{2+} exchanger type-1 is essential for the development of salt-sensitive hypertension

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Excessive salt intake is a major risk factor for hypertension. Here, we studied the role of Na^+/Ca^{2+} exchanger type 1 (NCX1) in salt-sensitive hypertension using SEA0400 (Matsuda et al. 2001), a specific inhibitor for the reverse mode of NCX1, and genetically engineered mice. SEA0400 lowered arterial blood pressure in salt-induced hypertensive rat models, but not in spontaneously hypertensive or normotensive rats. Infusion of SEA0400 into the femoral artery in salt-induced hypertensive rats increased the arterial blood flow, indicating direct vasodilation. SEA0400 reduced the ouabain-induced vasoconstriction or cytosolic Ca^{2+} elevation in mesenteric and femoral arteries. Furthermore, $NCX1^{+/-}$ mice showed low salt-sensitivity to blood pressure, whereas vascular NCX1.3-transgenic ($NCX1.3^{Tg/Tg}$) mice, but not cardiac $NCX1.1^{Tg/Tg}$ mice, displayed salt hypersensitivity. SEA0400 drastically lowered salt-induced hypertension in $NCX1.3^{Tg/Tg}$ mice, but not in NCX1.3-$G833C^{Tg/Tg}$ mice which overexpress an SEA0400-insensitive mutant. Our findings indicate that the reverse mode of vascular NCX1 is involved in the development of salt-sensitive hypertension and that it may be a new target for anti-hypertensive drug.

Abstract N° B64

Strong inhibition of Na^+–Ca^{2+} exchanger expression by RNAi does not prevent cardiac contraction

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The Na^+–Ca^{2+} exchanger (NCX) is the main mechanism for calcium efflux in the heart and is thought to be essential in cardiac excitation–contraction coupling. NCX1 gene disruption for the generation of knock-out mice is lethal during development. An alternative approach to study the lack of function of the exchanger in postnatal cardiomyocytes is the use of RNA interference (RNAi) for NCX silencing. The purpose of the present study, therefore, was to deplete NCX expression to directly evaluate the hypothesis that NCX plays a critical role in excitation–contraction coupling. We generated adenoviruses to deliver short hairpin RNAs (Ad-R) targeting different regions of NCX and one scrambled control. Protein levels were analyzed by Western blot and immunocytochemistry. NCX activity was measured using a ^{45}Ca uptake assay. Transduction of neonatal cardiomyocytes with Ad-R resulted in a ~94% decrease in NCX protein level compared to control. Surprisingly, cardiomyocytes with nearly depleted NCX still show spontaneous contractions (although at lower frequency) and respond to electrical stimulation. Intracellular calcium measurements in electrically stimulated cells showed that Na^+–Ca^{2+} exchanger depleted cells have depressed Ca^{2+} transient amplitude, depressed rate of Ca^{2+} rise and decline and, elevated diastolic [Ca^{2+}] compared to control cells. We observed a three times increase in sarcolemmal Ca^{2+} pump expression (the other calcium extrusion mechanism in the cells) as an adaptative mechanism to the lack of NCX. Thus, our data support an important but not critical role for the Na^+–Ca^{2+} exchanger in excitation–contraction coupling in the heart and demonstrates that RNA interference is a valuable tool for efficient downregulation of cardiac proteins.

This work was supported by the Heart and Stroke Foundation of Canada.

Abstract N° B65

Replacement of the endogenous NCX1.1 isoform of the Na–Ca exchanger by the NCX1.3 isoform in neonatal rat cardiomyocytes

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The sodium calcium exchanger NCX1 gene undergoes alternative splicing in a region that corresponds to a small region of the intracellular loop of the protein. NCX1.1, the

isoform found in the heart, contains exons A, C, D, E, F. NCX1.3, found in kidney cells, contains exons B,D. These two isoforms show different response to intracellular regulatory Na^+ and Ca^{2+}. To study the differences in calcium handling and regulation of both isoforms we designed a series of experiments that allow us to replace the endogenous 1.1 isoform by the 1.3 isoform in neonatal rat cardiomyocytes. First, we generated adenovirus constructs to downregulate the endogenous protein (NCX1.1 isoform) by RNAi. We observed a 94% decrease in protein level respect to control. We subsequently transduced the cells with another adenovirus expressing the NCX1.3 isoform (canine) or the NCX1.1 (canine) for control. We show that RNAi targeting a region 142 nucleotides from the start codon (where there are seven nucleotides in difference between rat and canine cDNA sequence) interferes with the expression of the exogenous 1.3 isoform and does not allow isoform replacement. However, RNAi targeting exon F (present in the 1.1 but not 1.3, either rat or canine) can successfully be used to perform the isoform replacement. We used adenoviruses coding NCX1.1/3 protein fussed to EGFP, the fusion protein runs at a different size respect to the endogenous protein in western blots and allows us to differentiate them. Characterization of calcium transients in cells expressing the 1.1 and 1.3 isoform show no differences in diastolic or systolic calcium. We found differences in the response of the cells to treatment with ouabain.

This work was supported by the Heart and Stroke Foundation of Canada.

Abstract N° B66
Response to simulated ischemia–reperfusion of HEK-293 cells expressing the cardiac or kidney isoform of the Na^+–Ca^{2+} exchanger

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The Na^+Ca^{2+} exchanger (NCX1) gene can generate different isoforms of the protein by alternative splicing. NCX1.1 is the isoform found in the heart whereas NCX1.3 is found in kidney cells. These isoforms show different response to intracellular regulatory Na^+ and Ca^{2+}. An increase in $Ca^{2+}{}_i$ is known to activate the Ca^{2+} entry mode and eliminate Na^+ inactivation in NCX1.1 but not in NCX1.3. Due to the intracellular $[Na^+]$ and $[Ca^{2+}]$ change during ischemia, the purpose of the present study was to determine if cells expressing either of the two isoforms respond in a different way to an ischemic insult. We generated stable transfected clones of 293 cells expressing similar levels of the NCX1.1 or NCX1.3. Protein levels were measured by western blot. We performed a protocol of simulated ischemia and reperfusion on the cells and measured changes in intracellular calcium and pH. We also studied non-transfected 293 cells that do not contain endogenous (NCX1). We found no differences in intracellular pH between groups before or during the ischemia–reperfusion protocol. Intracellular calcium was lower in the NCX1.1 before ischemia. During the ischemic insult, the not-transfected cells showed a small increase in $Ca^{2+}{}_i$ that has to take place through mechanisms other than the (NCX1). $Ca^{2+}{}_i$ was higher during ischemia in the NCX1.3 group compared to the non-transfected group and even higher in the NCX1.1 group. In summary, $[Ca^{2+}]_i$ was greater during simulated ischemia in HEK-293 cells when the 1.1 isoform of the NCX was present. The 1.3 NCX isoform was not as effective in inducing an increase in $[Ca^{2+}]_i$ during ischemia. Our results demonstrate that ionic regulation of NCX plays a role in calcium overload during ischemia–reperfusion. This work was supported by the Heart and Stroke Foundation of Canada.

Abstract N° B67
Cyanide inhibits Na^+–Ca^{2+} exchanger in single pacemaker cells isolated from sinus venosus of cane toad

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Dysfunction of the sinoatrial node is well recognised, particularly in ischaemic heart disease, and can cause a variety of arrhythmias. Recent studies have suggested that Ca^{2+} release from the sarcoplasmic reticulum plays an important role in determination of pacemaker rhythm by modulating Na^+–Ca^{2+} exchange current. To study whether Na^+–Ca^{2+} exchanger activity might be affected by ischaemia, we examined the effects of cyanide on Na^+–Ca^{2+} exchanger activity in isolated toad pacemaker cells. The reverse mode of exchanger activity was estimated by measuring the amplitude of $[Ca^{2+}]_i$ rise in response to extracellular Na^+ removal. After application of 2 mM NaCN for 3–5 min, the rise of $[Ca^{2+}]_i$ was significantly decreased from 377 ± 42 to 260 ± 46 nM (n = 8, $P < 0.01$). To study the forward mode of exchanger activity, we recorded the Li-sensitive tail current by using nystatin-perforated patch technique. The amplitude of tail currents was also significantly decreased by CN^- by 36 ± 2.6%. To investigate the intrinsic properties of Na^+–Ca^{2+} exchange during the metabolic inhibition, we simultaneously recorded $[Ca^{2+}]_i$ and Na^+–Ca^{2+} exchange current by using rapid caffeine application. Caffeine induced $[Ca^{2+}]_i$ signals were reduced from 741 ± 106 to 509 ± 57 nM, while the average inward current reduced from –121 ± 29 to –52 ± 12 pA. When current was plotted against $[Ca^{2+}]$, the slope of the decay phase was decreased in the present of CN^-, indicating that for a given $[Ca^{2+}]$, there was less current produced. The average slope was reduced to 44 ± 8% of control (n = 7, $P < 0.02$). These results indicate that CN^- inhibits Na^+–Ca^{2+} exchanger activity, and such inhibition is at least partially through the inhibition of the intrinsic properties of exchanger. Therefore, the heart rate changes during the ischaemia might be related to the changes of activity of Na^+–Ca^{2+} exchanger.

Abstract N° B68

Rise of intracellular Na^{2+} during cardiac ischaemia: the underlying mechanisms

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The importance of the intracellular sodium concentration ($[Na^+]_i$) in cardiac cells during and after ischaemia arises because the increase in $[Na^+]_i$ during ischaemia is removed by the Na^+–Ca^{2+} exchanger, which in turn increases $[Ca^{2+}]_i$, thereby promoting myocardial damage. There is a dispute over the mechanisms responsible for the increase of $[Na^+]_i$ in cardiac cells during ischaemia. One proposal is that the cardiac Na^+–H^+ exchanger 1 (NHE1) causes Na^+ entry in exchange for H^+ extrusion. Another proposal is that Na^+ entry is via the tetrodotoxin (TTX)-sensitive persistent Na^+ current. To distinguish these hypotheses, whole rat hearts were isolated and perfused. $[Na^+]_i$ was measured before, during and after ischaemia. In control experiments, $[Na^+]_i$ increased slowly during ischaemia and rapidly upon reperfusion. It was found that the rise of $[Na^+]_i$ during ischaemia was abolished by TTX (a Na^+ channel blocker; 0.3 μM) and amiloride (a low affinity NHE1 blocker; 0.1 mM), but not by zoniporide (a high affinity NHE1 blocker; 1 μM). The rise of $[Na^+]_i$ following reperfusion was blocked by amiloride and zoniporide. We propose that the rise of $[Na^+]_i$ during ischaemia is due to a TTX- and amiloride-sensitive inward Na^+ current. To test this hypothesis, single ventricular myocytes were isolated from rat hearts and prepared for patch clamping. Metabolic inhibition was produced by adding 2 mM CN^- to the pipette solution. Cells were then subjected to a voltage-step protocol, which allowed both transient inward Na^+ current and an inward Na^+ tail current to be measured. All other currents were suppressed. Inward tail currents of –15.6 ± 1.5 and –15.6 ± 1.7 pA were blocked by TTX and amiloride, respectively. However, the tail current blocked by zoniporide was significantly smaller (–6.9 ± 1.9 pA; $P < 0.005$). These results indicate that an inward tail current allows the entry of Na^+ into ventricular myocytes during ischaemia, which is a major cause of the rise in $[Na^+]_i$.

Abstract N° B69

Effects of NCX inhibitor on underperfusion injury in diabetic rat hearts

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We have reported that the diabetic (DM) than non-DM rat hearts were more susceptible to flow reduction and readily exhibited abnormal myocardial energy metabolism and increase in the left ventricular (LV) diastolic stiffness. Norepinephrine (NE) during underperfusion exacerbated the injury. In non-DM hearts, a pathophysiological role of the Na^+/Ca^{2+} exchanger (NCX) in myocardial ischemia–reperfusion injury has been proposed. In DM hearts, depression in NCX activity is reported. In the present study, therefore, the effect of a NCX inhibitor on the underperfusion injury was examined in isolated paced DM rat hearts without or with NE. KB-R7943 (3×10^{-6} M) was infused for 15 min before as well as during 60-min underperfusion (2 ml/min/g heart weight), and NE (10^{-6} M) during 55-min underperfusion. The abnormal transmural energy metabolism and the increase in LV stiffness during underperfusion without or with NE both were not definitely improved by KB-R7943. It rather tended to enhanced the injury with NE, particularly in the LV subendocardium. The results indicate that at least an increase in the outward Na^+/Ca^{2+} exchange may not appreciably contribute to the LV dysfunctions during 60-min underperfusion with NE in DM rat hearts.

Abstract N° B70

Engagement of Na^+–Ca^{2+} exchanger and Na^+–K^+ ATPase in the coronary artery relaxation induced by isosorbide dinitrate

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Isosorbide dinitrate (ISDN) is widely prescribed in management of ischemic heart diseases. Its vasodilating mechanism still remains to be elucidated. We investigated a role of Na^+–Ca^{2+} exchanger (NCX) and Na^+–K^+ ATPase in the ISDN-induced vasorelaxation, by simultaneously monitoring $[Ca^{2+}]_i$ and tension in the fura-2 loaded medial strips of porcine coronary artery. ISDN induced sustained decreases in $[Ca^{2+}]_i$ and tension during the contraction induced by U46619 (a thromboxane A_2 analogue) in the normal saline (137.3 mM Na^+). These decreases in $[Ca^{2+}]_i$ and tension were reduced by lowering $[Na^+]_o$ or by two different NCX inhibitors, KB-R7943 and 2′,4′-dichlorobenzamil. When Ca^{2+} was replaced by Ba^{2+} (which is not extruded by Ca^{2+} pumps but by NCX), ISDN also decreased $[Ba^{2+}]_i$ in the normal saline but not in low $[Na^+]$ saline. A large part of the ISDN induced decreases in $[Ca^{2+}]_i$ and tension were reversed by ouabain. This effect of ouabain was attenuated by either lowering $[Na^+]_o$ or diltiazem, and abolished by their combination. The ISDN-induced decreases in $[Ca^{2+}]_i$ and tension were abolished by ODQ (an inhibitor for guanylate cyclase). Rp-8-Br-cGMPs (an inhibitor of PKG) substantially inhibited the ISDN-induced decreases in $[Ca^{2+}]_i$ and tension, while Rp-8-Br-cAMPs (an inhibitor of PKA) was only partially effective. These results suggest that ISDN activates NCX mainly by activating Na^+–K^+ ATPase through a cGMP–PKG pathway and thereby induce vasorelaxation in the coronary artery.

Abstract N° B71

The value of heart-type fatty acid-binding protein for early detection of acute myocardial infarction

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To evaluate the diagnostic value of heart-type fatty acid-binding protein (H-FABP) for early detection of acute myo-

cardial infarction. Serum H-FABP of 126 healthy individuals and 53 AMI patients were measured by self-development ELISA. MYO, cTnI and CK-MB, traditional biochemistry diagnostic markers, were estimated in the same time. The dynamic changes of these myocardial indicators for AMI patients were analyzed, and the diagnostic sensitivity and specificity of them in the earlier period of AMI onset were evaluated. The plasma concentration of H-FABP began to increase at 1.84 ± 0.64 h, earlier than CK-MB and cTnI, after AMI onset. The time–concentration dynamic curves of H-FABP was similar to that of MYO and was in left compared with both CK-MB and cTnI. The sensitivity and specificity of H-FABP were 76.47% and 80.41% at 2 h after AMI; 89.16% and 91.26% at 4 h after AMI, respectively. H-FABP seems more sensitive and specificity in diagnosis of AMI early stage within 2 or 4 h after the onset. H-FABP probably will become an important myocardial marker for diagnosis of AMI in early stage.

Abstract N° B72

The effects of cholesterol on nuclear pore density

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The nuclear pore complexes (NPC) are channels found in the nuclear envelope through which proteins are carried. Protein movement into the nucleus can affect gene expression, cell proliferation and growth. Very little is known about factors involved in altering NPC density. In view of the altered cell proliferation that occurs under atherosclerotic conditions, we investigated the possibility that cholesterol may alter NPC density. New Zealand white rabbits were fed a 0.5% cholesterol diet for 8 weeks and control groups were fed a regular diet. Hepatic nuclei were isolated and nuclear protein was analyzed by western blots and immunocytochemistry using the antibody mAb414 that reacts to p62, an integral NPC protein. Immunostaining of the isolated nuclei for p62 displayed a high degree of punctate, fluorescent staining in the control group. The cholesterol fed rabbits showed a comparative decrease in immunostaining intensity. Western blotting for the nuclear pore protein showed a decrease in band intensity in the cholesterol group as compared to the control. These observations indicate a significant decrease in nuclear pore density in nuclei isolated from livers of cholesterol-fed rabbits when compared to a control group. Our data demonstrate that NPC expression is a dynamic adaptable structure. Changes in pore density may have important implications for nuclear protein import and, ultimately, gene expression.

Abstract N° B73

Cholesterol diet-induced hyperlipidemia influences gene expression pattern of rat hearts: a DNA microarray study

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Hyperlipidemia influences myocardial function and cardiac adaptation to ischemic stress via a yet unidentified, direct action on the myocardium (see for reviews: TIPS (1998); Br. J. Pharmacol. (2003)). To profile gene expression patterns involved in the direct myocardial effect of cholesterol-enriched diet-induced hyperlipidemia, we monitored global gene expression changes by DNA microarray analysis of 3200 genes in rat hearts. Twenty-six genes exhibited significant upregulation and 25 showed downregulation in hearts of rats fed 2% cholesterol-enriched diet for 8 weeks as compared to age-matched controls. The expression changes of 12 selected genes were also assessed by real-time quantitative PCR. Genes with altered expression in the heart due to hyperlipidemia included procollagen type III, cofilin/destrin, tensin, transcription repressor p66, synaptic vesicle protein 2B, Hsp86, chaperonin subunit 5-epsilon, metallothionein, glutathione *S*-transferase, protein kinase C inhibitor, ATP synthase subunit c, creatin kinase, chloride intracellular channel 4, NADH oxidoreductase and dehydrogenase, fibronectin receptor beta-chain, CD81 antigen, farnesyltransferase, calreticulin, disintegrin, p120 catenin, Smad7, etc. Although some of these genes have been suspected to be related to cardiovascular diseases, none of the genes have been previously shown to be involved in the mechanism of the cardiac effect of hyperlipidemia.

Abstract N° B74

The antiatherogenic effect of mouse recombinant leptin on high-fat diet induced oxidative stress

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Obesity, characterized by an excess of fat mass, is clearly associated with an increased risk of cardiovascular pathology. The link between increased adipogenesis, oxidative stress and development of atherosclerosis is still not well understood. Our aim in this study was to explore the tissue lipid peroxidation and antioxidant status in mice administered exogenous leptin along with high-fat diet (2% cholesterol, 0.125% bile salts and 5% peanut oil) for a period of 6 weeks. Significantly ($P < 0.05$) elevated levels of tissue thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and significantly lowered levels of superoxide dismutase (SOD), catalase (CAT), glutathione and its related enzymes were observed in the heart, liver and kidney of mice fed high-fat diet as compared with the control mice fed standard pellet diet. Subsequent to the treatment with high-fat diet (i.e. the initial period of 30 d) exogenous leptin (230 μg/kg body weight) was simultaneously administered every alternate day for 15 d along with the high-fat diet. Leptin administration significantly lowered the tissue levels of TBARS, CD and elevated the activities of SOD, CAT,

reduced glutathione (GSH), glutathione peroxidase (GPX) and glutathione *S*-transferase (GST) in both the control and high-fat diet fed mice. Food intake and average body weight at the end of the experimental period was significantly decreased on leptin administration. Thus, leptin administration was found to be effective in attenuating high-fat induced oxidative stress.

Abstract N° B75

Leptin downregulates lipid profile in mice fed a high-fat diet

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Dyslipidemia, atherosclerosis, diabetes, and obesity are among the many risk factors associated with coronary artery disease. The aim of our present study was to evaluate the effect of leptin on plasma and tissue lipids in an experimental model of atherosclerosis. Feeding a high-fat diet (2% cholesterol, 0.125% bile salts and 5% peanut oil) to 4-week-old healthy mice for a period of 45 d resulted in significantly elevated levels of plasma and tissue total cholesterol, phospholipids, free fatty acids and triglycerides as compared to control mice fed the standard pellet diet. Subsequently after 30 d, exogenous leptin (230 μg kg^{-1} body weight i.p.) was administered every alternate day for 15 d along with the high-fat diet to another group. Leptin administration significantly reduced body weight as well as the levels of total cholesterol, phospholipids, free fatty acids and triglycerides in the plasma, heart, liver and kidney of both the control and high-fat fed mice. Moreover leptin administration markedly reduced the levels of plasma low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and elevated plasma high-density lipoprotein (HDL) and the activity of lipoprotein lipase as compared with the untreated control and high-fat fed mice. Thus, leptin administration was found to reduce the risk of atherosclerosis markedly by virtue of its lipid lowering effects.

Abstract N° B76

Effect of obesity and hydralazine treatment on diurnal rhythms of blood pressure and heart rate

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Obesity results in both hypertension and loss of heart rate (HR) and blood pressure (BP) diurnal rhythms. Hypertension itself is associated with loss of HR and BP diurnal rhythms. Whether BP control in obesity restores diurnal rhythms is unknown. We tested whether BP control using hydralazine reversed loss of diurnal patterns during 12 weeks of developing obesity in rabbits. Female New Zealand white rabbits were divided into lean control (LC, $n = 8$), lean hydralazine treated (LH, $n = 10$), obese control (OC, $n = 8$) and obese hydralazine-treated (OH, $n = 11$) groups. Obese rabbits ate an ad lib high-fat diet; lean rabbits ate a maintenance diet. BP was monitored from 1100 to 0700 h daily using telemetry. Hydralazine treatment (6–14 mg/kg/d) began during week 2 of the dietary protocol. To evaluate diurnal rhythms, night (0200–0700 h) values were subtracted from day (1100–0400 h) values; groups were compared using one-way ANOVA.

Table 1
Day–night values

Group	Control		Week 12	
	BP	HR	BP	HR
LC	2.8 ± 1.2	49.0 ± 9.7	4.9 ± 0.9	53.5 ± 7.5
LH	5.8 ± 1.3	56.5 ± 3.5	3.6 ± 0.9	41.9 ± 7.7
OC	6.5 ± 1.7	59.1 ± 4.9	–0.6 ± 1.2	25.3 ± 10.5
OH	4.0 ± 1.2	45.0 ± 6.9	–0.8 ± 0.5	15.1 ± 5.1

Groups did not differ in day–night BP or HR during the control period. After 12 weeks of high-fat feeding, day–night BP was reduced in OC and OH, suggesting that there was no nighttime dipping of BP. Day–night HR was lower in OH compared with LC and LH. These data suggest that obesity's role in the loss of HR and BP diurnal rhythms is independent of hypertension.

Abstract N° B77

The relationship between angiotensin-converting enzyme gene polymorphism and peripheral arterial occlusive disease

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To investigate the relationship between angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and peripheral arterial occlusive disease (PAOD). ACE genotypes of 100 patients with hypertension complicated with PAOD and 150 hypertensive patients and 126 healthy people were detected by polymerase chain reaction (PCR) methods. To compare the differences of the distribution of ACE gene I/D polymorphism among the three groups. The frequencies of ACE DD genotype and deletion allele in hypertension complicated with PAOD group (0.23 and 0.44) were significantly higher than those in hypertension group (0.11 and 0.34, $P < 0.05$) and those in normal control group (0.10 and 0.33, $P < 0.01$). There was no significant differences between hypertension group and normal control group ($P < 0.05$). The D allele of ACE gene may be a susceptible gene to presence of PAOD. ACE gene I/D polymorphism may be not associated with presence of hypertension.

Abstract N° B78

The relationship between 4G/5G polymorphism located in the promoter region of plasminogen activator inhibitor-1 gene and peripheral arterial occulsive disease

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To explore the relationship between a single nucleo deletion/insertion (4G/5G) polymorphis located in the pro-

moter region of plasminogen activator inhibitor-1 (PAI-1) gene and peripheral arterial occulsive diseases (PAOD). The polymorphism of PAI-1 gene was analyzed by allele-specific polymerase chain reaction (AS-PCR) technique in 100 patients with hypertension, 100 patients with hypertension with PAOD, and 100 people as control subjects. In the meantime, blood glucose, blood lipids, blood pressure and blood fibrinogen, body mass index (BMI), and ankle-arm index (AAI) were studied. The frequency of 4G/4G genotype and 4G allele frequency of PAI-1 gene significantly increased in patients with hypertension with PAOD in comparison with patients with hypertension and control subjects ($P < 0.05$, respectively). While no significant difference was found between the patients with hypertension and control subjects. The levels of cholesterol and LDL were significantly increased in patients with hypertension with PAOD group compared with the other two groups ($P < 0.05$, respectively). In patients with hypertension with PAOD group, the 4G/4G genotype showed that the blood glucose, blood lipids (cholesterol, low-density lipoprotein, LDL and triglyceride, TG), blood fibrinogen, and BMI were higher than the 4G/5G genotype and the 5G/5G genotype, but there was no difference among them. The frequency of 4G/4G genotype and 4G allele frequency of PAI-1 gene were higher in patients with hypertension PAOD than that in patients with hypertension and control subjects, which suggest that 4G allele frequency of PAI-1 gene might be a risk factor of hypertension with PAOD.

Abstract N° B79

The prevalence of peripheral arterial occlusive disease and cardiovascular diseases in elderly population in Beijing

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To determine the prevalence of cardiovascular diseases (CVD) with peripheral arterial occlusive disease (PAOD) in elderly population. A cross-sectional survey for PAOD was made on the Wanshoulu area, Beijing. A total of 2124 subjects, males 943, females 1183, aged 60–95 (68.54 ± 5.43) years, were studied. PAOD was defined as an ankle–arm index (AAI) ≤ 0.9. The prevalence of PAOD was 15.91%, standardized 16.42% in Wanshoulu area. The prevalences of essential hypertension, hyperlipidemia, coronary heart disease (CHD) and diabetes in the population with PAOD were 55.8%, 54.3%, 41.0% and 25.4%, respectively, among which the prevalences of hypertension ($P < 0.01$), CHD ($P < 0.05$) and diabetes ($P < 0.05$) were significantly higher in the population with PAOD than those without PAOD, respectively. And the prevalences of hypertension in female PAOD ($P < 0.001$) and that of CHD in male PAOD population ($P < 0.01$) were higher than those without PAOD, respectively. The prevalence of CVD in the population with PAOD was 1.36–1.63 times higher than those in the population without PAOD. The prevalence of PAOD was 17.55% in the elderly population with single CVD risk factor $U = 3.8661$, $P < 0.001$, 20.33% in those with double risk factors and 22.14% in those with triple factors ($\chi^2 = 7.2408$, $P < 0.05$), respectively. The prevalence of PAOD in this study was similar to that in the developed countries and was increased significantly with the increase of cardiovascular risk factors. The prevalence of CVD in the population with PAOD was significantly higher than that without PAOD.

Abstract N° B81

Heart rate variability, parasympathetic action and cardiovascular risk

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Heart rate variability has been gaining increasing clinical importance during the last three decades. Its presence has been assumed to be an indication of appropriate adaptability of the organism to external challenges. Its absence or diminution on the other hand, is understood to indicate parasympathetic malfunction and to provide an indication of cardiovascular morbidity and mortality. However, the exact mechanism of parasympathetic action is not entirely clear. It is also not clear whether the relationship between the parasympathetic activity and the cardiovascular risk is causal or simply correlational. Although many authors still regard parasympathetic involvement as being achieved through a diminished parasympathetic tone, it is becoming increasingly clear that reflex parasympathetic mechanisms are involved. Evidence is available from both scientific and medical literature to show that parasympathetic reflex activity is complex and involves not only nervous reflexes, but also the spatiotemporal regulation of nitric oxide, production and release of insulin, glucose and hepatic insulin sensitizing substance (HISS) as well as regulation of mineral metabolism in the kidneys. Contrary to conventional belief, parasympathetic reflex activity becomes of great importance during increased states of activity, such as exercise or stress, when the parasympathetic tone is generally completely withdrawn. The neuronal pathways that are involved comprise intrinsic cardiac receptors and local neuronal circuits. There exist several positive feedback pathways within this complex system that includes the parasympathetic and sympathetic nerves, the vascular endothelium, the liver, pancreas and the kidneys. The presence of positive feedback reflexes may explain the relationship between parasympathetic activity and cardiovascular morbidity and mortality can be explained.

Abstract N° B82

Using of the controlled breath for studying the mechanisms of respiratory arrhythmia

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The aim is the studying of using the controlled-frequency breath for investigation of mechanisms of respiratory ar-

rhythmia. Twenty men and 15 women without the signs of cardiovascular and respiratory pathology were involved in the study. All the volunteers underwent tilt-test with controlled frequency of breathing (period of breath was 4–12 s). The depth and the phases ratio of controlled breathing did not differ from those under the spontaneous breathing. During the tilt-test the tachograms were registered for 3 min on each stage of the controlled breath. Spectrum of the heart rhythm was estimated using autoregressive algorithm. The intensity of frequency modulation of heart rhythm depends on the period of controlled breathing. More apparent modulation was observed under 8, 10 and 12 s period of breath. The maximal synchronization of the heart rhythm fluctuations and the phases of respiration was observed under 10 s period of breath. Supposing that there is the feedback system between the control centers of cardiac and respiratory rhythms, the coincidence of breathing frequency of internal oscillatory process may provoke resonance amplification of oscillations on the present frequency in the heart rhythm. In accordance with our data, the oscillation frequency in the feedback system between the control centers of heart and respiratory rhythms is about 0.1 Hz. The intensity of the frequency modulation of heart rhythm depends on the period of breathing. The frequency of synchronization mechanism, the respiratory phases and the heart rate fluctuations is about 0.1 Hz.

Abstract N° B83

Prevalence of diabetic retinopathy in diabetic populations: evaluating the ethnic differences

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Diabetic retinopathy (DR) is the fourth leading cause of world blindness, and is an ever-increasing problem in developing nations. The relative importance of genetic heritage, socio-economic status, culture, language and access to health care among ethnic groups, in relation to the prevalence of DR in diabetic populations, has remained unclear in the literature. This review aims to identify interacting or confounding variables associated with ethnic differences, and the trends in the prevalence of DR in diabetic populations of the United States (US), United Kingdom (UK), Southern India (SI), and Singapore. A MEDLINE search from 1975 to 2003 was conducted through the PubMed search system. Data extraction was based on risk factors, prevalence, determination of DR, and treatment strategies, followed by written critical appraisal of the literature. In the US, PIMA Indians, African Americans and Hispanics were at higher risk compared to their Caucasian counterparts. Migrant Asians, in particular, Indo-Chinese, were identified as high-risk groups in the UK. Certain communities in SI reportedly exhibited higher risk than other communities. Singaporean Indians and Malays were also found to be in the high-risk category. There is a strong evidence of ethnic disparities in the prevalence of DR in diabetic populations, and this has been found to be closely associated with variables such as socio-economic status, culture, genetic heritage, poor health education and awareness, and language barriers. It is now important for researchers and policy makers to better the causes of these disparities, to evaluate programs, and to then implement programs and policies necessary to address these disparities.

Abstract N° B84

A clustered randomised-controlled school community intervention to improve the dietary habits and cardiovascular risk factors of children and young adults: pilot study

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Multiple studies of children have demonstrated clearly that systolic blood pressure, total- and LDL-cholesterol, plasma insulin and obesity tend to cluster. A high likelihood exists that such cardiovascular risk factors present in obese children tend to track with age. Poor dietary habits developed in childhood have been found to contribute to obesity in children, and continue into adulthood. By aiming an intervention at children, whose dietary habits are in the process of being ingrained, the long-term benefits to adiposity and health overall are likely to be optimised. This study involved the participation of both parents and schools in (1) an intervention of providing healthy eating information, and serves of fruit daily in the classroom, over 8 weeks, (2) an assessment of children's dietary choices using 3-d-weighted food diaries, food frequency and lifestyle questionnaires administered during the intervention and post-intervention period, (3) measures of body mass index, lipid and glucose levels, and assessment of fitness levels of children, to evaluate any changes in children's adiposity and cardiovascular risk factors, which may have resulted during the course of this intervention and post-intervention. Results have suggested that baseline dietary habits were associated with cardiovascular risk factors in this younger aged population. Baseline dietary habits were also associated with parental demographic and dietary patterns. An increased number of children meeting Australian Dietary Guidelines would contribute to improvement in the overall health of the intervention population, and may help reduce the incidence of obesity and related disease burden.

Abstract N° B85

Atherogenic burden of hypertensive, diabetic and hypertensive–diabetic patients seen in Jos Nigeria

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The study set out to determine the atherogenic burden of hypertensive, diabetic and diabetic–hypertensive patients.

These diseases are known to accelerate atherosclerosis, which puts patients at risk for ischaemic events. Twenty-four, 28 and 24 diabetic, hypertensive and diabetic-hypertensive patients, respectively, were recruited. Fourteen apparently healthy subjects served as controls. Blood samples were collected and subjected to full lipid profile estimation. The patients were significantly older than the controls (diabetic vs. normal $P < 0.001$, hypertensive vs. normal $P < 0.001$, diabetic-hypertensive vs. normal $P < 0.001$). The mean body mass index (BMI) was significantly raised in diabetic-hypertensive patients compared with the control (37.4 kg/m^2 vs. 26.2 kg/m^2 $P < 0.001$). Both the diabetics and hypertensives did not significantly differ from the normals regarding BMI. All the patient groups had significantly raised total cholesterol levels (diabetic 6.0 mmol/l, hypertensive 6.4 mmol/l, diabetic-hypertensive 6.7 mmol/l). For the HDL fraction, which is protective, the hypertensives and diabetic-hypertensives had significantly higher levels than normals (hypertensive vs. normal $P < 0.02$, diabetic-hypertensive vs. normal $P < 0.02$). The diabetics had similar HDL levels with the normal subjects. For the LDL atherogenic sub fraction, only the diabetic-hypertensive had significantly raised levels (4.7 vs. 3.5 mmol/l $P < 0.05$). All groups are at risk of atherosclerosis given the high total cholesterol. The high HDL levels would seem to protect the hypertensives and diabetic-hypertensives. The diabetics from their low HDL levels are therefore at the greatest risk. This is also borne out by their TC/HDL ratio, which was the highest of all the groups.

Abstract N° B86

Peripartum cardiomyopathy: correlation between cholesterol, C-reactive protein and left ventricular dysfunction

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Heart failure is characterised by activation of inflammatory cytokines possibly due to increased endotoxin levels. Lipoproteins are non-specific endotoxin buffers. We analysed the correlation between serum cholesterol level, C-reactive protein (CRP) level and left ventricular dysfunction in patients with peripartum cardiomyopathy (PPCM). Single centre, prospective study of 100 patients with newly diagnosed PPCM. Clinical assessment, echocardiography and cardiac scintigraphy were done at baseline and after 6 months of treatment. CRP and serum cholesterol were measured at baseline only. Fifteen patients died and eight were not available for follow-up. Total serum cholesterol was 4.2 ± 0.9 mmol/l, while CRP was 10.8 ± 13.2 mg/l with 45% of patients having values >10 mg/l. We documented an inverse correlation between CRP and total serum cholesterol (rs = –0.29, $P = 0.01$). Total serum cholesterol also correlated inversely with left ventricular end-diastolic diameter (rs = –0.35, $P = 0.0009$) and end-systolic diameter (rs = –0.38, $P = 0.0001$). The correlation between baseline serum cholesterol and ejection fraction was positive (rs = 0.36, $P = 0.0006$). In PPCM low serum cholesterol levels are associated with elevated CRP levels at baseline and more severe left ventricular dysfunction after 6 months of treatment.

Abstract N° B87

Predictors of mortality in 100 prospectively studied patients with peripartum cardiomyopathy

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Peripartum cardiomyopathy (PPCM) is a disorder of unknown etiology. This analysis aims to identify predictors of death in PPCM. Single centre prospective study of 100 patients with newly diagnosed PPCM. Clinical assessment, echocardiography, cardiac scintigraphy and blood analysis were done at baseline and after 6 months of therapy. All patients received diuretics, angiotensin-converting enzyme inhibitors and carvedilol. Fifteen patients died and eight were not available for follow-up. Univariate analysis (Table 1) showed significant differences in New York Heart Association functional class (NYHA FC), systolic blood pressure (SBP), end-diastolic dimension (EDD), end-systolic dimension (ESD), left ventricular ejection fraction (EF), FAS/Apo-1 and aspartate aminotransferase (AST) between non-survivors (Group 1) and survivors (Group 2).

Table 1

	Group 1 (*n = 15*) *	Group 2 (*n = 77*) **	*P*
NYHA FC	3.3 ± 0.7	2.0 ± 0.8	0.04
SBP (mmHg)	102 ± 22	112 ± 17	0.002
EDD (mm)	64.9 ± 5.8	61.2 ± 7.2	0.032
ESD (mm)	57.7 ± 7.3	53.4 ± 8.0	0.032
EF (%)	22.8 ± 8.0	26.2 ± 8.3	0.04
FAS/apo-1 (U/ml)	9.8 ± 4.2	5.9 ± 3.9	0.002
AST (U/l)	50.4 ± 34.4	34.8 ± 24.0	0.036

* Non-survivors presented with higher NYHA FC and lower EF at baseline than survivors.

** FAS/apo-1 (a marker of programmed cell death) and higher NYHA FC at baseline were independent predictors of mortality.

Abstract N° B88

Troponin levels in patients with end-stage renal failure: assessment by three different assays

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Introduction. – ESRF has long been known as a confounding factor for the assessment of cardiac troponin (cTn), hampering the interpretation of cTn elevations for diagno-

sing myocardial infarction. We performed a study in a cohort of patients undergoing chronic haemodialysis in order to (i) compare three commercially available and widely used cTn assays in a population with ESRF and (ii) determine the effect of dialysis on cTn levels.

Methods. – Ninety-seven patients (71 males, mean age 62 years) on chronic haemodialysis were analyzed with blood samples on standardized time-points 1 h before and after haemodialysis. Eighty percent of the cohort had known hypertension (treated to 142 ± 22 mmHg), 34% had diabetes, and 24% had documented coronary artery disease, but none had symptoms, clinical signs or ECG alterations suggestive of myocardial ischemia. Fifty-two percent had left ventricular hypertrophy, and mean left ventricular ejection fraction was 51 ± 13%. The three cTn tests utilized were cTnT (Elecsys 2010, Roche Diagnostics), cTnI (AxSYM, Abbott Laboratories (Abb)) and cTnI (Access AccuTnI, Beckman Coulter (B-C)).

Results. – cTnT (Upper Reference Limit URL = 0.01 ng/ml) was elevated to 0.023 pre-dialysis and 0.017 post-dialysis (all median, $P < 0.001$; mean: 0.061 vs. 0.046); cTnI (Abb) (URL = 0.6 ng/ml) was 0.0 pre-dialysis and 0.0 post-dialysis (P = ns; mean: 0.196 vs. 0.20), and cTnI (B-C) (URL 0.02 ng/ml) was 0.017 pre-dialysis and 0.014 post-dialysis ($P = 0.007$; mean: 0.034 vs. 0.032). In expressing the measurements as percentage of patients reaching cTn elevations above the diagnostic cut-off for myocardial infarction (AMI), it was found that cTnT (cut-off > 0.1 ng/ml) diagnosed 16.5% of the pre-dialysis patients as AMI, cTnI (Abb) (cut-off > 1.0 ng/ml) diagnosed 5.9% as AMI, and cTnI (B-C) (cut-off >0.1 ng/ml) diagnosed only 4.0% of the patients as AMI. The corresponding post-dialysis numbers were 14.4% for cTnI, 4.9% for cTnI (Abb), and 5.0% for cTnI (B-C).

Conclusion. – In our cohort with no evidence suggestive of myocardial ischemia or myocardial infarction, cTnT levels were significantly more often false-positive than cTnI levels as assessed by two different cTnI assays. While the elevation of cTnT has been claimed to be a prognostic marker for cardiovascular events and mortality, the cTnI assays seem clearly superior for ruling out acute myocardial infarction in this population (with no difference found between the two assays). Dialysis decreases cTnT levels substantially, but does not seem to alter cTnI levels in a clinically relevant manner.

Abstract N° B90

High prevalence of chronic kidney disease in patients undergoing elective coronary angiography

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Chronic kidney disease (CKD) is one of the strongest predictors of outcomes in patients with coronary artery disease (CAD). In clinical cardiology practice serum creatinine measurement is the primary tool for assessment of kidney function, but it may underestimate the true prevalence of kidney disease. The aim of the study was estimation of the prevalence of CKD among patients referred for elective coronary angiography by using serum creatinine to calculate glomerular filtration rate (GFR) and assessing urine albumin-to-creatinine ratio. Data from study of 303 patients referred for elective coronary angiography selected to have apparently normal kidney function (serum creatinine <1.5 mg/dl and normal urinalysis) is presented. Medical history, clinical and laboratory tests, and angiographic severity of CAD were also evaluated. Presence and stages of CKD were assessed according to National Kidney Foundation Clinical Practice Guidelines. Average age at admission was 62 (±10) years; male/female ratio was 2.2:1. Overall prevalence of CKD (as defined by GFR < 60 ml/min/1.73 m^2 or presence of albuminuria) was 37% (95% confidence intervals (CI), 31–42%). Unadjusted for age, this value is 3.3 times higher than the National Health and Nutrition Examination Survey III estimate for adult population in the US (11%). Prevalence of albuminuria standardized by age was 1.4 times higher than for the general population (13% vs. 9%). Severity of CAD was positively associated with prevalence of CKD. The risk of having CKD in patients with angiographic presence of CAD was 2.4 (95% CI, 1.4–4.3, $P = 0.0003$) times higher compared to those without CAD. Moreover, 34% (95% CI, 29%–39%) of all patients with "normal" creatinine level (<1.5 mg/dl) had CKD. CKD is a very common condition in patients referred for elective coronary angiography even if markers (serum creatinine and urinalysis) of kidney function are normal by traditional criteria. It is important to recognize the high prevalence of CKD in adult cardiac patients, as this may allow better management strategies to avoid complications of cardiac procedures, as well as achieve better outcomes of treatment.

Abstract N° B92

Nuclear factor κB (NF-κb) inhibition reduces myocardial expression of macrophage colony-stimulating factor and of cell adhesion molecules after mitral valve surgery

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Transcription factors have become an increasingly attractive target for potential therapeutic intervention. Since NF-κB regulates gene expression of cell adhesion molecules (CAMs) and inflammatory mediators such as the macrophage colony-stimulating factor (MCSF-1), we studied whether pharmacological inhibition of NF-κB can prevent CAM upregulation after mitral valve surgery. Seven untreated rabbits were compared to 14 animals which underwent surgery, with a patch being sewed onto the mitral valve. Thirty minutes prior to surgery, seven of the latter animals had received 20 mg/kg pyrrolidine dithiocarbamate (PDTC) i.p. an NF-κB inhibitor. Six hours after surgery, left ventricular tissue was collected and relative mRNA levels were analyzed by RT-PCR using 18S RNA as internal control. Expression levels (% of control ± SD) were as follows:

	ELAM-1	PECAM-1	VCAM-1	MCSF-1
Control	100.0 ± 28.5	100.0 ± 41.9	100.0 ± 41.0	100.0 ± 25.0
Mitral valve surgery	166.1 ± 64.8	183.3 ± 40.6	120.7 ± 89.9	952.6 ± 586.7
PDTC pre-treatment	118.7 ± 82.8; ** P = 0.29*	81.5 ± 13.5; ** P < 0.001*	53.5 ± 40.7; ** P = 0.29*	576.3 ± 211.5; ** P = 0.165*

* Compared to mitral valve surgery group. PDTC pre-treatment clearly reduced CAM and MCSF expression in the left ventricle. Our data confirm that transcription of CAM and MCSF-1 mRNAs is controlled by NF-κB and suggest that in our animal model NF-κB inhibition effectively suppresses their transcription. Thus, NF-κB may be a promising target for pharmacological intervention in order to limit inflammatory processes during and after cardiac surgery.

Abstract N° B93

Coenzyme QI0 and l-carnitine alone and in combination in CHF

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Congestive heart failure (CHF) patients have a high mortality. l-Carnitine and coenzyme Q10 (CoQ10) both improve cardiac function by improving bioenergetics in failing heart. Both are known to benefit heart failure (HF) cases individually, but their combined long-term beneficial effect in CHF has not been fully evaluated. Over the last 4 years, 309 CHF cases (due to CAD, DCM and valvular lesions: NYHA Class II–IV), with global LVEF ≤30% on echocardiography were taken up for the study to evaluate and compare effects of long-term treatment in HF patients in the control group I (on conventional therapy of digoxin, diuretics, ACE inhibitors, etc.: group I, 150 cases) and the treatment group II (159 cases comprising three subgroups: subgroup IIa, 52 cases with addition of oral carnitine alone (3 g); subgroup IIb, 53 cases of CoQIO alone (120 mg), and addition of both drugs; subgroup IIc, 54 cases in addition to conventional treatment. Mean follow-up period was 18.9 months. As compared to the control group, patients on combined drug treatment, showed significant improvement in quality of life—68% vs. 34% in group II/I. Functional class improvement was 68% vs. 46% and reduction in hospitalizations 46% vs. 25%. There was insignificant reduction in incidence of mortality and SCD. Response was marginally better with addition of CoQ10 alone: group IIb, as compared to l-carnitine alone: group IIa. To conclude, both CoQ10 and l-carnitine improve cardiac contractile status in CHF. Their combination has additional beneficial effect on survival in HF as they act at different sites.

Abstract N° B94

Statins alone and in combination with coenzyme Q10 in dyslipidemias

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Statins reduce coenzyme Q10 (CQ10) blood levels by inhibiting mevalonoate synthesis, which is a substrate for synthesis of both cholesterol and CQ10.The 'statin-induced cardiomyopathy' and some other undesirable effects may be prevented by simultaneous use of CQ10. Diastolic LV function is highly ATP dependant. The statins induced diastolic LV dysfunction, fatigue, myalgia and myopathy and abnormal transaminases appear to be due to their CQ10 depletion effect. With this background 86 cases aged 37–74 years (mean 51.2 years) and male/female 62/24 (72.1%/27.9%) of hyperlipidemia were studied. Forty-nine/eighty-six (56.9%) were given 20–40 mg atorvastatin alone and 47/86 (43.1%) were given capsule CQ10—30 mg bd in addition. HF and unstable angina cases were excluded. Duration of study was 6 months. Reduction in cholesterol/LDL and levels of global LVEF were same in the two groups ($P = 0.02$). Baseline peak E, peak A, E/A ratio, deceleration time, IVRT (ms); and colour M-mode propagation velocity (cm/s) were much more affected with atorvastatin alone than when it was used with CQ10 ($P < 0.05$). Fatigue/myalgias and deranged liver function tests (LFTs) were seen in 10/49 (20.4%) vs. 3/47 (6.4%) cases ($P < 0.05$) in atorvastatin alone/combination therapy. No patient in either group developed myopathy or rhabdomyalinosis. Hence addition of CQ10 to statins may significantly reduce their undesirable effects particularly diastolic dysfunction, myalgic symptoms and abnormalities of LFTs.

Abstract N° B96

Impact of exercise training on plasma ET-1, BNP and TNF-alpha and automatic balance and quality of life in patients with severe heart failure and cor pulmonale

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Many studies have demonstrated that exercise training represents a safe and effective therapy and reduces brain natriuretic peptide (BNP), tumor necrosis factor-alpha (TNF-alpha), endothelin-1 (ET-1), and improves automatic balance in moderate heart failure. It remains unclear whether patients with severe ischemic heart failure and cor pulmonale (NYHA IIIb) also benefit from a training intervention. The aim of the present study was to evaluate the effect of an individual tailored exercise and ventilatory muscle training on cytokines. BNP and ET-1, heart rate variability (HRV) and quality of life score (QOL) in patients with severe ischemic heart failure (IHF) and cor pulmonale. Forty-two patients with stable IHF and cor pulmonale were randomized into an exercise training group (four times daily bicycle ergometer and ventilatory muscle training $n = 21$) or inactive control group ($n = 21$). Plasma rest and peak exercise BNP, TNF-alpha and ET-1, 24 h Holter monitoring for heart rate variability (HRV), a symptom-limited spiroegometry, left ventricular ejection fraction (LVEF) by echocardiography and Minnesota quality of life score (QOL) were assessed at randomization and after 6 months. In the exercise group, HRV analysis showed significant changes in SDNN and SDANN index ($P < 0.05$), but not in pNN50 and rMSSD, suggesting sympathetic activity reduction rather than para-

sympathetic activity increase. Decrease in rest and peak BNP, TNF-alpha and ET-1, increase of exercise capacity and improvement of QOL in exercise group were greater than those in control group ($P < 0.01$). Our data suggest that a carefully designed and individually tailored aerobic exercise and ventilatory muscle training in patients with stable ischemic heart failure and cor pulmonale is not only associated with an increase in exercise tolerance and improvement of QOL and autonomic balance but also decreased plasma rest and peak BNP, TNF-alpha and ET-1 levels.

Abstract N° B97

Impact of berberine on TNF-alpha and activity of aldose reductase in cardiorenal syndrome secondary to diabetic heart failure

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Circulating plasma TNF-alpha level and activity of aldose reductase (AR) is associated with development of heart failure and diabetic nephropathy. In our previous studies, berberine may decrease plasma TNF-alpha levels in patients with chronic heart failure and inhibit activity of AR of red cells in diabetic patients. We hypothesized that short-term berberine therapy may have beneficial effects in patients with cardiorenal syndrome in diabetic heart failure.

***Methods and results*.** – Sixty-nine patients with cardiorenal syndrome in diabetic heart failure were randomly divided into berberine (B 0.2 three times a day $n = 35$) or placebo (P $n = 34$). Clinical characteristics between two groups were same. Fasting blood glucose, plasma TNF-alpha, activity of AR in red cell.beta 2-MG, urine albumin excretive rate (UAER), 6 min walking distance and Minnesota Living with Heart Failure (MLWHF) score were assessed before randomization and 6 months after treatment. Plasma TNF-alpha.beta 2-MG in blood, activity of AR in red cell and UAER in B group decreased remarkably than in P group. Increase of 6 min walking distance and improvement of MLWHF in patients receiving B were remarkable than in P. Serum creatinine in B group had slightly lower but had increase in P.

Conclusions. – B may decrease plasma TNF-alpha and activity of AR and improve renal function, exercise capacity and quality of life in patients with cardiorenal syndrome secondary to diabetic heart failure during 6 month treatment.

Abstract N° B98

Clinical observations of effects of sheng mei for patient with profile L in chronic heart failure

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Patients with profile L, those with low cardiac output without clinical evidence of elevated filling pressure, often do not improve acutely with adjustments of oral therapy. No trials have targeted this small group. The aim of present study was to observe efficacy and safety of sheng mei for profile L in chronic heart failure.

Methods and results. – Clinical effects and safety of sheng mei in 112 patients with class II–III symptomatic and LVEF <40% with profile L of chronic heart failure secondary to coronary heart disease or dilated cardiomyopathy were observed by five medical centers in China. The patients were divided into sheng mei group (60 ml/d $n = 57$) and placebo ($n = 55$). Clinical characteristics between two groups were similar. Six minute walking test (6MWT), LVEF and Minnesota Living with Heart Failure (MLWHF) score were assessed at randomization and after 6 months. 6MWT, LVEF and MLWHF score in sheng mei group improved more remarkably than those in placebo group (see Table 1). There were no significant differences in side-effects between the two groups.

Table 1

	MLWHF	6MWT	LVEF
Sheng mei (%)	–16.4	16.6	5.6
Placebo (%)	–1.1	5.9	5.6
P	<0.005	<0.01	<0.05

Conclusions. – Sheng mei may improve quality of life and increase LVEF and exercise tolerance without side effects during 6 month of treatment in patients with profile L of chronic heart failure.

Abstract N° B99

Beneficial efficacy of losartan for ischemic heart failure with cor pulmonale

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***Objective*.** – The aim of study was to ascertain safety and efficacy of long-term losartan therapy on ischemic heart failure with cor pulmonale.

***Methods and results*.** – Two hundred seventeen patients with ischemic heart failure with cor pulmonale (CPHA) were randomly divided into two groups. All patients were given conventional therapy for CHF and anti-inflammatory drugs consisting of digoxin, nitrates and spironolactone. Patients in the treatment group ($n = 109$) were also given losartan 50 mg/day. The other 108 patients were given diltiazem. Symptoms, 6 min walk testing, left ventricular ejection fraction (LVEF), quality of life, and TNF-α, IL-1β, IL-6 and ET were assessed after 8 weeks of treatment and during a mean of 12 and 24 months follow-up. Improvement quality of life and exercise tolerance, decrease in plasma TNF-α, IL-I β, IL-6 and ET-I level and mortality in losartan group were more significant than those in diltiazem. And there were no apparent side effects in the treatment group.

***Conclusions*.** – Losartan may improve quality of life, exercise tolerance, decrease plasma cytokines and ET level and mortality during 2 years treatment.

Abstract N° B100

Atorvastatin for severe ischemic heart failure with cor pulmonale

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Heart failure and cor pulmonale are associated with inflammation and neurohormonal imbalance. The 3-hydroxy-3-methylglutaryl-3-CoA reductase inhibitors (statins) possess anti-inflammatory and vascular protective effects. We hypothesized that atorvastatin may be beneficial effect for severe ischemic heart failure with cor pulmonale since these diseases both cause inflammation and vascular endothelial dysfunction.

Methods and results. – One hundred twenty-three patients with severe ischemic heart failure combined with cor pulmonale were randomly divided into groups. One group received atorvastatin 10 mg/day ($n = 62$), and the other group received placebo ($n = 61$). The initial dose of atorvastatin was 5 mg/day, which was increased to 10 mg/day after 1 month. After 6 months, patients receiving atorvastatin exhibited a modest reduction in low-density lipoprotein (LDL)-C level compared with patients receiving placebo (126 ± 11 vs. 141 ± 17 $P < 0.01$), improvement of New York health association (NYHA) functional class in atorvastatin group was more remarkably than that in placebo group (2.16 ± 0.06 vs. 2.42 ± 0.08 $P < 0.01$). This corresponded to improved left ventricular ejection fraction (LVEF) in treatment group (21 ± 3 to 32 ± 5 $P < 0.01$) but not in the placebo group. And plasma TNF-alpha, interleukin (IL)-6, IL-18, ET-1 and brain natriuretic peptide were significantly lower than those in the atorvastatin group compared with the placebo group.

Conclusions. – Atorvastatin may improve cardiac function, neurohormonal imbalance, symptoms associated with severe ischemic heart failure and cor pulmonale and decrease circulating pro-inflammatory cytokine levels.

Abstract N° B101

Sulfaphenazole reduces post-ischemic vascular dysfunction

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We recently demonstrated that cytochrome p450 2C (CYP2C) inhibitors significantly reduce infarct size caused by cardiac ischemia and reperfusion (I/R) using a rat Langendorff perfusion model of global ischemia and a rabbit LAD occlusion model of regional ischemia (Proc. Natl. Acad. Sci. USA 101 (2004) 1321–1326). CYP2C inhibition reduced superoxide generation and augmented coronary flow. In this study, we hypothesize that CYP2C-mediated superoxide production results in nitric oxide scavenging, production of peroxynitrite and reduced endothelial-dependent, nitric oxide-mediated vasodilation. To test this hypothesis, rat hearts were perfused in Langendorff mode for 20 min in the presence, or absence, of the specific CYP2C9 inhibitor sulfaphenazole (10 μM) and then subjected to 30 min global no-flow ischemia followed by 15 min reperfusion. Septal coronary resistance arteries were isolated and mounted on glass cannulae for measurements of luminal diameter. Arterial tone was increased with U-46619 and tissues were exposed to increasing concentrations of acetylcholine (1–10 μM) to elicit endothelial-dependent, nitric oxide-mediated vasodilation. Acetylcholine caused near maximal dilation in control tissues not subjected to I/R. Following 30 min global ischemia and 15 min reperfusion, endothelial-dependent vasodilation occurred with reduced sensitivity and the maximal effect of acetylcholine was also attenuated. Pretreatment with sulfaphenazole restored both the sensitivity and maximal effect of acetylcholine. In summary, sulfaphenazole restored post-ischemic endothelium-dependent, nitric oxide-mediated vasodilation. This work was supported by grants from the CIHR and Michael Smith Foundation (MSF) DJG is a Tier II Canada Research Chair and MSFHR Scholar.

Abstract N° B102

Characterization of vascular reactivity of human umbilical artery in normal and reduced oxygen conditions

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This study investigates the responses of the human umbilical artery to various contracting and relaxing agents in order to determine the mechanisms involved in the regulation of the umbilical cord vascular tone. Rings of human umbilical arteries from full-term caesarian deliveries were suspended in tissue baths containing warm Krebs–Henseleit buffer under normal and reduced oxygen conditions. These rings were contracted with cumulative additions of potassium chloride, serotonin, bradykinin, endothelin-1, histamine and a thromboxane A_2 mimetic, U46619. Among these vasoconstrictors, only serotonin and U46619 elicited strong and sustained contractions at physiological concentrations in both oxygen conditions. Therefore, potassium chloride (50 mM), serotonin (10 μM) or U46619 (1 μM) were used to contract human umbilical arterial rings, which were then relaxed with the nitrovasodilator sodium nitroprusside, the potassium channel opener levcromakalim or the calcium antagonist amlodipine. Our data suggest that the responses of the human umbilical arteries to constricting agents, but not relaxing agents, were greater in the condition with a reduced oxygen supply. The nature of the constricting agents used did not affect the potency or efficacy of sodium nitroprusside, levcromakalim or amlodipine to relax human umbilical artery. While all three relaxing agents had similar potencies in this tissue preparation, sodium nitroprusside induced significantly smaller relaxation compared to levcromakalim and amlodi-

pine. These data suggest that serotonin and thromboxane A_2 effectively constrict human umbilical artery at physiological concentrations. Moreover, the smooth muscle of the human umbilical artery is less responsive to vasodilators that act via the nitric oxide pathway. As such, nitric oxide donors may not be effective therapeutic agents to reduce the elevated umbilical contractile tone in pathological conditions.

Abstract N° B103

Holistic preparation for cardiac surgery in high risk and elderly patients utilising a combination of metabolic, physical and mental therapy

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Cardiac surgery represents major stress, which in ageing patients is associated with increased production of reactive oxygen species. Previous studies examining individual effects of antioxidant therapy and physical and mental preparation have shown benefit but no studies investigated the combined effect. The methods we evaluated were a preoperative metabolic, physical and mental program. Antioxidants coenzyme Q_{10} (300 mg) and α-lipoic acid (300 mg), combined with magnesium orotate (1200 mg), and omega-3 fatty acids (1 g) were given daily with exercise and stress reduction therapy. A quality of life (QOL) questionnaire (SF-36) was administered before and after the program. A survey gauged gave satisfaction and benefit. QOL assessments before and after the program (pre-surgery) (n = 16) showed increase in: physical QOL from 33.5 ± 4.12 before, to 41.0 ± 4.5 after the program (P = 0.005); mental QOL from 44.3 ± 4.5 before, to 54.1 ± 5.3 afterwards (P = 0.006). QOL before and 1 month after surgery, also showed improvement in physical QOL in the MPM group from 37.7 ± 5.0 before, to 56.5 ± 7.0 afterwards (P = 0.01), the control group showed deterioration (P = 0.05). Mental QOL was similar: improvement in the MPM group from 48.4 ± 6.2 to 65.0 ± 5.7 (P = 0.048) and deterioration in the control group (P = 0.05). Malondialdehyde levels decreased from 23.9 ± 2.5 before the program to 13.2 ± 2.1 μM/ml afterwards (P = 0.026). The survey (n = 11) showed that 100% of patients rated satisfaction as excellent/very good with 68% and 60% benefiting from the exercise and mental component, respectively. Combined metabolic, physical and mental preparation prior to major surgery is satisfying to the patient, reduces oxidative stress and improves patient QOL.

Abstract N° B104

Beneficial effects of antioxidants on catecholamine-induced arrhythmias

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Oxidation products of catecholamines are known to cause arrhythmias. Since the mechanisms of this action are not clear, this study was designed to investigate the beneficial effects of antioxidants on catecholamine-induced arrhythmias. Rats were treated with or without different antioxidants such as vitamin A (30 mg/kg), vitamin E (20 mg/kg), vitamin C (100 mg/kg) and *N*-acetylcysteine (NAC, 200 mg/kg) for 2 d before studying the cumulative effects of epinephrine. Our results show that the minimum dose of epinephrine required for inducing arrhythmias in control was 4 μg/kg whereas the dose of epinephrine was 16 μg/kg in the treatment group. The onset time (s) of arrhythmias was 13.6 ± 1.1, 19.1 ± 0.6, 19.6 ± 1.3, 20.1 ± 0.8 and 21.4 ± 0.4 whereas the duration (s) of arrhythmias was 149 ± 11, 41 ± 5, 40 ± 6, 87 ± 4 and 30 ± 2 in control, vitamin A, vitamin E, vitamin C and NAC treated animals, respectively. In order to assess the status of oxidative stress, changes in malondialdehyde (MDA) (μmoles/l) were measured in the heart and plasma. Cardiac MDA and catecholamine levels were not changed in any of the groups; however, a significant difference in MDA levels was seen in plasma from control and antioxidant treated animals. On the other hand, epinephrine induced changes in hemodynamic parameters were not altered by any of the antioxidant treatment. These results thus support the view that epinephrine induced arrhythmias may be mediated by oxidation products of catecholamines and the beneficial effects of antioxidants may not be due to their direct effect on the heart.

(Supported by a grant from Heart and Stroke Foundation of Manitoba).

Abstract N° B105

The ability of macrophages from the blood of patients with ischaemic heart disease and healthy donors for LDL oxidation and uptake

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It is known that the contents of cytokines and lipid peroxidation products in the blood increase in the patients with ischaemic heart disease (IHD), and our earlier studies have shown that TNF-α promotes the priming or activation of macrophages (MP) in vitro, increasing their ability to oxidize low-density lipoprotein (LDL). The purpose of this work was to find out if the same activation of MP with IHD (MP_{IHD}) would take place in vivo. LDL from the blood of donors with low (LDLn) and high (LDLh) cholesterol (CH) levels were incubated with MP with healthy donors (MP_N) and MP_{IHD} cultures for 1–24 h under aerobic or ischaemic conditions. It was shown that the sensitivity of MP_{IHD} to LDLn and LDLh was expressed to a greater extent than that of MP_N, especially under ischaemia. The contents of TBARS in LDLn and LDLh gradually decreased after 1–24 h of incubation (to a lower level in LDLh than in LDLn), whereas LDL electrophoretic mobility increased, reaching a peak after 24 h. Accumulation of CH (ng/mg proteins) in MP_N and MP_{IHD} after incubation with LDLn and LDLh increased by a factor of 1.2–2.4 (to a higher level in LDL incubated with MP_{IHD}). Accumulation of CH in MP_N (ng/10^3 viable cells) after incu-

bation with LDLn and LDLh for 3–6 h increased by factors of 3.0–4.0 under aerobic and 2.7–6.9 under ischaemic conditions. The same parameter in MP_{IHD} after incubation with LDLn and LDLh for 3–6 h increased by factors of 4.7–7.8 and 5.7–9.1, under aerobic and ischemic conditions, respectively. This parameter in MP_{IHD} after incubation for 3–6 h exceeded the same parameter in MP_N in 1.5–3.4 times. Experimental data showed that IHD was accompanied by in vivo activation of blood MP, leading to their increased ability for LDL oxidation and uptake. This phenomenon was better expressed in the case of LDLh than LDLn and under ischaemic rather than aerobic conditions. Therefore, IHD, local ischaemia and high blood cholesterol stimulate LDL oxidation and uptake by MP and, hence, are responsible for specific localized atherosclerotic plaque formation. The study was supported by RFFR.

Abstract N° B106

Comparison of Cu^{2+}-, endothelial cell - and macrophage -induced oxidative modification of LDL under aerobic and ischaemic conditions

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The main factors responsible for low-density lipoprotein (LDL) oxidation in vivo are transition metals, endothelial cell (EC), and macrophage (MP), but their effects on LDL taken from healthy donors (LDLn) and patients with high cholesterol (HC) levels (LDLh) and on LDL incubated under aerobic or ischaemic conditions have not been compared. We studied the effect of these three oxidant models on LDLn and LDLh upon their co-incubation for 0.5–24 h. The accumulation of lipid peroxidation (LPO) products: TBARS and Schiff bases, and relative electrophoretic mobility (REM) in agarose gel were used as indices of LDL oxidation, and the accumulation of cholesterol in MP, as an index of LDL uptake. MP was taken from the blood of healthy donors. The results were as follows: (1) Incubation of LDLn and LDLh in vitro with $CuSO_4$, EC and MP resulted in the accumulation of LPO products. Their content was higher in LDLh than in LDLn, but this difference was significant only at the early stage of incubation. (2) Incubation of LDLn and LDLh with $CuSO_4$, EC, and MP entailed an increase in their REM. In all the models, the lag phase of change in REM was shorter in LDLn than in LDLh, which could be explained by LDL fragmentation and, in the MP model, also by more rapid LDL uptake. (3) With respect to the potential for LDLn and LDLh oxidation, the models could be arranged in the following series: $CuSO_4$ > EC > MP. (4) LDLn and LDLh oxidation by EC and their oxidation and uptake by MP were enhanced under ischaemia, compared to those under aerobic condition. (5) TNF-α (one or two small doses) promoted the priming or activation of MP, increasing their ability to oxidize LDL. (6) Antioxidants, such as K-phenozane, desferal and probucol as well as antihypoxant deltoran inhibited EC-, MP- and EC + MP-induced LDL oxidation. Therefore all the three factors caused LDL oxidation in vitro and in vivo; in the case of MP, this was accompanied by rapid LDL uptake. These factors, together with local ischaemia, and high cholesterol level, represent a very high risk of atherosclerotic plague formation. The study was supported by RFFR.

Abstract N° B107

Protective role of pentoxifylline in ischemia reperfsion induced heart injury in rats

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Although, pentoxifylline (PTXF), a methylxanthine phosphodiesterase inhibitor, is clinically used to prevent ischemic heart damage during cardiac surgery, the mechanism of its effect on ischemia reperfusion (I/R) induced heart injury is not clear. Since PTXF has been reported to prevent the production of TNF-α in heart failure and cardiomyopathy, we examined whether PTXF protected the heart from I/R injury due to its effect on depressing TNF-α synthesis and blocking NFκB activation. The isolated rat hearts were subjected to global ischemia for 30 min, followed by 30 min of reperfusion ; PTXF was given for 10 min before ischemia as well as for 30 min during reperfusion period. I/R caused significant change in cardiac contractile parameters, as indicated by a decrease in the rate of contraction (+ dP/dt), rate of relaxation (—dP/dt) and left ventricular developed pressure (LVDP), as well as an increase in left ventricular end diastolic pressure (LVEDP) ; these changes were significantly improved by PTXF treatment. An increase in TNF-α protein levels and an activation of NFκB in I/R group were also attenuated by PTXF treatment. In order to determine whether the beneficial effect of PTXF is related to alteration in intracellular Ca^{2+} concentration, Ca^{2+} paradox model was also used. PTXF treatment partially prevented changes caused by Ca^{2+} paradox in cardiac contractile parameter and TNF-α production as well as the activation of NFκB. These observations indicate that PTXF rendered cardioprotection by Ca^{2+} related mechanisms associated with reducing the TNF-α synthesis and blocking the activation of NFκB. (Supported by CIHR Group Experimental Cardiology).

Abstract N° B108

Involvement of LPA subtype-specific receptors expression in heart remodeling of AMI rat

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To investigate LPA receptor mediated signal transduction in left ventricle remodeling process, the relationship between the pattern of mRNA expression of LPA receptor subtypes and the myocardial hypertrophy and apoptosis in rat hearts after acute myocardial infarction (AMI) was studied. AMI model was established with the ligation of anterior descending coronary artery in Sprague–Dawley (SD) rat. 24 h after AMI, the 58 survivors were served as AMI (MI) group. Sham-operated (S) group was selected as control. Each

group was randomly divided to two subgroups, 48 hours (48h) and 4 weeks (4w) groups according to the time point observed. After the completion of hemodynamic study, pathological examination on the thickness of ventricular septum performed. Cardiomyocyte apoptosis was detected by TUNEL and DNA ladder assay. The mRNA expression of LPA receptor subtypes was determined by RT-PCR. Compared with S group, LVSP and ±d*p*/d*t* decreased and LVEDP elevated significantly in 48h and 4w group. The thickness of ventricular septum was obviously changed between MI and S group in 4w group but not in 48h group. Numerous TUNEL-positive cells and DNA ladder were found in tissue at 48h after AMI but apoptosis was prone to decreased at 4w MI group. Compared with S group, there was no remarkably difference in mRNA expression of LPA type 1 and type 2 receptor (LPA1 and LPA2) whereas mRNA level of LPA3 was increased by 40% at 48h after AMI, and the mRNA expression of LPA1 and LPA3 significantly increased by 98% and 120%, yet no difference was found in LPA2 mRNA levels in 4w group. At 48h after AMI, a remarkable cardiomyocyte apoptosis was accompanied with the notable increase of LPA3 mRNA expression suggesting that LPA3 may concern the signal pathway of apoptosis in early period after AMI. At 4w after AMI, notable increase of LPA1 and LPA3 mRNA level came with obvious cardiomyocyte hypertrophy and unconspicuous apoptosis, revealing that LPA1 may mainly mediate the signal pathway of cardiomyocyte hypertrophy while LPA3 might involve in apoptosis in the process of left ventricle remodeling after AMI in rats.

Abstract N° B109

Arachidonic acid inhibits hypoxia/reoxygenation-induced injury by upregulation of the phosphatase, MAPK-1, in cardiomyocytes

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We have previously demonstrated that hypoxia/reoxygenation-injury induced MAPK activation in neonatal cardiomyocytes and that arachidonic acid (ARA) protected the myocytes via attenuation of p38-MAPK activation. The aim of this study was to evaluate the putative role of the phosphatases in this phenomenon. Cultured neonatal cardiomyocytes were exposed to 20 μM ARA complexed to BSA for an hour before the onset of 60 min of hypoxia (chemical hypoxia was induced by deoxyglucose and KCN) followed by 30 min reoxygenation. To further investigate the mechanism of action of ARA on p38-MAPK inhibition, we employed okadaic acid (1 μM), an inhibitor of type 1 and type 2A serine/threonine phosphatases as well as orthovanadate (100 μM), a specific inhibitor of tyrosine phosphatases (including MAPK-1 (mitogen activated protein kinase phosphatase)). Vanadate, but not okadaic acid, significantly reduced ARA-induced inhibition of p38-MAPK activation. Thus, our data provide evidence for ARA-induced inhibition of p38-MAPK through activation of a tyrosine phosphatase during hypoxia/reoxygenation. We targeted MAPK-1 (a subclass of the tyrosine specific protein phosphatases and a member of the dual specific phosphatases, DSPs), and found ARA to cause a sixfold activation of MAPK-1 during hypoxia/reoxygenation. An in vitro dephosphorylation assay was used to demonstrate that it was the phosphatase which was responsible for inhibition of the kinase. We conclude that ARA exerts its beneficial effect during hypoxia/reoxygenation through activation of MAPK-1 causing dephosphorylation of p38-MAPK.

Abstract N° B110

Attenuation of isoproterenol-induced increase in intracellular calcium in cardiomyocytes isolated from ischemic–reperfused hearts

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Although isoproterenol is known to cause an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in control hearts, the status of Ca^{2+} mobilization by isoproterenol in ischemia–reperfused hearts is not fully understood. In order to examine the effect of ischemia–reperfusion on isoproterenol-mediated increase in $[Ca^{2+}]_i$, cardiomyocytes were isolated after 30 min of ischemia followed by 5, 15 and 30 min of reperfusion. Viability of cells at each of these time points was determined by tryphan blue exclusion assay. $[Ca^{2+}]_i$ was determined spectrofluorometrically in fura-2 loaded cardiomyocytes. Both ischemia and ischemia–reperfusion caused an increase in basal $[Ca^{2+}]_i$, which was associated with decreased cell viability and increased left ventricular end diastolic pressure. Pre-treatment of cardiomyocytes with isoproterenol (100 μM) augmented the increase in $[Ca^{2+}]_i$ due to KCl (30 mM), a known depolarizing agent, S(-)-Bay K 8644 (2 μM), a dihydropyridine calcium channel activator and ATP, (50 μM), a purinergic receptor agonist in control hearts. On the other hand, isoproterenol-induced increase in $[Ca^{2+}]_i$ due to these agents was attenuated in cardiomyocytes isolated from both ischemic and ischemic–reperfused hearts. These findings indicate that global ischemia for 30 min causes attenuation in isoproterenol-mediated augmentation of $[Ca^{2+}]_i$, which was not reversed by reperfusion up to 30 min.

(Supported by the CIHR Group in Experimental Cardiology).

Abstract N° B111

Modulation of doxorubicin-induced cardiac dysfunction in toll-like receptor-2 knockout mice

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Toll-like receptor (TLR)-2 is involved in inflammatory response and expressed in the heart. Recent studies have

demonstrated that TLRs are activated by endogenous signals such as oxidative stress, which contributes to doxorubicin (Dox)-induced cardiac dysfunction. Thus, we hypothesized that TLR-2 had potential role for pathogenesis in Dox-induced cardiac dysfunction. Myocardial lipid peroxidation was significantly increased after Dox (20 mg/kg, intraperitoneally injection), but no significant difference was found between wild-type (WT) and knockout (KO) mice. After Dox injection, NF-kappaB activation was decreased by 80% in KO mice compared with WT mice. Production of pro-inflammatory cytokines was attenuated in KO mice. Numbers of TUNEL positive nuclei and Dox-induced caspase-3 activation were decreased by 36.1% and 26.7%, respectively ($P < 0.01$), in KO mice compared with WT mice. Cardiac function evaluated by echocardiography was preserved in KO mice compared with WT mice. Consequently, survival rate was significantly higher in KO mice than in WT mice 10 d after Dox treatment (11% vs. 46%, $P < 0.05$). These findings suggest that TLR-2 may play an important role in the regulation of inflammatory and apoptotic mediators in the heart following Dox.

	WT-Con	WT-Dox	KO-Dox
Interleukin-6 (pg/mg protein)	1.0 ± 1.0	11.2 ± 3.3 **	2.0 ± 0.4 ##
TNF-alpha (pg/mg protein)	0.2 ± 0.2	3.0 ± 0.4 **	1.4 ± 0.8 *,##
Fractional shortening (%)	40.0 ± 0.4	27.7 ± 1.0 **	34.1 ± 1.6 **,##

Data were expressed mean ± SEM.

*$P < 0.05$.
**$P < 0.01$ vs. WT-control.
#$P < 0.05$.
##$P < 0.01$ vs. WT-Dox.

Abstract N° B112

Oxidant species are less effective in modulating pro-inflammatory cytokines turnover in ischemic heart disease

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Objectives. – We have previously demonstrated during blood recirculation that there is an increased production of oxidant species that are associated with a reduction in antioxidant capacity, and pro-inflammatory cytokines in blood from patients with ischemic heart disease (IHD) in contrast to that of healthy subjects. However, the effect of these specific oxidant species on the inflammatory response is still not well defined.

Methods. – To investigate the role of specific oxidant species on the production of three different types of pro-inflammatory cytokines in blood during extracorporeal circulation, radical scavengers NG-nitro-l-arginine methyl ester HCl (l-NAME), uric acid, SOD mimetic (manganese tetrakin porphyrin) and hydroxyl radical scavenger (*N*-2 mercaptopropinyl glycine) were used. Fifty milliliters of blood from 25 IHD patients with stable angina and 25 sex-age matched healthy controls were treated with specific scavengers and re-circulated at normothermia for 4 h. Samples were taken at 0 and 4 h and TNF-α, IL-6 and IL-8 were determined by sensitive ELISA assays.

Results. – Results are presented as mean ± S.D. with baseline levels of TNF-α, IL-6 and IL-8 found to be significantly greater in IHD than healthy controls, a trend that remained at 4 h. In all the scavengers only l-NAME and uric acid had significant effect on pro-inflammatory cytokines turnover. The inhibition of NO synthase activity by l-NAME significantly reduced the levels of TNF-α, only in IHD (66.4 ± 29.5 at 4 h; $P < 0.05$) in contrast to healthy controls (494.3 ± 120.0 at 4 h). Interestingly, in IHD, $ONOO^-$ scavenging by uric acid resulted in a significant elevation of IL-6 levels (7586.7 ± 167.7 vs. 112.5 ± 17.4 at 4 h; $P < 0.05$) but not TNF-α (342.6 ± 72.4 vs. 262.5 ± 98.6 at 4 h) and IL-8 levels (691.71 ± 30.54 vs. 366.3 ± 98.8 at 4 h; *P*=NS in both the cases).

Conclusion. – In conclusion, the blockade of NO metabolism attenuates TNF-α but enhances IL-6 turnover in IHD, suggesting a possible post-transcriptional regulation by these oxidant species. These results contribute to the understanding of the mechanism of extracorporeal blood circulation induced tissue damage and may represent a potential in targeting the inflammatory process in patients with IHD.

Abstract N° B113

Nitric oxide reduces ischemia-and reperfusion-induced DNA damage in rat ventricular myocytes

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Nitric oxide (NO) donors can mimic the protective effects of ischemic preconditioning (IPC) in ischemia- and reperfusion (I/R)-induced heart injury. Cardiac NO donors are synthesized primarily from l-arginine in a reaction that is catalyzed by endothelial and inducible NO synthases (eNOS and iNOS, respectively). A common mechanism of action of NO is the induction of a direct or indirect increase in tissue cGMP content, which activates protein kinase G (PKG). NO/cGMP/PKG signal transduction pathways are thought to reduce I/R-induced injury by activating both sarcolemmal and mitochondrial ATP-sensitive K^+ (K_{ATP}) channels during IPC. Here, we tested the hypothesis that intracellular signaling pathways that involve iNOS and K_{ATP} channels function in the reduction of I/R-induced oxidative damage during IPC. We estimated DNA strand breaks and oxidative damage in rat ventricular myocytes by means of single-cell gel electrophoresis and digestion with endonuclease III. In the I/R model, the level of DNA damage was significantly elevated. Three different preconditioning treatments, namely anoxia (5 min), diazoxide (100 μM), *S*-nitroso-*N*-acetylpenicillamine (SNAP, 300 μM), or 8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphate (8-pCPT-cGMP, 100 μM) protected cells against DNA damage that was induced by prolonged

anoxia (30 min). The protective effects were blocked by the concomitant presence of glibenclamide (50 μM), 5-hydroxydecanoate (5-HD, 100 μM), and an RP-isomer of cyclic GMP (Rp-CPT-cGMP, 100 μM). These results suggest that NO/cGMP/PKG signal transduction pathways contribute to the cardioprotective effects of K_{ATP} channels in rat ventricular myocytes. The increased expression of iNOS in the IPC model supports a role for this molecule in NO production wherein mitochondrial K_{ATP} channel opening is maintained, reducing injury due to ischemia.

Key words: Ischemic preconditioning; Inducible NO synthase; ATP-sensitive K^+ channels

Abstract N° B114

Role for reactive oxygen species in TNFα induced myocyte protection

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We have previously shown that TNFα is necessary and sufficient to induce preconditioning (PC) in both cells and the heart. However, the signalling pathways involved in this protection remain incompletely understood. One potential protective pathway involves the generation of reactive oxygen species (ROS), which can be produced by TNFα. We therefore hypothesized that TNFα promotes the production of ROS, and this leads to the protection seen in PC. To test this we measured TNFα-induced ROS production in C2C12 myotubes, using a concentration known to cause PC (0.5 ng/ml). ROS production and cell viability were measured in viable myotubes at 37 °C using a flow cytometer and the fluorometric dyes 2′,7′-dichlorofluorescin diacetate and propidium iodide, respectively. The ROS scavenger, *N*-acetyl-l-cysteine (NAC) was used to try to abolish the TNFα induced ROS production and PC. There was a rapid increase in ROS (dichlorofluorescin signal), following TNFα adminstration which peaked at 1 min (72.2 ± 23.8% increase). This initial burst of ROS lasted on average 3 min. A second increase in ROS was seen after 60 min. The antioxidant NAC abolished the ROS production following TNFα treatment. In addition the degree of protection afforded by TNFα was reduced by 27.7 ± 3.7%. These results indicate that TNFα treatment leads to a rapid burst of ROS production, and that this ROS plays a minor role in the protection afforded by TNFα.

Abstract N° B115

Asymptomatic myocardial ischemia in Mexican patients with diabetes mellitus type 2 and coronary artery disease

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Asymptomatic myocardial ischemia (AMI) is known to be associated with a poor prognosis. Patients with diabetes are at increased risk for coronary artery disease (CAD) and perhaps also for silent ischemia. A cross-sectional study was conducted to assess the effect of diabetes on the occurrence of AMI. Patients were derived from a clinical trial f for AMI. Two hundred and forty nine patients with CAD were screened with a 24-h Holter ECG, in order to diagnose silent ischemia. AMI was diagnosed according to the American Heart Association recommendations and diabetes according to the American Diabetes criteria. Odds ratios (OR) with 95% confidence intervals (CI 95%) were estimated to assess the strength of the association, and a chi-square for trend (χ^2_{trend}) was used to evaluate biologic gradient. Eighty-four diabetic patients were included (71 males and 13 females), mean age was 60.3 years. AMI was more frequent in diabetics (51%, 43 patients). Diabetic patients with longer duration of disease had double-fold higher risk of AMI (OR 2.5; CI 95% 2.7–9.2). Patients with triple-vessel disease had twofold higher risk of AMI (OR 2; CI 95% 0.6–7.3). The subjects with LVEF <40% had ninefold higher risk (OR 9.3; CI 95% 1.6–64.7); whereas those with a LVEF between 40% and 50% had a threefold higher risk of AMI (OR 2.6; CI 95% 0.6–12.8), due to a biologic gradient, major myocardial damage was related to major risk (χ^2_{trend} 8.4; $P = 0.03$) Patients with more severe CAD, greater myocardial damage and longer duration of diabetes had higher risk of AMI.

Abstract N° B116

The mechanism(s) of dexamethasone against ischemia and reperfusion-induced damage in isolated working hearts

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Dexamethasone (DX) treatment on the recovery of post-ischemic cardiac function and the development of reperfusion-induced ventricular fibrillation (VF) in isolated rat hearts (n = 6 in each group) were studied. Rats were treated with 2 mg/kg of intraperitoneal injection of DX, and 24 h later, hearts were isolated, and subjected to 30 min global ischemia followed by 120 min reperfusion. Cardiac function including heart rate (HR), coronary flow (CF), aortic flow (AF), and left ventricular developed pressure (LVDP) were recorded. In the DX treated hearts, a significant recovery in AF and LVDP were observed during reperfusion. Thus, after 60 and 120 min reperfusion, DX treatment improved the recovery of AF and LVDP from their control values of 10.7 ± 0.3 ml/min and 10.5 ± 0.3 kPa to 22.2 ± 0.3 ml/min ($P < 0.05$) and 14.3 ± 0.5 kPa ($P < 0.05$), 19.3 ± 0.3 ml/min ($P < 0.05$) and 12.3 ± 0.5 kPa ($P < 0.05$), respectively. HR and CF did not show a significant change during reperfusion. In rats treated with 0.5 mg/kg of actinomycin D (ActD), a protein synthesis inhibitor, injected i.v., 1 h before DX injection, suppressed the DX-induced cardiac protection. The incidence of VF, and cytochrome *c* release from the mitochondria into the cytosol were also monitored. Data demonstrate that DX pretreatment reduces the occurrence of VF.

Cytochrome *c* release was observed in the cytoplasm in the ischemic/reperfused and ActD-treated groups in contrast with the DX-treated group. The results suggest that inhibition of cytochrome *c* release is involved in DX-induced cardiac protection.

Abstract N° B117

Apomorphine decreases myocardial ischemia/reperfusion oxidative stress in the rat heart

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Apomorphine forms stable complexes with copper and with iron, and inhibits the copper-induced ascorbate oxidation. This study examined the hypothesis that low concentration of apomorphine improves post-ischemic hemodynamic and mitochondrial functions in the isolated rat heart model by attenuating oxidation of myocardial proteins. Control and apomorphine-treated isolated hearts were subjected to 35 min of perfusion, 25 min of no-flow normothermic global ischemia, and 60 min of reperfusion. Apomorphine (2 μM) was introduced into the perfusate for 20 min starting from the onset of reperfusion. Apomorphine significantly ($P < 0.05$) improved post-ischemic hemodynamic function. In particular, work index of heart (product of LVDP and heart rate) was twice as high in apomorphine-treated hearts as compared to controls at the end of reperfusion. Following isolation of cardiac mitochondria, the respiratory control ratio (RCR) was calculated from oxygen consumption rate of State 3 and State 4 respiration ratio. Apomorphine significantly improved post-ischemic RCR (87% of pre-ischemic value vs. 39% in control, $P < 0.05$). Using an immunoblot technique, carbonyl content of multiple myocardial proteins was observed to be elevated after global ischemia/reperfusion. Apomorphine significantly attenuated the increased protein oxidation observed at the end of reperfusion. These results support the conclusion that apomorphine is capable of preventing ischemia/reperfusion-induced oxidative stress by attenuating myocardial protein oxidation and preserving mitochondrial respiration function.

Abstract N° B118

Oral administration of geranylgeranylacetone blunts coronary endothelial dysfunction induced by ischemia/reperfusion in rat hearts

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Oral geranylgeranylacetone (GGA) induces expression of heat shock protein 72 (HSP72) and exhibits protection against ischemia/reperfusion injury in rat hearts. However, it remains unclear whether GGA preserves endothelial dysfunction induced by ischemia/reperfusion. Rats were orally administrated with GGA (200 mg/kg, GGA group) or vehicle (control, CNT group), and hearts were isolated 24 h after administration. We examined the effects of GGA on the left ventricular contractility, coronary endothelium-dependent vasodilation, and nitric oxide (NO) production before and during the 30 min of low-flow ischemia and following 30 min of reperfusion in Langendorff apparatus. Effects of a NO synthase (NOS) inhibitor, N^G-nitro-l-arginine methyl ester (L-NAME), a phosphatidyinositol 3 kinase inhibitor, LY294002, or a Rho kinase inhibitor, Y27632, were also investigated. Our results were as follows: (1) GGA augmented the postischemic functional recovery, which was abolished by L-NAME. (2) Ischemia/reperfusion caused a decrease in coronary perfusion pressure (CPP) in response to acetylcholine (ACh), which was preserved in GGA group. (3) The amount of NO in coronary effluents during the both ischemia/reperfusion period was increased in GGA group. (4) LY294002 abolished the preserved vasodilation in response to ACh and attenuated the augmented NO production by GGA while Y27632 augmented ACh-induced vasodilation and increased NO production in CNT group to comparable levels to those in GGA group. Our results indicate that GGA blunts the coronary endothelial dysfunction induced by ischemia/reperfusion injury, which may contribute, at least in a part, to the cardioprotective effects induced by GGA.

Abstract N° B119

Protective mechanism of taurine on ischemia-induced apoptosis in cultured cardiomyocytes

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To reveal the mechanism for the attenuation of ischemic injury by taurine in hearts, we examined the interaction between taurine and mitochondrial-mediated apoptosis using a cardiomyocyte model of simulated ischemia. Neonatal rat cardiomyocytes were cultured for 24–72 h in sealed flask, a condition that leads to simulated ischemia characterized by a decrease in the pH and oxygen content of the medium and catabolite accumulation. The frequency of apoptotic cells as determined by both the Hoechst 33258 staining and TUNEL method pattern was significantly reduced by 20 mM taurine for 24–72 h. In absence of taurine treatment, simulated ischemia led to mitochondrial dysfunction resulting in both cytochrome *c* releases from the mitochondria depolarization after a 24-h ischemic insults. It also promoted the apoptotic cascade initiated by the activation of caspase-9/-3 because their active forms were detected after a 30 h ischemic insult. The taurine loaded myocytes exhibited less mitochondrial dysfunction in response to ischemia. Interestingly, taurine inhibit ischemia-induced cleavages of caspase-9/-3, completely. Taurine loading also suppressed the formation of Apaf-1/caspase-9 apoptosomes formation, as evidenced by the immunoprecipitation study. These findings demonstrated that taurine effectively prevents myocardial ischemia-

induced apoptosis, by inhibiting Apaf-1/caspase-9 apoptosome formation.

Abstract N° B120

Urotensin II protectes rat heart from ischemia/reperfusion injury

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Urotensin II (UII) is a cyclic neuropeptide initially isolated from the caudal neurosecretory system of teleost fish and confers potent cardiovascular effects including depression of cardiac function and vessel contraction. The present study investigated the effects of human urotensin II (hUII) on myocardial ischemia/reperfusion (I/R) injury. In the I/R model of isolated perfused rat hearts, the effects of hUII on heart rate, VEDP, and ±d*P*/d*t* max were monitored with cardiac function software of MFL Lab200. ATP total calcium and MDA content in myocardium were detected. The coronary perfusion flow (CPF) was measured during reperfusion period. The results were that hUII pretreatment reduced leakage of LDH by 28% calcium overload by 27%, and lipid peroxidation by 24% and increased ATP content by 73%. hUII pretreatment also improved the cardiac function, and increased natrite/natrate content in myocardium and CPF. Thus, hUII attenuates I/R injury in isolated perfused rat hearts. The protective mechanism may be associated with NO-mediated coronary vasodilation.

Keywords: Peptide ischemic preconditioning; Myocardium

Abstract N° B121

Sustained cardioprotection afforded by A_{2A} adenosine receptors increases Hsp27 expression and CREB activation

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Introduction. – Stimulation of A_{2A} adenosine receptors ($A_{2A}R$) during reperfusion reduces infarct size. However, it has been shown that some therapeutic interventions may only delay cell death and extension of the reperfusion period results in loss of the cardioprotection.

Objective. – The objective of this study is to determine if the cardioprotection induced by $A_{2A}R$ stimulation is sustained and if heat shock protein 27 (Hsp27) and cAMP-response element binding (CREB) protein, known to be involved in long-term cardioprotection, is modulated by this therapeutic intervention.

Method. – Myocardial infarction was generated in rats by LAD occlusion (40 min) and reperfusion (72 h). Two groups were constituted: Control (Vehicle) and Treated ($A_{2A}R$ agonist: CGS21680 at 0.2 μg/kg/min for 120 min, starting 5 min prior to reperfusion). Infarct size is determined by triphenyltetrazolium chloride staining and Hsp27 expression along with CREB activation by western blotting.

Results. – Infarct size was reduced by 34% in the Treated group (36.2 ± 2.3%) compared to Control (54.6 ± 3.0%; *P* &λτ; 0.001). Hsp27 expression and CREB activation were significantly elevated in the Treated (305.7 ± 44.3% and 202.8 ± 34.1%, respectively, *P* &λτ; 0.01) compared to Control group.

Conclusion. – These results suggest that cardioprotection by $A_{2A}R$ stimulation is sustained and is accompanied with an enhanced Hsp27 expression and CREB activation.

Abstract N° B122

Atorvastatin at reperfusion attenuates myocardial infarction by the induction of heat shock protein 27 and p38 MAPK in the isolated perfused mouse heart

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Objectives. – Heat shock proteins (HSPs) are produced when cells are exposed to biological stress such as heat stress and chemical stress. This response is a highly conserved defense mechanism that can result in the activation of HSPs such as HSP27. This low-molecular weight HSP is recognised to act as a chaperone and can function in different unrelated cytoprotective processes such as RNA stabilisation, molecular chaperoning, stabilization of the cytoskeleton and ultimately restoring redox balance and inhibiting apoptosis. The phosphorylation of HSP27 is by the mitogen-activated protein kinase-2 (MAPK-2), a stress-sensitive kinase that is sequentially phosphorylated in a cascade of kinases involving p38 mitogen-activated protein kinase (p38 MAPK). Our aim was therefore to discover if atorvastatin is able to attenuate infarction via p38/HSP27 dependent pathways.

Methods. – We subjected an isolated Langendorff perfused mouse heart to 35 min global ischaemia and 30 min reperfusion, followed by either infarct size determination or western blot analysis for the phosphorylation of p38 MAPK and its down stream target HSP27. Fifty micromolars atorvastatin alone or atorvastatin with the specific inhibitor of p38 MAPK, SB203580 (10 μM) were administered in the perfusate at reperfusion.

Results. – There was a profound reduction of infarct size in the atorvastatin treated group (32.96 ± 3.41% ($n = 12$) vs. 51.2 ± 2.79% ($n = 11$) in controls $P < 0.005$). This protection was abrogated by the inhibitor of p38 SB203580. (49.37 ± 4.16% ($n = 9$) SB/Atorva vs. 49.40 ± 6.5% ($n = 6$) SB alone). Western blot analysis revealed that atorvastatin at reperfusion results in the rapid phosphorylation of p38MAPK which mediates the subsequent phosphorylation HSP27.

Conclusion. – Atorvastatin attenuates lethal reperfusion induced injury in an HSP27 dependent manner via the p38

MAPK cascade. This protection may be conferred by the ability of HSP27 to inhibit apoptosis.

Abstract N° B123

Reperfusion induced injury attenuated by atorvastatin in an experimental model of myocardial ischaemia/reperfusion: a role for p44/42 mapkinase and PI3kinase

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Objectives. – Statins are used in the treatment of hypercholesterolaemia. Several studies including WOSCOP and CARE suggest these compounds may have pleiotrophic effects including: plaque stabilisation, anti-inflammatory effects and stroke reduction, which are beyond their ability to lower cholesterol. Our previous animal studies have shown that atorvastatin attenuates lethal reperfusion injury via activation of the phosphatidyl inositol 3-kinase (PI3K) pro-survival signalling pathway. Using other agents we have also shown that phosphorylation of another pro-survival pathway, i.e the p44/42 MAPKinase pathway can also protect the myocardium from such injury. However this pathway has not been investigated in relation to statins. Therefore our aim was to elucidate if atorvastatin attenuates reperfusion injury not only via PI3K but also via p44/42 phosphorylation.

Methods. – We used an experimental model of Langendorff perfused mouse heart subjected to 35 min global ischaemia and 30 min reperfusion followed by either infarct quantification or western blot analysis (AKT and p44/42 phosphorylation). Fifty millimolars atorvastatin were administered during reperfusion. One hundred nanomolars Wortmannin were used to block PI3K, while 10 mM PD98059 or 10 mM U0126 were used to block the p44/42 pathway. The hearts were randomised into eight groups (n = 5–10): (a) control; (b) atorvastatin; (c) atorvastatin and Wortmannin; (d) Wortmannin alone; (e) atorvastatin and PD98059; (f) PD98059 alone; (g) atorvastatin and U0126; (h) U0126 alone.

Results. – Atorvastatin significantly reduced infarct size in the treated group: (36.0 ± 4.0% vs. 51.2 ± 3.1% in controls $P < 0.05$). This protection was abrogated by blocking PI3K with Wortmannin (48.4 ± 4.3%) and p44/42 with U0126 or PD98059 (52.6 ± 7.6% or 53.0 ± 8.0%), respectively. Western blot analysis confirmed phosphorylation of AKT and p44/42 by the administration of atorvastatin during reperfusion.

Conclusions. – Our data demonstrate that atorvastatin, given at reperfusion, significantly attenuated infarct size via both p44/42 and PI3K pathways, implying that these pro-survival pathways are involved in protection from reperfusion induced lethal injury.

Abstract N° B124

Detection and purification of protein sulphenic acids in H_2O_2 treated hearts

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Although post-translational modification of proteins by phosphorylation is well established, the reversible cysteine-targeted modifications are less well characterised. The principal product of the reaction between a protein cysteinyl thiol and H_2O_2 is a protein sulphenic acid (PSOH). Since PSOH formation is reversible, it provides a mechanism whereby redox status can control protein function. The deleterious effects of ischaemia and reperfusion in the heart have been attributed in part to oxidative stress and H_2O_2. At lower concentrations, however, H_2O_2 can act as a signalling molecule, and is implicated in genesis of ischaemic preconditioning. We have developed methods for the detection and purification of PSOH. Control (30 min aerobic stabilisation) or oxidised (0.05–1.00 mM H_2O_2 perfusion for 5 min) heart muscle was homogenised in an argon-gassed buffer containing the thiol alkylating agent maleimide (100 mM). SDS was added to denature proteins, allowing full access of the alkylation reagent and efficient blocking of protein thiols. Excess free maleimide was then removed by Superdex FPLC. Purified proteins were then treated with water (control) or 20 mM sodium arsenite for 30 min to specifically reduce PSOHs back to the free thiol groups. These free thiol groups were then labelled with 0.1 mM biotin–maleimide. Thus, the sulphenic group on cysteine is 'switched' for biotin, allowing detection by streptavidin–HRP and purification with streptavidin–agarose. Initial studies illustrate a multitude (possibly hundreds) of cardiac proteins form PSOH in response to H_2O_2. We have affinity purified these proteins, as a first step to their characterisation by LC-MS/MS. Cysteine containing peptides modified with maleimide can be excluded as sites of sulphenic acid formation, whereas those modified with biotin pinpoint where oxidation occurs. Preliminary experiments with a cysteine containing peptide control have validated this oxidation site mapping method. The mapping of cysteines that from sulphenic acid will enable more targeted studies aimed at understanding the effect of oxidation on protein function. An understanding of the physical consequences of increased cellular H_2O_2 should provide new insights into the mechanisms of injury, as well as stress adaptation.

Abstract N° B125

Effects of aging on cardiac antioxidant enzyme systems

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Thioredoxin reductase (Txnrd) and glutathione peroxidase (Gpx) are selenocysteine containing antioxidant enzymes involved in limiting oxidative stress. We have previously shown that upregulation of these enzymes is cardioprotective

and downregulation leaves hearts more susceptible to ischemia–reperfusion injury. Given that oxidative stress and cardiovascular disease are more prevalent in the older population we chose to investigate the effect of aging on these antioxidant enzyme systems. Hearts were isolated from 10 weeks (young) and 15 months (aged) male Wistar rats. RNA was extracted for quantitative real-time PCR analysis of gluatathione peroxidase (Gpx)-1and -4, glutathione reductase (Gsr), Txn, thioredoxin peroxidase-2 (Prdx2), and Txnrd-1 and -2. Txnrd, Gpx, and superoxide dismutase (SOD) activities were also measured in heart extracts. We found no difference in Txnrd activity (96 ± 5 vs. 90 ± 4 mol/min/mg protein) or Txnrd-1 mRNA between young and aged hearts, however there was a significant fourfold increase in Txnrd-2 transcription in aged hearts. Prdx2 mRNA was also significantly increased in aged hearts (~2.5-fold). Gpx activity was significantly reduced in aged hearts (256 ± 36 vs. 417 ± 48 μmol/min/mg protein in young) without changes in Gpx-1 or -4 transcription. There were no differences in SOD activity between age groups. These results suggest a reduced ratio between enzyme activities in relation to mRNA levels in aged hearts. This may result from decreased protein translation, reduced selenium incorporation into the active sites, or increased turnover caused by oxidative stress. This reduction in Gpx activity may also play a role in impaired tolerance to oxidative stress that is observed in older hearts.

Abstract N° B126

Effects of dietary selenium on post-ischemic expression of thioredoxin reductase and glutathione peroxidase antioxidant systems

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Cardiac ischemia–reperfusion injury results in oxidative stress and poor physiological recovery. Glutathione peroxidase (Gpx) and thioredoxin reductase (Txnrd) are selenocysteine dependent enzymes that protect against oxidative injury. Previously, we have shown selenium deficiency downregulates Txnrd and Gpx activity, impairing recovery from ischemia–reperfusion. Furthermore, selenium supplementation was found to be cardioprotective and lessened oxidative damage in reperfused rat hearts. We have now investigated the role of selenium in the mRNA expression of these and related antioxidant proteins post-ischemia–reperfusion. Male Wistar rats were fed varying doses of selenium for 5 weeks. Hearts were then isolated and perfused using the Langendorff method where they were subjected to 22.5 min ischemia and 45 min reperfusion. RNA was extracted for quantitative real-time PCR analysis of Gpx-1 and -4, glutathione reductase (Gsr), thioredoxin (Txn), thioredoxin peroxidase-2 (Prdx2) and Txnrd-1 and -2. Selenium deficiency produced significant reductions in Gpx-1, Gpx-4, Prdx2, Txnrd-1 and -2 gene expression. Conversely, selenium supplementation of 1000 μg selenium/kg food significantly upregulated Gpx-1, Gpx-4, Txn, Txnrd-1 and -2 transcription. These results indicate dietary selenium modulates the cardiac mRNA expression of antioxidant enzymes post-ischemia–reperfusion. This also suggests those consuming a low selenium diet may be less well equipped to cope with oxidative injury and selenium supplementation may be an effective method for reducing oxidative damage post-ischemia–reperfusion.

Abstract N° B127

PPAR agonists and vascular injury

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Patients with type 2 diabetes have a higher rate of restenosis following angioplasty. Peroxisome proliferator activator receptors (PPAR) ∀ and ligands, such as fenofibrate and rosiglitazone, have been shown to have protective effects on the vessel wall. We studied the effects of fenofibrate and rosiglitazone on intimal hyperplasia on the Zucker rat (a model for insulin resistance and type 2 diabetes) post-balloon catheter injury. Three groups of 13-week-old female fatty Zucker rats were administered either 3 mg/kg rosiglitazone ($n = 7$) or 150 mg/kg fenofibrate ($n = 6$) or served as controls ($n = 9$). In addition, two groups of 13-week-old female lean Zucker rats were either administered 3 mg/kg rosiglitazone ($n = 6$) or served as controls ($n = 6$). Carotid balloon injury was induced 1 week after starting drug administration, which was continued for 3 weeks. A 2.0 mm balloon catheter was introduced through the femoral artery to the left carotid. The balloon was inflated to 4 atm for 20 s and then deflated to 2 atm and dragged down to the aorta. Rats were sacrificed 3 weeks after injury. The carotid intima/media (I/M) ratio was calculated using Image-Pro software. Intimal hyperplasia after carotid balloon injury in the fatty Zucker rats was significantly reduced in the rosiglitazone treated (0.18∀0.29) as compared to untreated animals (0.97 ± 0.13; $P < 0.01$). Plasma glucose, triglyceride and insulin levels were elevated indicative of the presence of insulin resistance; rosiglitazone treatment significantly reduced insulin and triglyceride levels without decreasing glucose. Rosiglitazone treatment also reduced to a lesser extent the intimal hyperplasia in the lean Zucker rats (0.57 ± 0.10 vs. 1.06 ± 0.12 treated and untreated, respectively; $P < 0.01$). Rosiglitazone had no effect on insulin, triglyceride and glucose levels in this group. The intimal hyperplasia in the fenofibrate treated fatty Zucker rats was not reduced as compared to controls (0.84 ± 0.26 vs. 0.97 ± 0.13, respectively); insulin and triglyceride but not glucose levels were reduced by fenofibrate in these animals. The PPAR ligand rosiglitazone, but not PPAR∀ ligand fenofibrate decreases intimal hyperplasia following balloon injury. This effect of the PPAR ligand is independent of glycemia and is more pronounced in insulin-resistant rats.

Abstract N° B128

Estrogen induced changes in the β-adrenergic signal transduction in rat heart

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We have previously shown that estrogen deficient cause upregulation of β-adrenergic receptor (β-AR) which is correlated with higher susceptibility to ischemic damage in ovariectomized rat heart. However, the effect of ovariectomy on downstream β-AR signaling remains unknown. The purpose of the present study was to examine the effects of estrogen on the post receptor β-AR signaling cascade in the rat hearts from: (i) sham operated female Sprague–Dawley (SD) rats, (ii) female SD rats subjected to bilateral ovariectomy (Ovx), (iii) estrogen replaced Ovx rats (Ovx+E_2). It was found that there were no differences in the amount of $G_{s\beta}$ and G_{i1-3} proteins between the left ventricles in all groups of rats. In contrast, forskolin stimulated increase in electrically stimulated calcium transient ($E[Ca^{2+}]_i$) was significantly higher in Ovx than sham and Ovx+E_2 rats. However, forskolin stimulated increase in ventricular cAMP content was the same in all groups of rats. Activation of β-AR with isoproterenol (Iso) and treatment of cardiomyocytes with N6, 2′-*O*-dibutyryl adenosine cyclic monophosphate (DB-cAMP) more strongly potentiated the $E[Ca^{2+}]_i$ in the ventricular myocytes of Ovx rats than in the sham rats suggesting an enhanced Ca^{2+} response to cAMP in Ovx hearts. The basal activity of protein kinase A (PKA) in Ovx heart was also significantly higher than sham rats. The increased $E[Ca^{2+}]_i$ in Ovx rats was correlated with significantly higher $^{45}Ca^{2+}$ uptake through L-type Ca^{2+} channels activated by either Iso or DB-cAMP than in sham rats. Inhibition of PKA with its inhibitor, H-7, eliminated differences in the activity L-type Ca^{2+} channels between Ovx and sham rats. Taken together, besides affecting β-AR expression estrogen deficiency also impair reversibly one of the key stages of β-AR cascade in heart namely the PKA enzymatic activity. This abnormality is likely to target L-type Ca^{2+} channels and thereby modify both the $E[Ca^{2+}]_i$ and its potentiation by the agonist of β_1-AR. (Supported by the Research Grants Council, Hong Kong.)

Abstract N° B129

Oxidative stress in the infarcted heart: role of de novo angiotensin II production

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A beneficial role for angiotensin (Ang) II blockade on adverse structural remodeling of myocardium following infarction (MI) has been documented. During early stages of tissue repair that follow MI, macrophages express renin, ACE and AT1 receptors, thereby implicating locally generated AngII in the repair process. This possibility, however, has not been fully explored. In the present study, we hypothesized AngII induces oxidative stress (OS) with reactive oxygen species (ROS) production at the infarct site. Four groups (n = 12 rats/group) were studied: normal controls; MI; MI+AT1 receptor antagonist, losartan (Los, 10 mg/kg qd p.o.) and MI+antioxidant, *N*-acetylcysteine (NAC, 200 mg/kg qd i.p.). Hearts were collected on day 7 post-MI to monitor the expression of $gp91^{phox}$ (a subunit of NADPH oxidase involved in ROS generation) by immunohistochemistry and western blot; 3-nitrotyrosine (a marker of peroxynitrite formation) by immunohistochemistry; superoxide dismutase (SOD) expression by microarray and western blot; and macrophages by immunohistochemical ED1 labeling. We found: (1) $gp91^{phox}$ and 3-nitrotyroxine were highly expressed in the infarcted myocardium, but were not detected in normal myocardium; (2) cells expressing $gp91^{phox}$ and 3-nitrotyrosine at the infarct site were primarily macrophages; (3) compared to normal myocardium, the expression of SOD was reduced ($P < 0.05$) in infarcted myocardium in keeping with its consumption by ROS; (4) Los or NAC treatment reduced ($P < 0.05$) $gp91^{phox}$ expression in infarcted myocardium, while gene expression and activity of SOD were increased ($P < 0.05$) in treated as compared to untreated group. Thus, macrophage-derived AngII stimulates OS in an autocrine fashion by enhancing ROS production that leads to the consumption of antioxidant reserves. AT1 receptor blockade has the potential to protect the infarcted heart from further cardiac damage caused by OS and may reduce infarct size and structural remodeling.

Abstract N° B130

Cardiovascular remodelling of aged rats during chronic AT_1 receptor blockade involves AT_2 receptors

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Chronic administration of the angiotensin type 1 receptor (AT_1R) antagonist, candesartan cilexetil, is associated with increased circulating angiotensin II, and thus it has been suggested that a component of the cardiovascular effects of such drugs may be due to direct angiotensin type 2 receptor (AT_2R) stimulation. In addition, AT_2R expression is upregulated in hypertrophied hearts, and other components of the local renin–angiotensin system are also dramatically increased in the aging heart. Therefore the aim of this study was to determine the contribution of the AT_2R to the chronic antihypertensive and cardiovascular effects of AT_1R blockade in normotensive aged rats. Radiotelemetry probes were implanted into senescent (20 months) male Wistar–Kyoto (WKY) rats, and baseline recordings of mean arterial pressure (MAP) were made for 1 week. Candesartan cilexetil (2 mg/kg/d) was given in drinking water, while an additional group simultaneously received the AT_2R antagonist, PD123319 (10 mg/kg/d). At the end of the 4 weeks treatment period, animals were perfusion-fixed to enable histological

analysis of cardiovascular structure. MAP was decreased by candesartan cilexetil, however, this effect was not further influenced by PD123319. Cardiac hypertrophy and fibrosis, and aortic hypertrophy were all significantly reduced by candesartan cilexetil. Most interestingly, these structural changes were reversed by concomitant PD123319 administration, despite the lack of AT_2R-mediated effects on MAP. These results suggest that the AT_2R does not exert a significant influence on the antihypertensive effect of chronic AT_1R blockade in aged rats. However, PD123319 did reverse AT_1R-mediated regression of cardiovascular hypertrophy and fibrosis, highlighting the important role of the AT_2R on cardiovascular structure in the aging heart and vasculature.

Abstract N° B131

Angiotensin AT_2 receptor contributes to cardiovascular remodelling of aged SHR during chronic AT_1 receptor blockade

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Circulating angiotensin II levels rise as a consequence of treatment with the angiotensin type 1 receptor (AT_1R) antagonist, candesartan cilexetil, and it has been suggested that a component of the cardiovascular effects of such drugs may be due to direct angiotensin type 2 receptor (AT_2R) stimulation. In addition, AT_2R expression is upregulated with cardiac hypertrophy, and other components of the local renin–angiotensin system are also dramatically increased in the aging heart. Therefore the aim of this study was to determine the contribution of the AT_2R to chronic antihypertensive and remodelling effects of AT_1R blockade in aged hypertensive rats. Radiotelemetry probes were implanted into senescent (20 months) male spontaneously hypertensive rats (SHR) and baseline recordings of mean arterial pressure (MAP) were made for 1 week. Animals were then treated with either candesartan cilexetil (2 mg/kg/d), the AT_2R antagonist, PD123319 (10 mg/kg/d), or a combination of the two drugs. At the end of the 4 weeks treatment period, animals were perfusion-fixed to enable histological analysis of cardiovascular structure. MAP was markedly decreased by candesartan cilexetil, however, this effect was not further influenced by PD123319. Cardiac hypertrophy and fibrosis were significantly reduced by candesartan cilexetil, and interestingly, these structural changes were reversed by concomitant PD123319 administration. These results suggest that the antihypertensive effect of chronic AT_1R blockade is not significantly influenced by AT_2R stimulation in aged hypertensive rats. However, PD123319 did reverse AT_1R-mediated regression of cardiac hypertrophy and fibrosis, indicating that the AT_2R is involved in regulation of cardiovascular structure in the aging hypertensive heart.

Abstract N° B132

Long QT syndrome and APD_{90} prolongation in the cardiac Ang II-overproducing transgenic mouse model of heart failure

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Normotensive TG1306/1R (TG) mice, harbouring multiple copies of a cardiac-specific angiotensinogen transgene, develop dilated cardiomyopathy and exhibit a significant increase in mortality, associated with mechanical dysfunction and downregulation of SERCA2 protein levels. Here, we hypothesize that mechanical dysfunction is associated with changes in cardiac electrical activity and cardiomyocyte action potential (AP) duration in TG mice. Non-invasive surface-limb ECG measurements were recorded on 15–20- and 50–60-week-old male TG and WT mice under halothane anaesthesia. At a constant heart rate of ~450 beats per minute, QT and QTc intervals, but not P and QRS durations, were significantly prolonged in TG hearts relative to WT (QTc = 60.5 ± 1.4 vs. 50.0 ± 1.4 ms, respectively, at 50–60 weeks, $P < 0.001$). TG mice also showed an increased incidence of dysrhythmogenic events (extra systole), when compared to age-matched WT. Prolongation of the QT interval was associated with increased AP duration as measured in isolated ventricular myocytes by whole-cell patch clamp. While the resting potential and the amplitude of the AP remained unchanged, the APD_{90} was significantly increased in TG myocytes relative to WT (APD_{90} = 67 ± 5.0 vs. 48 ± 3.6 ms, respectively, at 50–60 weeks, $P < 0.01$). These data suggest that chronic overproduction of Ang II in the heart induces a long QT phenotype resulting from a prolongation of the cardiac and myocyte repolarization period. Patch-clamp analysis of repolarizing currents will determine which channel(s) may be accountable for such electrophysiological modifications.

Abstract N° B133

Impaired angiotensin II responses of cerebral artery in catecholamine-induced cardiac hypertrophy

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Catecholamines that accompany acute physiological stress are also involved in mediating the development of cardiac hypertrophy. Cardiac hypertrophy is an independent risk factor for cerebrovascular event. However, the cellular mechanisms involved in cerebrovascular impairment during cardiac hypertrophy are largely unknown. We therefore investigated the effects of cardiac hypertrophy, produced by isoprenaline, on cerebrovascular contractility and Ca^{2+} handling by angiotensin II (Ang II) in cerebral artery. We studied the contractile response to Ang II in pressured middle cerebral artery in vitro using a video imaging system to record diameter. Myocytes were also loaded with fura-2 to assess

$[Ca^{2+}]$i and SR Ca^{2+} content by Ang II. In control, vasomotion, which was qualitatively similar to that observed in hypertrophy. In contrast, the Ang II vasoconstrictor response in cerebral artery with cardiac hypertrophy was decreased compared to control. Ca^{2+} release from the SR was decreased, whereas resting $[Ca^{2+}]$i was unchanged in cerebral artery with cardiac hypertrophy compared to control. We conclude that treatment with Ang II impairs cerebrovascular contractility during cardiac hypertrophy through the modulation of Ca^{2+} sensitivity of the contractile elements.

Keywords: Cardiac hypertrophy; Cerebral artery; Ang II

Acknowledgements: This work was supported by Grants R05-2001-000-004113-0 and R05-2002-000-00905-0 and by the Biohealth Products Research Center of the Korea Science and Engineering Foundation.

Abstract N° B134

Angiotensin-related induction of immediate early genes in ventricle and cerebral artery during cardiac hypertrophy

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Angiotensin II (Ang II) controls not only the peripheral but also the brain circulation by stimulation of Ang II receptors and its receptors are defined as Ang II type 1 receptor (AT_1 receptor) and Ang II type 2 receptor (AT_2 receptor). In contrast to peripheral tissues, little is known about the stimulatory effects of Ang II receptors on the expression of immediately early genes (IEGs) in cerebral arteries. We have assessed whether Ang II receptors (AT_1 and AT_2) and Ang II-related biochemical signal pathways in cerebral arteries were altered during cardiac hypertrophy. New Zealand white rabbits were made cardiac hypertrophy by injection of isoproterenol (300 μg/kg body weight) for 7 d. In molecular biological analysis, the expression of Ang II (AT_1 and AT_2) receptors was lower in LVH than in control cerebral arteries. In contrast to increased level of IEGs in ventricle, the expressions of IEGs (c-fos, c-myc, and c-jun) were decreased in cerebral arteries during cardiac hypertrophy. The expressions of H-*ras* and *raf*-1 were significantly decreased in LVH cerebral arteries compared to controls. The levels of cAMP and PKA were decreased in LVH cerebral arteries compared to controls. We demonstrated a possible molecular mechanism that decreased expressions of H-*ras*/*raf*-1/c-*fos* and activities of cAMP/PKA as well as Ang II receptor would contribute to cerebrovascular dysfunction during cardiac hypertrophy.

Abstract N° B135

Improved cardiovascular function in ageing female SHR following chronic perindopril treatment

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The spontaneously hypertensive rat (SHR) is the most common animal model of human disease. Ageing male SHR develop cardiac hypertrophy and then heart failure from about 18 months of age as shown by decreased contractility, left ventricular dilatation, ventricular fibrosis and increased stiffness. We have shown that low-dose chronic perindopril (P) treatment (1 mg/kg/d po for 24 weeks) prevents the onset of heart failure in 15-month-old male SHR. In contrast, age-matched female SHR do not develop heart failure although hypertrophy and fibrosis are similar. This study has determined whether treatment of 15-month-old female SHR with P (1 mg/kg/d po for 24 weeks) changes cardiac structure and function using echocardiography and isolated Langendorff hearts ($n = 5–10$; $P < 0.05$). Administration of perindopril did not change systolic blood pressure (SHR: 189 ± 6 mmHg; SHR+P: 199 ± 5 mmHg) but decreased left ventricular wet weight (SHR: 4.34 ± 0.16 mg/g body weight; SHR+P: 3.42 ± 0.10* mg/g bodyweight). Left ventricular wall thickness at diastole was decreased (SHR: 2.38 ± 0.1 mm; SHR+P: 2.07 ± 0.1* mm) while fractional shortening (SHR: 58.2 ± 3.3%; SHR+P: 55.3 ± 5.3%) and E/A ratio (SHR: 1.68 ± 0.06; SHR+P: 1.73 ± 0.10) were unchanged. In the isolated Langendorff heart, cardiac stiffness was unchanged (SHR: 25.8 ± 1.0; SHR+P: 25.9 ± 0.9) but +dP/dt was improved (SHR: 1830 ± 70 mmHg/s; SHR+P: 2210 ± 110* mmHg/s). Thus, although ageing female SHR did not develop heart failure, chronic administration of the ACE inhibitor, perindopril, attenuates ventricular hypertrophy and improves ventricular function without decreasing systolic blood pressure.

Abstract N° B136

Perindopril reverses cardiovascular remodelling in young male spontaneously hypertensive rats

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ACE inhibitors may have beneficial effects on end-organ damage in hypertension independently of their effect on blood pressure per se. To further define this proposal, we investigated the effect of an oral 1 mg kg^{-1} daily dose of perindopril for 12 weeks on left ventricular structure and function in the 6–9-month-old male SHR. At this age, male SHR are hypertensive with ventricular hypertrophy and increased collagen deposition but no signs of heart failure when compared to age-matched normotensive male Wistar–Kyoto rats. Monthly measurement of blood pressure by tail-cuff plethysmography demonstrated marginal decreases in systolic blood pressure, with treated rats remaining significantly hypertensive. Echocardiographic assessment of in vivo cardiac function revealed that perindopril prevented the early development of diastolic dysfunction in the SHR over the course of the study. Isolated heart perfusion studies performed using the Langendorff technique showed perindopril

treatment normalised diastolic stiffness of the SHR left ventricle to control levels, with corresponding functional improvement such as an increased developed pressure compared to untreated hearts. Picrosirius red staining showed decreased interstitial collagen content of the left ventricle following treatment in the SHR. These results provide further evidence that ACE inhibition reverses cardiac remodelling and its functional consequences independent of lowering blood pressure, possibly related to inhibition of tissue rather than circulating ACE.

Abstract N° B137

Hypertrophic effects of angiotensin II and endothelin-I are mediated by leptin in rat neonatal ventricular myocytes

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Leptin is a 16 kDa product of the obesity gene secreted primarily by adipocytes. Leptin's main function was originally attributed to regulating energy homeostasis via its direct effect on the hypothalamus. We recently identified cardiomyocytes as a target for the direct hypertrophic effects of leptin and suggested that leptin may be a biological link between obesity and cardiovascular pathologies. Activation of the renin–angiotensin and endothelin systems is associated with development of cardiovascular diseases and plasma renin levels are elevated in obese individuals. We determined possible interaction between these factors in mediating hypertrophy in cultured neonatal rat ventricular myocytes. Twenty-four hours treatment with leptin (3.1 nM), angiotensin II (AngII, 100 nM) or endothelin-1 (ET-1, 10 nM) significantly increased cell area by 29%, 32% and 30%, respectively. Surprisingly, the hypertrophic effects of all three agents was prevented by leptin receptor (OBR) antibodies whereas the AT-1 receptor blocker (Sar^1–Ile^8)-AngII or the ETA receptor blocker BQ123 were ineffective against leptin-induced hypertrophy. Both AngII and ET-1 significantly increased leptin levels in the culture medium by three and fivefold, respectively. Moreover, gene expression of the long form of OBR (OBR-b) but not the short form (OBR-a) was increased by both AngII and ET-1 by 172% and 331%, respectively. In contrast, leptin had no effect on either AngII or ET-1 receptor mRNA levels. Our studies suggest that leptin and leptin receptors mediate at least in part, the hypertrophic effects of both AngII and ET-1 in cultured neonatal myocytes.

Abstract N° B138

Angiotensin converting enzyme {ACE} inhibitor {Lisinopril} improves semen quality : a preliminary report

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ACE inhibitors have many pharmacological effects. Little, if any, is manifested in the reproductive system. However in 1999, Mbah presented an anecdotal case report of the ability of Lisinopril to improve semen quality. He had in the course of treating 2 hypertensives having azoospermia and infertility with Lisinopril recorded improvement in semen quality, recording pregnancy in the wife of one.

We decided to conduct a prospective study on males with poor semen quality from infertile couples. They were given Zestril brand of Lisinopril at 2.5 mg daily for 3 months, and followed up for cardiovascular effects and changes in semen quality.

From 2001 when the study commenced, 15 cases were seen. 10 did not return for evaluation, but 2 came to report later that their wives had conceived and delivered. The 5 who returned for evaluation are the subject of this preliminary report. In all, there was no untoward cardiovascular effect. Positive changes in indices of semen quality were recorded in all, but none reported conception in their wives during the period. One of them was hypertensive and was continued on Zestril in higher dose for blood pressure control. His semen quality continued to improve until he reported conception in his spouse.

Infertility puts great stress on the the afflicted. We recommend further research to enunciate the mechanism of this effect and suggest that ACE inhibitors {Lisinopril} be given in low doses to oligospermic men in infertile couples.

Abstract N° B139

Detection of abnormalities in the diabetic heart using MRI

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Cardiovascular complications, including diabetic cardiomyopathy, are the major cause of fatalities in diabetes. Diabetic cardiomyopathy is expressed in part through fibrosis and left ventricular (LV) hypertrophy. This increases myocardial stiffness leading to heart failure. In order to search for curative interventions, precise evaluation of the diabetic heart pathology is extremely important. Magnetic resonance imaging (MRI) is a technique that is ideally suited for the assessment of heart disorders due to its dimensional accuracy, high resolution, and 3D properties. Streptozotocin injected Sprague–Dawley rats were used as a model of type I diabetes. Changes in heart rate and stress test measures confirmed cardiac abnormalities in the diabetic animals. Increases in heart to body weight ratio and in EKG R wave heights were suggestive of LV hypertrophy. Masson trichrome staining revealed significant accumulation of collagen, an indication of fibrosis, in diabetic rats compared to non-diabetics. To further characterize the LV changes in

diabetic rats we performed high resolution cardiac MRI using a 9.4 T scanner. In diabetic rats compared to controls, MRIs demonstrated increased LV wall volume, suggestive of LV hypertrophy; increased LV wall pixel intensity, suggestive of the presence of fibrosis; and decreased T2 relaxation time, suggestive of biomechanical changes in the diabetic tissue, perhaps due to presence of fibrosis. Also, the diameters of LV chamber in completely collapsed hearts were dramatically increased in diabetes indicating decreased compliance of tissue. Together the data suggest that LV hypertrophy and fibrosis may be a major factor underlying structural and functional abnormalities of diabetic heart.

Abstract N° B140

Signalling pathways involved in development of diabetes-induced cardiac dysfunction in the globally ischemic heart

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We studied the roles of Ras-GTPase, tyrosine kinases (TKs) including epidermal growth factor receptor (EGFR), and PI3-kinase in global ischemia and reperfusion (I/R) in a perfused diabetic rat heart model. Diabetes was induced by a single intraperitoneal injection of 55 mg/kg body weight streptozotocin. Recovery of cardiac function, measured as left ventricular developed pressure (P_{max}) and left ventricular end-diastolic pressure (LVEDP), after 40 min episode of global ischemia followed by a 30 min reperfusion was significantly worse in perfused diabetic rat hearts as compared to non-diabetic controls. Pre-treatment of rats with Ras-GTPase inhibitor FPTIII (232 ng/min for 6 d), TKs inhibitor genistein (1 mg/kg/d for 6 d), or PI3-kinase inhibitor LY294002 (1 mg/kg/d for 6 d) significantly enhanced cardiac recovery in terms of left ventricular contractility. In contrast, hearts from diabetic rats pre-treated with EGFR inhibitor AG1478 (578 ng/min for 6 d) produced detrimental effects on recovery of cardiac function:

	P_{max} (mm Hg) (% recovery)	LVEDP(mm Hg) (% recovery)
Non-diabetic	47 ± 4	532 ± 28
Diabetic	15 ± 2 *	1050 ± 49 *
Diabetic + FPTIII	25 ± 2 **	782 ± 23 **
Diabetic + LY294002	29 ± 3 **	734 ± 41 **
Diabetic + genistein	37 ± 2 **	544 ± 34 **
Diabetic + AG1478	9 ± 2 **	975 ± 51

Data (mean ± SEM) recorded at 30 min reperfusion

* Significant as compared to non-diabetic.
** Significant as compared to diabetic.

These data suggest that EGFR is involved in signaling pathways leading to recovery from cardiac ischemia, whereas activation of Ras-GTPase-, TKs-, or PI3-kinase-mediated signaling pathways are critical in the development of cardiac dysfunction due to I/R in the diabetic heart.

Abstract N° B141

Atrial natriuretic peptide levels in experimental diabetes

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Diabetes mellitus may lead to a cluster of cardiovascular complications including altered cardiac endocrine activity. In the present study, we aimed to investigate levels of atrial natriuretic peptide (ANP) and amino terminal fragment of proANP (NTproANP) in systemic plasma and pericardial fluid of diabetic dogs. Metabolically healthy (H, $n = 9$) and alloxan-diabetic (i.v. 560 μmol/kg; DM, $n = 6$) mongrel dogs of either sex were used. The chest was opened under pentobarbital (133 μmol/kg nembutal, i.v.) anesthesia and an inflatable balloon was placed into the right atrium. As a stimulus for ANP secretion, right atrial pressure was increased by balloon inflation (by 7–9 cm H_2O for 20 min). Systemic blood and pericardial fluid samples were taken at baseline and every 10 min during the 60-min study period. Natriuretic peptide levels were determined by radioimmunoassay. Pericardial ANP levels proved to be higher than systemic plasma levels (2.2-fold in DM, 2.7-fold in H). Elevated baseline NT-proANP levels were measured in plasma of diabetic dogs compared to metabolically healthy controls (DM: 0.47 ± 0.03, $P < 0.01$ vs. H: 0.31 ± 0.03 pmol/l). In response to elevation in right atrial pressure, ANP and NT-proANP levels increased (ANP: 26.34 ± 3.31 $P < 0.01$ vs. 39.37 ± 6.01 pmol/l and NT-proANP: 0.31 ± 0.03 $P < 0.05$ vs. 0.42 ± 0.04 pmol/l) in metabolically healthy controls only. However, we failed to prove significant differences in pressure-stimulated peptide levels between the two investigative groups. The above results may lead to the conclusion that elevated basal NT-proANP levels and diminished natriuretic peptide secretory responsiveness indicate a deterioration in cardiac function in diabetes.

Abstract N° B142

Expression of connective tissue growth factor is increased in the diabetic myocardium and associated with increased left ventricular chamber stiffness

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Upregulation of the profibrotic protein connective tissue growth factor (CTGF) may lead to left ventricular (LV) diastolic dysfunction in diabetes. However the relationship between CTGF expression and LV chamber stiffness has not been determined. Hence, we sought to characterise this relationship in experimental diabetes. Open-chest anaesthetised sheep were instrumented to determine the LV pressure–vol-

ume relationship following 12 months untreated streptozotocin-diabetes. Chamber stiffness was calculated as the slope of the linearised LV end-diastolic pressure–volume relationship, myocardial collagen content was determined by hydroxyproline assay and CTGF expression was determined by blinded semi-quantitative analysis of immunostained sections. Diabetes increased the slope of the linearised LV end-diastolic pressure–volume relationship (0.00024 ± 0.00010 diabetes vs. 0.00016 ± 0.000032 control $P < 0.05$) reflecting increased chamber stiffness. LV collagen content was significantly higher in diabetic animals (31.0 ± 3.1 μg/mg dry tissue) compared to controls (23.9 ± 5.0 μg/mg dry tissue) ($P < 0.01$). CTGF expression increased 29.8% in diabetes ($P = 0.002$) and correlated with chamber stiffness ($r = 0.62$, $P < 0.05$). This study demonstrated for the first time a clear relationship between increased chamber stiffness and CTGF upregulation in experimental diabetes. Combined with its known profibrotic effect, this data further implicates CTGF in the induction of diastolic dysfunction by diabetes and substantiates CTGF as a potential molecular target to prevent and treat diabetic cardiomyopathy.

Abstract N° B143

Effects of experimental diabetes on the endothelin-induced ventricular arrhythmias in dogs

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Endothelin (ET) is known to have direct arrhythmogenic effect in the mammalian heart. Diabetes mellitus (DM) is accompanied by a series of endothelial and cardiac dysfunctions, however little is known about ET-induced direct arrhythmias in DM. Therefore, we infused ET (33 pmol/min) into the left anterior descending coronary artery of 28 mongrel dogs, and measured basic hemodynamic parameters, coronary flow and ECG. DM was induced by alloxan and experiments were done 8 weeks later. Metabolically healthy dogs served as controls (C). In a further control group, local hyperglycemia (HG) was induced by intracoronary glucose infusion (HG). The electrophysiological parameters were comparable between the groups. This was followed by the occurrence of ventricular premature beats, coupled extrabeats and later sustained ventricular tachycardia. Most of the experiments were terminated by ventricular fibrillation. The onset of arrhythmias was shorter in DM dogs as compared to C and HG dogs (18 ± 8 min vs. 24 ± 8 and 30 ± 28 min, $P < 0.05$). However, there was no difference in the number of ventricular fibrillations, and the total elapsed time until the termination of the experiments. Therefore, the diabetic heart seems to be more prone to ET-induced arrhytmias and this is probably not a result of locally high glucose concentrations.

Abstract N° B144

Blockade or abrogation of angiotensin II AT1 receptor suppressed accelerated diabetic atherosclerosis in streptozotosin-induced diabetic apolipoprotein E-deficient mice

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Background. – Atherosclerotic complications represent a leading cause of morbidity and mortality in diabetic patients. Recent evidence suggests increased angiotensin II generation and its type 1 (AT1) receptor function in the arterial wall of diabetic animals and humans. However, role of angiotensin II AT1 receptor in the mechanism of accelerated diabetic atherosclerosis has not been clearly addressed.

Methods and results. – Diabetes was induced by injection of streptozotosin in 8 to 9-week-old apolipoprotein (Apo)E-deficient mice. These mice were treated with or without angiotensin II receptor blocker (ARB), telmisartan, or olmesrtan for 6 weeks. Non-diabetic ApoE-deficient mice were used as controls. Diabetic animals showed a fourfold increase in atherosclerotic lesions in the aorta (2.7 ± 0.5%, 8.7 ± 2.3% ($P < 0.01$ vs. control), and 3.7 ± 0.4% ($P < 0.01$ vs. diabetic), in the control, diabetic, and diabetic+ARB mice, respectively). This was associated with increased expression of AT1 receptor and inflammation-promoting molecules such as monocyte chemoattractant protein-1 in lipid- and macrophage-rich lesions. Treatment with ARB did not affect serum lipid profile, blood glucose, or blood pressure, but did suppressed atherosclerotic plaque formation and decreased inflammation and lipid deposition. Interestingly, AT1 receptor lacking ApoE-deficient mice displayed similar suppression of accelerated diabetic atherosclerosis and inflammation.

Conclusion. – This study demonstrated the pivotal role of angiotensisn II AT1 receptor in the mechanisms of diabetes-associated acceleration of atherosclerosis. Blockade of AT1 receptor may function as anti-inflammatory therapy beyond glycemic control.

Abstract N° B145

Improvement of vasorelaxation by endothelin receptor antagonist CPU 0213 is mediated by suppression on preproET-1 of the vascular wall in diabetic rats

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Aim. – The endothelial lesion in diabetes could be related to an excess of ET-1 which causes dysfunction of vasorelaxation. A novel endothelin receptor antagonist CPU 0213 a dual blockader is tested to recover the impairment of vasorelaxation in the STZ induced diabetes.

Methods. – Rats were injected with STZ 60 mg/kg i.p. developed sustained hyperglycemia in 4 weeks and treated with CPU 0213 80 mg/kg p.o. and aminoguanidine (AGD) 100 mg/kg p.o. for 28 d, separately. The vasorelaxation to acetylcholine, measurement of ET-1, mRNA of preproET-1

in myocardium and thoracic aorta, iNOS and NO in serum were conducted.

Results. – ET-1 raised significantly in serum in diabetic rat by 39.5 and a reduction in ET-1 level was significant by CPU 0213 (–40.4%) and AGD (–30.0%). In the diabetic model the mRNA abundance of preproET-1 was increased dramatically in the myocardium (104%) and aortic wall (581%), respectively. CPU 0213 and AGD reduced preproET-1 mRNA in myocardium (–42% and –28%, respectively) and thoracic aorta (–83% and –80%). The iNOS mRNA increased in diabetic aorta (305%) and was reduced by 0213 and AGD (–70% and –38%). The iNOS activity in serum was elevated in the model (865%), and reduced by 0213 and AGD (–81% and –51%). The NO in serum was increased in model significantly (96%) and reduced by 0213 and AGD (–16% and –18%). The maximal vasorelaxation was reduced in the model (–69%), and improved by 0213 and AGD (151% and 125%).

Conclusion. – The novel endothelin receptor antagonist CPU 0213 which improves significantly vasorelaxation of the diabetic thoracic aorta is more effective to suppress overexpression of preproET-1 mRNA in thoracic wall. (Supported by a key project of National Science Foundation of China No: 30230170.)

Abstract N° B146

PPAR-γ agonist rosiglitazone reduces clinical inflammatory responses in type 2 diabetes with coronary artery disease after coronary angioplasty

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Type 2 diabetes mellitus patients are at an increased risk for coronary artery disease (CAD) and coronary restenosis after angioplasty or stenting. Persistent chronic inflammatory condition and metabolic disorder contribute importantly to the pathogenesis of atherosclerosis and media many stages of atheroma development from leukocyte recruitment to rupture of plaque and restenotic lesions. Rosiglitazone (RSG), an agonist of peroxisome proliferators activated receptor-γ (PPAR-γ), is insulin-sensitizing antidiabetic agent and inhibits restenosis in animal blood vessels. However, whether RSG was benefit for diabetes with CAD patients after percutaneous coronary intervension (PCI), and if so, to explore the mediating in a clinical trial. The diabetic CAD patients 70 undergone PCI were randomized receive RSG (4 mg/d) treatment or served as control for 6 months. After 6 months RSG treatment, the levels of fasting plasma glucose, fasting plasma insulin, HbA1c and HOMA-IR were significantly decreased in RSG group when compared with baseline values and control group. In addition, plasma levels of MCP-1, CRP and hyper-responsiveness of low-dose LPS-induced MCP-1 secretion from patient monocytes were also reduced compared with baseline levels and control group. Furthermore, coronary events were significantly decreased in RSG group when compared with control group after 6 months follow-up. In conclusion RSG not only improves metabolic disorders, but also reduces proinflammatory responses in diabetic with CAD patients after PCI. Most importantly is that RSG significantly reduces coronary events in study subjects. These results indicate that PPAR-γ agonist RSG may protect vascular wall by anti-inflammation and anti-metabolic disorders in diabetic CAD patients after PCI.

Abstract N° B147

Cardiovascular changes in the ageing growth hormone deficient rat

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Exogenous growth hormone has been implicated as a potential novel therapy for patients with congestive heart failure. This project has characterised the Lewis Dwarf (LD) growth hormone deficient rat model from 6 to 18 months (M) of age using echocardiography, isolated Langendorff heart preparations, single-cell microelectrode electrophysiological recordings and responses from isolated thoracic aortic rings to determine if growth hormone deficiency initiates heart failure. LD rats were moderately hypertensive (6 M, 148 ± 3*; 12 M, 157 ± 4*; 15 M, 162 ± 3*; 18 M, 161 ± 4* mmHg) compared to age-matched Wistar (W) controls (6 M, 121 ± 3; 12 M, 126 ± 3; 15 M, 133 ± 2; 18 M, 129 ± 6 mmHg) with decreased left ventricular internal dimensions in diastole (LD: 6 M, 5.4 ± 0.3*; 12 M, 5.3 ± 0.1*; 15 M, 6.1 ± 0.1*; 18 M, 6.8 ± 0.2* mm; W: 6 M, 7.1 ± 0.1; 12 M, 8.2 ± 0.2; 15 M, 7.8 ± 0.3; 18 M, 7.9 ± 0.3 mm) and increased left ventricular posterior wall thicknesses (LD: 6M, 1.87 ± 0.08*; 12 M, 1.87 ± 0.07*; 15 M, 1.89 ± 0.08*; 18 M, 1.76 ± 0.1* mm; W: 6 M, 1.76 ± 0.08; 12 M, 1.72 ± 0.07; 15 M, 1.77 ± 0.08; 18 M, 1.89 ± 0.08 mm) indicative of concentric cardiac hypertrophy. Fractional shortening, ejection fraction, maximum ascending aortic blood flow velocity and maximum +dP/dt were all increased in the LD rat showing improved systolic function. LD rats showed prolonged action potential duration (APD90: LD: 6 M, 47.5 ± 5.9*; 12 M, 52.9 ± 4.3; 15 M, 60.2 ± 5.3*; 18 M, 76.5 ± 7.2* mm; W: 6 M, 33.1 ± 3.7; 12 M, 47.8 ± 5.9; 15 M, 49.9 ± 4.1; 18 M, 53.7 ± 5.6 ms). Additionally, maximal responses to noradrenaline, acetylcholine and sodium nitroprusside were significantly reduced in LD rats. Diastolic stiffness was unaltered between the groups but increased with age. Thus, chronic growth hormone deficiency produces compensated concentric cardiac hypertrophy with improved left ventricular function without symptoms of heart failure but vascular function is dramatically reduced in the LD rat.

Abstract N° B148

Rosiglitazone, a PPARγ-activator, improves cardiomyocytes contractile response to insulin in spontaneously hypertensive rats

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To compare the effects of insulin on cardiomyocytes of normal Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), and further investigate whether rosiglitazone (ROSI), a peroxisome proliferators-activated receptor γ (PPARγ) activator, modifies the sensitivity of SHR myocytes to insulin. Male SHR were randomized to receive ROSI (3 mg/kg/d, o.p.) or vehicle for 14 d. Single ventricular myocytes were enzymatically isolated and field stimulated. Myocyte shortening and intracellular Ca^{2+} transient were assessed and compared with those of age- and sex-matched WKY by a video-based motion edge detection system. Insulin concentration dependently (at 10^{-6}–10^{-8} mol/l) increased the myocyte shortening of both WKY and SHR. At 10^{-7} mol/l, insulin increased the peak twitch amplitude (PTA) of WKY myocytes by 20.5% (n = 10, $P < 0.05$ vs. vehicle). Compared with WKY, however, the response of SHR myocytes to insulin reduced significantly as evidenced by decreased PTA (7.83 ± 0.55% vs. 10.17 ± 0.48% of WKY, n = 10, $P < 0.01$) and decreased maximal velocity of shortening or relengthening (±dL/dt, n = 10, $P < 0.05$). After the treatment with ROSI, the sensitivity of SHR myocytes to insulin was augmented remarkably: PTA, +dL/dt and –dL/dt increased by 31.2% (n = 10, $P < 0.01$), 23.7% and 21.6% (n = 10, $P < 0.05$), respectively. In addition, the Ca^{2+} transient (fura-2 fluorescence intensity change) in ROSI-treated SHR myocytes to insulin increased from 0.27 ± 0.02 to 0.43 ± 0.03 (n = 10, $P < 0.01$). Insulin has positive inotropic action on normal rat cardiomyocytes, which is significantly blunted in SHR. PPARγ-activator ROSI enhances the insulin-induced contractile effect on cardiomyocytes of SHR by increasing intracellular Ca^{2+} transient.

Abstract N° B149

Mechanism of heart failure resulting from chronic β-adrenergic stimulation

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As the heart hypertrophies and fails, metabolic energy stores become depleted through unknown mechanisms. We have used non-invasive magnetic resonance imaging (MRI) and isolated heart techniques to determine whether the energy depletion results from increased workload or from decreased mitochondrial ATP production. Male Wistar rats (n = 16) were injected with the β-adrenergic agonist, isoproterenol (Iso), 5 mg/kg for 7 d, to induce cardiac hypertrophy. In vivo cine MRI showed that the hearts of treated animals had 25% increased left ventricular weight and 24% lower cardiac output, with no changes in ejection fraction or stroke volume. Plasma levels of glucose and triglycerides were decreased by 38% and 36%, respectively, but the non-esterified fatty acid levels were unchanged in Iso-treated animals. Hearts were isolated and perfused in working mode with Krebs–Henseleit buffer containing 11 mM glucose. The hydraulic work performed by Iso-treated rat hearts was 42% lower than controls. Protein levels of the insulin responsive glucose transporter protein, GLUT4, were decreased by 26% and levels of the mitochondrial uncoupling protein, UCP3, were increased by 38% in the Iso-treated rat hearts compared with controls. The observed decrease in GLUT4 and the lower work performed by hearts perfused with glucose alone suggest that the hypertrophied hearts were insulin resistant. The increased UCP3 levels suggest that there would be increased mitochondrial uncoupling and a consequent decrease in cardiac efficiency in the presence of plasma fatty acids. Thus the decreased energy stores in hypertrophied hearts are probably due to decreased ATP production that limits the cardiac work.

Abstract N° C1

Cardioprotective efficacy of zoniporide, a potent and selective inhibitor of Na^+/H^+ exchanger isoform 1, in an experimental model of cardiopulmonary bypass

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We determined (1) the inhibitory potency of zoniporide against the native Na^+/H^+ exchanger isoform 1 (NHE1) expressed in adult rat ventricular myocytes and platelets, and (2) the cardioprotective efficacy of zoniporide in isolated, blood-perfused adult rat hearts subjected to cardioplegic arrest, hypothermic ischaemia (150 min at 25 °C) and normothermic reperfusion (60 min at 37 °C). In isolated myocytes, zoniporide produced a dose-dependent inhibition of NHE1 activity, as measured by the rate of H^+ efflux following intracellular acidification (IC_{50} 73 nM at 25 °C). A comparable NHE1-inhibitory potency was retained at 37 °C. In platelets, the rate of cell swelling, a surrogate index of NHE1 activity, was also inhibited by zoniporide (IC_{50} 67 nM at 25 °C). In the isolated heart model, administration of zoniporide (loading bolus of 1 mg kg^{-1} i.v. plus continuous infusion of 1.98 mg kg^{-1} h^{-1} i.v.) to the support animal achieved a free drug concentration of ≥1 μM. At this dose, relative to vehicle treatment, zoniporide afforded improved preservation of left ventricular end-diastolic and developed pressures and coronary perfusion pressure during reperfusion. Myocardial myeloperoxidase activity was also attenuated by zoniporide treatment, indicating reduced neutrophil accumulation. These data show that zoniporide (1) is a potent inhibitor of native NHE1 activity in ventricular myocytes and platelets, and (2) affords significant cardioprotective benefit during ischaemia and reperfusion in an experimental model that

mimics several distinctive features of human cardioplegic arrest with cardiopulmonary bypass.

Abstract N° C2

Myocardial protection by Na^+/H^+ exchanger inhibition and ischemic preconditioning of rat heart

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This study was designed to compare the cardioprotective efficacy of BIIB-722, a novel selective Na^+/H^+ exchanger inhibitor (Boehringer Ingelheim Pharma, KG), and ischemic preconditioning (IP) in a rat model of myocardial infarction. The left anterior descending coronary artery (LAD) was ligated for 40 min and then reperfused for 1 h (control). BIIB-722 was infused intravenously (3 mg/kg) for 5 min before LAD occlusion or at the first minute of reperfusion ($BIIB_i$ or $BIIB_r$ group, respectively). IP was induced by two cycles of 5-min LAD occlusion and 5-min reperfusion prior to sustained ischemia. Myocardial infarct (MI) size was determined by staining slices with triphenyltetrazolium chloride. Metabolic state and cell membrane damage of the area at risk (AR) were assessed by ATP, phosphocreatine (PCr), total creatine (ΣCr = PCr+Cr) and lactate contents after reperfusion. The AR to the left ventricular weight ratio was on average 32 ± 4% in all studied groups. The percentage MI/AR ratios in $BIIB_r$ and IP groups did not differ and were significantly lower than in the control (17 ± 2% and 15 ± 3%, respectively vs. 43 ± 5%). This index was significantly higher in $BIIB_i$ group (25 ± 2%) comparing with IP and $BIIB_r$ groups but 1.7-fold lower than in the control. MI size limitation was combined with better ΣCr preservation in $BIIB_r$ and IP groups than in $BIIB_i$ one at the end of reperfusion (70 ± 5%, 75 ± 4% and 63 ± 5% of the initial value, respectively). ΣCr content in the control was 58 ± 3% of the preischemic value indicating pronounced sarcolemmal rupture. Both $BIIB_r$ and IP groups showed significantly higher ATP and PCr levels and lower lactate content comparing with these indices in the control. BIIB-722 administration before sustained ischemia but did not affect metabolic state of the AR. The results indicate that administration of Na^+/H^+ exchanger inhibitor on early reperfusion is much effective than before ischemia. We believe that IP may inhibit Na^+/H^+ exchanger during reperfusion to prevent myocardial damage.

Abstract N° C3

Intracellular acidosis stimulates ERK activity in adult myocytes through a GPCR-, PKC- and Src-independent mechanism

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Intracellular acidosis has a profound effect on myocyte function and viability. We have shown previously that intracellular acidosis activates the ERK pathway in neonatal rat ventricular myocytes (J Biol Chem 2003;278:31676–84). Here, we examined acidosis-induced activation of the ERK pathway and potential upstream mediators of this response in adult rat ventricular myocytes (ARVM). After overnight culture, ARVM were transferred to bicarbonate-free Tyrode solution for 90 min, and then exposed to 30 mM NH_4Cl for 4 min. Intracellular acidosis was induced and maintained by NH_4Cl washout with Tyrode solution containing the NHE inhibitor cariporide (3 μM). Intracellular acidosis induced a robust (10-fold) increase in ERK activity (as determined by western blotting with an antibody which recognises the phosphorylation of ERK1/2 by their upstream activator, MEK1) in a rapid (peak within 3 min) and transient (return to basal by 10 min) manner. Acidosis-induced ERK activation was unaffected by: (1) Adenovirus-mediated overexpression of regulator of G protein signalling (RGS) proteins, to inhibit signalling via G_q, G_i and $G_{12/13}$ proteins. (2) Pharmacological inhibition of protein kinase C with GF109203X (10 μM). (3) Pharmacological inhibition of Src tyrosine kinases with PP2 (10 μM). In contrast, ERK activation was abolished by pharmacological inhibition of MEK1 (U0126, 3 μM) or adenovirus-mediated expression of a kinase-inactive dominant negative MEK1 mutant. Our data indicate that, in ARVM, intracellular acidosis increases ERK activity through a GPCR-, PKC and Src-independent mechanism mediated by MEK1.

Abstract N° C4

Caveolar Na/K-ATPase moonlights by interacting with protein tyrosine kinases

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Binding of ouabain to Na/K-ATPase activates multiple signaling pathways by stimulation of Src and other tyrosine kinases. Since the α subunit of the pump contains two conserved caveolin-binding motif, we aimed to test if the caveolar Na/K-ATPase is involved in transmitting extracellular ouabain signal. GST pull-down assay showed that the α subunit bound to the N-terminus of caveolin-1. Significantly, ouabain regulated this interaction in a time- and dose-dependent manner and stimulated tyrosine phosphorylation of caveolin-1. When added to the isolated membrane fractions, ouabain increased tyrosine phosphorylation of proteins from the isolated caveolae, but not other membrane fractions. Consistently, ouabain induced the formation of a Na/K-ATPase/Src/caveolin complex in the isolated caveolae preparations as it did in live cells. Finally, depletion of either cholesterol by methyl β-cyclodextrin or caveolin-1 by siRNA significantly reduced the caveolar Na/K-ATPase and Src. Concomitantly, cholesterol depletion abolished ouabain-induced recruitment of Src to the signaling pump. Like depletion of caveolin-1, it also blocked the effect of ouabain on ERKs, which was restored after cholesterol repletion. Clearly, the caveolar Na/K-ATPase represents the signaling pool of the pump that interacts with Src and transmits the ouabain signals. Supported by NIH grants HL-36573, HL-67963, and HL-63238.

Abstract N° C5

Contractile function is depressed in the phospholemman knockout mouse heart

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Phospholemman (PLM) is the primary cardiac sarcolemmal substrate for PKA and PKC, and may regulate cell volume. Like other FXYD proteins, PLM has also been implicated in the modulation of Na/K-ATPase activity. We have recently shown that unphosphorylated PLM may exert a tonic inhibition on Na/K-ATPase activity. We have investigated cardiac function in PLM knockout (KO) mice. Isolated hearts were aerobically perfused in Langendorff mode for 30 min and left ventricular developed pressure (LVDP) measured. PLM KO hearts exhibited a significantly reduced LVDP compared with wild type (29 ± 9 vs. 96 ± 5 mmHg; $n = 4$, $P < 0.05$), with no difference in coronary flow. This depressed contractility was also seen in vivo. In anaethetised, ventilated open-chest mice ($n = 8$/group), an LV Millar conductance catheter was inserted via the cardiac apex and pressure–volume loops recorded at various preloads.

	Wild type	KO
d*P/dt max (mmHg/s)*	8032 ± 302	4548 ± 230 *
d*P/dt min (mmHg/s)*	–7928 ± 227	–5033 ± 233 *
Ejection fraction (%)	73.5 ± 4.7	59.6 ± 6.0
Stroke work (mmHg·µl)	598 ± 55	396 ± 70 *
Tau-Weiss (ms)	7.64 ± 0.13	9.67 ± 0.25 *
Ventricle/body weight ratio ($\times 10^2$)	2.78 ± 0.07	3.27 ± 0.17 *

* $P < 0.05$.

These studies suggest PLM plays a significant role in the regulation of cardiac contractile function and are consistent with an elevated Na/K-ATPase activity in the KO heart. This in turn may reduce intracellular sodium concentration and hence indirectly reduce intracellular calcium concentration and contractility.

Abstract N° C6

Isoform specific regulation of cardiac Na/K ATPase by phospholemman

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Regulation of the Na/K ATPase (NKA) by protein kinases is tissue and model specific. There is a well-established functional link between PKA and cardiac NKA, but the NKA α1 subunit is not a PKA substrate in vivo. The aim of this study was to investigate the relationship between NKA, PKA and phospholemman (PLM, the primary substrate for PKA and PKC in cardiac sarcolemma) in isolated guinea pig ventricular myocytes and homogenates of Langendorff-perfused rat hearts. NKA activity in homogenates and subcellular fractions of hearts perfused aerobically and made ischemic for up to 30 min was determined by measuring ouabain-sensitive phosphate generation from ATP at 37 °C. NKA activity in isolated myocytes was measured using the perforated patch technique, and currents due to NKA α1 and α2 ($I\alpha1$ and $I\alpha2$) distinguished by their sensitivity to dihydroouabain. In sarcolemmal membranes prepared from Langendorff-perfused hearts, ischemia caused a substantial increase in NKA activity (from 2.4 ± 0.4 in aerobic controls to 7.2 ± 0.5 µmol phosphate/min/g wet weight after 30 min ischemia, $n = 5$), which was blocked by treatment with the PKA selective inhibitor H89 (1 µmol/l) prior to ischemia. In voltage clamped myocytes, PKA activation with forskolin (1 µmol/l) caused a significant H89-sensitive increase in Iα1 of 36 ± 15% ($n = 6$), but no change in Iα2. PLM was phosphorylated but NKA α1 subunit was not following PKA activation in ischemic Langendorff-perfused hearts and forskolin-treated myocytes. PLM was found associated with NKA α1 and β1 but not α2 subunits by both co-immunoprecipitation and immunofluorescence. Therefore, PLM is an integral part of the NKA enzyme complex in the heart and provides a functional link between PKA and NKA. Its role is analogous to that of phospholamban in regulating the sarcoplasmic reticulum calcium ATPase: phosphorylation of PLM leads to disinhibition of NKA.

Abstract N° C7

Dobutamine stimulates rubidium ion uptake in pig hearts in vivo: ^{87}Rb-MRS study

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We have previously shown using 87-rubidium MRI that the adrenergic agonist, dobutamine (Dob), stimulate Rb^+ uptake in isolated pig hearts. In the present work we studied whether Dob can increase Rb^+ uptake rate in vivo. Open-chest domestic pigs ($n = 14$) were used under general anaesthesia. The surface coil was placed against the anterior left ventricular wall to obtain ^{87}Rb spectra. RbCl (188 mM) was infused at the rate of 1.35 ± 0.14 mmol/kg/h without or with Dob (0.6 mg/kg/h) over a 60-min period and then infusions were terminated for 60 min. Hearts were arrested, excised and analysed for RbCl content. ^{87}Rb spectra were obtained every 5 min using a Bruker Avance spectrometer interfaced with Magnex 7T, 40-cm horizontal bore magnet. Dob increased the rate constant and Rb^+ influx rate (twofold) at the similar plasma [Rb^+] (Table 1) and tissue/plasma Rb ratios (38 ± 9).

Table 1

Group	$k \times 10^3$ (min^{-1})	Flux (%/min)	Plasma [Rb^+] (mM)
Dob	36 ± 11.7	4.8	0.51 ± 0.19
Control	13 ± 2.4	2.5	0.73 ± 0.24

Heart rate (HR) and blood systolic pressure (BSP) increased by 52 and 19% from 106 ± 9 bpm and 78 ± 7 mmHg, respectively. Stimulation of Rb^+ uptake by Dob is consistent

with the activation of Na^+/K^+ ATPase previously observed in isolated hearts. However the 50% increase in HR and doubling of coronary flow (BSP increase and vasodilatation) could also contribute to this effect.

Abstract N° C8

ANP activates the sarcolemmal sodium pump via nitric oxide synthase

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We reported that atrial natriuretic peptide (ANP) at physiological cardiac tissue concentration (~10 nM) activates the Na^+–K^+ pump in rabbit ventricular myocytes by activating cGMP dependent protein kinase-G. Given a reported absence of particulate guanylyl cyclase (GC) in ventricular myocytes, we tested the hypothesis that the ANP effect on the pump was mediated by soluble guanylyl cyclase (sGC). We examined the effect of 10 nM ANP on the cardiac sarcolemmal Na^+–K^+ pump by voltage clamping single rabbit ventricular myocytes. Electrogenic Na^+–K^+ pump current (I_p) was identified as the shift in holding current induced by 100 μM ouabain. Pipette solutions included Na^+ in a concentration of 10 mM. I_p of control myocytes was 0.35 ± 0.02 pA/pF ($n = 9$) while I_p of myocytes exposed to 10 nM ANP was 0.54 ± 0.03 pA/pF ($n = 7$, P &λτ; 0.001). We inhibited sGC by enclosing 10 μM 1h-[1,2,4]oxadiazole [4,3-a]quinoxaline-1-one (ODQ). ODQ abolishes ANP-induced pump stimulation ($I_p = 0.28 \pm 0.02$ pA/pF, $n = 7$). We examined if nitric oxide synthase (NOS) links ANP to the Na^+–K^+ pump by including 10 μM of the NOS-inhibitor L-*N*-monomethyl-arginine (L-NAME) in pipette solutions. L-NAME induced a decrease in I_p in control myocytes not exposed to ANP ($I_p = 0.20 \pm 0.2$ pA/pF, $n = 5$) and abolished ANP-induced pump stimulation ($I_p = 0.20 \pm 0.06$ pA/pF, $n = 5$). We concluded that ANP activates the Na^+–K^+ pump by a mechanism that involves activation of sGC by NO as a result of NOS activation.

Abstract N° C9

Camp regulation of HERG K^+ channel expression

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The timing and rate of cardiac repolarization is governed by alterations of K^+ channels including the rapidly activating delayed rectifier current (IKr) which is produced by the HERG gene. cAMP signaling is likely to provide a link between stress and arrhythmias (hereditary long QT syndrome (LQTs) and acquired) by regulating of HERG K^+ channel activity. Although HERG currents regulated by cAMP have been studied extensively, little is known about its effect on channel proteins involved in trafficking and expression. In the present study, we found that cAMP increased HERG K^+ channel abundance dose dependently in HEK293 which stably expressed HERG K^+ channel. Treatment of other transfected ion channels such as KvLQT1, Kir2.1, Kv1.4 and Kv1.5 with the same CMV promoter with 50 μM cpt-cAMP for 24 h did not change their abundances, suggesting that the cAMP effect is specific to the HERG channel protein. To determine whether the effect of cAMP depends on de novo protein synthesis or not, we investigate whether the new synthesized nuclear factors are required for the effect of cpt-cAMP on the transcription of HERG gene. Treated with 500 μg/ml cycloheximide (CHX) for 30 min to inhibit protein synthesis did not abolish the effect of cpt-cAMP on HERG abundance. Mutation of PKA phosphorylation sites in HERG channel attenuated, but did not abolish, the effect of cAMP, suggesting that PKA phosphorylation of HERG K^+ channel may, at least partially, contribute to the channel trafficking and expression. Moreover, treatment of rabbit cardiac myocyte with cpt-cAMP for 6 h also significantly increased ERG/Ikr expression. H-89, a PKA inhibitor, significantly attenuated the effect of cAMP. These data suggested that elevation of intracellular cAMP level in cardiac myocyte may also alter cardiac ERG/Ikr expression. So in summary, in the present study we demonstrated for the first time that cAMP/PKA activity is a potential post-translational regulator of cardiac HERG expression.

Abstract N° C10

Role of I_{kur} for action potential shape and contractility in human atrial tissue from patients in atrial fibrillation or in sinus rhythm

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The ultrarapid outward current I_{Kur} is a major repolarizing current in human atrium and a potential target for selectively treating atrial arrhythmias. Here we have investigated the effects of selective block of I_{Kur} by low concentrations of 4-aminopyridine (4-AP) or the biphenyl derivative AVE-0118 on right atrial action potentials (AP) in trabeculae from patients in sinus rhythm (SR) or with chronic atrial fibrillation (AF). AP duration at 90% of repolarization (APD_{90}) was shorter in AF than in SR (300 ± 16 ms, $n = 6$ vs. 414 ± 10 ms, $n = 15$), whereas APD_{20} was longer (35 ± 9 ms in AF vs. 5 ± 2 ms in SR, $P < 0.05$ for both). Exposure of trabeculae to 4-AP, 5 μM, elevated the plateau phase to more positive potentials from –21 ± 3 to –6 ± 3 mV in SR and from 0 ± 3 to +12 ± 3 mV in AF The same concentration shortened APD_{90} from 414 ± 10 to 350 ± 10 ms in SR but prolonged APD_{90} from 300 ± 16 to 320 ± 13 ms in AF. AVE 0118 induced similar effects. Computer simulations of selective I_{Kur} block in human atrial APs predicted substantial secondary increases in $I_{Ca,L}$ and in the outward rectifiers I_{Kr} and I_{Ks} with smaller changes in AF than in SR. The indirect effect on $I_{Ca,L}$ was supported by a concentration-dependent positive inotropic response to 4-AP in the absence of direct effects on $I_{Ca,L}$.

In accordance with the model predictions block of I_{Kr} with E-4031 converted APD shortening effects of selective I_{Kur} block in SR into APD prolongation like in AF. We conclude that whether inhibition of I_{Kur} prolongs or shortens APD in human atria depends on the disease status of the atria and involves conductance of I_{Kr}.

Abstract N° C11

Transient opening of the mitochondrial K_{Ca} channel is protective in the human adult but not neonatal myocardium

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We have investigated whether transient exposure to NS1619 (a K_{Ca} opener) can protect both the human adult and neonatal myocardium and if K_{Ca} channels play a role in ischemic preconditioning (IPC). Right atrial trabeculae (n = 6/group), were obtained from adult (65 ± 12 years) and neonatal (<1 month) patients undergoing cardiac surgery. Trabeculae were isolated and superfused at 37 °C, field stimulated at 1 Hz, stretched to I_{max} and allowed to stabilise for 120 min. Ischemia was simulated by switching to hypoxic, substrate-free buffer and rapidly pacing at 3 Hz. Trabeculae were subjected to either 60 min simulated ischemia/120 min reperfusion (I/R) or this protocol preceded by IPC (3 min I/15 min R) or pretreatment protocols. Pretreatments included: NS1619 (30 μM) in the presence or absence of the K_{Ca} antagonist paxilline (PAX, 1 μM) or the antioxidant *N*-acetyl cysteine (NAC, 4 mM). Post-ischemic recovery of function (PIR; % baseline) was measured. In separate studies, mitochondrial flavoprotein oxidation (MFO) was measured as an indirect index of mitochondrial K_{Ca} channel opening. Following I/R alone, PIR was 19 ± 3% and 48 ± 7% in the adult and neonates, respectively. This was significantly ($P < 0.05$) improved in the adult by both IPC (PIR 70 ± 6%) and NS1619 (53 ± 4%) but not in the neonatal myocardium (51 ± 5% and 50 ± 3% with IPC and NS1619, respectively). In the adult both NAC and PAX, abolished this protection and NS1619 induced a reversible increase in MFO from 12 ± 3% to 64 ± 11%. Thus we have shown a novel mechanism by which the human adult but not neonatal myocardium can be 'preconditioned' by the transient activation of mitochondrial Ca-activated K channels using NS1619, a process not implicated in IPC. This protection appears to be mediated via mitochondrial oxidation and a free-radical-dependent mechanism. Understanding this pathway may provide alternate therapeutic options.

Abstract N° C12

Sex and strain differences in adult mouse cardiac repolarization: importance of androgens

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We previously demonstrated that compared to CD-1 male, both female and castrated male mice of the same strain display prolonged repolarization time associated with a specific decrease in the ultrarapid delayed rectifier K^+ current density that can be explained by a lower expression of Kv1.5. These data strongly suggested that gender differences observed in mice are mainly due to the action of androgens. Since previous studies have reported that male mice of the C57BL strain are considered chronically androgen deficient, we compared testosterone levels in different strains of mice and observed that effectively only the mice of the C57BL/6 strain had very low levels of testosterone; the others strains examined (FVB, C3H and CD-1) having normal levels of male sex hormones. We therefore took advantage of this particularity of the C57BL/6 mice to further confirm the role of male sex hormones in the regulation of ventricular repolarization. We verified that C57BL/6 male mice exhibit similar ventricular repolarization than female mice but delayed repolarization compared to the CD-1 male mice which have physiological levels of male sex hormones. Furthermore, we showed that androgen replacement in the C57BL/6 male mice as well as in the castrated CD-1 male mice fastens ventricular repolarization. Results obtained in this study provide strong evidence that androgen is a major regulatory factor of cardiac repolarization and that special attention should be paid to the hormonal status of the animal used when studying hormonal regulation of cardiac repolarization.

Abstract N° C13

Molecular mapping of the mammalian sinoatrial node

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The sinoatrial node (SAN) is known to be heterogeneous, and can be divided into centre and periphery. Our aim was to delineate these two regions, and to construct an anatomically and molecularly detailed 3-D model of the SAN. Techniques used were: (1) Fluorescent optical imaging for detection of the leading pacemaker. (2) Histological staining for detailed anatomy and cell orientation. (3) Immunocytochemistry, confocal imaging and quantitative real-time PCR for distribution of: (a) markers (neurofilament, NF-M, and atrial natuiretic peptide, ANP), (b) connexins responsible for electrical coupling (Cx40, Cx43, Cx45), (c) pacemaking channel subunits responsible for I_f (HCN1 and HCN4), (d) the Na^+ channel subunit responsible for I_{Na} ($Na_V1.5$), and (e) an inward rectifier K^+ channel subunit responsible for $I_{K,1}$ (Kir2.1). (4) Computer modeling. Our results show that in the centre, cells are not uniformly arranged (as compared to atrial muscle), and they do not express ANP, Cx43 and $Na_V1.5$, but they do express NF-M, HCN1 and HCN4. In the centre, Cx45 is highly, but Cx40 is poorly, expressed. In the periphery, there is intermingling and interdigitation of SAN and atrial cells, and ANP is not expressed, but NF-M, Cx40,

Cx43, Cx45, $Na_V1.5$, HCN1 and HCN4 are expressed. Our preliminary data also show that Kir2.1 is expressed in the periphery, but not in the centre. The regional differences in the distribution of connexins and ion channels reflect the different functions of the SAN: the function of the centre is to be the leading pacemaker, and the function of the periphery is to drive the atrial muscle.

Abstract N° C14

I_f channel inhibitor ivabradine lowers heart rate in mice with enhanced sympathoadrenergic activities

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Ivabradine selectively reduces heart rate (HR) by inhibiting the cardiac pacemaker I_f current, thus prolonging the duration of spontaneous depolarisation in the sinus node. HR-reducing activity of ivabradine under conditions of an enhanced sympathoadrenergic drive were investigated by testing the effects of repeated oral administration in mice with sympathoadrenergic activation due to restrain-associated stress, cardiac-restricted overexpression of β_2-adrenergic receptors (β_2AR), or β-agonist administration. HR and left ventricular fractional shortening (FS) were determined by echocardiography. Initial experiments showed that the conscious restrained state was associated with stress-mediated sympathetic activation whilst sympathetic withdrawal occurred under anesthetized conditions. In non-transgenic (NTG) mice, oral treatment with ivabradine lead to dose-related increase in the plasma level of ivabradine. Ivabradine at 10 and 20 mg/kg/d reduced HR by about 10–20% under both conscious (stressed) and anaesthetised states while FS was unchanged by the treatment. Ivabradine at 10 and 20 mg/kg/d was similarly effective in reducing HR by about 15% in the β_2AR transgenic (TG) mice. Further, in anesthetized NTG mice, ivabradine at 10 mg/kg/d reduced the maximal HR increase in response to the β-agonist isoproterenol at 4 μg/kg i.p. (428 ± 26 vs. 580 ± 15 bpm, $P < 0.01$) without modifying the response of contractile parameters (FS: 55 ± 2% vs. 61 ± 2%, P = NS). These data indicate that oral administration of ivabradine in mice reduces HR while ventricular performance is maintained. This specific HR reducing action of ivabradine is well preserved under conditions that are associated with significant activation of the sympathoadrenergic system.

Abstract N° C15

Signal-averaged P-wave after cardioversion of atrial fibrillation: a marker of electrophysiological remodeling

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Prolonged P wave in the P wave signal-averaged electrocardiogram (PSAECG) may indicate an impaired intra- and interatrial conduction. The aim of the study was to evaluate the influence of the duration of an atrial fibrillation (AF) episode on the parameters of PSAECG just after the cardioversion (CV) to sinus rhythm and the relation of the duration to the basic echocardiographic parameters. We evaluated 38 consecutive patients (29 months, age 62 ± 12 years) after successful electrical CV of AF persistent more than 1 month (Group A) and 22 patients (16M, age 63 ± 14 years) after CV of acute AF with duration less than 48 h (Group B). We measured the signal-averaged P wave duration (SA-P) and the root mean square voltage of the terminal 20 ms of the signal-averaged P wave (RMS 20), using MAC 5000, Marquette. The measurements were performed 0.5–2 h after CV. Left atrial diameter (LAD) and left ventricular ejection fraction (LVEF) were evaluated by 2D-echocardiography.

	Group A	Group B	*P value*
LAD (mm)	44.8 ± 6.2	37.3 ± 5.1	<0.001
SA-P (ms)	173.3 ± 46.8	132.2 ± 21.3	<0.001
RMS 20 (ms)	3.2 ± 1.8	4.1 ± 3.2	NS
Noise (uV)	0.48 ± 0.21	0.46 ± 0.16	NS

Both groups were clinically comparable in the LVEF, amiodarone treatment and presence of mitral valve disease. Longer duration of an AF episode leads to longer duration of the P wave in PSAECG after the CV, what can reflect more profound remodeling of the atria caused by AF. Question remains, whether SA-P is an independent variable or if it reflects greater diameter of the left atrium after longer episodes of AF.

Abstract N° C16

Drug-induced ventricular defibrillation

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Ventricular fibrillation (VF) is a menacing and often fatal arrhythmia that requires instant defibrillation. Presently, the sole method of defibrillation is electric shock. We searched for alternative means, using young domestic pigs, and focussed on drug-induced ventricular defibrillation (DVD). Landrace pigs aged 5–10 d (n = 12) were anaesthetized with pentobarbitone, i.v. and ventilated. Following sternotomy, the heart was exposed in a pericardial cradle. ECG and aortic pressure were recorded. VF was induced by bipolar electrical stimulation of the ventricular epicardium (100 Hz, 15 V, 0.1 ms duration). Three conditions were studied, A: no treatment, B: intravenous infusion of vehicle and C: infusion of a drug mix, consisting of dibenzepine (7.5 mg/kg) and adrenaline (2.5 μg/kg) dissolved in 2 ml isotonic saline. Vehicle or drug were infused 15–75 s after the onset of VF and distributed by cardiac massage. In control experiments with or without infusion of vehicle, none of the animals survived. As VF ensued, blood pressure fell precipitously and never recovered. However, demise could be consistently averted by

infusing a defibrillatory drug mix consisting of dibenzepine and adrenaline. During and following drug infusion, ventricular electrical activity slowed and synchronized until VF ceased and sinus rhythm resumed. Electric shock was not required. In the wake of DVD, the VF threshold increased markedly. When VF was induced repeatedly in the same pig, it was of short duration and sinus rhythm ensued spontaneously within 2–3 min. DVD succeeded in all pigs studied. The present study provides the first report of consistent DVD in young domestic pigs, indicating that VF is a potentially reversible condition. Complementary experiments revealed that the prototypic drug regimen, consisting of the tricyclic antidepressant dibenzepine and adrenaline, may promote spontaneous VD by increasing sarcoplasmic reticulum Ca^{2+} uptake.

Abstract N° C17

Long term follow up of patients undergoing electrophysiological study on amiodarone

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We conducted a retrospective study comparing outcomes in patients who were inducible vs. non-inducible for ventricular tachycardia (VT) at electrophysiological study (EPS) whilst on amiodarone. EPS involved one to three extrastimuli at two right ventricular sites during two paced cycle lengths. Episodes were defined as documented VT/ventricular fibrillation (VF), appropriate defibrillator therapy or sudden cardiac death. Mean follow up was 48 months. Thirty-four patients had inducible VT at EPS and 15 patients were non-inducible; 30/34 patients in the inducible group received an automatic cardioverter defibrillator as did 3/15 patients in the non-inducible group, two of whom had episodes. There was no significant difference between the two groups in amiodarone dose, aetiology of cardiac disease, left ventricular function, or angiotensin converting enzyme inhibitor use. Beta blocker use was higher in inducible VT group ($P = 0.47$). The inducible VT group had significantly higher incidence of episodes than the non-inducible group (27/34 patients vs. 3/15 patients, $P < 0.001$). Sudden cardiac death occurred in one patient in the non-inducible group. There was no difference in mortality between the two groups (8/34 inducible patients died vs. 3/15 non-inducible patients). In patients on amiodarone inducible VT is a good predictor of future episodes. Non-inducibility defines a group at lower risk of future arrhythmic events.

Abstract N° C18

Nickel and ryanodine effects on sinus venosus pacemaker activity of the heart in young and adult frogs

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The aim of the work was to investigate the role of current via Na–Ca exchangers and sarcoplasmic reticulum (SR) in the modulation of generation rate of action potentials (APs) in the cells of sinus venosus of young (3-months old, group I) and adult (>1.5-years old, group II) frogs, *Rana temporaria.* Comparative analysis of the electrophysiological parameters of AP cells showed that pacemaker cells of sinus venosus differ according to frequency: 57 ± 4 beats per minute (bpm, group I, $n = 15$) and 44 ± 3 bpm (group II, $n = 14$) at 20 °C. In a control solution, the amplitude of APs cells in the adults was larger by 20 mV, threshold potential was larger by 16 mV and diastole duration (DD) was 21% longer than in young frogs ($P < 0.05$). Nickel (100 μmol/l) did not change the rate of spontaneous AP generation in the adult hearts. IC_{50} was observed at 200 ± 50 μmol/l ($n = 7$) and a block of AP was observed at Ni^{2+} equal to 400 ± 50 μmol/l ($n = 6$). In the young, IC_{50} for Ni^{2+} was registered at 500 ± 50 μmol/l according to a 15 min exposure. After 25 min of exposure, ryanodine (Ry, 3 μmol/l) decreased the frequency of AP generation in the adults by 43% and the young ones showed an 11% increase compared to the control. Negative chronotropic effect in adult frogs was observed due to the increase of DD by 46% and DD velocity reduction from 16 to 7 mV/s. Increase of the external Ry concentration (20 μmol/l) completely blocked APs generation in the adults at the 7th min of exposure. In young frogs, the decrease of AP rate by 5% was registered at the 25th min. It was followed by the increase of APs amplitude by 15 mV, APD_{90} showed a 19% decrease and DD increased by 18% in the average. To conclude, in sinus venosus of the young current via Na–Ca-exchanger channels and Ca^{2+} releasing from SR are less dominating factors in the modulation of AP generation rate than in adult frogs.

Work was supported by Russian Fund BR project 01-04-49668.302.

Abstract N° C19

GLUT1 and GLUT4 localisation by immunogold electron microscopy

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Western blotting provides the relative distribution of transporters between endosomal and plasma membrane fractions, while immunogold electron microscopy enables the exact localisation of the transporters within the tissue to be visualised. Hearts ($n = 3$) were perfused with Krebs buffer for 2 h. Western blots of GLUT1 and GLUT4 showed relative distributions of 86.3 + 6.4% and 28.5 + 8.3% (mean + SEM) in plasma membranes, respectively. Such data have previously been assumed to represent sarcolemmal GLUT content. A second group of hearts ($n = 3$) was perfused for 2 h and then fixed by perfusion with 2% formaldehyde + 0.2% glutaraldehyde. Cryosections of 70 nm were cut and double labelled with primary antibodies against GLUT1 and GLUT4, followed by secondary antibodies with 10 and 15 nm gold particles attached for each transporter, respectively. Labelling was quantified using stereological techniques

to count gold distribution relative to membrane length or compartment size.

	Location	Labelling density (mean + SEM, *n = 3)*
GLUT1	Capillary membrane	0.43 + 0.09 (golds μm^{-1})
	Sarcolemma	0.19 + 0.07 (golds μm^{-1})
	T-tubule membrane	0.04 + 0.02 (golds μm^{-1})
GLUT4	Capillary membrane	Non-detectable
	Sarcolemma	0.12 + 0.09 (golds μm^{-1})
	T-tubule membrane	0.07 + 0.01 (golds μm^{-1})
	Membrane-associated vesicles	3.16 + 0.42 (golds μm^{-2})

From the data above, we have shown that 65% of GLUT1 resides in the capillary plasma membrane, where GLUT4 is absent; GLUT4 was mainly found in vesicles associated with the T-tubules. These data suggest that, in heart, GLUT1 is responsible for the transport of glucose from the bloodstream into the interstitium; GLUT4 and the remaining GLUT1 are responsible for the transport of this glucose into the myocyte via the T-tubules and sarcolemma.

Abstract N° C20

Effect of clenbuterol treatment on cardiac contractility in rats

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The β_2-adrenoceptor agonist clenbuterol has a marked anabolic effect in skeletal muscle caused by protein accretion. However, in cardiac muscle, clenbuterol has been shown to cause cardiac hypertrophy, but the functional changes in cardiac contractility have not been reported previously. Male Wistar rats (commencing at 80 g body weight) were administered clenbuterol in feed at a rate of 25 mg/kg feed for 10 d. This dose caused an increased body growth rate, ventricular hypertrophy and tachycardia relative to control rats ($P < 0.05$). In tissue bath studies, the isolated right atria had a higher basal rate of contraction and lowered potency to (–)-isoprenaline ($P < 0.05$) indicative of β-adrenoceptor downregulation. In the left atria, (–)-isoprenaline had a lower potency, but a higher efficacy paralleled by an increased efficacy to calcium ($P < 0.05$) relative to left atria in control rats. In contrast, left ventricular papillary muscles from clenbuterol treated rats showed a lowered potency and efficacy to (–)-isoprenaline, accompanied by a reduced efficacy to calcium ($P < 0.05$). Normalisation of the (–)-isoprenaline responses relative to the calcium responses revealed no difference in the efficacy of (–)-isoprenaline between clenbuterol treated and control rats. In conclusion, clenbuterol causes differential regulation of contractility in different cardiac chambers with enhance atrial, but impaired ventricular contractility.

Abstract N° C21

Effects of interleukin-2 on the mechanical restitution and post-rest contraction in rat ventricular papillary muscle

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Previous studies demonstrated that interleukin-2 (IL-2) has negatively inotropic effect on cardiomyocyte. To determine whether application of IL-2 alters function of sarcoplasmic reticulum, we measured mechanical restitution and post-rest potentiation in isolated rat papillary muscles, which were paced at 0.5 Hz. Mechanical restitution curves were constructed by interpolating extra-systoles at different test intervals following a train of steady-state beats. Post-rest potentiation was obtained after pause of 5, 10, 30, 60, 120 and 300 s. In control group, the maximal post-rest potentiation was reached after 60–120 s of rest and the maximal potentiation factor was 2.36 ± 0.23. IL-2 at 200 U/ml decreased the steady-state force of contraction to 56.4 ± 7.2% of pre-drug control. But the time constant of recovery of steady-state force was not altered following administration of IL-2. Post-rest potentiation in relation to the pre-rest control value in the presence of IL-2 was similar to that in the absence of IL-2, but the potentiation was enhanced when compared with the pre-drug control value. In papillary muscle treated with IL-2, the maximal post-rest potentiation occurred later and the potentiation factor after 300 s was 4.72 ± 0.58 times that at the steady state. Recirculation fraction of calcium which was calculated from the decay of post-rest potentiation was 0.78 ± 0.09 in control and 0.59 ± 0.08 after IL-2 treatment. We conclude that IL-2 decreases the function of sarcoplasmic reticulum, which suggests that an impaired function of sarcoplasmic reticulum may contribute to the negative inotropic effect of IL-2.

Abstract N° C22

Involvement of calcium, calmodulin and PLC in the epidermal growth factor-induced activation of Ral-STAT3 pathway in smooth muscle cells

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Epidermal growth factor (EGF) activates c-Src through the Ras/RalGEF/Ral pathway, leading to activation of STAT3. Since Ral activation is partly Ca^{2+}/calmodulin-dependent, we used rat aorta smooth muscle cells (A7r5) to study if EGF-induced activation of Ral, c-Src and STAT3 is Ca^{2+}/calmodulin-dependent, and if Ca^{2+}/calmodulin-regulated Pyk-2 is involved in this pathway. Pull-down of activated Ral showed that the EGF-induced activation of Ral is Ca^{2+}-, calmodulin- and PLC-dependent in A7r5 cells.

Immunoblotting of cell lysates with antibodies to phosphorylated Pyk-2, c-Src and STAT3 showed that Pyk-2 is activated by EGF, and that EGF-induced activation of Pyk-2, c-Src and STAT3 in A7r5 cells depends on calmodulin and PLC, but only minimally on calcium. Therefore, Pyk-2 is involved in novel signal transduction pathways, and calmodulin plays an essential role in growth factor-mediated stimulation of STAT3-regulated gene transcription.

Supported by CIHR.

Abstract N° C23

The response of the neonatal cardiac myocyte to endotoxin exposure

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Exposure of the heart to bacteriological agents, exemplified by endotoxin, leads to myocyte and cardiac dysfunction and death. The mechanisms involved in this response involve the induction of inflammation with subsequent cardiac and whole body responses necessary to resolve the inflammatory response. We have observed that the neonatal cardiac myocyte has an innate resistance to cell death and accompanying cardiac dysfunction associated with lipopolysaccharide (LPS) exposure. Exposure of neonatal rat cardiac myocytes to low, non-toxic doses of endotoxin leads to a rapid degradation of IκBα and a translocation of NFκB to the nucleus where it can activate cardioprotective genes like iNO synthase. Production of TNFα by the myocytes is also increased in response to endotoxin. Activity of cyclooxygenase-2 (COX-2) increases rapidly and falls to baseline within 2 h. Lipoxygenase (5-LOX) rapidly falls in the neonatal myocyte in response to endotoxin. Thus, there appears to be a limited, rapidly resolved, inflammatory response to endotoxin by the neonatal cardiac myocyte. The role of cytochrome P450 in detoxifying pro-inflammatory prostaglandins is well known and has been implicated in protective mechanisms following endotoxin exposure of other tissues. Cytochrome P450s of the CYP4 gene subfamily are functionally conserved and selective for omega hydroxylation of arachidonic acid, synthesizing the biologically actives HETEs and EETs. We have observed that expression of CYP4F isoform increases in the neonatal rat cardiac myocyte in response to endotoxin (50-fold within 1.5 h) showing a temporal pattern inverse to that observed for COX-2 and 5-LOX suggesting that CYP4F is inactivating inflammatory leukotrienes and prostaglandins.

Supported by the US Army (TATRC) DREAMS project.

Abstract N° C24

Modulation of human endothelial cell function by HIV-1 protease inhibitors

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HIV-1 protease inhibitors (PIs) as part of the highly active antiretroviral therapy (HAART) have been shown to induce insulin resistance and cardiovascular diseases (CVD) in HIV-1-infected patients. However, the molecular mechanisms involved in PI-induced endothelial dysfunction have not been delineated. We have hypothesized that PI-induced inhibition of insulin signaling can decrease eNOS gene expression and production of NO coupled with an increase in ROS production within the vasculature, which might play a significant role in chronic endothelial injury. Furthermore, we have hypothesized that the presence of inflammatory cytokine, TNFα, associated with HIV-1 infection, exacerbates the PI-induced effects. A combination of indinavir, zidovudine and efavirenz at therapeutic concentrations induced a significant decrease in eNOS gene expression in human aortic endothelial cells (HAECs) within 24 h. This inhibition was synergized in the presence of TNFα (0.2 U/ml) and was reversed upon stimulation with insulin (100 mU/ml). The intracellular NO levels were decreased by approximately 80% in the presence of TNFα (1 U/ml). Indinavir alone or in HAART combination induced leukocyte recruitment (1.5–2.5-fold) and increased cell surface expression (2–5 fold) of cell adhesion molecules (CAMs). CAM-specific antibodies inhibited TNFα-induced endothelial-leukocyte adhesion. These data suggest that the pathogenic consequences of endothelial cell activation and leukocyte infiltration due to inhibition of NO production can result in a local inflammatory response leading to chronic endothelial injury and CVD in HIV-1-infected patients.

Abstract N° C25

Effect of roxithromycin in patients following coronary angioplasty and stenting: a case control study

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Chronic inflammation is the underlying pathologic process in atherosclerotic coronary artery disease (CAD). Various infectious agents are found to be associated with chronic inflammation—*Chlamydia pneumoniae* is one among them. We studied the 6 months clinical and angiographic follow-up of CAD patients, who underwent coronary angioplasty ± stenting and treated with roxithromycin—an anti-*Chlamydia* antibiotic. Thirty patients each of acute coronary syndrome, who had at least two coronary lesions on coronary angiography—one is ≥70%, dilated with angioplasty ± stenting and other <70%, no intervention done—were included in both case and control groups. All patients in case group were given roxithromycin 150 mg twice a day for 6 weeks, in addition to conventional treatment, while in control group no antibiotic was given. At 6 months of follow-up, all patients were assessed for acute coronary syndrome, stent restenosis and progression of insignificant coronary lesions. Twenty-seven cases and 30 controls underwent follow-up coronary angiography at 6 months. Three patients in case group were excluded from the study—two had side effect of roxithromycin and one died on day 3 of myocardial infraction (MI) because of ventricular septal disease (VSD).

	≥70% Lesions	<70% Lesions	ACS at 6 months (% patients)	Stent restenosis (% stents)	>0.4 mm progression in <70% lesions (%)
Case *n = 27*	38 lesion 36 stent 1 PTCA	41 lesion	4 (14.8%)	9 (25%)	11
Control *n = 30*	43 lesion 40 stent 2 PTCA	40 lesion	10 (33.3%)	11 (27.5%)	15
P value	–	–	>0.1	>0.1	>0.1

Six weeks of roxithromycin therapy has favorable but insignificant effects on ACS at 6 months of follow-up in patients, who underwent angioplasty ± stenting with no significant effect on stent restenosis or lesion progression. The favorable effect may be a result of plaque stabilization.

Abstract N° C26

Study of invasiveness of Group A *Streptococcus* in epithelial and endothelial cells: proposal for a novel in vitro model

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Attachment of Group A Streptococci (GAS) to the host cell is the most important step in colonization of understanding of pathogenic mechanisms in the disease. Our study was carried out with some rheumatogenic strains of GAS and their interaction with epithelial cell line, HEp-2, and the endothelial cell line, Endo. The adhesion assay was performed by incubation of bacteria on monolayer of the cells, which were then lysed and plated to look for colonies formed by adhering bacteria. The invasion assay was performed similarly except for the treatment with antibiotics to remove adhering live bacteria. The GAS are capable of invading endothelial cells several times more than the epithelial cells. These observations are being given a new direction by creating a new experimental model of rheumatic heart disease in vitro. Porcine aortic/pulmonary valves are being acellularized by using Triton-X. Endothelial cells as well as myofibroblast cells will then be isolated in parallel from human saphenous veins and expanded in vitro. Specimens of the surface of the acellular matrix will be seeded with myofibroblast cells and endothelial cells. The tissue engineered valve will then be cultured with GAS, sensitized serum and lymphocytes of acute rheumatic fever/rheumatic heart disease patients to see the pathological lesions.

Abstract N° C27

Seroprevalence of anti-*Chlamydia pnemoniae* and anti-*Helicobacter pylori* IgG antibodies in coronary artery disease patients

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Recent studies have shown the association of *Chlamydia pnemoniae* (CP) and *Helicobacter pylori* (HP) infection with coronary artery disease (CAD). We correlated the seroprevalence of IgG antibody titers for CP and HP in angiographically proven CAD cases and compared it with controls. IgG antibody titers were determined by ELISA method for HP in 90 cases and 30 controls, and also for CP in 75 cases and 15 controls. Both the groups were matched for demographic variables and conventional CAD risk factors. HP group: the IgG titer for HP was positive in 42.2% cases and 23.3% controls. The difference was found to be marginally significant ($P = 0.06$) with likelihood ratio of 3.60. Multifactorial logistic regression analysis after considering conventional CAD risk factors showed statistically significant ($P < 0.04$) association with correlation coefficient of 1.11 and odds ratio of 3.05. CP group: the IgG titer for CP was positive in 52.0% cases and 20.0% controls. The difference was statistically significant ($P = 0.02$) with likelihood ratio of 5.5. Multifactorial logistic regression analysis after considering conventional CAD risk factors showed statistically significant ($P = 0.03$) association with correlation coefficient of 1.52 and odds ratio of 4.58. The seropositivity in both groups was not related with the type of clinical presentation like unstable angina, chronic stable angina and acute myocardial infarction. Logistic regression analysis between two groups showed independent association of anti-CP and anti-HP antibodies with CAD (correlation coefficient 0.03, $P = 0.78$). Serologically, infection with HP and CP is significantly and individually associated with CAD.

Abstract N° C28

Identification of genes associated with persistent atrial fibrillation using oligonucleotide microarrays

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High-density microarrays capable of evaluating thousands of mRNAs are ideal for transcriptome analysis and identification of genes implicated in disease. Our aim was to compare the gene expression pattern in right atria of patients suffering from persistent atrial fibrillation (AF) (>6 months, $n = 17$) to that of patients in sinus rhythm (SR, $n = 20$). Right atrial appendages were obtained during cardiac surgery and total RNA was isolated and subsequently converted into cDNA. Then, Cy3- and Cy5-labeled cRNA was generated from both patient groups and hybridized to Pan 10K microarrays (MWG Biotech, Germany; with dye reversal), contai-

ning 9850 gene-specific oligonucleotides. We have identified differential expression of certain key genes previously found to be linked with persistent AF as well as new, potentially important genes involved in AF pathophysiology. The most intriguing changes among the upregulated genes in AF atria were observed as with genes associated with extracellular matrix remodeling (collagen genes COL3A1 and COL1A2, MMP-9, osteopontin), cell survival (BH3 interacting domain death agonist; serine/threonine kinase 17a apoptosis inducing; secreted frizzled-related protein 4), the contractile apparatus (myosin genes MYH7 and MYL3) and the CXC-chemokine receptor 4. Among the downregulated genes were several interferon-stimulated proteins (ISG15, IFIT1, IFIT4, MX1), protein kinase isoforms (myosin light chain kinase isoform 6; protein kinase c epsilon) and MMP-25. To independently validate expression ratios obtained by microarrays, a subset of genes was selected to measure relative expression levels by semiquantitative RT-PCR, which confirmed the array results. This is the first study to define at the global level the transcriptome profile of atrial tissue in patients with persistent AF and provides new insights into the pathophysiology of this most common arrhythmia.

Abstract N° C29

Function of ADP-ribosylation factor 1 gene in cardiac hypertrophy

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ADP-ribosylation factor 1 (ARF1) is a member of small G-protein family that plays a role in vesicular trafficking. In the present study, we showed that the expression of the ARF1 was increased in the hypertrophied hearts caused abdominal aortic banding, an in vivo model for cardiac hypertrophy. To understand the biological significances of such increase, the *ARF1* gene was over-expressed in cardiomyocytes by an adenoviral vector. We demonstrated that the over-expression of *ARF1* gene promoted the cardiac myocytes growth by increases of total protein contents and cardiomyocytes areas, increased expressions of PKC-ϵ, PKC-β1 and PKC-α, and activated the proliferation of cardiac fibroblasts without stretch. Such effects were further augmented when stretch was applied. On the other hand, the opposite effects were observed when the ARF1 expression was decreased by antisense against ARF1, or when ARF1 activity was prohibited by brefeldin A treatment. The expressions of PKC-β2, PKC-δ, ERK1/2, JNK1/2, p38, p53, Bax, Bcl2 or Fas were not affected by over-expression or down-regulation of ARF1. Thus, our data suggested that ARF1 promoted cardiomyocytes growth via the branches of PKC signaling pathway, likely, PKC-ϵ, PKC-β1 and PKC-α, during cardiac hypertrophy.

Abstract N° C30

Arrhythmogenic effects of arsenic trioxide in patients with acute promyelocytic leukemia and electro-physiological study in isolated guinea pig papillary muscles

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Arsenic trioxide (As_2O_3) is a new promising regimen for relapsed acute promyelocytic leukemia (APL). Poisoning of As_2O_3 has been shown to cause arrhythmia, such as torsade de pointes (TdP). This study was carried out in order to evaluate an incidence of arrhythmias during As_2O_3 therapy, and clarify the mechanism of arrhythmogenesis. ECGs were monitored throughout As_2O_3 therapy in 20 patients. As_2O_3 (0.15 mg/kg) increased the QTc interval (445 ± 7 to 517 ± 17 ms, $P < 0.01$), the QTc dispersion (43 ± 4 to 73 ± 9 ms, $P < 0.01$) and the transmural dispersion of repolarization (110 ± 5 to 122 ± 6 ms, $P < 0.05$). Non-sustained ventricular tachycardias and a TdP were observed in five and one patients, respectively. Action potential and isometric contraction were measured from guinea pig right ventricular papillary muscles. As_2O_3 (350 μM) prolonged action potential duration (APD_{90}: 150 ± 11 to 195 ± 12 ms, $P < 0.01$, $n = 5$) and increased developed tension (28 ± 8 to 43 ± 14 mg, $n = 5$). APD prolongation showed the dose- and reverse-use dependency. In addition, prolonged exposure to As_2O_3 caused rigor contracture, after contraction, triggered activity and electro-mechanical alternans. Lowering extracellular K^+ concentration (3.0 mM) and lowering rate of stimulation rate (0.1 Hz) caused marked prolongation of APD, leading to early after depolarization and triggered activity. Tetrodotoxin (3 μM) and butylated hydroxytoluene (50 μM), an inhibitor of lipid peroxidation, prevented APD prolongation partially. The prolongation of APD and Ca^{2+} overload by As_2O_3 can explain the prolongation of QT interval and arrhythmogenesis. The effects of APD prolongation by As_2O_3 might be, at least, due to block I_{kr} and increase I_{Na} for its reverse-use dependent effect and inhibition by tetrodotoxin, respectively. The lipid peroxidation could be responsible for these effects.

Abstract N° C32

Modulation of doxorubicin-induced cardiac dysfunction in toll-like receptor-2 knockout mice

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Toll-like receptor-2 is involved in inflammatory response and expressed in the heart. Recent studies have demonstrated that TLRs are activated by endogenous signals such as oxidative stress, which contributes to doxorubicin (Dox)-induced cardiac dysfunction. Thus, we hypothesized that TLR-2 had potential role for pathogenesis in Dox-induced cardiac dysfunction. Myocardial lipid peroxidation was significantly increased after Dox (Dox 20 mg/kg, intraperito-

neally injection), but no significant difference was found between WT and KO mice. After Dox-injection, NF-kappaB activation was decreased by 80 % in KO mice compared with WT mice. production of pro-inflammatory cytokines was attenuated in KO mice. Numbers of TUNEL positive nuclei and Dox-induced caspase-3 activation were decreased by 36.1 % and 26.7 %, respectively ($p < 0.01$), in KO mice comprared with WT mce. Cardiac function evaluated by echocardiography was preserved in KO mice compared with WT mice. Consequently, survival rate was significantly higher in KO mice than in WT mice 10 days after Dox treatment (11 % vs. 46 %, $p < 0.05$). These findings suggest that toll-like receptor-2 may play an important role in the regulation of inflammatory and apoptotic mediators in the heart following doxorubicin.

	WT-Con	WT-Dox	KO-Dox
Interleukin-6 (pg/mg protein)	1.0 ± 1.0	11.2 ± 3.3 **	2.0 ± 0.4 ##
TNF-alpha (pg/mg protein)	0.2 ± 0.2	3.0 ± 0.4 **	1.4 ± 0.8 * ##
Fractional shortening (%)	40.0 ± 0.4	27.7 ± 1.0 **	34.1 ± 1.6 ** ##

Data were expressed mean ± SEM. * $P < 0.05$, ** $P < 0.01$ vs WT-Con. # $P < 0.05$, ## $P < 0.01$ vs WT-Dox.

Abstract N° C33

Doxorubicin-induced cardiac dysfunction: the nitric oxide-peroxynitrite-poly(ADP-ribose) polymerase connection

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Dysregulation of nitric oxide and increased oxidative stress have been implicated in the cardiotoxicity of doxorubicin (DOX), a commonly used antitumor agent. Peroxynitrite is a reactive oxidant produced from nitric oxide and superoxide in various forms of cardiac injury. Oxidant-induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP), which in turn contributes to cardiac and vascular dysfunction in various pathophysiological conditions including diabetes, reperfusion injury, heart failure and circulatory shock. Recently we have demonstrated increased oxidative and nitrosative stress, metalloproteinase and PARP activation in the heart following DOX administration. Here we show the effect of a novel peroxynitrite decomposition catalyst and ultrapotent PARP inhibitor, on DOX-induced cardiac dysfunction. Mice received a single injection of DOX (25 mg/kg i.p.) or three equal doses of 9 mg/kg every 10 d. Left ventricular function was measured 5 or 25 d after DOX administration using Millar's new Aria pressure–volume conductance system. Both acute and chronic administration of DOX-induced high mortality and a significant decrease in mean BP, left ventricular systolic pressure, $+dP/dt$, $-dP/dt$, stroke volume, stroke work, ejection fraction and cardiac output. Treatment with a novel peroxynitrite decomposition catalyst or ultrapotent PARP inhibitor reduced DOX-induced mortality and markedly improved cardiac function. Peroxynitrite neutralization or PARP inhibition did not interfere with DOX's antitumor effect. Thus, peroxynitrite and PARP activation plays a key role in the pathogenesis of DOX-induced cardiac failure. Targeting peroxynitrite formation or PARP may represent a new cardioprotective strategy after DOX exposure, or in other conditions which are associated with peroxynitrite formation and PARP activation, including myocardial ischemic/reperfusion injury.

Abstract N° C34

A novel ultrapotent poly(ADP-ribose) polymerase inhibitor improves cardiac and endothelial dysfunction associated with aging-induced heart failure

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Increased production of reactive oxygen and nitrogen species has recently been implicated in the pathogenesis of cardiac and endothelial dysfunction associated with atherosclerosis, hypertension and aging. Oxidant-induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP), which in turn contributes to cardiac and vascular dysfunction in various pathophysiological conditions including diabetes, reperfusion injury, circulatory shock and aging. Here we investigated the effect of a novel ultrapotent PARP inhibitor, INO1001, on cardiac and endothelial dysfunction associated with aging using Millar's new Aria pressure–volume conductance system and isolated aortic rings. Young (3-months old) and aging (24-months old) Fischer rats were treated for 2 months with vehicle or the ultrapotent PARP inhibitor INO1001. In the vehicle-treated aging animals there was a marked reduction of both systolic and diastolic cardiac function and loss of endothelial relaxant responsiveness of aortic rings to acetylcholine. Treatment with INO1001 remarkably improved cardiac performance in aging animals and also Ach-induced, NO-mediated vascular relaxation. In addition to the beneficial effects of chronic treatment with PARP inhibitor, 1-h in vitro incubation of aortic rings from aging rats with INO1001 was also able to improve the endothelial dysfunction. Thus, pharmacological inhibition of PARP may represent a novel approach to improve cardiac and endothelial dysfunction associated with aging.

Abstract N° C35

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Thus, pharmacological inhibition of PARP may represent a novel approach to improve cardiac and endothelial dysfunction associated with aging.

Abstract N° C36

Prognostic value and pathologic correlation of echocardiographic indices in mice with cardiomyopathy

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Echocardiography is commonly used in studies on mice but its prognostic value and correlation with morphometric measures are yet to be assessed. We studied male non-transgenic (NTG, $n = 26$) and transgenic (TG $n = 72$) mice with cardiomyopathy due to cardiac overexpression of b_2-adrenergic receptors (b_2AR) by performing echocardiography at 12 months of age and then monitor them for survival for a further 3-month period, a time period when heart failure (HF) and premature death occur in this model. We determined left ventricular (LV) dimension (r), fractional shortening (FS), wall thickness (h), LV mass and Doppler-derived aortic flow (AF) velocity and cardiac output. Autopsy was performed to determine organ weights and pathological events. HF was considered by chronic atrial thrombi, chest fluid accumulation and increased wet weights of lungs, atria and the right ventricle (RV). Weights of lungs, RV and atria were correlated well with each other ($r = 0.79$~0.83). TG mice had reduced FS (0.22 vs. 0.36, $P < 0.01$) and LV dilatation (diastole: 5.0 vs. 4.2 mm, systole: 4.0 vs. 2.7 mm, both $P < 0.01$). During the 12–15-month period, none of NTG but 71% of TG animals died of either HF or non-HF reason (arrhythmia). To explore the use of echo indices in predicting survival and HF death, TG mice were re-grouped based on various echo indices. Using the ratio of (FS × (h/r)), we identified a subgroup of mice with the lowest ratio (1.5 ± 0.4 vs. 6.2 ± 1.7 in NTG, mean ± S.D.) and the highest total mortality and HF death. M-mode-derived parameters (FS, r) were correlated well with weights of RV, atria and lungs ($r =$ 0.48~0.66) whereas correlations between the organ weights and Doppler AF parameters were poor. The correlation between M-mode and AF-derived indices was in general poor. In conclusion, M-mode echo parameters are able to identify cardiomyopathy mice with severe HF and poor prognosis but Doppler flow measurements have limited value.

Abstract N° C37

Effects of ACEI and ARB on changes in dystrophin-related glycoproteins in the failing heart

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Dystrophin-related glycoproteins (DRG) are considered to play an important role in the regulation of myocardial contractility. Recently, we have shown that a-sarcoglycan (SG) and dystrophin (DSP) of the viable left ventricle (LV) in the failing rat heart decreased after myocardial infarction (MI). In the present study, effects of an angiotensin I-converting enzyme inhibitor (ACEI) trandolapril (Tra) and an angiotensin II type 1 receptor blocker (ARB) candesartan (Can) on the changes in DRG were examined. MI was induced by permanent occlusion of the left coronary artery. Tra (3 mg/kg/d) or Can (1 mg/kg/d) was administered from the 2nd to 8th week after MI. Long-term treatment with Tra or Can improved hemodynamic parameters of the MI rat at the 8th week, associated with preservation of a-SG and DSP. Other SGs in the viable LV did not alter throughout the experiment, regardless of treatment with or without these agents. MI also induced a sustained increase in both m- and m-calpains. Tra and Can attenuated increases in these proteases of the viable LV after MI. In contrast, Tra and Can did not affect mRNA expression of DRG. m-calpain activity of cytosolic fraction of the viable LV increased. Tra and Can attenuated MI-induced increase in the protease activity. These results suggest that ACEI and ARB preserve myocardial DRG after MI via an attenuation of the increase in protease activity in the failing heart, leading to an improvement of cardiac contractile function after MI.

Abstract N° C38

Pitavastatin inhibits cardiac hypertrophy in rat model of progressive renal injury

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Cardiac hypertrophy, observed in many patients with chronic renal failure, is a major risk factor for cardiovascular death. The aim of the present study was to examine the

effects of pitavastatin (PITAVA) on cardiac hypertrophy in a progressive renal injury rat model by subtotal nephrectomy (SNx). Since we previously reported that angiotensin II (AII) played a pivotal role in cardiac hypertrophy of SNx rats, we first investigated the effects of PITAVA on AII-induced activation of extracellular-signal regulated kinase (ERK) and serum response element (SRE) DNA-binding activity using neonatal rat cardiomyocytes. The phosphorylation activity of ERK was measured by western blotting. DNA-binding activity of SRE, found in the promoter of several immediate-early response gene, was measured by dual-luciferase assay. The ERK activation by AII (10 μM) was attenuated by the pre-treatment with PITAVA (10 μM) for 1 h (2.19 ± 0.33-fold vs. 1.31 ± 0.30-fold, $P < 0.05$). AII-induced increase of SRE DNA-binding activity was also inhibited by PITAVA (2.60 ± 0.62-fold vs. 1.34 ± 0.13-fold, $P < 0.05$). We next examined the effect of PITAVA on hypertrophied heart of SNx rats. Treatment with PITAVA (3 mg/kg/d) prevented ERK activation and cardiac hypertrophy in SNx rats without changes in blood pressure. These data suggest that PITAVA has a beneficial effect on cardiac hypertrophy in renal failure through preventing the activation of ERK.

	LVW/TL (mg/mm)	IVSd (mm)	PWd (mm)	ERK activity
Sham	16.5 ± 0.44	1.69 ± 0.05	1.64 ± 0.04	1.00 ± 0.14
SNx	23.3 ± 1.26*	2.11 ± 0.06 *	2.07 ± 0.04 *	2.27 ± 0.32 *
SNx + PITAVA	18.4 ± 0.93 **	1.90 ± 0.05 **	1.69 ± 0.06 **	1.21 ± 0.35 **

LVW/TL: left ventricular weight to tibial length ratio; IVSd: diastolic interventricular septal thickness; PWd: diastolic posterior wall thickness. ERK activity is shown as fold increase over sham. Data are reported from seven animals for each.
* $P < 0.01$ vs. sham.
** $P < 0.05$ vs. SNx.

Abstract N° C39

Time course and regional distribution of myocyte hypertrophy after myocardial infarction in rabbits

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Compensatory hypertrophy of myocytes (CHM) involves post-infarct ventricular remodelling. However, it has not been described the temporal and regional distribution of this hypertrophy. For this reason, we evaluate temporal and regional distribution of the CHM in remote myocardium of the infarcted hearts. The left coronary artery of 40 rabbits was ligated and rabbits were sacrificed at 2, 4, 6, 8, 12, 14, 18, 26, 35 and 56 d post-ligature ($n = 4$ each group). Two rabbits were used as control and four were sham operated. Transversal diameter and cross-sectional area of myocyte were measured using an image analyser software. We evaluated 70 myocytes from each group. Septum remote zones and right ventricle (RV) were evaluated in two slices: One slice included the infarct area and in the other slice the infarct area was absent. The average infarct size was 25.1 ± 3.8%. The diameter (μ) and the cross sectional area (μ) of the myocytes in the remotes zones of the septum increased in both slices, but they were significantly higher in the slice with infarct and peaked in the 26th day (21.9 ± 0.54 and 289 ± 8 in the slice with infarct vs. 16.8 ± 0.48 and 195 ± 5 in the slice without infarct, $P < 0.05$). After that these measurements started to decrease slowly. Diameter and the cross-sectional area in both slices of the RV were significantly lower than in the septum slice (14.8 ± 0.2 and 173 ± 4 in the RV of the slice with infarct, and 12.9 ± 0.3 and 119 ± 6 in the RV of the slice without infarct, $P <$ 0.05 vs. septum slice wit infarct). Our data suggests that development of compensatory hypertrophy in myocardial infarction includes the participation of at least two components: (1) the time course of the infarct and (2) the regional-loading conditions.

Abstract N° C40

Effects of acute and chronic oral administration of amiodarone on arrhythmias induced by ischemia/reperfusion in canine model

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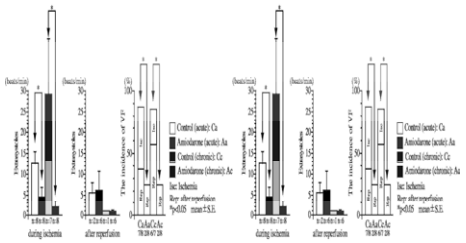
We previously reported that intravenous infusion of amiodarone (6.67 mg/kg/h) did not suppress the ventricular fibrillation (VF) induced by coronary ligation/reperfusion (ischemia/reperfusion, IR) in in vivo dogs. In this study, we examined effects of acute and chronic oral administration of amiodarone on arrhythmias induced by IR in in vivo dogs. Thirty-one female beagle dogs (8.0–12.5 kg/mg) were divided into four groups: amiodarone (40 mg/kg, p.o., 2 h before the operation)-treated, placebo (p.o., 2 h before the operation)-treated, amiodarone (40 mg/kg, once daily for 4 weeks, p.o.)-treated, and placebo (once daily for 4 weeks, p.o.)-treated groups. Dogs were anesthetized with pentobarbital sodium and artificially ventilated. The chest was opened, and the left anterior descending coronary artery was ligated for 30 min, and then reperfused. The occurrence of arrhythmias was examined during the ischemic and reperfusion period. As shown in Fig. 1, both acute and chronic oral administration of amiodarone suppressed the extrasystoles induced by coronary ligation ($P < 0.05$) and VF induced by IR ($P < 0.05$). In conclusion, acute and chronic oral administration of amiodarone are effective in suppressing arrhythmias induced by IR.



Abstract N° C41

Dietary supplementation of vitamin E and α-lipoic acid upregulates cell growth and signaling genes in rat myocardium

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The efficacy of antioxidant supplementation in the prevention of cardiovascular disease appears equivocal, however the use of a more potent antioxidant combination than those traditionally used may exert a more positive effect. We have shown previously that supplementation of vitamin E and α-lipoic acid increases cardiac performance during post-ischemia reperfusion in older rats and increases Bcl-2 levels in endothelial cells. The purpose of this study was to examine the effect of vitamin E and α-lipoic acid supplementation on expression of genes associated with cell signaling and growth in the myocardium. Young male rats received either a control ($n = 7$) or vitamin E and α-lipoic acid supplemented diet ($n = 8$) for 14 weeks. RNA from myocardial tissue was then amplified and samples were pooled within groups and competitively hybridized to 8.5 K oligonucleotide rat microarrays. The relative expression and variance of each gene was then compared to the control sample. Animals that received the antioxidant-supplemented diet exhibited upregulation (>1.5×) of 13 genes in the myocardium with two genes downregulated. Upregulated genes include those involved in cell growth and maintenance (LynB, Csf1r, Akt2, Tp53), transcription regulation (Dbp), cell signaling (LynB, Csf1r) and signal transduction (Pacsin2, Csf1r). Downregulated genes encode thyroid (Thrsp) and F-actin binding proteins (Nexilin). Therefore, supplementation of rats with vitamin E and α-lipoic acid has an effect on myocardial genes; further experiments will determine the implications of these findings.

Abstract N° C42

Chronic effects of probucol on development of right ventricular failure

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Background. – The treatment of right heart failure (RHF) is challenging due to the limited treatment options available. In fact for these patients there is no cure available in modern medicine. Since heart failure evolves through time, one of the solutions lies in targeting molecular mechanism involved in this process. Oxidative stress is known to play a role in the pathogenesis of heart failure. We have already shown a cause and effect relationship between RHF and oxidative stress. In this study, we examined the chronic effects of probucol, an antioxidant and lipid lowering drug on oxidative stress and development of RHF.

Methods. – Pulmonary hypertension (PH) and secondary RHF was created in rats by monocrotaline (MCT; 60 mg/kg i.p.). This is similar to clinical condition in patients with RHF secondary to PH. Probucol (cumulative dose, 120 mg/kg body weight) was given (i.p.) in 12 equal injections after MCT over 2 weeks. At 6 weeks post-treatment, animals in all groups were assessed by echocardiography, including measurements of pulmonary artery (PA) flow velocity; right ventricular (RV) outflow tract dimensions/aortic dimensions ratios and hemodynamics (RV systolic pressure, RVSP). Myocardial antioxidant enzymes and lipid peroxidation were also analyzed.

Results. – Based on the Doppler echocardiography at 6 weeks the ratio of RV outflow tract dimensions to aortic dimensions, RVSP and PA velocity (PV) increased in the MCT group compared with the controls. These echocardiographic and hemodynamic findings were correlated with interventricular septum (IVS) wall thickness and leftward displacement of IVS without change in left ventricle (LV) free wall. MCT treatment decreased glutathione peroxidase activity and increased lipid peroxidation. Probucol treatment prevented: sever bulging of IVS into LV; increase in the ratio of the (RV) outflow tract dimensions to aortic dimensions; increase in RVSP and increase in PV. Probucol improved glutathione peroxidase activity and reduced lipid peroxidation.

Conclusion. – Considering the cause and effect relationship between oxidative stress and development of RHF and that probucol effectively prevent PH and RHF in an experimental model of RHF, it is suggested that probucol may prove effective for the treatment of RHF in patients. (Supported by CIHR.)

Abstract N° C43

Cardiovascular structural and functional adaptations to group IIA $sPLA_2$ inhibition

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Group IIA phospholipase A2 ($sPLA_2$) enzymes are involved in inflammation by initiating the release of arachidonic acid from the cell membrane. $sPLA_2$ enzymes and other components of the arachidonic acid cascade have been linked to both replacement and reactive fibrosis, without direct evidence of their involvement. This study has treated male spontaneously hypertensive rats (SHR) and normotensive age-matched Wistar-Kyoto rats (WKY) from 4-12 weeks of age with KHO64 (5 mg/kg/day po), a selective group IIA $sPLA_2$ inhibitor to determine whether products of the arachidonic acid cascade play a role in the cardiovascular changes associated with the development of genetic hypertension. The $sPLA_2$ inhibitor prevented the increases in perivascular (WKY 12 wk 18.2 ± 1.4, n = 6 ; SHR 12 wk 26.9 ± 1.3, n = 5 ; + KH064 18.8 ± 2.1 %, n = 5) and interstitial fibrosis (WKY 12 wk 4.8 ± 0.4, n = 6 ; SHR 12 wk 8.5 ± 0.88, n = 5 ; + KH064 5.3 ± 0.4 %, n = 6) that are associated with hypertension. KHO64 had no effect on cardiac hypertrophy

as shown by echocardiographic analysis of left ventricle posterior wall thickness (WKY 12wk 1.53 ± 0.06, n = 12 ; SHR 12 wk 1.77 ± 0.08, n = 11 ; + KHO64 1.81 ± 0.09 mm n = 9) and chamber diameter (WKY 12 wk 7.15 ± 0.16, n = 12 ; SHR 12 wk 6.78 ± 0.21, n = 12 ; + KH064 6.67 ± 0.25 mm, n = 8). Increased systolic blood pressure was partially prevented in SHR rats by KHO64 (WKY 12 wk 142 ± 3, n = 14 ; SHR 12 wk 199 ± 5, n = 10 ; + KH064 169 ± 5 mmHg, n = 9). Endothelial function was reduced in KHO64 treated rats as shown by a reduced maximal response to acetylcholine in isolated thoracic aortic rings. Therefore, the arachidonic acid cascade may be important in the development of fibrosis in the heart but not in the development of cardiac hypertrophy.

Abstract N° C44

Blockade of anandamide hydrolysis reverses hypertension through activation of cardiac and vascular CB_1 receptors

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Background. – Endocannabinoids are novel lipid mediators with hypotensive activity that have been implicated in cardiovascular regulation. Here we document an endocannabinergic tone in hypertension, and show that potentiation of this tone by inhibiting the metabolic inactivation of the endocannabinoid anandamide normalizes the elevated blood pressure and cardiac contractility of hypertensive rats.

Methods and results. – Using the Millar pressure–volume system in rats with three different models of hypertension we find that cannabinoid-1 receptor (CB_1) antagonists markedly increase blood pressure and left ventricular contractile performance. In contrast, preventing anandamide inactivation by treatment with an inhibitor of fatty acid amidohydrolase or anandamide transport reduces blood pressure, cardiac contractility and vascular resistance to levels in normotensive rats, and these effects are reversed by CB_1 antagonists. In normotensive control rats, the same parameters remain unaffected by any of these treatments. CB_1 agonists lower blood pressure much more in spontaneously hypertensive rats (SHR) than in normotensive Wistar Kyoto rats (WKY), and the expression of CB_1 is increased in heart and aortic endothelium of SHR compared to WKY.

Conclusion. – We conclude that upregulation of vascular and cardiac CB_1 in hypertension unmasks an endocannabinergic negative inotropic and vasodilator tone, and enhancing this tone by blocking anandamide hydrolysis can normalize blood pressure. Targeting the endocannabinoid system offers novel therapeutic strategies in the treatment of hypertension.

Abstract N° C45

PKC activates NAD(P)H oxidase in human failing heart

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NAD(P)H oxidase is one of the major sources of superoxide anion (O_2^-) in cardiovascular system. In recent studies, we have found that O_2^- determined by lucigenin (5 μM) chemiluminescence is increased in the homogenate of human failing hearts (FH; $n = 7$) vs. non-FH by fivefold. superoxide dismutase (SOD) (200 U/ml) and DPI (10 μM), a flavoprotein inhibitor, both reduced O_2^- by 25–40%. Further, characterization indicated that O_2^- was derived from flavoprotein-dependent enzymes, which mainly consumed NADPH, because addition of NADPH (100 μM) and NADH (100 μM) increased O_2^- by 60- and 15-fold, respectively, in an apocynin (100 μM) and DPI (10 μM) inhibitable manner. Incubation of FH homogenates with a PKC inhibitor, staurosporine (10 μM), almost completely abolished O_2^- production. Thus, these data suggest that PKC activated NAD(P)H oxidases and O_2^- generation in heart failure.

Abstract N° C46

Evidence for a role of reactive oxygen species in angiotensin II-induced left ventricular hypertrophy in vivo

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In vitro studies have suggested that reactive oxygen species (ROS) are important mediators of cardiomyocyte hypertrophy. We studied the effect of dimethylthiourea (DMTU), a hydroxyl radical scavenger, on angiotensin II (Ang II)-induced left ventricular hypertrophy. Male Sprague–Dawley rats (n = 27) were infused with Ang II (33 μg/kg/h) via subcutaneously implantated osmotic mini-pumps in the presence and absence of a daily intraperitoneal injection of DMTU (50 mg/kg/d) for 6 d. Total RNA was isolated from left ventricular tissue and mRNA levels were measured by northern blot. Ang II infusion increased left ventricular weight/body weight (LVW/BW) ratio (2.63 ± 0.18 vs. 2.09 ± 0.11; Ang II vs. control, $P < 0.01$). DMTU treatment significantly attenuated the effect of Ang II on LVW/BW ratio (2.37 ± 0.16, $P < 0.01$). In contrast to the attenuation of the increase in LVW/BW ratio, DMTU had no significant effect on Ang II induced increases in the mRNA levels of atrial natriuretic peptide (12.7-fold vs. 15.7-fold; Ang II vs. Ang II + DMTU; P = NS), B-type natriuretic peptide (3.5-fold vs. 4.5-fold; P = NS), skeletal alpha-actin (3.9-fold vs. 3.7-fold; P = NS), collagen I (1.8-fold vs. 2.2-fold; P = NS), and fibronectin (1.4-fold vs. 1.8-fold; P = NS). The present results suggest that ROS significantly contributes to the development of left ventricular hypertrophy in vivo as the Ang II induced increase in LVW/BW ratio was substantially attenuated by DMTU. Moreover, the hydroxyl radical scavenger appears to

have a selective effect on protein synthesis since it did not affect the increased expression of key genes associated with cardiac hypertrophy.

Abstract N° C47

Dietary fish oil attenuates cardiac hypertrophy and improves cardiac function in a murine model of systemic carnitine deficiency

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The juvenile visceral steatosis (JVS) mouse, a murine model of systemic carnitine deficiency, develops cardiac hypertrophy with lipid accumulation. Fish oil (FO) or n-3 fatty acids may exert their beneficial effects on the pathological cardiac hypertrophy by modifying the fatty acid composition of myocardial 1,2-diacylglycerol (DAG), a lipid second messenger that activates protein kinase C (PKC). We investigated the effect of dietary FO on the hypertrophied heart in JVS mice. Both JVS and control mice were fed a FO diet (containing 10% FO) or a standard diet from 4 weeks of age. At 8 weeks of age, the ventricular-to-body weight ratio in JVS mice was 2.7-fold higher than that in controls ($P < 0.01$) and was reduced by 22% by dietary FO ($P < 0.01$ vs. JVS mice). In JVS mice, myocardial DAG levels were 2.3-fold higher than in controls ($P < 0.01$) with an increase in C18:1n-7,9 and C18:2n-6 fatty acids (both $P < 0.01$ vs. controls); dietary FO reduced both fatty acid species ($P < 0.05$ and $P < 0.01$ vs. JVS mice, respectively) without changing the total DAG levels. Membrane translocation of cardiac PKCa, b2, and e in JVS mice was greater than that in controls ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively), and was reduced to control levels by dietary FO ($P < 0.01$, $P < 0.05$, and $P < 0.05$ vs. JVS mice, respectively). From 8 to 10 weeks of age, JVS mice exhibited a progressive decline in left ventricular fractional shortening, which was maintained in FO treated JVS mice ($P < 0.01$). In conclusion, dietary FO may attenuate cardiac hypertrophy with improvement of cardiac function in JVS mice via modification of molecular species composition of myocardial DAG and consequent inhibition of PKC translocation.

Abstract N° C48

Limits to the beneficial effects of dietary flaxseed in prolonged hypercholesterolemic conditions

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We have shown that dietary flaxseed supplementation preserves vascular function and reduces the progression of atherosclerotic lesions during 6 and 8 weeks of hypercholesterolemic conditions. It is unclear however, if flaxseed remains beneficial in more prolonged hypercholesterolemic conditions. Male New Zealand White (NZW) rabbits were assigned to a control group (regular chow), a 10% flaxseed supplemented diet, a 0.5% cholesterol supplemented diet, or a 0.5% and 10% flaxseed supplemented diet for 16 weeks of feeding. The aorta and carotids were analyzed en face for atherosclerotic lesion coverage. Aortic cross-sections were stained with oil red O and Hematoxylin and analyzed for thickness of intimal lesions. Aortic vascular response to KCl, norepinephrine (NE), and acetylcholine (Ach) was examined. Plasma cholesterol levels were significantly increased with cholesterol feeding. Vascular contractile activity was impaired by cholesterol. The cholesterol-flax and cholesterol groups had decreased contractile responses to KCl and NE, and a decreased relaxation response to ACh as compared to the control and flax groups. Dietary cholesterol stimulated atherogenesis. Flaxseed added to the high cholesterol diet significantly increased the area of aortic and carotid atherosclerotic lesions as compared to the three other groups. The cholesterol-flax group also had increased intimal plaque thickness as compared to lesions in the cholesterol fed group. In conclusion, the combination of dietary flaxseed with cholesterol did not deter atherogenesis or correct the contractile abnormalities. Dietary flaxseed does not provide a protective effect in prolonged conditions of severe hypercholesterolemia.

This study was supported by CIHR, the Heart and Stroke Foundation of Canada, NSERC, and Polar Foods, Inc.

Abstract N° C49

Platelet aggregation in rats fed a hempseed supplemented diet

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N-3 polyunsaturated fatty acids (PUFA) are known to be antithrombotic because they reduce platelet aggregation. However, n-6 PUFAs are suggested to have pro-thrombotic effects. Hempseed is a unique food enriched in both linoleic acid (LA), an n-6 PUFA, and alpha linolenic acid (ALA), an n-3 PUFA. The purpose of our study was to determine if chronic dietary supplementation with hempseed can alter platelet aggregation. Sprague–Dawley (S–D) rats were administered a regular diet or one supplemented with either 5% hempseed, 10% hempseed, 10% hempseed cake (lipid depleted), or 1% palm oil for 6 or 12 weeks. The extent and rate of ADP-induced platelet aggregation was recorded with a chrono-log aggregometer. LA and ALA levels in the plasma and diets following 6 weeks of feeding were analyzed by gas chromatography. Proximate analysis of the diets was also conducted. Dietary and plasma LA and ALA levels were significantly increased in the two hempseed supplemented groups. After 6 weeks of dietary intervention, there were no differences in the extent and rate of ADP-induced platelet

aggregation in any of the groups. However, after 12 weeks of dietary intervention, hempseed supplementation reduced the extent and rate of platelet aggregation in comparison to rats consuming a regular diet. In summary, our data would argue against a detrimental effect of elevated n-6 fatty acids on platelet aggregation. Extended dietary supplementation with hempseed provides protective effects against platelet aggregation.

This study was supported by CIHR, the Heart and Stroke Foundation of Canada, NSERC, and Hemp Oil Canada.

Abstract N° C50

Dietary flaxseed limits atherosclerotic development in the LDLr-KO mouse

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Flaxseed, the richest plant source of the omega-3 fatty acid alpha-linolenic acid (ALA), has been reported to have inhibitory effects on atherosclerosis in rabbits. The mechanism for this beneficial effect is unclear. The purpose of our work was to re-examine the anti-atherogenic capacity of flaxseed in a more optimal model—the low-density-lipoprotein receptor deficient (LDLr-KO) mouse. LDLr-KO mice were administered a regular diet (RG), a 10% flaxseed supplemented diet (FX), or an atherogenic diet containing 2% cholesterol, alone (CH) or supplemented with 10% flaxseed (CF), 5% flaxseed (CF5), 1% flaxseed (CF1), 10% low-ALA flaxseed (CX), or 5% coconut oil (CS) for 24 weeks. Plasma cholesterol and triglyceride levels were measured enzymatically. The whole aorta and cross-sections at the aortic sinus were analyzed for atherosclerotic lesion coverage. Tissue sections were stained with oil red O and hematoxylin. Dietary cholesterol supplementation elevated plasma cholesterol levels. The CH group had significantly greater plasma cholesterol levels than all groups. CF5 had lower plasma cholesterol levels than all other atherogenic groups. The addition of 5% and 10% flaxseed to an atherogenic diet reduced plasma triglyceride levels as compared to the CS group. Dietary cholesterol stimulated atherogenesis. The CF and CF5 groups had a reduced area of aortic atherosclerotic lesions. The CF5 group also had less severe lesions at the aortic sinus. In summary, the addition of 5% and 10% dietary flaxseed to an atherogenic diet reduced plasma cholesterol and triglyceride levels and deterred the development of atherogenesis. The high ALA content of flaxseed appears to be providing these beneficial effects. Dietary flaxseed provides preventative effects on atherosclerotic development.

This study was supported by CIHR, the Heart and Stroke Foundation of Canada, NSERC, and Polar Foods Inc.

Abstract N° C51

Influence of dietary hempseed on arrhythmia generation by ischemia/reperfusion

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Polyunsaturated fatty acids (PUFAs) have been suggested to induce both beneficial and detrimental effects on the cardiovascular system. Specifically, the omega-3 family has been identified for its anti-arrhythmic effects while the omega-6 family has been suggested to be pro-arrhythmic. Hempseed contains a ratio of 3:1 of omega-6: omega-3 PUFA. It is not yet known what influence the two kinds of PUFAs have on arrhythmia generation when consumed as a dietary supplement. In the present study, male New Zealand White (NZW) rabbits were maintained for 8 weeks on a regular diet or a diet supplemented with one of either 10% hempseed (HP), 0.5% cholesterol (OL), 10% hempseed + 0.5% cholesterol (OLHP), 10% delipidated hempseed (SC) or 5% coconut oil (CO). At 8 weeks, blood samples were collected by venipuncture and plasma analyzed by gas chromatography for the lipid profile. The highest levels of linoleic and alpha-linolenic acid were found in the OLHP group. The hearts were excised and subjected to ischemia/reperfusion by Langendorff technique. During global ischemia, the incidence of arrhythmias was highest in the OL group. The duration of ischemia-induced tachycardia was longest in the OLHP and SC groups. The duration of fibrillation was longest in the OL and SC groups. The incidence of reperfusion-induced arrhythmias was highest in the SC group. The duration of reperfusion-induced tachycardia was longest in the OLHP group while fibrillation was longest in the HP group. These results demonstrate the influence dietary hempseed has on arrhythmia generation which may be achieved thorough its unique PUFA composition.

Supported by CIHR, Heart and Stroke Foundation, and Hemp Oil, Canada.

Abstract N° C52

The effects of dietary hempseed on platelet aggregation under hypercholesterolemic conditions

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Hempseed is a unique food enriched in both omega-6 and omega-3 polyunsaturated fatty acids (PUFA), such as linoleic acid (LA) and alpha-linolenic acid (ALA), respectively. The purpose of our study was to determine if chronic dietary supplementation with hempseed would alter platelet aggregation in animals with high circulating cholesterol levels. Male New Zealand White (NZW) rabbits were assigned to a regular diet (RG), or a diet supplemented with either 10%

hempseed (HP), 0.5% cholesterol (OL), 0.5% cholesterol + 10% hempseed (OLHP), 10% delipidated hempseed (SC), or 5% coconut oil (CO) for 8 weeks. The extent and rate of both collagen- and adenosine diphosphate (ADP)-induced platelet aggregation was recorded with a chrono-log aggregometer. Plasma LA and ALA levels were analyzed by gas chromatography. Plasma LA and ALA levels were significantly increased with hempseed supplementation (HP and OLHP). Cholesterol feeding enhanced plasma LA and ALA levels in the OLHP group. The extent and rate of platelet aggregation induced by both ADP and collagen was significantly lower with hempseed supplementation (HP and OLHP) than with cholesterol feeding. Hempseed cake supplementation did not reduce platelet aggregation. The combination of cholesterol and hempseed resulted in less collagen-induced aggregation than the OL, SC, HP, and CO groups. In summary, hempseed supplementation increases plasma PUFA levels. Hempseed reduces the extent and rate of platelet aggregation even in the presence of high circulating cholesterol. Delipidated hempseed does not elevate plasma PUFA levels or prevent platelet aggregation, suggesting that the PUFA content in hempseed provides the beneficial antithrombotic effects.

Supported by CIHR, NSERC, Heart and Stroke Foundation of Canada and Hemp Oil Canada.

Abstract N° C54

A flaxseed-supplemented diet greatly enhances omega-3 fatty acid content in rabbit tissues

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Epidemiological studies relate diets rich in omega (ω)-3 polyunsaturated fatty acids (PUFA) to decreased incidences of cardiovascular disease and mortality. The extent of incorporation of these beneficial fats into body tissues through food consumption is uncertain. In this study, male New Zealand White rabbits were fed 125 g/d of either regular rabbit chow or a diet containing 10% ground flaxseed—a seed highly enriched with the ω-3 PUFA, α-linolenic acid (ALA). Heart, aorta, liver, kidney, brain and blood were collected from the animals after 8 weeks of feeding. Fatty acids were extracted from tissues and analyzed by gas chromatography. Incorporation of ALA and its metabolic products eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was most significant in the heart, which had 4.9 ± 1.2 mg ALA/g tissue compared to only 0.8 ± 0.1 mg ALA/g tissue in the control group. Similar trends were observed in all other tissues, but to a much lesser extent in the brain. Arachidonic acid (ω-6) was decreased in all tissues obtained from the flax-supplemented group and the ratio of ω-6:ω-3 fatty acids was dramatically lowered in the tissues compared to controls. Thus, consumption of dietary flaxseed appears to be an effective means to increase ω-3 content in body tissues. These tissue stores of ω-3 fatty acids could protect against arrhythmias, thrombosis, and other potentially fatal events.

This study was supported by CIHR, Heart and Stroke Foundation of Canada, Flax Council of Canada and Polar Foods, Inc.

Abstract N° C55

Dietary fatty acids alter phospholipid composition in a murine model of AngII induced cardiac hypertrophy

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Elevated endogenous angiotensin II (AngII) is associated with cardiac growth (independent of blood pressure) and impaired cardiac performance. In various contexts cardioprotection can be enhanced by increasing the myocardial phospholipid omega-3:omega-6 polyunsaturated fatty acid (ω-3:ω-6 PUFA) ratio. Membrane fatty acid composition impacts on lipid–protein interactions, and alteration of membrane structure and fluidity affects membrane protein function and membrane mediator-derived signalling. The goal of this study was to determine (1) if AngII induced hypertrophy alters membrane phospholipid fatty acid composition and (2) whether dietary lipid manipulation can impact on membrane phospholipid fatty acid composition to increase the ω-3:ω-6 ratio in this murine model. Mature female (34–38 weeks) wildtype (WT) and transgenic cardiac AngII-overexpressing mice (TG) were fed a diet enriched with either ω-3 PUFA (7% fish oil + 3% olive oil) or ω-6 PUFA (5% sunflower oil + 5% olive oil) for 4 weeks. The percentage of ω-3 and ω-6 PUFA and the ratio of ω-3:ω-6 PUFA in cardiac phospholipids were analysed by gas chromatography (see Table 1). These findings indicate that cardiac AngII induced growth results in altered phospholipid composition which can be advantageously modulated by dietary manipulation to increase the ω-3:ω-6 ratio.

Table 1

	ω-6 diet		ω-3 diet	
	WT	TG	WT	TG
% w-3	24.5 ± 0.7	21.9 ± 1.3 *	48.7 ± 0.5 #	46.5 ± 0.6 *,#
% w-6	27.0 ± 0.5	33.1 ± 0.8 *	8.8 ± 0.08 #	7.4 ± 0.2 *,#
ω-3:w-6	0.91 ± 0.04	0.66 ± 0.05 *	5.9 ± 0.2 #	6.32 ± 0.2 *,#

* $P < 0.05$ when compared to WT.

# $P < 0.05$ when compare to ω-6 diet.

Abstract N° C56

Dietary modification of hypertension induced cardiac damage

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A cardioprotective role for n-3 fatty acids has been well established by epidemiological, experimental and clinical research. Moderate reductions in the blood pressure (BP) of hypertensive patients have been reported but decreased reinfarction, increased survival from cardiac events and improved peripheral flow response are consistently observed, hence the effects of n-3 fatty acids on hypertension may be achieved by end organ protection. Hypothesis: n-3 fatty acids have a time dependent effect on target organ damage with longer feeding times related to higher levels of protection. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) offer different levels of protection. Ten-week-old stroke prone spontaneously hypertensive rats (SHRSP) were fed either an olive oil (OO) control diet for 12 weeks or one of five test diets (1) 8 weeks OO 4 weeks fish oil (FO), (2) 4 weeks OO 8 weeks FO, (3) 12 weeks FO, (4) 12 weeks EPA and (5) 12 weeks DHA. Histological analysis of myocyte hypertrophy, coronary vasculature hypertrophy and collagen deposition was performed. All FO fed animals showed moderate time dependent decreases in BP. No significant reduction was seen in cardiac hypertrophy. FO fed rats showed time dependent reductions in collagen deposition, myocyte cross-section area (CSA) and coronary artery hypertrophy. Comparison of EPA and DHA showed greater protective effect of DHA on collagen deposition and myocyte CSA.

	OO	12 FO	EPA	DHA
BP (mmHg)	241 ± 1 *	219 ± 2.5	217 ± 2.2	218 ± 1.6
CSA (mm^2)	0.43 ± 0.01 *	0.36 ± 0.02	0.36 ± 0.03	0.34 ± 0.02 **
Collagen (%)	23.01 ± 3.20 *	14.70 ± 4.93	13.39 ± 3.59	10.28 ± 2.28 ***

* $P < 0.05$ compared to OO.
** $P = 0.059$.
*** $P < 0.05$ compared to FO.

This study shows n-3 fatty acids provide cardio-protection in hypertensive animals independently of BP change. Protection is time dependent and greater with DHA alone than FO or EPA.

Abstract N° C57

A comparative effect of fish oil, flaxseed oil, and hemp seed oil supplementation on selected parameters of cardiovascular health in healthy volunteers

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The effects of three dietary oils (fish oil, flaxseed oil, and hemp oil supplementation) given in concentrations expected to be self-administered in the general population were examined in healthy volunteers in terms of providing potential cardiovascular health benefits. Eighty-six healthy male and female volunteers completed a 12-week double blinded, placebo controlled, trial. They were randomly assigned to any one of the four groups. Subjects were orally supplemented with two 1-g capsules of placebo, fish oil, flaxseed oil or hemp seed oil per day for 12 weeks. The lipid parameters (TC, HDL-C, LDL-C and TG) did not show any significant differences among the four groups. Oxidative modification of LDL showed no increase in lag time over the 12-week period. Both collagen and thrombin induced platelet aggregation showed no statistical difference amongst the four groups studied. Similarly C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) levels at baseline and after supplementation showed no significant changes. From a consumer's perspective, ingesting two capsules of any of these oils in an attempt to achieve cardiovascular health benefits may not provide the desired or expected result over a 3-month period.

Supported by CIHR and NSERC.

Abstract N° C58

Molecular mechanisms for cardioprotective effects of *Curcuma longa* on myocardial apoptosis, cardiac function and antioxidant milieu in an ischemia reperfusion model of myocardial infarction

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The present study investigated the effect of *Curcuma longa* (Cl), a medicinal herb on myocardial injury, apoptosis, antioxidant status and cardiac functional recovery in rats subjected to 45 min of ischemia followed by 1 h of reperfusion. In the control ischemia reperfusion (I/R) group, significant myocardial injury, cardiac apoptosis as evidenced by TUNEL-positive nuclear staining, depressed hemodynamics, decline in myocardial antioxidant status and elevation in lipid peroxidation was observed as compared to sham control. However, myocardial injury produced after I/R was significantly reduced in the Cl-treated group. Cl treatment maintained the antioxidant reserve of the myocardium by augmenting endogenous antioxidant enzyme activities, inhibiting lipid peroxidation and restoration of the antioxidant status of the myocardium. Our results indicate that cardioselective overexpression of bcl-2 and downregulation of bax protein exerts a cardioprotective effect after myocardial I/R, and this effect is probably mediated via an anti-apoptotic action of Cl. The beneficial effects also translated into the functional recovery of the heart. Histopathological examination reconfirmed the protective effects of Cl on the myocardium. The results of the present study demonstrate that administration of Cl provided significant protection against myocardial injury and this may be attributed to its antioxidant and anti-apoptotic properties.

Abstract N° C59

Effects of sour cherry seed extract in isolated ischemic/reperfused mouse heart

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The effects of sour cherry seed flavonoids were evaluated for their contribution to post-ischemic recovery in isolated mouse hearts subjected to ischemia/reperfusion. Mice were orally treated with various doses (1, 5, 10, and 30 mg/kg/d) of flavonoid-rich extract of sour cherry seeds for 10 d. One day after the last treatment, animals were anesthetized, hearts were isolated in "working mode" and subjected to 30 min ischemia followed by 2 h reperfusion. The recovery of post-ischemic cardiac function including heart rate (HR), coronary flow (CF), aortic flow (AF), and aortic pressure (AOP) were registered. In mice treated with 10 and 30 mg/kg/d of sour cherry flavonoid-rich extract, after 60 min of reperfusion, AOP was significantly improved from its untreated control value of 75 ± 4 to 98 ± 6 mmHg ($P < 0.05$) and 119 ± 6 mmHg ($P < 0.5$), respectively. The same improvement in post-ischemic recovery of HR, CF, and AF was also observed. Lower concentrations (1 and 5 mg/kg/d) of the extract failed to significantly improve the recovery of cardiac function. The incidence (%) of reperfusion-induced ventricular fibrillation (VF) was also reduced from its drug-free value of 83–50% and 33% in mice treated with 10 and 30 mg/kg/d of extract, respectively. However, this protection against VF, because of the relatively low numbers of hearts ($n = 6$, and nonparametric distribution) in each group, was not at a significant level. In summary, sour cherry seed flavonoid-rich extract possesses protective effect against reperfusion-induced injury through its abilities to improve post-ischemic cardiac function.

Abstract N° C60

Relationship between clinical effects of injection sheng mei on chronic heart failure and its concentration in plasma

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Background. – In the previous studies, we found that sheng mei may be of value for long-term treatment in patients with chronic heart failure. In order to improve its clinical effect and to reduce side effects, it is necessary to study the relationship between its effect and its concentration in plasma in patients with congestive heart failure (CHF).

Methods and results. – The study in 59 patients with class III–IV chronic heart failure was performed. All patients were given conventional therapy for CHF consisting digitalis, diuretics, nitrates, beta blockers and ACE inhibitors for 2 weeks, and were randomly divided into three groups: sheng mei 60 ml/d ($n = 20$) or sheng mei 30 ml/d ($n = 20$) or placebo ($n = 19$). After 4 week sheng mei therapy, data of left ventricular ejection fraction (LVEF) 6 min walking test and Mennesota Living with Heart Failure score were assessed again and sheng mei concentration in plasma was measured by HPLC. Plasma samples were pretreated by protein precipitation with methanol and centrifuged at 17 000 rpm for 10 min. Re of sheng mei was determined in 60 or 30 ml/d group using a uB and pank C18 column (10u, 300 3.9 mm I.D.) with a mobile phase of acetonitrile mixed with 0.02 mol/l phosphoric acid water solution in plasma were 8 ng/ml after injection of 50 µl of sample. The average recovery of gengsen Re of sheng mei in plasma was 96.5%. The results showed that increase in LVEF and exercise capacity and improvement of quality of life in 60 ml group were more remarkabe than those in 30 ml group which corresponded to change plasma concentration in sheng mei (0.33 µg/ml vs. 0.1 µg/ml, $P < 0.01$). And increase in exercise capacity and improvement of quality of life in 30 ml group were more apparent than that in placebo. There was not any apparent side effect among three groups.

Conclusions. – Monitoring sheng mei concentration in plasma may be helpful in treating the patients with chronic heart failure by using sheng mei.

Abstract N° C61

Evaluation of cardiovascular and cardioprotective effects of *Terminalia arjuna*

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The bark of *Terminalia arjuna* (TA) possesses beneficial cardiovascular effects, Hence, the present work was undertaken to screen the effect of aqueous extract (AqE) of TA on the isolated-perfused rabbit's heart preparation and isoproterenol-induced myocardial necrosis in rats, The effect of different doses of the AqE in the dose range 1–2048 µg was observed at different time intervals of the isolated-perfused rabbit's heart preparation. The AqE increased the force of contraction and decreased the heart rate at and above the dose of 64 µg and increases the coronary flow rate above the dose of 512 µg significantly. The cardioprotective effect of AqE of TA was evaluated in the Wistar rats. The rats were divided into four groups of 10 each. Group 1 was the saline control. In groups 2, 3 and 4, myocardial necrosis was induced with isoproterenol (ISO; 85 mg/kg, s.c.) on the last 2 d of pretreatment of animals with saline (i.p.), propranolol (2 mg/kg, i.p.) or TA (150 mg/kg, p.o.) respectively for 9 d. The effect of treatment on electrocardiographic changes and blood pressure in all the groups was recorded 24 h after the last injection of ISO. AqE of TA produced significant hypotension (60.25 ± 1.1 vs. 98.2 ± 2.03 mm of Hg in group 1; $P < 0.001$). A significant reduction in the J-point elevation was also noted (0.68 ± 0.1 vs. 1.36 ± 0.19 mm in group 2; $P < 0.02$). However, ISO led to a significant increase in the heart rate in AqE-treated rats (301.5 ± 7.19 vs. 252.8 ± 4.75 beat/min in group 1; $P < 0.001$). The results of the present study indicate that the AqE of TA enhances coronary flow, lowers blood pressure and also reduces the intensity of ISO-induced myocardial necrosis in experimental animals.

Abstract N° C62

Cardioprotective effects of hydro-alcoholic extract of *Withania somnifera* on isoproterenol-induced myocardial infarction in rats

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The present study evaluated the cardioprotective potential of hydro-alcoholic extract of *Withania somnifera* (Ws) an Indian medicinal plant against isoproterenol (ISP)-induced myocardial infarction in rats. Rats were administered Ws at doses of 25, 50 and 100 mg/kg, orally for 4 weeks and randomly divided into three groups: sham, ISP control and Ws treatment. On 29th and 30th day ISP (85 mg/kg) was administered subcutaneously to the ISP control and Ws treatment groups. On the 31st day after recording hemodynamics, hearts were processed for histopathological and biochemical studies. In ISP group left ventricular dysfunction as evidenced by decrease in heart rate, left ventricular peak positive and negative pressure change and elevated left ventricular end-diastolic pressure was observed. In addition a significant decrease in glutathione (GSH), activities of superoxide dismutase (SOD), catalase (CAT), creatinine phosphokinase (CPK) ($P < 0.05$) and lactate dehydrogenase (LDH) ($P < 0.01$) as well as increase in lipid peroxidation marker malondialdehyde (MDA) level ($P < 0.01$) was observed in ISP control as compared to sham group. The treatment with Ws significantly reduced the myocardial injury subsequent to ISP-induced necrosis. The cardioprotection may be due to augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of the altered hemodynamics. Histopathological assessment further confirmed the cardioprotective effects of Ws. Ws at dose of 50 mg/kg was found to be most effective in restoring hemodynamic and biochemical alterations as compared to dose of 25 and 100 mg/kg of Ws.

Abstract N° C63

Dietary substitution of sesame oil regulates changes in lipid composition and hematological indices in hypertensive patients

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The current study was conducted to find out the effectiveness of sesame oil as sole edible on lipid composition and hematological indices in hypertensive patients medicated with nifedipine, a calcium channel blocker. The study group comprised of 200 hypertensive patients administered with nifedipine (10–30 mg/d) as antihypertensive medication. Of 200 patients, 125 patients were supplied with sesame oil (idhayam gingelly oil), which comes to 35 g of oil/d/person and directed to use the oil in place of other edible oils for 60 d. Seventy-five patients were treated only with nifedipine for 60 d. Lipid profile (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C ratio and triglycerides), plasma and RBC membrane fatty acid composition and hematological parameters (RBC, WBC, hemoglobin, platelets and prothrombin time) were analyzed at basal and after substitution of sesame oil and the drug, nifedipine for 60 d. Plasma level of TC, LDL-C and TC reduced significantly while HDL-C elevated significantly upon the substitution of sesame oil. TC/HDL-C also reduced. Saturated fatty acids content of plasma and RBC reduced where as monounsaturated fatty acids and polyunsaturated fatty acids content increased significantly. Hemoglobin levels showed significant elevation. Platelets and prothrombin time altered significantly during the oil substitution. These results indicate that sesame oil influenced beneficially in the modulation of lipids and hematological parameters, suggesting that dietary substitution of sesame oil is added advantageous to hypertensive patients.

Abstract N° C64

Myocardial salvaging effect of *Moringa oleifera* in isoproterenol induced myocardial infarction

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The present study evaluated cardioprotective potential of hydro-alcoholic extract of *Moringa oleifera* (Mo) on the basis of histopathological and biochemical parameters against isoproterenol (ISP)-induced myocardial infarction in rats. Wistar albino rats (150–200 g) were divided into five groups: sham control, ISP control, and Mo treatment groups. Mo was administered at doses of 100, 200 and 400 mg/kg, orally for 30 d. On day 29th and 30th the rats of ISP control and Mo treatment groups were administered ISP (85 mg/kg), subcutaneously at an interval of 24 h. On the 31st d hemodynamic parameters were recorded and the hearts were subsequently removed and processed for histopathological and biochemical studies. Myocardial damage determination was done by direct staining using tri-phenyl tetrazolium chloride (TTC) in phosphate buffer saline. Myocardial enzyme lactate dehydrogenase (LDH), markers of oxidative stress: reduced glutathione (GSH), superoxide dismutase (SOD), and thiobarbituric-acid reactive substances (TBARS) were estimated. Histopathological examination was done for all groups. On morphological examination normal saline control group exhibited myocardial tissue stained brick red (Formazan of TTC and LDH) in contrast to ISP group which showed patches of unstained regions (leakage of LDH from myocardial tissue due to oxidative stress). Infarct like lesions were produced in ISP-treated group with the accompanying fall in LDH, GSH, SOD and rise in lipid peroxidation marker TBARS as compared to normal saline group, which is statistically significant ($P < 0.05$). Significant improvement ($P < 0.05$) of biochemical parameters and histopathological assessment in Mo-treated groups confirmed the protective effects of Mo on the myocardium. Mo at 200 mg/kg dose was found to be the most optimal in functional recovery of the heart and favorable restoration of biochemical and histopathological alterations.

Abstract N° C65

Cardioprotective effect of an indigenous drug: glycyrrhiza glabra in experimentally induced myocardial infarction

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In the present study cardioprotective potential of glycyrrhiza glabra (Gg) has been evaluated against isoproterenol (ISP)-induced myocardial ischemia. Gg was administered at doses of 100, 300, 600 and 900 mg/kg orally for 30 d to Wistar albino male rats randomly divided in sham and ISP control and Gg treatment groups using lisinopril as standard. On 29th and 30th d, ISP (85 mg/kg) was administered subcutaneously at an interval of 24 h. On 31st day, hemodynamic parameters were recorded and the hearts were subsequently excised and processed for biochemical and histopathological studies. A significant decrease in glutathione (GSH) ($P < 0.05$), activities of SOD, catalase (CAT), creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) ($P < 0.01$) as well as increase in malondialdehyde (MDA) level ($P < 0.01$) was observed to be restored by Gg treatment in comparison to the ISP and sham-treated groups. However, no significant change was observed in the activity of GSHPx and protein levels. Left ventricular dysfunction was evidenced as a decrease in heart rate, left ventricular rate of peak positive and negative pressure change and elevated left ventricular end-diastolic pressure in the ISP control group. Gg significantly restored heart rate and left ventricular function in comparison to ISP and lisinopril group. The significance of these results are implicated in relation to cardioprotective effects of Gg in ISP induced myocardial ischemia. Among the different doses studied, Gg at 100 mg/kg dose produced maximum cardioprotective effect. Restoration of endogenous antioxidants as well as significant restoration of the altered hemodynamic parameters may contribute to its cardioprotective effect.

Abstract N° C66

Cardiovascular effects of danshen extract and its magnesium tanshinoate B enriched form

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The Chinese medicine, danshen, is the dried root and rhizome of *Salvia miltiorrhiza* Bunge (Labiatae) and has been used as a health supplement to protect against the development of hypertension and myocardial infarction. One of its major active water-soluble components is magnesium tanshinoate B (MTB). The present study examines and compares the cardiovascular effects of the water-soluble extract of danshen (SME) and its MTB enriched form containing 70% of MTB (MTB70) in anaesthetized rats. Rats were subjected to intravenous infusion of saline and phenylephrine to produce a basal state of normal and elevated blood pressure, respectively. Different doses of SME, MTB70 or its vehicle were then injected intravenously in rats and their effects on blood pressure were monitored. Our results indicate that both SME and MTB70 transiently reduce blood pressure in a dose-dependent manner. Independent of the initial blood pressure state, SME caused a smaller reduction in blood pressure than MTB70. While the effect of SME was similar in rats infused with saline or phenylephrine, MTB70 reduced blood pressure to a greater extent in rats infused with phenylephrine compared to those infused with saline. From these findings, it appears that MTB is one of the major components responsible for the cardiovascular effects of danshen, and its beneficial cardiovascular effect is enhanced in conditions with elevated blood pressure. In view of the highly variable content of danshen extract, standardization of danshen preparation is essential to ensure consistent pharmacological actions. Therefore, our data suggests that the amount of MTB might be employed as a standard reference for the preparation of danshen as a therapeutic agent, especially in the treatment of cardiovascular disorders.

Abstract N° C67

Comparison of vasodilatation between quercetin and rutin in the isolated rat thoracic aorta

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To determine the possible difference in vascular effect of rutin and quercetin (Que), we investigated the vasodilatation of rutin and Que on the isolated rat thoracic aorta rings. Rutin at range of 10–150 μmol/l caused dose-dependent vasorelaxation in endothelium-intact rings preconstricted with phenylephrine (PE, 10 μmol/l), but had no effect in aorta rings without endothelium. Que (10–150 μmol/l) caused endothelium-independent relaxation of aorta rings preconstricted with PE in a dose-dependent manner. Pretreatment with l-NAME (0.1 mmol/l), an inhibitor of nitric oxide synthase, abolished the vasorelaxation by rutin, but did not influence the vasodilating effect of Que in endothelium-intact rings. Pretreatment with methylene blue (10μ mol/l) as well as 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one (ODQ, 100 μmol/l), inhibitors of guanylyl cyclase, canceled the vasorelaxation both by Que and rutin. Administration of indomethacin (10 μmol/l), an inhibitor of cyclooxygenase, attenuated the vasodilatation of Que, but did not affect the vascular effect of rutin. The results indicate that the vasorelaxation by rutin in rat aorta ring is mediated by nitric oxide-guanylyl cyclase pathway, while the vascular effect of Que is via cyclooxygenase-dependent pathway, suggesting glycosyl groups in the structure of rutin may be the determinant factor in the different vascular effect. (Supported by TCM Bureau of Zhejiang Province, G20010358.)

Abstract N° C68

Mechanisms responsible for the acute relaxation of porcine left anterior descending coronary artery by diosgenin

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The objective of this study was to determine the in vitro vasodilating effect of diosgenin, a plant-derived sapogenin (phytoestrogen) that is structurally similar to progesterone, in porcine resistance left anterior descending coronary artery (tertiary branch, O.D. ~ 500 μm). In 5-hydroxytryptamine (3 μM) pre-contracted preparation, diosgenin caused a concentration-dependent, endothelium-independent relaxation (0.03–1 μM) with a maximum relaxation of ~72% at 1 μM. Diosgenin-elicited relaxation was not altered by ICI 182,780 and mifepristone. The iberiotoxin-sensitive, Ca^{2+}-activated K^+ (BK_{Ca}) current of single vascular myocytes record was markedly enhanced by diosgenin, 17β-oestradiol and progesterone. KT 5823 (300 nM) pre-treatment eradicated the enhancement of BK_{Ca} amplitude by these three compounds. In oestrogen competition essay using human breast cancer cell (MCF-7 cells), diosgenin (0.001–10 μM) did not interact with nuclear oestrogen receptor and no competition with ^{3}[H]-oestradiol binding was observed. In conclusion, diosgenin possesses an endothelium-independent coronary artery relaxation effect via the protein kinase G signalling cascade and activation of the BK_{Ca} channel. In addition, neither the oestrogen nor progesterone receptors are involved in mediating diosgenin-elicited vascular response.

Abstract N° C69

Cholesterol-enriched diet leads to increased oxidative stress and cardiac dysfunction in apoB100 transgenic mice

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Hypercholesterolemia is a major factor in the development of arteriosclerosis, however, its direct myocardial effects are poorly understood. To determine the direct effect of hypercholesterolemia on cardiac performance and oxidative stress, wild type and apoB100 transgenic mice were fed 2% cholesterol-enriched or normal diet for 18 weeks. Cardiac performance was assessed in isolated working hearts. There was no significant difference in aortic flow between wild-type and apoB100 transgenic mice fed normal diet (0.24 ± 0.02 vs. 0.26 ± 0.02 ml/min/g body weight). In cholesterol-fed groups, aortic flow was not affected in wild types, however, it was decreased in apoB100 transgenic (0.22 ± 0.02 vs. 0.14 ± 0.03 ml/min/g body weight, $P < 0.05$). Cholesterol-enriched diet increased cardiac superoxide generation assessed by lucigenin-enhanced chemiluminescence assay in apoB100 transgenic mice (from 23 ± 9 to 73 ± 19 cpm/mg, $P < 0.05$) but not in wild types (22 ± 7 vs. 26 ± 5 cpm/mg). The peroxynitrite decomposition catalyst FeTPPS (20 mg/kg i.p. injections 24 and 1 h prior to isolation of the heart) attenuated the decrease in aortic flow in apoB100 transgenic mice fed cholesterol-enriched diet (0.23 ± 0.02 ml/min/g body weight). We conclude that hypercholesterolemia induced by cholesterol-enriched diet leads to increased oxidative stress and subsequent cardiac dysfunction in the hearts of apoB100 transgenic mice.

Abstract N° C70

Effect of chronic carnitine supplementation on myocardial metabolism and function in uraemia

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Cardiac complications are the major cause of mortality in patients with chronic renal failure. Secondary carnitine deficiency frequently occurs in chronic uraemia and can lead to impaired myocardial fatty acid metabolism. In addition, myocardial carnitine deficiency has been associated with cardiac hypertrophy and heart failure. The aim of this study was to determine the impact of chronic carnitine supplementation on cardiac function and oxidative fluxes in experimental uraemia using ^{13}C NMR spectroscopy. Uraemia was induced in male Sprague–Dawley rats via a two-stage 5/6 nephrectomy. l-Carnitine was administered (5 mM, 0.25 μl/h) over a 3-week period by means of subcutaneous mini-osmotic pumps. Three weeks after surgery, hearts were perfused in the isovolumic mode with Krebs–Henseleit buffer containing physiological concentrations of substrates including 5 mM 1-^{13}C glucose and 0.3 mM U-^{13}C palmitate. Following carnitine supplementation, palmitate oxidation increased significantly in uraemic hearts compared to its controls, and was associated with a small increase in rate pressure product (RPP). Carnitine supplementation also resulted in a significant improvement in haematocrit in uraemia. In conclusion, chronic carnitine supplementation improves long chain fatty acid oxidation and function in the uraemic heart.

	Baseline		Chronic carnitine	
	Control (*n = 10*)	Uraemic (*n = 10*)	Control (*n = 6*)	Uraemic (*n = 6*)
RPP ($\times 10^3$) (mmHg beats/min)	30.2 ± 13	24.9 ± 6.3	30.4 ± 6.2	29.9 ± 2.4
Palmitate oxidation (%)	26.3 ± 8.1	29.9 ± 7.7	22.3 ± 4.2	31.1 ± 7.3 *
Haematocrit (%)	39.1 ± 2.2	31.4 ± 3.1	39 ± 4.2	36 ± 3.4 **

* Compared to its control; $P \leq 0.05$.
** Compared to baseline uraemic.

Abstract N° C71

Acute carnitine supplementation modifies cardiac efficiency

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Cardiac complications are the major cause of mortality in patients with chronic renal failure. Secondary carnitine deficiency frequently occurs in chronic uraemia and can impair myocardial fatty acid metabolism. In addition, myocardial carnitine deficiency has been associated with cardiac hypertrophy and heart failure. The aim of this study was to determine the impact of acute carnitine supplementation on cardiac function and oxidative fluxes in experimental uraemia using ^{13}C NMR spectroscopy. Uraemia was induced in male Sprague–Dawley rats via a two-stage five or six nephrectomy. Six-weeks post surgery, hearts were perfused in the isovolumic mode with Krebs–Henseleit buffer containing physiological concentrations of substrates including 0.3 mM U-^{13}C palmitate, ± 5 mM l-carnitine. Despite comparable cardiac function and substrate selection, myocardial oxygen consumption (MVO_2) was significantly lower in both control and uraemic hearts, following acute carnitine supplementation. This resulted in a marked increase in cardiac efficiency (rate pressure product (RPP)/MVO_2). In conclusion, acute carnitine supplementation enhances cardiac efficiency in both control and uraemic hearts.

	Baseline		Acute carnitine	
	Control (*n = 6*)	Uraemic (*n = 6*)	Control (*n = 6*)	Uraemic (*n = 6*)
RPP ($\times 10^3$) (mmHg beats/min)	24.2 ± 0.4	21.6 ± 0.2	28.3 ± 2.2	23.6 ± 3.0
MVO_2 (μmol/min/g dry heart weight)	17.7 ± 0.8	16.9 ± 2.9	11.5 ± 1.2 *	10.9 ± 2.2 *
Cardiac efficiency $\times 10^3$	1.4 ± 0.2	1.4 ± 0.2	2.6 ± 0.3 *	2.6 ± 0.4 *

* $P < 0.05$ compared to corresponding baseline group.

Abstract N° C72

PPARα, free fatty acids and mitochondrial UCP3 in mouse heart

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Diabetic patients have been shown to have abnormal cardiac energetics, which correlated with high levels of plasma free fatty acids (FFAs). We hypothesized that the high plasma FFAs had increased uncoupling protein 3 (UCP3) levels in heart by activating the nuclear transcription factor peroxisome proliferator-activated receptor α (PPARα). To test this hypothesis we used western blotting to measure UCP3 in mitochondria isolated from the hearts of PPARα null mice and mouse models of diabetes. In the PPARα null mouse, cardiac levels of UCP3 were 33% lower than in controls, suggesting that cardiac UCP3 was indeed under PPARα control. In confirmation, treatment with the PPARα-specific agonist, WY-14,643, did not alter UCP3 levels in PPARα null mice, but increased UCP3 levels in control mice by 54%. In the type 2 diabetic (d*b*/d*b*) mouse, which had 50% higher plasma FFA levels than control mice, cardiac levels of UCP3 were increased 38%. Streptozotocin (STZ) injection, which increased circulating FFAs by 91%, increased cardiac UCP3 levels by 50% in control mice, but not in PPARα null mice. We conclude that cardiac UCP3 levels were increased by high concentrations of plasma FFAs, controlled via PPARα. High levels of UCP3 decrease cardiac efficiency (work/O_2 consumption) and may underlie the energetic deficiency of the diabetic heart.

Mouse model (*n = 5*)	Plasma FFAs (mM)	Relative cardiac levels of UCP3
Control	0.35 ± 0.03	1 ± 0.13
PPARa null	0.27 ± 0.05	0.67 ± 0.05 *
Control + WY-14,643	0.43 ± 0.01	1.54 ± 0.18 *
PPARa null + WY-14,643	0.38 ± 0.02	0.60 ± 0.08 *
Type 2 diabetic (d*b*/*db*)	0.53 ± 0.02 **	1.38 ± 0.03 **
Control + STZ	0.67 ± 0.07 **	1.5 ± 0.04 *
PPARa null + STZ	0.80 ± 0.06 **	0.67 ± 0.05 *

* $P < 0.05$ compared with control.
** $P < 0.01$ compared with control.

Abstract N° C73

Plasma free fatty acids and energy depletion in failing human hearts

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In vivo ^{31}P NMR spectroscopy of the failing human heart has shown a decreased phosphocreatine/ATP ratio, indicating a lowered energy status. The extent of this energy deficit correlated inversely with plasma free fatty acid (FFA) levels. We propose that the energetic defect in heart failure originates from increased mitochondrial uncoupling and decreased substrate availability caused by high circulating FFA concentrations, which increase mitochondrial uncoupling protein (UCP) and decrease insulin-sensitive glucose transporter, GLUT4, levels in human heart. Plasma samples, for the measurement of FFA concentrations, and right atrial appendage and skeletal muscle biopsies were obtained from 39 patients undergoing coronary artery bypass surgery. Immunoblotting was used to quantify UCP2 and UCP3 levels in isolated cardiac mitochondria and GLUT4 protein levels in whole tissue. Positive correlations were found between fasting plasma FFA concentrations and cardiac UCP2 ($r = 0.64$; $P < 0.0001$) and UCP3 ($r = 0.46$; $P < 0.001$). Negative

correlations were found between plasma FFA concentrations and GLUT4 in cardiac ($r = 0.58$; $P < 0.0001$) and skeletal muscle ($r = 0.53$; $P < 0.001$). This study indicates that the energy deficient state, characteristic of human heart failure, may result from two main factors; firstly, increased mitochondrial UCPs that cause less efficient ATP synthesis, and secondly, depleted GLUT4 that decreases the availability of glucose as an energy-producing substrate for the heart.

Abstract N° C74

Translocation of glucose transporters and hexokinase in response to insulin and no-flow ischaemia in the heart

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Immunogold electron microscopy (IGEM) provides spatial information on intracellular protein location beyond the distributions provided by western blotting. We have used IGEM to characterise the response of myocardial GLUT 1 and 4 and hexokinase (HK) I and II to ischaemia and insulin. Hearts ($n = 3$/group) were perfused with Krebs buffer for 1 h, followed by a further 30 min of: control perfusion; insulin stimulation (1IU/l); or no-flow ischaemia. Hearts were perfusion-fixed (2% formaldehyde + 0.2% glutaraldehyde), and cryosections were double-labelled for GLUT1 and 4 or HKI and II. Labelling was quantified relative to membrane length or compartment volume to give labelling density (LD). HKI and GLUT1 remained unchanged in all groups. Capillary membranes contained the majority of GLUT1 (0.41 ± 0.01 golds μm^{-1}) while sarcolemmal and T-tubule membranes each contained 0.12 ± 0.05 golds μm^{-1} (mean ± SEM). HKI was located on the mitochondrial membranes with an LD of 0.05 ± 0.01 golds μm^{-1}. GLUT4 and HKII distributions are summarised below:

Location	Control LD	Insulin LD	Ischaemia LD
GLUT4 (golds μm^{-1})			
Sarcolemma	0.02 ± 0.02	0.05 ±0.04	0.16 ±0.04
T-tubule membranes	0.06 ± 0.01	0.15 ±0.01	0.17 ± 0.04
Vesicles (golds μm^{-2})	3.16 ± 0.52	1.36 ± 0.12	1.11 ± 0.20
HKII (golds μm^{-1})			
Mitochondrial membranes	0.04 ± 0.04	0.08 ± 0.01	0.14 ± 0.05
Vesicles (golds μm^{-2})	9.25 ± 1.77	6.00 ± 1.91	6.92 ± 1.17

Insulin- and ischaemia-mediated HKII binding to the mitochondrial membrane allows HK direct access to mitochondrial ATP. The close proximity of many of the mitochondria to either the T-tubules or the sarcolemma means that the GLUT4-transported glucose can be delivered extremely rapidly to the mitochondrial bound HKs.

Abstract N° C76

Insulin preserves endothelial-dependent coronary function in a canine model of myocardial ischemia and reperfusion

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Accumulating evidence has suggested that insulin is an important endogenous vasomotion modulator in addition to its classical actions to coordinate glucose homeostasis. The objective of the present study was to evaluate whether insulin exerts protective effect against reperfusion-induced coronary endothelial dysfunction in dogs. Anesthetized dogs were subjected to 45 min myocardial ischemia (MI) followed by 3 h of reperfusion (R), and randomized to receive vehicle (i.v. infusion at the rate of 1.5 ml/kg/h beginning at 5 min before R), glucose–insulin–potassium (GIK) or GK. At the end of R, 1.5–2 mm coronary segments distal to the site of occlusion were removed, mounted and perfused in wire myographs. Endothelium-dependent relaxations to acetylcholine (ACh, 10^{-12}–10^{-6} M) were determined in arteries pre-contracted by endothelin (10^{-8} M). I/R induced a marked decrease in the coronary responses to ACh (maximal relaxations: sham: 62 ± 7%, $n = 5$; I/R + vehicle: 40 ± 8%, $n = 8$, $P < 0.05$). This impaired response was partially restored by the treatment with GIK (53 ± 6%, $P < 0.05$ vs. vehicle, $n = 10$), but not GK (37 ± 5%, $P > 0.05$ vs. vehicle, $n = 10$). Western blot showed the enhanced phospho-eNOS expression ($P < 0.01$ vs. vehicle, $n = 3$) of coronary arteries. Moreover, incubation of coronary arterial segments with insulin (10^{-10} to 10^{-7} M) elicited a concentration-dependent increase of nitric oxide production in culture medium, with maximal increase of 132% ($P < 0.01$, $n = 5$). Taken together, these data shows that GIK/insulin preserves the endothelial-dependent coronary function during MI/R which is, partly at least, attributed to the insulin-induced increase of endothelial release of nitric oxide.

Abstract N° C77

Palmitate oxidation does not jeopardise function in the ageing heart

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Recent evidence has shown that ageing predisposes the heart to failure and an enhanced susceptibility to stress injury. However, the underlying cellular mechanisms are unclear. Metabolic studies in the failing hypertrophied heart have identified a shift in substrate utilisation from fatty acids to glucose. Adaptations in the ageing heart are thought to parallel closely those observed in cardiac hypertrophy. Therefore, the aim of this study was to investigate palmitate oxidation in the ageing heart and determine whether metabolic adaptations predispose the ageing heart to stress injury. Oxidative flux using ^{13}C NMR and cardiac function were investigated in hearts from 6 months ($n = 29$) and 24 months

(n = 23) rats. Hearts were perfused in an isovolumic mode with buffer containing 3% albumin and a physiological mixture of substrates including low (0.3 mM) (LP) or high (1.2 mM) (HP) U-^{13}C palmitate, ± 40 μg/l dobutamine (D). Cardiac function and efficiency were preserved with age under baseline conditions, HP and HP+D. However, there was a significant decline (–18.0 ± 11.0%, $P < 0.05$) in efficiency in the 6 month hearts perfused with HP+D with a concurrent fall in developed pressure. Palmitate oxidation was significantly greater in 24 month hearts perfused with LP relative to 6 months, but at HP, palmitate oxidation was significantly lower in the 24 month hearts. HP+D further increased palmitate oxidation in the aged hearts.

	% Palmitate oxidation			
	n	0.3 mM (LP)	1.2 mM (HP)	1.2 mM (HP+D)
24 months	5	38.0 ± 8.7	63.6 ± 4	74.5 ± 6.9
6 months	6	25.4 ± 7.1 *	82.3 ± 7.6	n.d.

*$P < 0.05$, 6 m vs. 24 m LP; $P < 0.001$, 6 m vs. 24 m HP; * n = 11.*

Thus, alterations in palmitate oxidation do not jeopardise cardiac function nor enhance susceptibility to dobutamine induced stress in ageing hearts.

Abstract N° C78

Pyruvate augments phosphorylation potential and glutathione redox state of arrested myocardium

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Purpose. – The natural carbohydrate pyruvate increases energy state, antioxidant defenses and contractile performance of isolated and in situ hearts. We tested whether pyruvate could restore energy and antioxidant reserves of arrested myocardium during internal CPR and following recovery of spontaneous circulation (ROSC).

Methods. – Open-chest, anesthetized beagles were subjected to 5 min cardiac arrest and 5 min cardiac massage (80 compressions/min; aortic pressure 60–70 mm Hg), then defibrillated (5 J epicardial DC countershocks). Pyruvate (pyr) or NaCl control was infused i.v. (0.125 mmol/kg/min) from 4 min arrest to 25 min ROSC. Energy metabolites (phosphocreatine, creatine and inorganic phosphate), glutathione (GSH) and glutathione disulfide (GSSG) were measured in snap-frozen biopsies of the anterior wall to determine phosphocreatine phosphorylation potential (~PCr; M^{-1}), a measure of myocardial energy reserve, and glutathione redox state (GSH/GSSG).

Results. – (Table 1: mean ± SEM, n = 6–8; *$P < 0.05$ vs. pre-arrest; $^{\S}P < 0.05$ vs. NaCl): Cardiac arrest severely depleted myocardial energy and GSH antioxidant reserves. CPR partially restored energy and GSH redox states; pyruvate increased energy state even further. At 25 min ROSC, pyruvate augmented myocardial contractility (+ dP/dt, mm Hg/s) and GSH redox state.

Conclusion. – Pyruvate accelerates myocardial energetic and antioxidant recovery during CPR and ROSC.

	+d*P*/*dt*	~PCr	GSH/GSSG
Pre-arrest	3200 ± 130	174 ± 19	23 ± 2
5 min arrest	–	3.0 ± 0.8 *	9.3 ± 0.9 *
CPR + NaCl	–	30 ± 6 *	15 ± 2 *
CPR + pyr	–	63 ± 15 *·**	20 ± 1
ROSC + NaCl	2410 ± 170 *	176 ± 31	20 ± 3
ROSC + pyr	3290 ± 260 **	238 ± 24 *	32 ± 3 **

Abstract N° C79

Pyruvate supplementation ameliorates ex vivo cardiac dysfunction in GLUT4 deficient mice

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Deficiency of the insulin-stimulated GLUT4 transporter impairs cardiomyocyte glucose uptake. Reduced availability of glucose in the heart has been associated with development of cardiac hypertrophy and contractile dysfunction in insulin resistance. Pyruvate, a metabolic product of glycolysis and an oxidizable fuel in the heart elicits a positive inotropic response when administered after ischemia. The goal of this study was to investigate if pyruvate could ameliorate cardiac contractile dysfunction associated with GLUT4 deficiency in the basal state. We investigated the GLUT4 knock-out (GLUT4-KO) mouse model (cardiac specific) and littermate controls, which show a global GLUT4 knock-down (GLUT4-KD), and wild-type C56Bl/6 control mice (WT). Left ventricular function was measured ex vivo in Langendorff-mode perfused hearts using a fluid-filled balloon and pressure transducer (MLT884) (Krebs–Henseleit buffer, 37 °C). Under standardized diastolic pressure conditions GLUT4-KO hearts showed significantly reduced developed pressure and rate of pressure decline compared to GLUT4-KD and WT hearts (54 ± 12, 104 ± 8, 160 ± 6 mmHg, –1.9 ± 0.5, –3.4 ± 0.4, –4.67 ± 0.2 mmHg/ms, $P <$ 0.05). Heart rate and rate of pressure development were significantly reduced in GLUT4-KO compared to WT hearts only (313 ± 33 vs. 413 ± 11 bpm, 3.9 ± 0.5 vs. 6.2 ± 0.4 mmHg/ms, $P < 0.05$). GLUT4-KO response to pyruvate supplementation (5 mM) was significantly enhanced compared to GLUT4-KD and WT mice. GLUT4-KO mice showed a 22% increase in developed pressure, a 12% increase in rate of pressure development and a 32% increase in rate of pressure decline on addition of pyruvate. Our findings suggest pyruvate supplementation ameliorates cardiac dysfunction associated with GLUT4 deficiency, by bypassing the glycolysis pathway and restoring cardiac energy reserves and subsequently enhancing cardiac performance.

Abstract N° C80

Aspartic acid and mannitol enhance efficacy of the St. Thomas cardioplegic solution

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The aim of this work was to assess effects of a modified St. Thomas' Hospital cardioplegic solution (MSTS) on postis-

chemic metabolic and functional recovery of isolated rat heart. The St. Thomas' Hospital cardioplegic solution no. 2 (pH 7.8 ± 0.1 at 22 °C) was used as a control (STS). MSTS additionally contained 21.5 mM aspartic acid and 20.0 mM mannitol, pH was 7.6 ± 0.1 at 22 °C. Osmolarity of both cardioplegic solutions was 340 ± 5 mOsm. After 20 min initial perfusion according to Neely the hearts were subjected to 40-min normothermal total ischemia followed by 30-min reperfusion. Cardioplegic solutions were infused prior to ischemia at rate of the initial coronary flow for 5 min at room temperature. After ischemia MSTS treated hearts exhibited 1.4-fold less lactate accumulation and significantly better preservation of ATP and phosphocreatine (PCr) compared with these indices in control. By the end of reperfusion the hearts of MSTS group completely recovered coronary flow (to 98 ± 3% of the initial value vs. 77 ± 3% in the control, $P < 0.02$) and showed better restoration of cardiac work and pressure–rate product (to 64 ± 3% and 72 ± 6% of the initial values, respectively, vs. 12 ± 2% and 24 ± 5% and in the control, $P < 0.01$). The hearts treated with MSTS recovered contents of PCr and lactate to the preischemic values and restored ATP level up to 65.2 ± 4.6% of initial one comparing with 40.5 ± 3.2% in the control at the end of reperfusion. The hearts protected by MSTS showed a markedly reduced formation of (5,5′-dimethyl-1-pyrroline-*N*-oxid)-OH, a hydroxyl radical adduct at the early reperfusion assessed by spin-trap measurements. Therefore in an isolated rat heart model MSTS provides substantially superior protection against ischemia/reperfusion stress compared to STS.

Abstract N° C81

Measurement of IMP in purine mixtures

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Ischaemia induced catabolism of adenine and guanine nucleotides produces an array of purine nucleotides, nucleosides and bases, analysis of which provides information about the enzymatic reactions involved. Most purines can be quantitated by reversed phase high performance liquid chromatography (RP-HPLC) with UV absorbance. A problem is the measurement of small amounts of inosine 5′-monophosphate (IMP) in the presence of ATP as both nucleotides co-elute. We searched for a method that allows accurate assay of IMP without changing the HPLC conditions. We noticed that ATP and ADP are acid labile. Both nucleotides were readily dephosphorylated in 6% perchloric acid (PCA). Acid hydrolysis affected primarily the gamma and beta phosphates. Under the conditions chosen, the monophosphates AMP and IMP were stable. The AMP content of acidic extracts increased with time due to hydrolysis of its precursor ADP. The IMP content remained constant. IMP was neither formed nor degraded in acid milieu. Importantly, deamination of AMP–IMP did not occur. The acid hydrolysis of ATP was temperature dependent. Dephosphorylation was complete after 8 h at 37 °C and 24 h at ambient temperature. These observations facilitate the measurement of IMP in tissue extracts by RP-HPLC. The following procedure is recommended. Specimens were extracted in 6% PCA. Following centrifugation, the acid supernatants were divided. One part was neutralized with 5 N KOH. The other part was kept 24 h at room temperature. Analysis was by RP-HPLC. The neutralized supernatant was analysed directly (all purines) and the acid extract next day (IMP). The present study provides a method for the measurement of IMP in complex purine mixtures by RP-HPLC, which is known for deficient separations of ATP and IMP. To obviate modifications of the chromatographic conditions, the co-eluting ATP was hydrolysed with 6% PCA prior to HPLC. As PCA is commonly used for tissue extractions, it is important to recognize that the preservation of acid labile nucleotides for subsequent analysis requires instant neutralisation.

Abstract N° C82

Inihibition of aldose reductase prevents carotid artery restensosis in diabetic rats

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Diabetes is a significant risk factor for cardiovascular disease and a predictor of restenosis, but the mechanisms by which diabetes affects vascular response to injury remain unknown. We tested the hypothesis that aldose reductase (AR) activity is a key determinant of diabetic vascular responses to injury. The study group consisted of 20 non-diabetic and 20 diabetic rats. Diabetes was induced by a single injection of streptozotocin (55 mg/kg, i.p.). Six weeks after streptozotocin treatment left carotid artery of non-diabetic and diabetic animals were injured by balloon withdrawal. One day before the injury and throughout the observation time, 10 rats in each group were gavage-fed with AR-inhibitor tolrestat. Ten days after the injury, the injured arteries of streptozotocin-diabetic rats displayed >35% greater abundance of AR as compared to the injured carotid arteries of non-diabetic rats. In diabetic rats, intimal expansion was >30% higher at 10 d of injury and >50 higher at 21 d of injury as compared to the corresponding non-diabetic rats. Ten days after the injury, a greater number of cells (30–50%) in 10-d old diabetic neointima were stained with antibodies against proliferating cell nuclear antigen (PCNA), α-actin, and activated NF-ϰB. At 21 d after the injury, PCNA, α-actin and AR staining of the diabetic neointima were comparable to non-diabetic rats. Treatment with the AR inhibitor tolrestat decreased neointima formation and the number of PCNA, α-actin, and activated NF-κB-positive cells in diabetic lesions. In injured carotid arteries, tolrestat treatment increased the abundance of protein adducts with the lipid peroxidation product—4-hydroxy *trans*-2-nonenal. These results suggest that vascular inflammation and responses to injury are exaggerated during diabetes and that these responses are prevented by inhibiting AR.

Abstract N° C83

NRF1 Inhibits USF1- dependent induction of the cardiac isoform of acetyl-CoA carboxylase

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Previous studies have shown that 5′-AMP activated protein kinase (AMPK) is upregulated with hypoxia. Moreover, AMPK inhibits the cardiac-enriched acetyl-CoA carboxylase isoform (ACCβ), an indirect inhibitor of mitochondrial fatty acid uptake. To further delineate its regulation in the heart, we measured in vivo ACCβ gene expression with hypoxia and found it was significantly reduced ($P < 0.05$). However, the transcriptional mechanisms directing ACCβ gene expression are poorly understood. Since AMPK activates nuclear respiratory factor 1 (NRF1), we hypothesized that hypoxia diminishes ACCβ gene expression by NRF1 activation. To evaluate this, we transiently transfected a 1317 bp human ACCβ promoter-luciferase construct (pPβII-1317) with an NRF1 expression vector into H9c2 myoblasts. Interestingly, NRF1 co-transfection diminished basal pPβII-1317 induction by 55 ± 6% ($P < 0.001$). To rule out interference of endogenous muscle-type trans-activators, we co-transfected pPβII-1317 and upstream stimulatory factor 1 (USF1), a putative trans-activator of ACCβ, into null-background CV-1 fibroblasts and HepG2 hepatic cell lines. Here, NRF1 inhibited USF1-dependent induction of pPβII-1317 by ≥65% ($P < 0.01$). Moreover, transfection studies using deletion constructs suggest that a putative NRF1 regulatory region is situated between +65 and –93 bp of the ACCβ promoter region. We therefore propose that NRF1 may be a novel repressor of ACCβ gene expression during hypoxia.

Abstract N° C84

Low flow myocardial ischaemia: a metabolic balancing act

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The energy balance involved in maintaining cell viability during myocardial hibernation remains poorly understood; increased ^{18}FDG6P seen in clinical PET scans suggests that glucose flux is crucial. Using PANDA (*P*ET *A*nd *N*MR *D*ual *A*cquisition) and immunogold electron microscopy (IGEM), we have constructed an "energetic profile" of an isolated heart model that, we have shown previously, exhibits many of the clinical hallmarks of hibernation. Rat hearts ($n = 4$/group) were perfused (Krebs +250 MBq ^{18}FDG +11 mM glucose +/– 0.4 mM oleate) for 40 min at 100% flow and 3 h at 10% flow; ^{18}FDG scans and ^{31}P NMR spectra were acquired throughout. Hearts were then fixed and the distribution of GLUT 1, GLUT 4 and hexokinase I and II determined by IGEM. The rate of ^{18}FDG6P accumulation in ischaemic tissue was the same as (glucose only, G) or higher than (glucose+oleate, G + O) control tissue despite receiving 10% of the flow (23 ± 2 vs. 11 ± 2 cps/g/min, G + O vs. control, $P < 0.05$). In G hearts, pH decreased to 6.76 ± 0.02 and did not recover; PCr decreased to 287 ± 9%, recovering to 39 ± 7% at 3 h and P_i increased to 467 ± 88% of preischaemic values. In G + O hearts, pH decreased to 6.88 ± 0.02, but recovered to 6.98 ± 0.05; PCr decreased to 42 ± 14% but recovered to 68 ± 12% and P_i increased to 220 ± 53%. In response to ischaemia, GLUT1 did not move but GLUT4 moved from the cytosolic vesicles to the sarcolemma and the T-tubules in all hearts, with a larger change in the presence of oleate. In G + O hearts, ischaemia increased sarcolemmal and T-tubule GLUT 4, from undetectable to 0.18 ± 0.06, and from 0.05 ± 0.02 to 0.15 ± 0.04 golds μm^{-1}, respectively. Concomitantly, GLUT4 in cytosolic vesicles decreased from 4.38 ± 0.84 to 1.88 ± 0.30 golds μm^{-2}. In G hearts, ischaemia translocated hexokinase II to the mitochondria, thus increasing glycolytic through put; this effect was not observed in the presence of oleate. We conclude that there is sufficient O_2, even at 10% flow, to enable fatty acid oxidation; this contributes significantly to the maintenance of both cellular energy levels and intracellular pH.

Abstract N° C85

Preischemic intracellular glycogen as a determinant of cardioprotective effect of ischemic preconditioning

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Relation between preischemic intracellular glycogen (PIG) and ischemic preconditioning (IPC) effect was assessed in isolated Langendorff-perfused rabbit (1.7 kg body weight) hearts. Hearts were subjected to 45 min global ischemia (I) followed by 120 min reperfusion (R), with IPC (5 min I and 10 min R, $n = 10$) or not (control, $n = 9$). PIG content (μmol/g tissue, mean ± S.E.), left ventricular function, lactate, high-energy phosphate derivatives (HEPd) and cell viability (assessed by formazan production) considered for statistical analyses. Baseline PIG before 45 min I and before IPC were 16.12 ± 73 and 15.34 ± 63 mg/l, respectively; lactate and HEPd were not significantly different between groups. IPC increased recovery of LV function and cell viability. However, these cardioprotective effect of IPC was abolished by concurrent low PIG (10.00 ± 50 by 5 mg/l sodium acetate, $n = 10$, $P < 05$ vs. baseline) or high PIG (23.58 ± 1.41 by 1 unit/l insulin, $n = 10$, $P < 01$ vs. baseline) before IPC. These results indicate that PIG before IPC may be a key determinant for the cardioprotective effect of IPC.

Abstract N° C86

Diabetes, the hexosamine biosynthesis pathway and protein *O*-glycsoylation in the heart

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The hexosamine biosynthesis pathway (HBP) consumes 2–4% of the glucose transported into the cell leading to protein *O*-glycosylation. Increased flux through the HBP as

well as increased protein *O*-glycosylation have been implicated in diabetic complications; however, little is known about the HBP in the heart. Therefore, we measured the level UDP-GlcNAc, a metabolite of the HBP, in hearts from 12-week-old male Zucker diabetic fatty (ZDF) rats and Lean non-diabetic littermates. UDP-GlcNAc levels are increased from 60 ± 7 in Lean to 112 ± 7 arbitrary units in the ZDF group ($P < 0.05$). To evaluate the level of protein *O*-glycosylation, we used CTD110 an antibody that is specific for *O*-GlcNAc, on protein extracts from ZDF and lean hearts. The level of *O*-GlcNAc in ZDF group was clearly increased compared to Lean (88 ± 8 vs. 65 ± 5 O.D. units; $P < 0.05$). To determine the impact of altered HBP flux, hearts from ZDF rats were perfused ± azaserine (Aza, 20 μM), an HBP inhibitor, and hearts from Lean rats were perfused ± glucosamine (GlcN, 5 mM), which increases HBP flux. All hearts were subjected to low flow ischemia (30 min, 0.3 ml/min) and 60 min reperfusion. In the absence of treatment, functional recovery as % of pre-ischemic level was higher in the ZDF group compared to the Lean group (90 ± 7% vs. 74 ± 8%; $P < 0.05$). However, Aza reduced recovery of the ZDF group (69 ± 6%; $P < 0.05$) whereas GlcN improved recovery of the Lean group (94 ± 7%; $P < 0.05$). These data show that diabetes increases HBP flux and *O*-glycosylation in the heart and that this may account for the increased recovery of function in the ZDF group following ischemia.

Abstract N° C87

Increased carbohydrate oxidation and decreased fatty acid oxidation do not improve cardiac function after ischemia

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A shift from fatty acid to carbohydrate oxidation in the heart has been reported to decrease ischemic injury. However, many of these reports are based on studies where glucose is the only available carbohydrate. Therefore, we investigated whether increasing carbohydrate oxidation was protective when physiologically relevant concentrations of lactate (1 mM) and pyruvate (0.1 mM) as well as glucose and palmitate (0.3 mM) were available. Hearts from male Sprague–Dawley rats were isolated, perfused and assigned to one of three groups: (1) Control, glucose 5 mM, insulin 50 μU/ml; (2) +dichloroacetate (DCA), 5 mM; and (3) glucose 30 mM and insulin 1000 μU/ml (HG/HI). All hearts were subjected to low flow ischemia (30 min, 0.3 ml/min) and 60 min reperfusion. Substrate oxidation rates were determined during reperfusion using 13C-NMR glutamate isotopomer analysis. Total carbohydrate oxidation increased from 45 ± 4% of total substrate oxidation in control group to 86 ± 3% in the DCA group ($P < 0.01$). However, the recovery of rate pressure product (RPP) was similar in both Control and DCA groups (73 ± 11% and 74 ± 5%, respectively). Surprisingly in the HG/HI group carbohydrate oxidation was only 46 ± 2%, which was not significantly different from control. However, % recovery of RPP was improved to 86 ± 2% ($P < 0.05$ vs. control). These data suggests that in the presence of other carbohydrates, a shift from fatty acid to carbohydrate oxidation may not be associated with improved recovery of function following LFI.

Abstract N° C88

Oxidative and non-oxidative metabolic substrate fluxes in normal and ischemic rat heart

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During acute occlusion of a coronary artery, collateral vessels may provide 10–20% of baseline flow in the infarct zone. However, our understanding of energy metabolism during such low flow ischemia is relatively limited. Therefore, we developed a method using $^{1}H/^{13}C$ NMR spectroscopy and oximetry to quantify the oxidation of up to four exogenous substrates plus oxidative and non-oxidative glycogen metabolism, during severe low flow ischemia (LFI, 0.3 ml/min). Isolated rat hearts were perfused for 30 min under control and LFI conditions with insulin and ^{13}C-labeled lactate, pyruvate, palmitate, and glucose. The results (see Table 1) show that despite a ~50-fold reduction in substrate delivery and oxygen consumption, during LFI oxidative metabolism accounted for ~30% of total ATP synthesis. During control perfusion, lactate oxidation was the major source of ATP; however in LFI this shifted to a combination of oxidative and non-oxidative glycogen metabolism. These results demonstrate the importance of oxidative energy metabolism for ATP production even during very LFI. We believe that this approach will be valuable for studies into mechanisms related to the protective effect of increasing cardiac carbohydrate utilization.

Table 1
Total, non-oxidative and oxidative, ATP synthesis rates (μmol/min/g wet weight) in the control (con) and LFI groups

	Total	Non-oxidative	Oxidative	% oxidative
Control	21 ± 1	1.3 ± 0.2	20 ± 1	97 ± 9
LFI	1.5 ± 0.3	1.0 ± 0.2	0.5 ± 0.1	33 ± 7

Abstract N° C89

Effects of simultaneous antegrade and retrograde cardioplegia on myocardial water content, cellular compartments, and energy metabolism

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We have demonstrated in our previous study that simultaneous antegrade/retrograde cardioplegia (SARC) improves myocardial blood perfusion in jeopardised regions as well as in normal region of the myocardium. The present study was to assess the effects of SARC on myocardial water content, the volumes of the intracellular and extracellular compartments, and myocardial energy metabolism. Four isolated pig hearts were subjected to 20-min control perfusion and 2-h

SARC. Throughout the experiment, myocardial water content was monitored using near infrared (NIR) spectroscopy with a NIR probe positioned over the anterior wall of the left ventricular wall. The volume of the intracellular and extracellular compartments and high-energy phosphates of the hearts were followed using ^{31}P MR spectroscopy in conjunction with the chemical markers for the total water space (dimethyl methylphosphonate, DMMP) and extracellular space (phenylphosphonic acid, PPA). NIR spectra showed a significant increase in water peak (62 ± 15%) during the 2-h SARC, suggesting severe tissue edema. ^{31}P MR spectra exhibited significant increase in the levels of DMMP and PPA, which translates to significant expansion of both intracellular (35%) and extracellular (50%) compartments. However, the levels of high-energy phosphates (ATP and PCr) remained at normal levels throughout 2-h SARC. We conclude that although SARC increases tissue water content and enlarges the cellular compartments, it sustains normal myocardial energy metabolism. The effect of SARC-induced edema on cardiac contractile function, however, remains to be investigated.

Abstract N° C90

Regional flow, oxygenation and optical tracer kinetics assessed by near-infrared imaging in the in vivo pig hearts

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We used near-infrared spectroscopic (NIRS) imaging to assess flow dependence of heart oxygenation, mapping oxy- to deoxy-(hemoglobin (Hb) + myoglobin (Mb)) ratios (O/D) and intravascular tracer, indocyanine green (ICG). In open-chest pigs nominal flow through the left anterior descending artery was reduced to 0 (n = 6), 20 ± 1 and 44 ± 5% by variable 90-min occlusion and restored afterwards (to 219 ± 71%) for 120 min. ECG-gated NIRS images of the heart were obtained using a CCD-array camera with a liquid crystal tunable filter, which acquired reflectance spectra in the range of 650–1050 nm for each of 256 × 256 pixels (0.4 mm^2). Deoxy-, and oxy-(Hb+Mb) contents were calculated using a spectral fitting algorithm based on their absorption spectra between 650–890 nm. To visualize flow distribution ICG bolus (8.3 mg/5 ml) was injected i.v. at each step of the protocol and gated images were acquired at 800 nm every beat over 60 s period. The ratio of ICG wash-in velocity to equilibrium absorbance (V/A_{tail}) was calculated. O/D and V/A_{tail} decreased as flow reduced in the area at risk (Table 1). Thus oxy/deoxy measurements detected regional flow below 44% while ICG kinetics detected flow below 20%.

Step	(n)	Flow (%)	D(O/D) vs. 0 flow	V/A_{tail}
Occlusion	(5)	20	0.10 ± 0.28	0.32 ± 0.34
Occlusion	(4)	44	0.76 ± 0.32	1.20 ± 0.39
Normal	(9)	100	1.63 ± 0.58	1.60 ± 0.49
Reflow	(9)	219	2.25 ± 0.92	1.80 ± 0.58

Abstract N° C91

Regional oxidative metabolism in complete left bundle branch block

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The recent reports discuss about that homogenization of myocardial glucose and oxidative metabolism results in successful cardiac resynchronization therapy (CRT) in heart failure (HF) with complete left bundle branch block (CLBBB). We have three responders to CRT, who also showed homogenization of oxidative metabolism. Inhomogeneity of myocardial metabolism might be the independent predictor of cardiac reverse remodeling. On the other hand, regional metabolism in isolated CLBBB has been uncertain. We evaluate the oxidative regional metabolism in patients with CLBBB by measuring the monoexponential clearance rate of [^{11}C] acetate (k_{mono}) with positron emission tomography (PET). Serial CLBBB patients with HF (group A, seven cases, five males, age 68 ± 9 years), CLBBB without HF (group B, 12 cases, five males, age 68 ± 10 years) and HF patients without CLBBB (group C, seven cases, five males, age 66 ± 18 years) that consulted our hospital from January to September 2003 underwent PET estimation. We also did PET estimation in five volunteers (group D, five males, age 35 ± 5 years). The k_{mono} lateral to septum ratio of group A and B were similar (1.18 ± 0.42 and 1.16 ± 0.10) but higher than group C and D (0.96 ± 0.08 and 1.00 ± 0.13). Patients with CLBBB have inhomogeneity of myocardial oxidative metabolism despite of whether they have HF or not. So further work should be necessary to clarify the relation between improvement of HF and metabolic homogenization that CRT results in.

Abstract N° C92

Glucagon-like peptide-1 mediated cardiac protection against ischemia/reperfusion injury involves multiple pro survival pathways

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We have shown previously that GLP-1 protects against myocardial infarction in both in vivo and in vitro rat model. The present study aimed to elucidate the mechanisms by which such protection occurred. We used a Langendorff perfused rat heart model of 30 min regional ischemia and 120 min reperfusion. The end point was the measurement of the infarction developed in the risk zone using triphenyltetrazolium chloride. The values were expressed as percentages of the area at risk (I/R%); 0.3 nM GLP-1 and 20 mg/l valine pyrolidide (VP), an inhibitor of GLP-1 breakdown, were added 15 min prior to ischemia and continued throughout the experiment. Our results showed that GLP-1 significantly reduced infarct size (26.7 ± 2) vs. control (58.7 ± 4.1) and VP alone (52.6 ± 4.7) ($P < 0.001$). This protection was

abolished by the GLP-1 receptor antagonist Exendin-9-39 (57.3 ± 3.8, $P < 0.001$) suggesting a receptor mediated trigger. The c-AMP inhibitor Rp-cAMP also abolished the protection (57.5 ± 5.0) suggesting the involvement of a cAMP dependent pathway. Furthermore the protection was abolished by the PI3kinase inhibitor LY294002 (43.4 ± 3.9) as well as by the p42/44 MAPK inhibitor UO126 (48.3 ± 8.6) implicating both these pro survival pathways in the cardioprotection mediated by GLP-1. This study demonstrates that GLP-1 protects the myocardium from ischemia reperfusion injury via activation of a number of cellular pro survival signaling pathways.

Abstract N° C93

Differential generation of superoxide and cytosolic nadph by bovine pulmonary vs. coronary artery

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Systemic and pulmonary circulatory systems have been known to show physiologically different phenotypic characteristics. However, the underlying biochemical differences have not been sorted out. In this study, we demonstrate that bovine pulmonary artery (PA) generated twofold more lucigenin (5 μM) chemiluminescence detectable superoxide than coronary artery (CA). A thromboxane receptor agonist, U46619 (100 nM) which activates protein kinase C (PKC), increased superoxide by 1.3-fold and 1.5-fold in the CA and PA, respectively. Furthermore PDBu (10 μM), a PKC agonist, elevated the superoxide production by fivefold in CA vs. 12-fold in PA. In addition, we have found that PA generates 4.2-fold more NADPH, which is one of the co-factor for NAD(P)H oxidase, and 7.5-fold more $NADP^+$ than the CA. The pentose phosphate pathway (PPP) is the primary source of cytosolic NADPH in many cell types and glucose-6-phosphate dehydrogenase (G-6-PD) is a rate limiting enzyme of PPP. Thus, examination of G-6-PD activity in PA and CA indicated that in the PA G-6-PD was 1.5-fold more active than in CA. Similarly, G-6-PD protein was 1.6-fold greater in the PA than CA. Thus, PA differ from CA in that they have higher levels of G-6-PD activity, NADPH and superoxide.

Abstract N° C94

Agonist-mediated control of adenosine receptor transcripts in multiple cell types

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Adenosine receptors are crucial in cardiovascular control and cardioprotection. However, nothing is known regarding control of their cellular expression. Using quantitative real-time PCR we investigated agonist-mediated control of adenosine receptor (*ADOR*) gene expression in Jurkat T-cells and U397 and THP-1 monocyte cultures. Data reveal that adenosine selectively modulates *ADOR* levels. Adenosine (50 μM) or A_1AR agonist (50 nM CPA) both reduced *ADORA1* and *ADORA2A* expression (2–3-fold) in Jurkat cells after 4 h of stimulation. A_3AR agonism (100 nM Cl-IB-MECA) down-regulated *ADORA1* and *ADORA2A* levels (12- and 4-fold, respectively), whereas A_{2A}AR agonism (10 nM CGS21680) down-regulated *ADORA1* (~3-fold) without modifying *ADORA2A*. Conversely, *ADORA2B* expression was modestly enhanced by all agonists. Unfortunately accurate *ADORA3* quantitation in these cell types was limited due to its low expression. Both phosphotidylinositide 3-kinase (PI3-K) and MEK/ERK dependent pathways maintain *ADORA1* and *ADORA2A* transcription, since inhibition (100 nM wortmannin, 50 μM PD98059) virtually abolished *ADORA1* and *ADORA2A* expression. In contrast, MEK/ERK represses *ADORA3*, since PD98059 enhanced expression >4-fold. MEK/ERK signalling also counters stimulatory effects of adenosine on *ADORA2B* and *ADORA3*. These data indicate that: (i) *ADOR* mRNA levels are differentially regulated via receptor agonism; (ii) PI3-K and MEK/ERK dependent paths maintain *ADORA1* and *ADORA2A* expression; (iii) MEK/ERK activity represses *ADORA3* expression and counters induction of *ADORA2B* and *ADORA3* by adenosine. Further studies with PKC, PKA, Raf-1 and tyrosine kinase inhibitors, and in U937 and THP-1 cells, reveal transcriptional responses involve multiple signalling paths and vary in different cell types.

Abstract N° C95

Targeted deletion of A_{2A} adenosine receptors attenuates post-ischemic coronary flow without altering cardiac contractility

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The relative roles of A_2 adenosine receptor subtypes in regulation of coronary flow (CF) and myocardial contractility following ischemia–reperfusion remain incompletely understood. To examine the effects of targeted deletion of A_{2A} adenosine receptors (A_{2A}AR) on coronary vascular and functional responses to ischemia–reperfusion, constant-pressure perfused isolated hearts from A_{2A}AR knockout (A_{2A}KO) mice and their respective wild-type (WT) littermates underwent 20 min of global ischemia followed by 40 min of reperfusion. In the first 3 min of reperfusion A_{2A}KO and WT hearts had equal post-ischemic hyperemia (181 ± 17 and 168 ± 13% change from pre-ischemic baseline, respectively, $n = 4$, n.s.). However, beginning at 5 min of reperfusion, CF in A_{2A}KO hearts was ~35% lower than WT hearts and this reduction was sustained for the duration of reperfusion (CF at 40 min 82 ± 5 and 127 ± 17% of baseline, respectively, $n = 4$, $P < 0.05$). Left ventricular developed pressure (LVDP) and diastolic pressure (DP) were the same in A_{2A}KO and WT hearts throughout reperfusion (LVDP at 40 min 79 ± 4 and 73 ± 4% of baseline, respectively, $n = 4$, n.s.; DP at 40 min 6 ± 3

and 13 ± 7 mmHg, $n = 4$, n.s.). Taken together, these data support the conclusion that targeted deletion of $A_{2A}AR$ attenuates post-ischemic CF without altering contractile function in isolated murine hearts. That recovery of LVDP and DP are preserved independent of attenuated CF in $A_{2A}KO$ hearts raises the possibility that contractile function is supported by alternative adenosine receptor subtype(s) in this model.

Supported by NIH KO8 HL074001 and HL 27339.

Abstract N° C96

Mechanisms of postischemic cardiac release of TNFα: insights from knockout mice

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Reperfusion of the human heart after bypass surgery is accompanied by release of tumor necrosis factor-alpha (TNFα) in two phases: acutely (first min of reperfusion) and delayed (after about 60 min). An identical time-course is seen when TNFα is liberated from isolated hearts of rat or mouse. This study assessed TNFα release in isolated hearts of mice after 15 min of global ischemia, comparing IL-$6^{-/-}$ (H. Drexler, MHH Hannover), MMP-$7^{-/-}$ (L. Matrisian, Nashville, TN), mast-cell deficient $kit^{w/w-v}$ (L. Hueltner, GSF Munich), TNFα-$R1^{-/-}$ (K. Pfeffer, TU Munich) and wild-type mice, the latter without and with infusion of cycloheximide or matrixmetalloprotease(MMP)-inhibitor II. Samples of coronary effluent and interstitial transudate were analyzed for cytokine by ELISA. Cytokine release from TNFα-$R1^{-/-}$ hearts showed a similar two-peak pattern to wild-type controls, so that autostimulation via TNF-receptor 1 does not seem obligatory. Immunohistology demonstrated TNFα predominantly in cardiac mast cells of wild-type mice. Release of TNFα from mast cell-deficient hearts was absent during early reperfusion but increased with duration of reperfusion. Pertinently, $kit^{w/w-v}$ mice revealed TNFα localized to tissue macrophages and endothelial cells in the heart. Therefore, mast cells supply most of the TNFα released immediately upon reperfusion, but are not the only source in the murine heart. Surprisingly, the pattern of TNFα release of IL-$6^{-/-}$ mice was similar to that of $kit^{w/w-v}$ mice, i.e. there was no early peak. This may be explained by the fact that IL-6 is an important factor for the maturation of mast cells. Indeed, the mast cells in IL-$6^{-/-}$ hearts stained only poorly. MMP-$7^{-/-}$ mice lacked the late TNFα peak. Infusion of MMP-inhibitor II, a specific inhibitor of the metalloproteases 1, 3, 7 and 9, or of cycloheximide, a protein synthesis inhibitor, likewise suppressed the late peak. Thus, the second phase of TNFα release after about 1 h of reperfusion is due to de-novo synthesis, and may involve cleavage of membrane bound TNFα by MMP-7. Experiments using regional ischemia suggest that cardiomyocytes and not mast cells are the source.

Supported by the Deutsche Forschungsgemeinschaft (SFB 469).

Abstract N° C97

Over pressure load induce production of tumor necrosis factor-α

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To observe whether TNF-α is involved in the process of myocardium hypertrophy induced by overpressure in vivo. Coarctation of the abdominal aorta was performed on male Sprague–Dawley rats weighing 180–200 g. Sham-operated controls underwent an identical procedure except for the placement of the silver clamp. Cardiac hypertrophy was evaluated by IVSTd and LVPWd at 1 and 3 months after coartation by M dimensional echocardiography. Immunohistochemistry study was used to detect TNF-α expression level in myocardium. Electrophoretic motif shift assay EMSA was used to screen for the probable promoter of TNF-α. Left ventricular (LV) wall thickness was assessed by echocardiography once a month after the operation. Three months later, aorta coarctation rats develop significantly left ventricular hypertrophy compared with those of sham operation (IVSTd: 2.68 ± 0.40 and 2.46 ± 0.37 mm, respectively; LVPWd: 2.70 ± 0.37 and 2.29 ± 0.36 mm, respectively; $P < 0.05$). But left ventricular wall thickness had no difference between 1 month coarctation and sham operation rats (IVSTd: 2.68 ± 0.40 and 2.46 ± 0.37 mm, respectively; LVPWd: 2.70 ± 0.37 and 2.29 ± 0.36 mm, respectively; $P > 0.05$). The myocardium TNF-α was negative in non-coarctation rats' myocardium, but it was persistently positive in all over-pressure load rats' hearts despite of hypertrophy or not. EMSAs showed that nuclear extract of all overload myocardium specifically bound with two upstream oligonucleotides of TNF-α, which is –619 to –591cat tcc ctc tgg ggc tgc ccc att ctc ctc and –508 to –481ctc aga caa ggg ggc ttt ccc tcc tca ac. Aortic coarctation increase heart pressure load, as well it activate neuroendocrine system. The expression of TNF-α is earlier than the morphorism hypertrophy and persistently exists in the hypertrophy myocardium, suggesting TNF-α participate in the remodeling process. The sequences of –619 ~ –591 and –508 ~ –481 in the upstream of TNF-α contain the potential sites that switch on the expression of TNF-α in over-pressured myocardium.

Abstract N° C98

Angiotensin II stimulate the production of TNF-α in cultured cardiac myocytes of neonatal rat

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To observe the production of TNF-α in cardiac myocytes stimulated with Angiotensin II. In primary cultured rat myocytes, we use 10–7 M Angiotensin II) (AngII)) or 100 ng/ml lipopolysaccharide (LPS) to stimulate the cardiac myocytes for 24 or 48 h. Using immunohistochemistry to observe TNF-α in situ. We performed RT-PCR to detect TNF-α

mRNA in different myocytes. TNF-α immunohistochemistry staining was negative in control myocytes, but it was positive in myocytes treated with AngII or LPS. With the prolongation of the treating time, there was a tendency that the staining granule was more obvious. TNF-α mRNA level was detected by RT-PCR, same tendency was noticed that the optical dense ratio TNF-α PCR product was obviously increased in AngII or LPS treated myocytes, and the quantity was elevated with the continuation of the stimulation. Both protein level and mRNA levels of TNF-α were very low in the control cultured newborn rat myocytes but they were obviously increased in AngII treated myocytes, which suggested that TNF-α may be a effector of AngII in myocytes.

Abstract N° C99

Investigation of urotensin-II converting enzyme activity in human cells and blood

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Human urotensin-II (UII) is an 11 amino acid peptide produced by cleavage of 124 and 139 amino acid residue isoforms of a prohormone. While "urotensin converting enzyme" (UCE) activity is required for biological activity, the endogenous UCE has not been investigated. This study investigated UCE in human cultured epicardial mesothelial cells obtained from the right atrium of patients with coronary artery disease ($n = 3$) and venous blood from healthy individuals ($n = 3$). Cells were incubated in medium containing Tris–HCl with or without $CaCl_2$ (1 mM) and EDTA (2 mM) (37 °C for 3 h). UCE activity was determined by measuring conversion of a 25 amino acid C-terminal fragment of the prohormone (CTF-pUII) to UII using mass spectrometry. The putative cleavage site contains dibasic residues, a common recognition sequence for broad range convertases, and a recognition sequence for the trans-Golgi endoprotease, furin. UII was readily detected at pH 7.0 in the superperfusate of cells incubated with 10 μM CTF-pUII and permeabilised with 0.1% triton X-100, but not in the superperfusate of non-permeabilised cells, indicating the presence of intracellular enzyme(s). UCE activity was inhibited at pH 5.0, and at pH 7.0 when cells were incubated in Ca^{2+}-free medium in the presence of EDTA. The characteristics of UCE activity in cells was similar to that determined for 2 U/ml recombinant furin in a cell-free system. UCE activity was detected in blood samples incubated with CTF-pUII, and was inhibited by 35 μM aprotinin. Similar aprotinin-sensitive activity was observed when CTF-pUII was incubated with 0.05 mg/ml trypsin in a cell-free system. These findings identified UCE activity in human epicardial mesothelial cells that was mainly associated within intracellular compartments, and resembled the characteristics of furin. Aprotinin-sensitive UCE activity was detected in blood, consistent with the presence of a serine protease such as trypsin.

Abstract N° C100

Urotensin II regulates mesenteric micro-circulation in rats

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Urotensin II, a cyclic neuropeptide, confers potent cardiovascular effects including depression of cardiac function and vessel contraction. To investigate the effects of human urotensin II on in vivo mesenteric microcirculation, 24 adult SD rats were randomly divided into the following groups: control, human urotensin II (10^{-6} mol/l), noradrenaline(10^{-6} mol/l), and human urotensin II (10^{-6} mol/l) + NA(10^{-6} mol/l). The intestinal loop was mounted on the stage of an intravital microscope equipped with a TV camera. Video images of microcirculation were stored by a video cassette recorder. Changes of internal diameter and microcirculatory velocity of microvessels were measured using the ImagePro software. The blood flow in intestinal wall was measured with PIMII laser Doppler perfusion imager. The findings were that the internal diameters of arterioles and venules decreased by 34.1% and 41.7% in human urotensin treated rats, respectively. The blood flow in intestinal wall increased 1 min after treated with human urotensin II and up to high peak at 5 min (6.4 ± 1.1 perfusion unit vs. control 4.2 ± 0.9 ,$P < 0.05$). However, microcirculatory velocities of arterioles and venules have no significant changes in human urotensin II group. The study shows that urotensin II contracts mesenteric microvessels and increase microcirculatory blood perfusion to intestinal wall in rats.

Keywords: Peptide; Microcirculation; Small intestine

Abstract N° C101

Effects of human urotensin II on pia mater microcirculation in rats

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Urotensin II is a cyclic neuropeptide confers potent cardiovascular effects including depression of cardiac function and vessel contraction. To determine whether human Urotensin II has effects on cerebral microcirculation, we separated 32 rats into four groups received either normal saline, human Urotensin II , noradrenaline , and Urotensin II with noradrenaline groups. For recording of microcirculation images in pia mater , skull windows were performed and mounted on the stage of an intravital microscope equipped with a TV camera. Video images of microcirculation were stored by a video cassette recorder. Temporal changes in internal diameter and microcirculatory velocity of microvessels were measured using the ImagePro software. Blood flow in cerebral tissue was measured with PIMII laser Doppler perfusion imager. It was found that human Urotensin II decreased the internal diameters of arterioles and venules by 27.7% and 42.4%, respectively. The blood flow in meninges increased by 52% in human Urotensin II treated rats. However, both

microcirculatory velocities in arterioles and venules have no significant changes in human Urotensin II group. We conclude that human Urotensin II contracts microvesseles in pia mater and increases microcirculatory blood perfusion to cerebral tissue in rats. We suggested that Urotensin II may regulate cerebral microcirculation.

Keywords: Peptide; Microcirculation; Brain

Abstract N° C102

Macrophage colony-stimulating factor MCSF-1 and proto-oncogene *c-fms* expression on mitral valves during experimental bacterial endocarditis

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MCSF-1 and its receptor *c-fms* are essential factors for mobilizing and activating mononuclear phagocytes in the inflammatory response. Only little information is available about MCSF-1-triggered activation of monocytes via the *c-fms*-pathway on diseased heart valves. In an experimental model mitral valves of seven untreated rabbits were compared to seven animals after mitral valve surgery and to seven animals after mitral valve surgery plus bacteremia with *Staphylococcus aureus*. The surgical intervention consisted of a Dacron patch being sewed to the anterior leaflet of the valve causing an endothelial lesion and a mild valve insufficiency. Six hours after the intervention, valve tissues were explanted and mRNA expression was analyzed by RT-PCR. The results of the control group were set as 100% and up- or downregulation in the surgery groups are presented as relative values. After mitral valve surgery MCSF-1 mRNA increased to 166 ± 29% ($P < 0.001$), whereas *c-fms* was downregulated to 67 ± 13% ($P < 0.05$) compared to control. After mitral valve surgery in combination with bacteremia MCSF-1 mRNA expression further increased to 263 ± 69% ($P < 0.01$) compared to the surgery group. The *c-fms* mRNA nearly reached baseline values after surgery plus bacteremia (81 ± 31%, n.s.). MCSF-1 upregulation promotes monocyte activation in traumatized heart valve tissues. A further upregulation was documented during the development of bacterial endocarditis with *S. aureus*. We for the first time demonstrated basal *c-fms* expression on heart valve tissue. The downregulation after surgical manipulation may be interpreted as negative feedback loop to locally increased MCSF-1 concentrations. The mechanism of the MCSF-1/*c-fms* pathway in heart valve tissue during inflammatory processes has to be further evaluated in detail.

Abstract N° C103

Cardioprotective mechanisms of melatonin against adriamycin-induced cardiotoxicity in rats

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In the present study, we evaluated the effect of melatonin administration on adriamycin-induced cardiotoxicity in rat. We measured malondialdehyde (MDA) content as an index of lipid peroxidation and lactate dehydrogenase (LDH) release as an indicator of lethal cell injury. Serious adriamycin-induced lethality was observed in rat with a single intraperitoneal injection in a dose-dependent manner. A single injection of adriamycin (25 mg/kg, intraperitoneal injection) resulted in a lethality rate of 86%, which was reduced to 20% with melatonin treatment (10 mg/kg subcutaneous injection for 6 d). The severe body weight loss caused by adriamycin was also significantly attenuated by melatonin treatment. The adriamycin-induced MDA formation and LDH release were also significantly prevented by melatonin. A cell damage induced by adriamycin was not evident after melatonin treatment. Melatonin prevented adriamycin-induced nuclear DNA fragmentation, mitochondrial depolarization and glutathion depletion without changes of reactive oxygen species content. These data indicate that melatonin prevents adriamycin-induced cardiotoxicity and thus it may be used in combination with adriamycin to reduce the free radical-mediated cardiotoxicity.

Abstract N° C104

Peripartum cardiomyopathy: correlation between cholesterol, C-reactive protein and left ventricular dysfunction

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Heart failure is characterised by activation of inflammatory cytokines possibly due to increased endotoxin levels. Lipoproteins are non-specific endotoxin buffers. We analysed the correlation between serum cholesterol level, C-reactive protein level and left ventricular dysfunction in patients with peripartum cardiomyopathy (PPCM). Single centre, prospective study of 100 patients with newly diagnosed PPCM. Clinical assessment, echocardiography and cardiac scintigraphy were done at baseline and after 6 months of treatment. CRP and serum cholesterol were measured at baseline only. Fifteen patients died and eight were not available for follow up. Total serum cholesterol was 4.2 ± 0.9 mmol/l, while CRP was 10.8 ± 13.2 mg/l with 45% of patients having values >10 mg/l. We documented an inverse correlation between CRP and total serum cholesterol (rs = –0.29, $P = 0.01$). Total serum cholesterol also correlated inversely with left ventricular end-diastolic diameter (rs = –0.35, $P = 0.0009$) and end-systolic diameter (rs = –0.38, $P = 0.0001$). The correlation between baseline serum cholesterol and ejection fraction was positive (rs = 0.36, $P = 0.0006$). In PPCM low serum cholesterol levels are associated with elevated CRP levels at baseline and more severe left ventricular dysfunction after 6 months of treatment.

Abstract N° C105

A lack of effects of nicotinic acid on plasma lipids and atherosclerosis in apo E-KO mice

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Pharmacological doses of nicotinic acid may reduce coronary events due to its beneficial effects on plasma lipoprotein profiles. The aim of the present study was to investigate whether administration of nicotinic acid beneficially modifies plasma lipoprotein profiles and subsequently prevents atherosclerosis in apolipoprotein E deficient mice (apo E-KO). Two groups of mice were fed with a cholesterol-enriched diet (0.2% w/w) with (treated group (n = 8) or without (control group, n = 7) supplementation with nicotinic acid (0.5% w/w) for 4 months. Plasma lipid levels (total cholesterol, triglycerides) were measured using standard enzymatic methods. At the end of the study, the hearts and aortas were collected, and the extent and severity of atherosclerotic lesions in the aortic roots were examined by a number of histological and morphometrical techniques. Treatment with nicotinic acid neither significantly changed plasma lipid levels nor reduced the extent of atherosclerosis in the aortic roots, as compared to controls. Supported by Manitoba Medical Services Foundation and NCARM.

Abstract N° C106

Ankle brachial blood flow index—a possible physiological tool to predict atheroslerosis

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Forearm hyperemia, carotid intima thickness, Ankle Brachial Pressure Index are subclinical markers associated with coronary artery disease. Ankle Brachial Index is the ratio of systolic pressure as measured at the ankle and arm by the Doppler.It has been used to detect atherosclerosis. In the present study Imdedance Cardiovasograph (BARC, Mumbai) has been used to measure Blood Flow Index (BFI) at ankle and upper arm by Impedance Plethysmography. In our study there is 90% sensitivity, 96% specificity and a positive predictive value of 94% for atherosclerosis and a significant ($P < 0.0001$) association with coronary artery disease. This method being non-invasive, quick and easy has a high patient acceptability and is an accurate and reliable indicator of atherosclerosis.

Abstract N° C107

Role of various adhesion and activation molecules in patients with coronary artery disease

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Chronic inflammation is the underlying pathologic process in development of atherosclerotic coronary artery disease (CAD). Chronic inflammatory response is associated with the generation of cytokines, which results in increased expression of various adhesion and activation molecules. We estimated levels of various adhesion and activation molecules in peripheral and coronary sinus blood samples in angiographically proven CAD cases and compared it with normal coronary arteries controls. Various molecules like CD3 (T cell surface receptor), CD11 (receptor for ICAM), CD19 (B cell surface receptor), CD25 (interleukin-2 receptor), CD54 (intercellular adhesion molecule-1), and CD69 (early activation marker), were estimated in peripheral and coronary sinus blood samples in 20 cases and compared it with 20 controls. Both groups were matched for demographics and conventional CAD risk factors. Significant elevation of CD3, CD11b, CD25, CD54 and CD69 were found both in coronary sinus and peripheral blood samples in cases compared to controls ($P < 0.05$). No significant difference was noted for CD11a, CD11c and CD19 ($P > 0.1$). Only CD11b, CD11c, CD54 and CD69 were elevated in coronary sinus compared to peripheral blood in cases ($P < 0.05$), while in controls CD11a, CD11b, CD25, CD54 and CD69 were elevated in coronary sinus compared to peripheral blood ($P < 0.05$). Adhesion and activation molecules like CD25, CD54, CD69, CD3, and CD11b were elevated in CAD patients. There is not much of incremental benefit in estimating various molecules in coronary sinus compared to peripheral blood.

Abstract N° C108

Fenofibrate paradoxically increases plasma total cholesterol levels but not HDL-cholesterol levels in apo E-KO mice

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Fibrates may reduce coronary events due to beneficial effects on plasma lipoprotein profiles. The aim of the present study was to investigate whether administration of fenofibrate beneficially modifies plasma lipoprotein profiles and subsequently prevents atherosclerosis in apolipoprotein E deficient mice (apo E-KO). Two groups of mice received a cholesterol-enriched diet (0.2% w/w) with (treated group (n = 8) or without (control group, n = 7) supplementation with fenofibrate (0.1% w/w) for 4 months. Plasma concentrations of total cholesterol, HDL-cholesterol, and triglyceride levels were measured at the baseline and 4-week intervals during the study period; non-HDL-cholesterol levels were calculated by subtracting HDL-cholesterol values from total cholesterol values. The hearts and aortas were collected, and the extent and severity of atherosclerotic lesions in the aortic roots were examined by a number of histological and morphometrical techniques. Fenofibrate significantly increased plasma concentrations of total cholesterol without reducing plasma triglyceride or increasing HDL-cholesterol concen-

trations as compared to controls. Treatment with fenofibrate did not result in the prevention of atherosclerosis in the aortic roots of the mice.

Supported by Manitoba Medical Service Foundation and NCARM.

Abstract N° C109

Genetic polymorphism of apolipoprotein E in coronary artery disease patients of north India

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Genetic predisposition is considered to be an important risk factor for coronary artery disease (CAD). We studied the genetic polymorphism of apolipoprotein E in patients with established coronary artery disease in north India. A total of 150 subjects in three groups were studied: group I—50 patients with established CAD; group IIA—50 age and sex matched controls without any family history of CAD; group IIB—age and sex matched controls, who were first degree relatives of patients with CAD. Fasting blood samples were collected from all subjects and lipid profile was analyzed. The genomic DNA was isolated from the leukocytes and amplified. The Apo E genotyping was carried out by Hixon and Vernier method. Group I and IIB subjects had higher total cholesterol (TC) and low density cholesterol (LDL) compared to group IIA. Group I patients had significantly lower high density cholesterol (HDL) than group IIA and IIB subjects. There was no significant difference in triglycerides (TG) levels or in distribution of apo E genotypes between the three Groups. The apo E 3/3 genotype was the most common among all the three groups. The apo E 4/3 genotype was significantly higher in group I compared to group IIA (18% vs. 4%). Among the alleles, ϵ3 allele carriers were the highest in all the three groups. The ϵ4 allele carriers were more than doubled in CAD (20%) compared to ϵ3 allele carriers (8.2%) and ϵ2 allele carriers (10.9%), but the value did not reach statistical significance. The ϵ4 allele was more prevalent in group I (CAD) than group IIA or group IIB. There was no positive correlation between ϵ4 allele and elevated TC or LDL-C. ϵ4 allele carriers had significantly higher TG levels than ϵ3 or ϵ2 allele carriers. The ϵ4 allele carriers had 3.5 times higher odds (95% CI- 1.2–9.4) to develop CAD compared to subjects without ϵ4 allele. Subjects with CAD had higher prevalence of apo E4/3 genotype and ϵ4 allele. There was no positive correlation between ϵ4 allele and elevated total cholesterol or LDL cholesterol.

Abstract N° C110

Profile of North Indian patients with dilated cardiomyopathy and MyBPC: a preliminary study

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The study was aimed at a 25 base pair deletion in MyBPC, which has been described in the Indian population but is not present in Caucasians. This polymorphism results in alternate splicing of exon 33 and therefore can be a prevalence of the 25 base pair deletion in intron 32 of MyBPC in these patients. Patients with left ventricular ejection fraction less than 40% in the absence of a secondary cause were included in this study. DNA was extracted from whole blood and PCR of intron 32 of MyBPC carried out. The PCR product was run on 2.5% agarose gel to detect the presence of the deletion. Thirty-five patients with idiopathic dilated cardiomyopathy were studied. The mean age was 43.2 ± 15.4 years (range 13–77 years), 22 were females. Out of these, four each were hypertensive (11%) and diabetic (11%). Two patients (6%) had peripartum cardiomyopathy and one had a history dilated cardiomyopathy affecting several members of family. Three (8.6%) patients were in NYHA Class I, 22 (62.9%) in Class II, eight (22.9%) in Class III and two (5.7%) in Class IV. Two (6%) patients were in atrial fibrillation, eight (23%) met the voltage criteria for left ventricular hypertrophy, six (17%) had left bundle branch block, four (11%) had bifascicular block, two (6%) had complete heart block, one (3%) had right bundle branch block while only one (3%) had a completely normal ECG. Echocardiogram was carried out in all patients. The left ventricular ejection fraction was 30.9 ± 6.9% (range 18–40%). Twenty of these patients were investigated for a 25 base pair deletion in intron 32 of MyBPC. This deletion has only been reported in Indians. One (5%) was found to carry the polymorphism. Seventy control subjects were also studied, of these three (4.2%) were found to carry this deletion. This polymorphism has a prevalence of 4.2% in control subjects; it is therefore unlikely to cause dilated cardiomyopathy but its role, as a disease-modifying factor needs further studies.

Abstract N° C111

Acute treatment with statins not chronic treatment attenuates infarct in an isolated rat heart model

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Chronic treatment with statins (HMG Co-A reductase inhibitors) have been shown to improve survival following myocardial infarction. This benefit has been primarily attributed to its cholesterol lowering effects. However patients treated chronically with statins have been shown to have a lower incidence of heart disease than individuals with comparable cholesterol levels. Statins are known to have a number of pleiotropic effects that may be beneficial in vascular occlusive disease such as inhibition of smooth muscle proliferation and platelet aggregation, anti-inflammatory effects, enhancement of endothelial function. One other pleiotropic

effect is its ability to recruit the phosphatidyl inositol 3-OH kinase (PI3 kinase)/Akt pathway, one of the pro-survival pathways. In this regard, it has also been recently shown that atorvastatin can limit infarct size if given prior to the point of reperfusion an effect which is mediated via its ability to recruit the PI3 kinase/Akt/eNOS cascade. The aims of this study were to determine whether chronic treatment with statins could limit infarct size and secondly to determine whether additional acute statin treatment would be beneficial in the presence of chronic statin therapy. Sprague–Dawley rats (SDR) were gavaged daily for 2 weeks with either 1% methylcellulose vehicle (group 1), atorvastatin 20 mg/kg (group 2), atorvastatin 40 mg/kg (group 3) or atorvastatin 80 mg/kg (group 4) after which time the hearts were isolated and subjected to 35 min of regional ischaemia on a Langendorff apparatus followed by 120 min of reperfusion. Two further groups, group 5 and 6, consisted of SDR gavaged for 2 weeks with 20 mg/kg atorvastatin as with group 2 (group 5) and 80 mg/kg atorvastatin as with group 4 (group 6) and also subjected to 35 min of ischaemia with a further 50 μmol of atorvastatin given acutely for the first 15 min of reperfusion. The results of this study shows that atorvastatin given chronically did not limit infarct size measured by infarct to risk (I/R) ratio compared to controls (I/R ratio 54.6% ± 6.4%) at 20, 40 or 80 mg/kg (I/R ratio 58.4 ± 3.3%, 42.4 ± 8.5% and 58.4 ± 5.1%, respectively). A further acute dose of 50 μmol produced a significant reduction in infarct size ($P < 0.05$) after chronic gavage with 20 and 80 mg/kg of atorvastatin (I/R ratio 25.67 ± 5.4% and 24 ± 6.6%, respectively). We conclude from these results that although chronic treatment with statins confers long-term protection in cardiovascular disease, this does not appear to be mediated via a limitation in infarct size. However, a further acute dose of statin at reperfusion reduces infarct size despite chronic treatment with a statin. This may provide us with a new and novel therapeutic use of statins.

Abstract N° C112

Atorvastatin reduces infarct size acutely in an isolated rat heart model

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Statins have been shown to be of great benefit in the treatment of coronary heart disease. This has been attributed mainly to their cholesterol lowering effect. However, statins are known to have pleiotropic effects. One such effect may occur as a result of their ability to recruit the PI3-kinase/Akt pathway, the pro-survival pathway. This pathway is central to the protection afforded by ischaemic preconditioning. We therefore hypothesized that statins could reduce infarct size if used as a preconditioning mimetic.

Methods. – Male Sprague–Dawley rats were anaesthetised, the hearts isolated and mounted on a Langendorff apparatus. They were then stabilized for 35 min during which time they received 50 μmol atorvastatin for 10 min followed by 10 min of washout prior to ischaemia/reperfusion (Group A). There were three further groups: Group B received 10 min of vehicle followed by washout; Group C received atorvastatin with wortmannin, the PI3-kinase inhibitor, and Group D received wortmannin alone. Atorvastatin given acutely at a dose of 50 μmol was found to reduce infarct size significantly compared to controls reducing the infarct to risk (I/R) ratio from 44.3 ± 2.5% to 22.2 ± 6.6%. This protective effect was abrogated by the PI3K inhibitor wortmannin (I/R ratio 53.0% ± 2.6%). Wortmannin alone had no effect on infarct size (I/R ratio 58.0 ± 6.3%). Atorvastatin given as a preconditioning mimetic reduces infarct size in an isolated perfused rat heart model of ischaemia/reperfusion injury and this protection is mediated via the PI3-kinase signalling pathway. This is the first time that a statin has been shown to precondition the myocardium and could lead to new and novel uses of these agents in the treatment of ischaemic heart disease.

Abstract N° C113

Short-term pre-treatment with atorvastatin reduces infarct size in an isolated rat heart model of ischaemia/reperfusion injury

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Statins have been shown in numerous studies to be of benefit in the long-term treatment of ischaemic heart disease. However, a number of studies have shown that short-term treatment with statins can be beneficial. This beneficial effect is thought to be due to inhibition of inflammation and endothelial dysfunction by preventing activation and extravasation of neutrophils. The aim of this study was to observe the effect of pre-treatment with statins on the heart in isolation. Male Sprague–Dawley rats were gavaged for 1 d with methylcellulose 1% vehicle (Group 1), 1 d with 20 mg/kg atorvastatin (Group 2), 3 d with methylcellulose 1% (Group 3) or 3 d with atorvastatin 20 mg/kg. After 2 weeks they were anaesthetised, the hearts isolated and mounted on a Langendorff apparatus. After a period of stabilisation, the hearts were subjected to 35 min of regional ischaemia followed by 2 h of reperfusion. Infarct size was then determined using methylene blue and TTC staining. Infarct size was significantly reduced ($P < 0.05$) after oral gavage for 1 d with atorvastatin from an infarct to risk zone ratio (I/R) of 56.4% ± 2.3% after 1 d of methylcellulose gavage to 38.9 ± 3.1%. The same was true after 3 d gavage with infarct reduced from 61.3 ± 3.8% after methylcellulose gavage to 39.3 ± 2.4% after gavage with atorvastatin ($P < 0.05$). Short-term pre-treatment with statins reduces infarct size in an isolated rat heart model of ischaemia/reperfusion suggesting that the protective effect of this treatment is not entirely due to a reduction in inflammation but may be in part due to activation of an intrinsic protective mechanism within the myocardium.

Abstract N° C114

Rosuvastatin prevents cardiovascular remodelling without lowering blood pressure in DOCA-salt hypertensive rats

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The pleiotropic responses of the statins represent novel mechanisms for the treatment of hypertension and heart failure, as well as hyperlipidaemia. This project investigated cardiac remodelling in DOCA-salt hypertensive rats treated with rosuvastatin (20 mg/kg/d in 10% Tween 20) by oral gavage for 32 d, commencing 4 d before surgery. Male 8 week old Wistar rats were uninephrectomised and treated with deoxycorticosterone acetate (DOCA, 25 mg every 4th day sc) and 1% NaCl in the drinking water for 4 weeks; uninephrectomised (UNX) rats served as controls (n > 6/group). Rosuvastatin did not alter systolic blood pressure, but decreased left ventricular weight/body weight in DOCA rats (3.05 ± 0.10 vs. 2.68 ± 0.10; UNX: 1.81 ± 0.04 vs. 1.92 ± 04 mg/kg). Diastolic stiffness was lowered from 24.4 ± 0.6 to 21.5 ± 0.5 in treated DOCA-salt rats, whilst UNX rats remained unchanged (21.4 ± 0.4 vs. 21.9 ± 0.4). Rosuvastatin treatment normalised left ventricular interstitial collagen content in DOCA-salt rats (DOCA: 4.39 ± 0.59 vs. 2.61 ± 0.39; UNX: 2.58 ± 0.16 vs. 2.38 ± 0.31% area). Action potential duration at 90% of repolarisation in isolated papillary muscle preparations was reduced from 114.4 ± 3.3 to 95.2 ± 3.1 ms with treatment in DOCA-salt rats (UNX: 45.99 ± 0.99 vs. 51.36 ± 3.91 ms). Thus, rosuvastatin prevented increases in cardiac stiffness and collagen deposition and attenuated both cardiac hypertrophy and action potential prolongation in the DOCA-salt model of hypertension in rats without altering systolic blood pressure.

Abstract N° C115

Preconditioning ischemia favorably attenuates platelet aggregation: insight into molecular mechanisms

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There is extensive evidence that brief antecedent preconditioning (PC) ischemia limits infarct size. Recent studies have further revealed that PC ischemia also improves vessel patency in models of coronary artery injury + stenosis (I + S) mimicking unstable angina, presumably by as-yet unknown, favorable effects of the PC stimulus on subsequent platelet aggregation. To address this issue, anesthetized dogs received 10 min PC ischemia + 10 min reflow or a matched control period, after which I + S (resulting in cyclic variations in coronary flow, caused by repeated formation/dislodgement of platelet thrombi) was initiated. Coronary flow was monitored for 3 h following I + S, and blood samples were obtained at baseline and 2 h post I + S for blinded measurement, by flow cytometry, of multiple archetypal molecular indices of platelet activation/aggregation, including formation of neutrophil-platelet aggregates (NPAs), fibrinogen binding (FB), and surface expression of P-selectin (P-sel).

	Controls (n = 9)			Preconditioned (n = 9)		
	Baseline	Post I + S	%	Baseline	Post I + S	%
NPAs	6.5 ± 0.8	9.5 ± 1.1	158 ± 21	6.9 ± 0.9	7.0 ± 0.7	107 ± 8 *
FB	1.8 ± 0.3	2.9 ± 0.4	177 ± 24	2.3 ± 0.3	2.8 ± 0.6	112 ± 14 *
P-sel	2.3 ± 0.3	4.1 ± 0.4	197 ± 23	2.6 ± 0.6	3.4 ± 0.5	148 ± 14 **

Formation of neutrophil-platelet aggregates and fibrinogen binding were both abrogated (* P = 0.03), P-selectin expression was attenuated (** P = 0.09), and, as expected, coronary flow was better maintained (mean of 53 ± 5% vs. 23 ± 5% of baseline; P < 0.01) in the PC group vs. controls. These data provide the first molecular insight into the mechanisms by which brief antecedent PC ischemia favorably attenuates subsequent platelet activation and, thus, improves coronary patency in the unstable angina model.

Abstract N° C116

Increased platelet-derived microparticles in the coronary circulation of patients undergoing coronary angioplasty

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Platelet-derived microparticles (PMPs) that are produced during platelet activation are capable of adhesion and aggregation. Endothelial trauma which occurs during percutaneous transluminal coronary angioplasty (PTCA) may support PMP adhesion and contribute to subsequent development of restenosis. We have previously reported an increase in platelet-derived microparticles in peripheral arterial blood with PTCA. This finding raised concerns regarding the role of PMPs in restenosis, and therefore in this study, we have monitored PMP levels in the coronary circulation. The study population consisted of 19 angioplasty patients. Paired coronary arterial and sinus samples were obtained following heparinisation, following contrast administration, and subsequent to all coronary arterial intervention. PMPs were identified with an anti-CD61 (glycoprotein IIIa) antibody using flow cytometry. There was a significant decrease in arterial platelet-derived microparticles from heparinisation to contrast administration (P = 0.001), followed by a significant increase to the end of PTCA (P = 0.004). However, there was no significant change throughout the venous samples. These results indicate that the higher level of PMPs after PTCA in arterial blood remained in the coronary circulation. This may have implications for the development of coronary restenosis post-PTCA, although this remains to be determined.

Abstract N° C117

Effect of roxythromycin on arterial vasoactivity

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Background. – Inflammatory disease can damage vascular functioning and advance atherosclerosis. Coronary events and a high flow of the brachial artery have been reported in patients treated with macrolides.

Objective. – To determine the function of the macrolide, roxythromycin (RX) on arterial vasoactivity.

Methods. – Vascular rings were obtained from human and rat internal mammary arteries (IMA), attached to a force transducer and immersed into organ chambers with oxygenated Krebs–Henseleit (KH) solution. Vascular integrity in response to norepinephrine (NE), acetylcholine (ACh), sodium nitroprusside (SNP) was investigated. After restabilization, 10^{-6} M NE, 10^{-7}–10^{-4} M RX, followed by 10^{-5} M SNP, were added. The mechanism of RX action was tested in rats using solutions containing KH; KH + L-NAME, nitric oxide (NO) production inhibitor; KH + calcium ionophore (Ca); KH + indomethacin, a prostaglandin inhibitor; KH + glibenclamide; KH + 5-hydroxydecanoic acid, membrane and mitochondrial K channel inhibitors (10^{-6} M each).

Results. – IMA and rat aorta exhibited similar contraction and relaxation rates in response to NE, RX, and ACh. RX relaxation (4 ± 1 to 15 ± 3%) was dose dependent and similar to ACh, lower than SNP. Relaxation was significantly reduced in the presence of Ca, L-NAME, 5-HD ($P < 0.005$). Glibenclamide and indomethacin had no effect on relaxation.

Conclusion. – RX relaxation is mediated by calcium, mitochondrial K ATP channels and NO production. Thus, RX offers a therapeutic advantage, for cardiac patients needing macrolide treatment.

Abstract N° C118

Would aging impair cerebral oxygenation during orthostatic stress?

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The purpose of this study was to test the hypothesis that cerebral perfusion during orthostatic stress was compromised by aging. Lower-body negative pressure (LBNP) –15, –30, and –50 Torr was applied to simulate orthostatic stress in eight elderly (E, 71 ± 2 years, 74.5 ± 5.4 kg and 1.68 ± 0.03 m) and seven young (Y, 27 ± 2 years, 70.2 ± 5.9 kg and 1.72 ± 0.03 m) healthy volunteers. During the test stroke volume (SV), cardiac output (CO, thoracic impedance), systemic arterial pressure (SAP, Tonometer), systemic oxygen saturation (SO_2S, Oximeter), and regional cerebral oxygen saturation (RO_2S, near-infrared spectroscopy) were continuously recorded. Baseline SAP was significantly higher in E (132 ± 4/73 ± 4 mmHg) than Y (117 ± 3/63 ± 2 mmHg), whereas SV and CO were significantly lower in E (54.5 ± 4.0 ml and 3.21 ± 0.30 l/min) than Y (84.2 ± 7.1 ml and 5.44 ± 0.51 l/min). Though SO_2S was significantly lower in E than Y (95.0 ± 0.6 vs. 96.6 ± 0.6%), there was no statistical difference in RO_2S between the groups (E vs. Y: 67.0 ± 3.8 vs. 69.2 ± 3.3%). LBNP significantly decreased SV and CO in both groups, indicating a central hypovolemia during orthostatic stress. During LBNP SO_2S remained constant, whereas RO_2S significantly decreased (to 61.6 ± 2.4 and 63.6 ± 3.9% in E and Y, respectively, at –50 Torr), suggesting cerebral under-perfusion. Decrease in RO_2S in terms of unit decrease in CO or SV appeared to be greater ($P < 0.05$) in E than Y (5.71 ± 0.75 vs. 3.04 ± 0.14%/l/min or 0.25 ± 0.04 vs. 0.15 ± 0.01%/ml). We concluded that aging impaired cerebral oxygenation during central hypovolemia, which would explain orthostatic intolerance of the elderly.

(This Study is supported in part by NIH grant R01-HL65613).

Abstract N° C119

Acute negative inotropic effect of β_2AR-blockers through p38-MAPK signaling pathway in human ventricular myocytes

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Our previous studies have shown that β_2AR-blockers could acutely depress the contraction of failing human myocytes and that the effect was mediated primarily through the inhibitory guanine nucleotide binding protein, Gi. The present work continued to investigate the mechanism of this effect. Consistent with our previous results, acute treatment with 3 μM ICI 118,551—a specific β_2AR blocker—produced a significant depression of contraction in both failing human myocytes and β_2AR-overexpressing rat myocytes. This negative inotropic effect could be prevented by pretreatment with SB 203580 (10 μM), a specific p38-MAPK inhibitor (basal 100%; ICI 64.8 ± 3.6%, $P < 0.01$ vs. basal; SB 106 ± 3%; SB + ICI 102 ± 5%; n = 14, 14, 6, 6 human myocytes, respectively, 6–8 mM Ca^{2+}). In addition, western blot showed that the phosphorylated p38-MAPK protein levels increased significantly in ICI 118,551-treated samples from CABG patients (arbitrary units: –ICI: 10.2 ± 2.4; +ICI: 17.8 ± 2.5, $P < 0.01$; n = 6). Overexpression of β_2AR for 48 h using adenovirus (Ad.β_2AR-GFP, 500 MOI) in rat myocytes raised basal cAMP levels but the basal contraction amplitude was lower than control (Ad.GFP). Amplitude in β_2AR-overexpressing cells could be restored to levels even higher than those of control cells following treatment with PTX, which indicates that the negative effect is Gi dependent. The negative effect of β_2AR overexpression was not reduced by the p38-MAPK inhibitor (% cell shortening: control 5.04 ± 0.58%; +β_2AR 3.08 ± 0.31%, β_2AR + SB 3.08 ± 0.36%, $P < 0.05$, vs. control; n = 10 cells). We have therefore demonstrated that the p38-MAPK signaling pathway plays an important role in the acute negative inotropic

effect of β_2AR-Gi coupling in rat and human ventricle. However, the negative inotropic effect of β_2AR overexpression per se in rat is Gi- but not p38-MAPK-dependent and occurs despite an increase in cyclic AMP.

Abstract N° C120

Opposing roles of p38 MAPK and ERKS pathway in β-AR-mediated secretion of IL-6 in mouse cardiac fibroblasts

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We recently reported that cardiac fibroblasts, but not cardiomyocytes, are served as the predominant source of IL-6 in response to β-adrenoceptor (β-AR) stimulation in mouse myocardium. The present study investigated the molecular mechanism of β-AR-mediated secretion of IL-6 in mouse cardiac fibroblasts. Treatment of cells with isoproternol (ISO, β-AR agonist) induced a time-dependent accumulation of IL-6 secretion, which was completely inhibited by pretreatment with ICI118551 (β_2-AR antagonist). Cholera toxin and foskolin treatment for 12 h significantly induced IL-6 secretion. PDE inhibitor also elevated β_2-AR-mediated IL-6 production significantly. However, no effect was observed by protein kinase A (PKA) inhibitors. ISO-induced IL-6 production was also markedly reduced by pretreatment with an L-type Ca^{2+} channel antagonist nifedipine. Moreover, ISO-induced secretion of IL-6 was significantly elevated by inhibiting G_i, phosphoinositide 3-kinase (PI3K), or MEK1 with pertussis toxin (PTX), LY294002, or PD98059, respectively, but was completely abolished by inhibiting p38 MAPK with SB203580. Most importantly, SB203580 pretreatment also completely reverse the elevated levels of IL-6 mediated by PTX, LY294002 and PD98059. Meanwhile, the phosphorylation of p38 MAPK induced by β-AR stimulation was significantly enhanced by pretreatment with PTX and LY294002. Taken together, the present study shows for the first time that cAMP and calcium influx, but not PKA, are critically involved in β_2-AR-mediated secretion of IL-6 in mouse cardiac fibroblasts, and that the secretion of IL-6 is regulated positively by p38 MAPK and negatively by G_i-PI3K-ERKs signaling cascade. This work was supported by the Major State Basic Research Development Program of PR China (G2000056906) and by grant from the National Science Foundation of China (30270540).

Abstract N° C121

Ceramide-dependent inhibition of nuclear protein import in vascular smooth muscle cells

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Ceramide has anti-mitogenic actions. The purpose of this study was to determine if the anti-proliferative effects of ceramide may be due to an effect on nuclear protein import. Nuclear protein import is a process critical to cell growth. The permeabilized cell assay was used to examine nuclear protein import in vascular smooth muscle cells (VSMCs). Exogenous cytosol pre-treated with 1 and 10 μM ceramide significantly inhibited nuclear import. This inhibition was reversed upon the addition of SB-202190, a p38 MAP kinase blocker, and PD-98059, a MEK antagonist. Nuclear protein import was also significantly inhibited in situ after microinjection of ceramide into vascular smooth muscle cells. This was again reversed by the addition of SB-202190. CAS is a critical protein that modulates nuclear protein import by regulating the nuclear/cytoplasmic cycling of importin alpha in the cell. Ceramide treatment eliminated the localization of CAS at the nuclear periphery in control cells. Treatment of cells with ceramide ± SB-202190 or 20 μM PD-98059 caused a redistribution of CAS back to the nuclear rim. This was also observed for importin alpha. In conclusion, ceramide inhibits nuclear protein import in VSMCs via an inhibition of p38 and MEK kinase activity. CAS appears be a target of MAP kinase activity and it mediates the inhibitory effects of ceramide on nuclear protein import. Supported by CIHR and NSERC.

Abstract N° C122

Lysophosphatidylcholine stimulates nuclear protein import

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Lysophosphatidylcholine (LPC) stimulates vascular smooth muscle cell proliferation, but its mechanism of action is unclear. Import of proteins from the cytoplasm into the nucleus of a given cell is integral to the regulation of gene expression. We hypothesized that LPC exerts its effects through alterations in nuclear protein import. A cytosolic nuclear import cocktail was treated with LPC for varying lengths of time. LPC caused a dose- and time-dependent increase in import. This effect was not observed with other lysophosphatidyl species. Lysoplasmenylcholine also stimulated import. LPC did not stimulate import when incubated with the nuclei, suggesting the nuclear pore complex itself did not directly mediate its effects. Instead, the cytosolic mechanism of action of LPC was found to be through an augmentation of GTP hydrolysis via activation of the MAP kinase pathway. LPC stimulated RanGAP, a RanGTPase activating protein and a critical regulatory component of nuclear protein import. We conclude that LPC may alter gene expression and cell proliferation through striking effects on nuclear protein import via cytoplasmic activation of RanGAP. Supported by CIHR and NSERC.

Abstract N° C123

MAPKS in adriamycin cardiomyopathy and heart failure and their modulation by probucol

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Phosphorylation of ERK1/2, p38 and JNK mitogen-activated protein kinases (MAPKs) in adriamycin-induced cardiomyopathy (AIC), along with the modulatory effects of antioxidant, probucol (PROB) was studied in rats. Male rats were administered with adriamycin (ADR, cumulative dose, 15 mg/kg) with and without PROB (120 mg/kg). The animals were assessed clinically and hemodynamically and the hearts were collected at different post-treatment durations. Phosphorylation of ERK1/2, showed a biphasic response with an increase to 167% at 1 h, 209% at 2 h and peaking at 513% at 4 h. At 24 h, the activity decreased to 197% of control and at 3 weeks, was 66.8% of the control. Phosphorylation of p38 showed a steady increase through 2, 4, 24 h and 3 weeks (119%, 138%, 140% and 148%). Increase in phosphorylation of JNK also showed a graduate rise through 1, 2, 4, 24 h and 3 weeks (116%, 124%, 127%, 141% and 148%). PROB modulated these ADR-induced changes in all three MAPKs. ADR depressed myocardial function, caused heart failure as well as mortality. These changes were also completely prevented by PROB. It is suggested that all three MAPKs are involved in the AIC in both early and late stages. Modulation of these changes in signaling pathways, as well as complete prevention of AIC by antioxidant PROB may suggest that these changes may be mediated by adriamycin-induced oxidative stress (Supported by Heart and Stroke Foundation).

Abstract N° C124

Fibroblast growth factor 2 mediates isoproterenol-induced cardiac hypertrophy through specific MAPK and PKC pathways

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Fibroblast growth factor 2 (FGF2) has been shown to affect growth and differentiation in some tissues and to be required for cardiac hypertrophy in vivo. FGF2 has been shown in vitro to signal throught the mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) pathways to affect cell survival and growth. In order to ascertain which of these pathways might mediate FGF2's role in cardiac hypertrophy in vivo, we have utilized mice with a targeted ablation of the FGF2 gene (FGF2 KO). FGF2 KO and wildtype mice at 10–12 weeks of age were treated with isoproterenol (60 mg/kg/d) or saline via subcutaneous mini-osmotic pump implants for 2 weeks to induce a hypertrophic response. Isoproterenol-treated wildtype animals exhibited marked cardiac hypertrophy, whereas this response was significantly attenuated in FGF2 KO mice. Echocardiography revealed significantly decreased fractional shortening in isoproterenol-treated wildtype mice but not in FGF2 KO mice suggesting that the absence of FGF2 protects against the maladaptive cardiac dysfunction seen in cardiac hypertrophy. Western blot analysis of the MAPK and PKC pathways was then performed on isoproterenol or saline-treated FGF2 KO or wildtype mouse hearts. These studies revealed ERK-MAPK activation in isoproterenol-treated wildtype hearts but not in isoproterenol-treated FGF2 KO hearts. Similar results were obtained for some PKC isoforms. Together these data suggest that FGF2 is integral to the hypertrophic response induced by isoproterenol and that this response is mediated through specific MAPK and PKC pathways.

Abstract N° C125

Inhibition of phenylephrine-induced cardiac hypertrophy by a carbonic anhydrase inhibitor: a pathological pathway linking CAII, NHE1 and AE3fl

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Cardiac hypertrophy (CH) contributes to heart failure, a major cause of death by cardiovascular disease. Carbonic anhydrase II (CAII), which catalyses the reversible CO_2 hydration, is expressed in early stages of cardiac development. CAII activates transport activity of Na^+/H^+ exchanger 1 (NHE1) and anion exchangers (AE). Stimulation of α-adrenergic receptors (α1R) also activates NHE1 transport activity in isolated cardiomyocytes (CM). AE3fl (SLC4) and SLC26A6 (SLC26) are the predominant anion exchangers in the heart. The effect of the α1R agonist, phenylephrine (PHE) on anion transport mediated by AE3fl and SLC26A6 was studied in HEK293 cells individually co-transfected with AE3fl/α1aR, or SLC26A6/α1aR, cDNAs. Cells were loaded with BCECF-AM and intracellular pH measured. PHE (10 μM) increased anion exchange activity of AE3fl by 70% ± 9 ($n = 3$; $P < 0.05$), but inhibited anion transport by SLC26A6 by 65% ± 3 ($n = 4$; $P < 0.05$). Cardiomyocyte hypertrophy (CMH) in cultured neonatal rat cardiomyocytes was induced by PHE (10 μM). PHE increased cell surface area by 42% ± 6; atrial natriuretic factor (ANF) and CAII, mRNA expression increased by 66% ± 9, and 61% ± 9, respectively ($n = 4$; $P < 0.05$). The CA inhibitor, 6-ethoxyzolamide (ETZ, 100 μM), completely abolished the PHE-induced CMH (cell size 94% ± 2 of control, $n = 4$). ETZ also normalized ANF and CAII expression. Taken together, we conclude that CAII, AE3fl and NHE1 are associated in the hypertrophic phenomenon in cardiomyocytes.

Abstract N° C126

Crucial roles of cardiac alpha1B-adrenoceptor on contractile functions in both physiological and pathophysiological conditions

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Currently, the cardiovascular phenotype of alpha1B-adrenoceptor (1B-AR) single knockout (KO) mice has revealed no significant alterations in cardiovascular parameters. To address the functional roles of cardiac 1B, we evaluated the phenotype of 1BKO mice. As compared with wild-type (WT) control, the basal blood pressure and HR of 1BKO were similar, whereas fractional shortening and cardiac output were low when assessed by echocardiography. Under anesthesia the measurement by a direct catheterization method revealed lower LVDP and d*P*/d*t* in this KO mouse. LV mass index by echocardiography, actual heart weight, and cross-sectional area of cardiomyocytes of the 1BKO were smaller than those of WT. Although cardiac hypertrophy was evident after pressure overload stress, there was no significant difference in contractile and morphological parameters. In the isolated perfused hearts, phenylephrine-induced positive inotropic response was comparable, whereas the postischemic recovery of LVDP was low in the 1BKO hearts. The results provide evidence that cardiac 1B-AR plays an important role in the maintenances of heart function under both physiological and pathophysiological status.

Abstract N° C127

Alterations in alpha adrenoreceptor density and localization after mechanical left ventricular unloading with the Jarvik flowmaker assist device

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Left ventricular assist devices can improve myocardial performance. We hypothesized that mechanical unloading would result in favorable alterations in alpha one adrenoreceptor (α_1AR) density and distribution in patients with low-output syndrome and left ventricular failure.

Methods. – Myocardial α_1AR density and localization were compared at the time of Jarvik flowmaker insertion and removal in 13 patients with heart failure. Snap frozen sections were probed with fluorescent markers for α_1ARs. Deconvolution microscopy produced planar images, which were reconstructed as three-dimensional renditions. Samples were examined with tagged Prazosin for visualization of α_1ARs. Receptor density, determined by pixel numbers, was measured, and localization of receptors was determined in reconstructed, deconvoluted and stacked sections.

Results. – We found a statistically significant increase ($P < 0.05$) in left ventricular α_1AR density after mechanical left ventricular unloading with a continuous-flow pump. These findings were complemented by alterations in the distribution of the α_1AR (i.e. perivascular vs. intra-myocytic) from a clumped pattern before LVAD insertion to a homogenous appearance at the time of LVAD explantation. The alterations were more consistent with the normal physiological state of ventricular myocardium.

Conclusion. – Mechanical unloading of the heart with a continuous-flow pump significantly increases α_1AR density and normalizes their distribution.

Abstract N° C128

Two alpha1-adrenoceptors regulating vasopressor response have differential roles in postural hypotension

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Alpha1-adrenoceptors (ARs) have a prominent role in regulating vascular tone and are targets for antihypertensive therapy. They contain three subtypes (1A, 1B and 1D-AR), and functional role of each AR subtype is still uncertain. Although non-selective AR blockers have been widely used to control hypertension, their adverse effect, postural hypotension, limits their safe use and subtype-selective AR blockers could reduce such an unwanted effect. To study the role of individual AR subtypes, we created mice lacking the 1B- and/or 1D-AR and studied hemodynamic and vasocontractile responses. Both 1D knockout (1DKO) and 1BD-AR double KO (BDKO), but not the 1BKO mice, had a significantly lower level of basal blood pressure than did wild-type (WT) mice. All mutants showed reduced catecholamine-induced pressor and vasoconstriction responses. However, a tilt-induced postural hypotensive response was more prominent in the 1BKO and WT mice treated with prazosin than in mice lacking the 1D. We show that both 1B and 1D-ARs mediate vasopressor responses to catecholamines, but 1B-AR is more closely related to the tilt-induced postural change in blood pressure. Our study provides evidence for the clinical efficacy of drugs that are highly selective for the different ARs subtypes.

Abstract N° C129

Effects of aging in mouse heart expressing constitutively active α1B-adrenergic receptors

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Cardiac-directed overexpression of wild-type α1B-adrenergic receptor (AR) (26–43-fold) results in dilated cardiomyopathy and premature death at 9 months of age and suppression of β1-AR signalling. To investigate whether this heart failure phenotype is due to chronic activation of the α1B-ARs, transgenic mice (Milano et al. PNAS 1994;91:10109–13) expressing constitutively active α1B-AR by twofold in the heart (TG) and their non-transgenic (NTG) littermates were non-invasively studied at 6, 9, 12 and 15 months of age using M-mode and Doppler

echocardiography. Fractional shortening (FS) was significantly increased in TG vs. NTG mice (Table). Notably, the ratio of left ventricular (LV) early and atrial filling flow velocities (E/A) was reduced and the deceleration time (DT) of the E-wave was prolonged in TG mice. This LV diastolic dysfunction was evident at 6 months of age and persisted at the advanced ages. LV mass, estimated via echocardiography and normalised for body mass (LVM/BM), was not significantly different between TG and NTG mice, a finding that was verified at autopsy. Catheterisation experiments at 15 months revealed unchanged LV contractility at baseline and blunted responses to β-agonist stimulation for heart rate and –d*P*/d*t* in TGs. In summary, unlike cardiac overexpression of wild-type α1B-ARs, expression of constitutively active α1B-AR does not impart detrimental effects leading to premature death.

Group	E/A ratio	DT (ms)	FS (%)	LVM/BM (mg/g)
NTG				
6 months	2.11 ± 0.11	32 ± 1	35 ± 1	3.3 ± 0.1
12 months	2.09 ± 0.19	33 ± 2	35 ± 1	3.6 ± 0.1
TG				
6 months	1.31 ± 0.06 *	48 ± 2 *	44 ± 2 *	3.6 ± 0.1
12 months	1.26 ± 0.05 *	44 ± 1 *	40 ± 2 *	3.8 ± 0.2

*$P < 0.05$ vs. age-matched NTG group.

Abstract N° C130

mTld alters subcellular localization of α_{1A}-adrenergic receptors

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It has been recognized that non-G-proteins interact with G-protein-coupled receptors. However, whether such interaction occurs to α_1-adrenergic receptors (α_1AR) remains unclear. We screened pretransformed human cDNA library using α_{1A}AR C terminal as bait, and obtained the CUB domain of mTld, known as an extracellular matrix protease. We then studied the interaction between α_{1A}AR and mTld both in yeast and mammalian cells. In yeast cells, the colony-lift filter assay (X-gal) indicated that CUB5 domain of mTld interacted well with the C terminal of α_{1A}AR, but not the cytoplasmic tails of either α_{1B}AR or α_{1D}AR. Immunoprecipitation also confirmed the coupling of α_{1A}AR and mTld in HEK293 cells. With immunofluorescence assay, we observed that α_{1A}AR expressed both on the cell surface and intracellularly in HEK293 cells in the absence of mTld, whereas in cells with mTld, α_{1A}AR were accumulated in certain cytoplasmic compartments and could not be detected on the cell surface. Radioligand binding showed that the density and affinity of α_{1A}AR were not affected by mTld. However, whole-cell ELISA assay revealed that the cell surface α_{1A}AR in HEK293 cells in the presence of mTld were only 56 ± 6% of that in cells transfected with a control vector, LacZ. Furthermore, in cells without mTld, there was a rapid agonist-induced internalization of cell surface receptor. In cells co-expressing mTld, however, the cell surface α_{1A}AR increased initially followed by progressive decline in response to α_{1A}AR stimulation. Collectively, this study is the first to demonstrate a specific interaction of mTld with α_{1A}AR. Such interaction does not change receptor expression, but significantly alters the subcellular localization of α_{1A}AR and the time-course of agonist-induced α_{1A}AR internalization.

Abstract N° C131

Cardiac myocyte intracellular calcium handling and contractility in alpha 1_A-adrenergic receptor overexpressing mice

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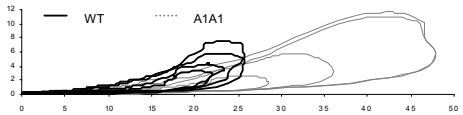
Previous in vivo studies have shown that transgenic mice which overexpress the α_{1A}-adrenergic receptor (A1A1) have enhanced cardiac contractility but no evidence of hypertrophy. However the contractile profile of cardiac myocytes in these mice is unknown. Intracellular calcium (Ca^{2+}_i) handling and contractility (% cell shortening) were compared in single cardiac myocytes from wild-type (WT, $n = 7$) and A1A1 ($n = 7$) mice using indo-1 loaded, electrically paced cells. Responses to increasing concentrations of phenylephrine (PE) were also measured. Basal calcium transients were not different between WT and A1A1, but cell shortening was significantly reduced in A1A1 (WT, 6 ± 1% vs. A1A1, 3 ± 2%, $P < 0.01$). With increasing [PE], $[Ca^{2+}]_i$ and cell shortening increased significantly in A1A1 but WT cells were unresponsive. Plotting fractional cell shortening against fractional change in $[Ca^{2+}]_i$ indicated that the increased cell shortening at higher [PE] in A1A1 is a result of increased $[Ca^{2+}]_i$, and not due to increased sensitivity to calcium. These



data suggest that the in vivo phenotype can be explained by the increased density of α_{1A}-adrenergic receptors in the presence of circulating catecholamines.

Abstract N° C132

Sex dependent differences in cardiac myocyte hypertrophy

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Cardiac hypertrophy is a major risk factor for cardiovascular-related morbidity and mortality. There is evidence for sex differences in the incidence of cardiac hyper-

trophy, and the presence of oestrogen and androgen receptors in cardiac myocytes raises the possibility that sex hormones may influence how the heart responds to conditions that lead to the development of cardiac hypertrophy. The aim of the present study was to examine possible sex differences in the response of isolated cardiac myocytes to hypertrophic agents. Cultures of both adult and neonatal rat cardiac myocytes were studied, with the hypertrophic response to angiotensin-II (AngII), endothelin-1 (ET-1) and phenylephrine (PE) assessed by increases in protein synthesis. Cardiac myocytes from either males or females were separately harvested by enzymatic digestion and established in culture, prior to treatment with the hypertrophic agents in [^{3}H]-phenylalanine spiked media. [^{3}H]-phenylalanine incorporation into protein (a marker of myocyte hypertrophy) was normalised to total DNA to account for differences in cell numbers between cultures. In cultures of adult cardiac myocytes there was a significant difference between male and female cells in the hypertrophic response to AngII and PE, with the male cells exhibiting a greater response. The response to ET-1 was not significantly different between the sexes. In contrast, all three hypertrophic stimuli elicited robust responses in neonatal cardiac myocytes, with no significant sex differences. These results suggest that in the neonate, prior to exposure of the heart to circulating sex steroids, there are no sex differences in the heart's hypertrophic responsiveness. In the adult, however, after exposure to the normal circulating sex hormone levels of the sexually mature animal, sexual dimorphism in hypertrophic responsiveness develops. This has important clinical implications, in that development of, and optimal treatment for, cardiac hypertrophy may differ between the sexes.

Abstract N° C133

Gonadectomy and testosterone replacement influence intracellular Ca^{2+} in male rat cardiac myocytes

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It is becoming increasingly evident that many aspects of cardiac phenotype show important physiological differences between the sexes, suggesting that sex steroid hormones may serve as long-term modulators of myocardial function. The present study investigated the effects of 4 weeks gonadectomy (GDX), with or without testosterone (T) replacement, on intracellular Ca^{2+} ($[Ca^{2+}]_i$) in male rat cardiac myocytes. Cardiac myocytes were freshly isolated by collagenase digestion and loaded with fura-2 for monitoring $[Ca^{2+}]_i$. Values for the amplitude of the Ca^{2+} transient recorded in myocytes stimulated at 0.5 Hz in 1.5 mM Ca^{2+} solution are given in the table, along with the Ca^{2+} transient decay time constant and extent of shortening (*denotes value is significantly different from sham, †denotes value is significantly different from testosterone replaced animals; n = 12–14 cells; N = 5–7 animals in each group).

	Sham	GDX	GDX + T
Ca^{2+} transient amplitude (nM)	179 ± 17	102 ± 18 *,†	187 ± 20
Decay time constant (ms)	279 ± 23	455 ± 80 *,†	277 ± 19
Extent of shortening (%)	7.8 ± 1.8	2.7 ± 0.6 *,†	7.2 ± 1.3

These results show that testosterone promotes increased levels of intracellular Ca^{2+} and contractility in cardiac myocytes. This raises the possibility that testosterone may, through influencing intracellular Ca^{2+} handling, play a role in modulating cardiac performance in males and thereby contribute to sex-dependent differences in cardiac function and disease.

Abstract N° C134

Effect of sex hormone levels on the non-genomic action of 17β-estradiol in rat mesenteric arteries

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The lower incidence of cardiovascular events in women has been attributed to the beneficial effects of the female sex hormone estrogen. The effects of estrogen on the cardiovascular system include both genomic and non-genomic mechanisms. However, how these two effects interact is not well understood. Sprague–Dawley rats (8 weeks old) were orchiectomized (male), ovariectomized (female) or sham-operated and treated with vehicle or 17β-estradiol for 14 d. Rats were then sacrificed and isometric tension of the mesenteric arteries were measured in organ bath setup. Acute administration (30 min) of a physiological concentration of 17β-estradiol (1 nM) caused a decrease in contraction to phenylephrine in male but not in female rat mesenteric arteries. However, this non-genomic effect was present when female rats were ovariectomized and treated with vehicle. In both sham-operated and ovariectomized female rats treated with 17β-estradiol, the non-genomic effect was not present. Sham-operated, vehicle-treated rats exhibited this non-genomic effect. However, orchiectomy with vehicle treatment abolished this effect. The non-genomic effect was also lost in sham-operated and orchiectomized male rats treated with 17-estradiol. The present study suggests that the reduction of contraction in the arteries by acute administration of 17β-estradiol is dependent on the levels of chronic circulating sex hormones. This non-genomic effect appears to be enhanced by testosterone and inhibited by estrogen. The rapidity of this action and the low concentration required (1 nM) to elicit this effect suggest that it is a receptor-mediated response.

Abstract N° C135

Effect of testosterone on the secretion of adrenomedullin and endothelin in cultured endothelial cells

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To investigate the effect of testosterone on the secretion of adrenomedullin (ADM) and endothelin (ET) in cultured endothelial cells. Incubated endothelial cells with different concentration of testosterone (0, 2, 10, 50 and 100 ng/ml) for 24 h, then collected the cells and cultured medium respectively, radioimmunoassay was carried out to measure the content of ADM and ET in cells and in cultured medium respectively.

Results. – (1) The levels of ADM in cultured medium in testosterone groups (2, 10, 50 and 100 ng/ml) were 34.29 ± 4.2 (vs. control group, $P < 0.05$), 44.65 ± 4.8 (vs. control group, $P < 0.001$), 78.29 ± 8.07 (vs. control group, $P < 0.001$), 70.98 ± 10 pg/ml (vs. control group, $P < 0.001$) respectively, but the content of ADM in cells in any groups were no statistically different. (2) Likely ADM, ET in cultured medium was also higher than in control group except at concentration 2 ng/ml of testosteorne group, the content of ET in cells was no difference between these groups.

Conclusion. – Testosterone can promote the secretion of ADM and ET (testosterone's concentration higher than 2 ng/ml) in cultured endothelial cells, through which testosterone may have an effect on the blood pressure.

Abstract N° C136

Effects of exogenous testosterone on atherosclerosis in castrated rabbits

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To study the effect of serum testosterone level on experimental atherosclerosis in castrated rabbits. Forty-five male New Zealand white rabbits were randomized and then the castrated rabbits were randomized to hypotestosteronemia group, physitestosteronemia group, hypertestosteronemia group, placebo group and sham operation group. The castrated rabbits in the first three groups were intramuscularly injected testosterone undecanoate 3, 6 and 12 mg/kg once every 2 weeks, respectively, since the operation, whereas the placebo group and the sham-operated rabbits did not receive any testosterone. All animals were fed a cholesterol-rich diet during the 16-week treatment period. Blood samples were collected for determination of serum levels of total testosterone (TT), estradiol (E_2), lipids, lipoproteins and apolipoproteins at the 1st week before treatment and 16th week after injection. At the end of study, the aortic of the second part rabbits was analyzed morphmetrically and immunohistologically and the aortic cholesterol content were determined chemically. The average serum lipids, atherogenic lipoproteins and apolipoproteins, such as total of cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), etc. and the cholesterol content (mmol/g) and intimal thickening in the proximal aortic arch were significantly higher in the placebo, exogenous hypotestosteronemia and hypertestosteronemia groups than those in the sham operated and the exogenous hysitestosteronemia groups. Exogenous testosterone, at physiological level seems have a good effect on the serum lipids, lipoproteins, lipoproteins and atherosclerosis in castrated male rabbits, whereas the endogenous or enexogenous hypotestosteronemia and hypertestosteronemia may have not.

Abstract N° C137

Set recruitment and chromatin remodeling are epigenetic components responsible for myocardial gene profiles in the hypertrophied and failing heart

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Histone modifications remodel chromatin structure and transcription activity. Hypertrophied and failing myocardium are characterized by profound changes in gene transcription and molecular remodeling and it was not known whether an epigenetic program is involved in myocardial gene regulation during heart failure. We studied acetylation/deacetylation or methylation states of histone tails and correlated these marks with transcriptional state of selected genes in hypertrophied and failing hearts of C57B6 mice with transverse aorta constriction (TAC) for 16 weeks. Mice with TAC developed severe left ventricular hypertrophy (179 ± 11 vs. 95 ± 4 mg), chamber dilatation (4.8 ± 0.3 vs. 3.4 ± 0.1 mm) and failure (fractional shortening: 22 ± 3 vs. 36 ± 2%, all $P < 0.01$ vs. sham-operated group, SHAM). Real-time RT-PCR showed enhanced (α-MHC, ANF) or suppressed (α-MHC, SERCA2, α_1AR) expression. Soluble chromatin fractions derived from cross-linked SHAM and TAC hearts were immunoprecipitated with antibodies against acetyl-H3, acetyl-H4, histone deacetylase 1 (HDAC1), or histone H3-K4 methylation using specific chromatin immunoprecipitation (ChIP) assays and real time-PCR to determine particular promoter sites. Chromatin from active gene sets were enriched in acetyl-H3 and H4 together with a concomitant release of HDAC1 in TAC vs. SHAM hearts, and the opposite occurred for the down-regulated gene sets. H3-K4 is known to activate transcription. We observed that the H3-K4 histone methyltransferase Set7 is mobilised onto transcriptionally competent genes (α-MHC and ANF). Interestingly, these epigenetic changes are reversible 6 weeks following removal of aorta banding. In summary, our results suggest that a coordinated and ordered response to hypertrophy and failing heart is the active remodeling of chromatin, and that mobilization of the epigenome is critical in regulating gene activity in heart disease.

Abstract N° C138

Gene expression profiling reveals distinct sets of genes altered during hormonally and metabolically induced cardiac hypertrophies

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Today there is still a lack of diagnostic indicators and prognostic markers that might direct individualized clinical management of cardiac diseases induced by multifactorial metabolic and neuro-humoral disturbances. cDNA high-density microarray assays were used to explore changes in cardiac expression of ~15,000 clones in: (1) the cardiac angiotensin II-overproducing transgenic TG1306/1R mouse and (2) the insulin-resistant cardiomyopathic muscle-specific GLUT4-KO (knock-out) mouse. In TG1306/1R, we report the (>2-fold) upregulation of 51 known genes and 26 ESTs (e.g. Hsd17b4, Tia1, Aogn, Col15a1, Mmp7, Calr, Rab18), with a (>2-fold) downregulation of 11 known genes and 14 ESTs (e.g. Nxf2, Grid2). In GLUT4-KO, we report the (>2-fold) upregulation of 24 known genes and 17 ESTs (e.g. Ndr4, Nppb, Gapd, S100a6, Cdh22, Ftl1), with a (>2-fold) downregulation of 25 known genes and 11 ESTs (e.g. Atp2a2, Tpm1, Atp5, Fabp3). Among the genes altered in both mouse strains, we found Esterase 22, mitochondrial ribosomal protein S16 and cytochrome *c* oxidase I (downregulated in both mouse strains), as well as Hcapg, ICOS ligand and CD2 antigen binding protein 2 (downregulated in TG1306/1R, but upregulated in GLUT4-KO). Altered expression of the genes identified in this study may assist to systematically characterize molecular events pertaining to complex multifactorial diseases such as diabetic cardiomyopathy and hormonally-induced cardiac hypertrophy.

Abstract N° C139

Gene expression profiling of human heart failure

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Heart failure (HF) is a major cause of mortality in the Western world. Despite our increasing understanding of the development and progression of HF from human studies and animal models, the molecular mechanisms underlying this disease are still not understood. In order to investigate these molecular mechanisms we have examined gene expression profiles of 58 patients with end-stage HF and 24 non-diseased, donor hearts using Affymetrix Human Genome U133 2.0 plus gene arrays and cardiovascular-specific CardioChip cDNA arrays. HF patients with different aetiologies, including: idiopathic dilated cardiomyopathy (13 cases); viral-induced dilated cardiomyopathy (seven); familial dilated cardiomyopathy (five); familial hypertrophic cardiomyopathy (six); post-partum dilated cardiomyopathy (five); ischemic cardiomyopathy (22); and anthracycline-induced cardiomyopathy (six) were examined. This enabled us to observe changes in gene expression due to different aetiologies of HF, and characterized distinct sets of genetic and biological pathways to provide an insight in understanding the pathways involving HF. We have also distinguished the genomic profiles of the seven types of HF using hierarchical cluster analysis. One of challenges we face when examining human tissues compared to animal models is that individual genetic background differences and epigenetic factors, such as history of smoking, diet, drinking, and medications, may affect gene expression as much as the disease itself. To address this problem, detailed clinical data were obtained and the effects of epigenetic factors on the gene expression were examined. This is the largest gene expression profiling analysis undertaken to date and will provide valuable information on the nature of human HF.

Abstract N° C140

Relationship between MTHFR gene polymorphism and peripheral arterial occlusive disease

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Methylenetetrahydrofolate reductase (MTHFR) is a speed-limiting enzyme of homocysteine (Hcy). The decrease of MTHFR activity, which was caused by C677T mutation in MTHFR gene, may lead to an elevated level of plasma Hcy. Hyperhomocysteinemia may speed up the progress to arteriosclerosis and appears to be an independent risk factor for peripheral vascular occlusive disease. This study aims at exploring the relationship between MTHFR gene polymorphism and the risk to peripheral arterial occlusive disease (POAD) in the elderly of Beijing community. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was carried out. One hundred control subjects (NC group), 83 patients with peripheral arterial occlusive disease (POAD group) were screened to assess the polymorphism of MTHFR gene. The frequencies of three genotypes were C/C (homozygous normal), 31%; C/T (heterozygous), 50%; T/T (homozygous mutant), 19% in NC group and 13.3%;51.8%;34.9% in POAD group respectively. The frequencies of allele T were 44% and 60.8%, respectively. The frequencies of homozygous T/T and allele T in POAD group were significantly higher than those in NC. C677T mutation in MTHFR gene is associated with the risk to peripheral arterial occlusive disease in the elderly of Beijing community.

Abstract N° C142

Monte Carlo simulation demonstrating the regression dilution bias of the risk estimation of high blood pressure on cardiovascular events and mortality

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The random fluctuations in systolic blood pressure (SBP) or diastolic blood pressure (DBP) may lead to underestimation of the odds ratio of developing disease, associated with blood pressure, using logistic regression. For example, the true association between SBP and cardiovascular (CV) mortality may be underestimated using only one measurement of blood pressure. A correction of the estimates can be granted, given at least two measurements of blood pressure (BP) i.e. risk estimations are more precise based on at least two measurements of BP than based on only one measurement.

The Israeli Ischemic Heart Disease (IIHD) project was a longitudinal investigation of cardiovascular disease among 10,059 male civil servant and municipal employees in Israel. Participants underwent clinical evaluation in 1963, 1965 and 1968. Using bootstrapping we randomly divided the population 1,000 times into two sets, termed the in-sample set and the out-sample set. We calculated the 10^{th} and 90^{th} percentiles of the SBP measured in 1963 in the in-sample set, and estimated the odds ratio (OR) of the cardiovascular mortality rate in the two sets, using the same 10^{th} and 90^{th} percentiles of the SBP in the out-sample set. We found that OR for CV mortality, based on the in-sample set, underestimated the true OR obtained in the out-sample set. The correction of the regression dilution factor estimate was 1.64 (CI = 1.61-1.64). Specifically, the OR for CV mortality over a 21-year follow-up, associated with an increase of 20 mmHg of SBP was estimated based on one SBP measurement to be 1.51 (CI = 1.46-1.55), whereas the —true » OR for CV mortality over a 21-year follow-up was 1.96 (CI = 1.94-1.98). This underestimation can be corrected using at least two measurements of SBP. In addition we demonstrate the regression dilution bias using Monte Carlo simulation.

Abstract N° L1

Sarcomeric proteins as centers of multiplex functions in signaling and mechano-transduction in the myocardium

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Apart from their critical role in generating tension and shortening, cardiac sarcomeres actively participate in important mechanisms that determine cardiac function in health and disease. I will present experiments aimed at testing the following three hypotheses : 1) Modifications of the sarcomeric response to calcium are essential elements in the control of the extent and rate of tension development by cardiac myocytes, and their rates of shortening and relengthening. 2) Signaling and functional changes at the level of the sarcomere are integral elements in the cascade of reactions associated with compensated hypertrophy and the transition to failure. 3) The Z-disk functions not only as a physical anchor for the myofilaments, cytoskeletal proteins, and membrane proteins, but also as a pivot point for the reception, transduction, and transmission of mechanical and biochemical signals. Approaches to these hypotheses include mutagenesis, transgenesis, and in vitro exchange of sarcomeric proteins. This lecture honors the memory of Keith Reimer, who did seminal work in ischemic injury. I will present data demonstrating that increased myofilament response to calcium is able to blunt effects of reperfusion injury.

Abstract N° L2

The damaged heart

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Cardiac stem cells and early committed cells (CSCs-ECCs) are present throughout the myocardium, express c-Met and IGF-1 receptors and through a feed-back loop respond by secreting the corresponding ligands, HGF and IGF-1. HGF mobilizes CSCs-ECCs and IGF-1 promotes their survival and proliferation. Therefore, HGF and IGF-1 were injected in the heart of infarcted mice to favor, respectively, translocation of CSCs-ECCs from the surrounding myocardium to the dead tissue, and to enhance their viability, growth and differentiation within the damaged area. The new myocardium contained arterioles, capillaries and functionally competent myocytes, which with time increased in size, ameliorating ventricular performance at healing and long thereafter. Myocardial regeneration induced by growth factors rescued animals with infarcts up to 86 % of the ventricle, which are commonly incompatible with life and improved survival. Thus, the heart has an endogenous reserve of CSCs-ECCs that can be activated to reconstitute lost myocardium after infarction and obviate the need to introduce exogenous stem cells.

Abstract N° L3

Use of gene therapy for cardioprotection

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The heart has evolved an adaptive response of stress, termed late preconditioning (PC), which confers powerful and sustained protection against ischemia/hyperfusion injury. Previous work by us and others has shown that late PC is underlain by a genetic reprogramming of the heart, i.e., by the upregulation of a cluster of stress-responsive cardioprotective proteins, such as iNOS, HO-1, COX-2 and ecSOD. Based upon this work, we tested the feasibility of transferring these genes to the heart to achieve a permanent preconditioned-like state.

From the inception of our work on late PC, our goal has been to exploit the mechanism of late PC to achieve a chronic preconditioning-like state that could afford long-term, possibly permanent, cardioprotection in patients at risk for coronary artery disease. A number of studies performed in our laboratory now show that cardiac transfer of iNOS, COX-2, HO-1 and ecSOD affords robust and sustained protection against lethal ischemia/reperfusion injury in mice and rabbits. While most of these studies were performed with first-generation adenoviral vectors, we are now using recombinant adeno-associated viral vectors (rAAV) to overcome the two major limitations of first-generation vectors, namely, the short duration of expression and the inflammatory response. Our data show that rAAV-mediated gene transfer is feasible and results in long-term genetic reprogramming of the heart, which confers a protective phenotype. These studies demonstrate that gene therapy can be used to achieve chronic prophylactic cardioprotection and lay the groundwork for novel therapeutic approaches to myocardial ischemia/reperfusion injury predicated upon the over expression of the proteins that are responsible for late PC.

Abstract N° L4

Cardiac channelopathy : a bridge from the gene to clinical practice

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Recent advances in molecular genetics and electrophysiologic method have disclosed the genetic abnormalities in cardiac ion channels as pathological basis for inherited arrhythmic disorders : cardiac channelopathy. Congenital long QT syndrome is caused by mutations of ion channel genes. At present, seven chromosomal loci and mostly ion channel genes have been identified. They are classified as LQT1 — LQT7. LQT1 is the most frequent form and is caused by mutations in *KvLQT1* (or *KCNQ1*), which encodes Iks. LQT2 is the next prevalent type and is caused by mutations in *HERG* (*KCNH2*), encoding Ikr. Mutations of these genes underlie various degrees of current suppression (loss of function). We have observed different sites of mutations in *HERG* demonstrating various mechanisms of current suppression in Japanese LQT2 patients including activation and inactivation abnormalities, and trafficking defects. LQT3 is far less frequent than the other two forms and is caused by mutations in *SCN5A*, encoding human cardiac Na^+ channels. Mutations of *SCN5A* exhibit persistent Na^+ current providing the gain of function to prolong QT intervals. Other forms (LQT4-LQT7) are quite rare. Brugada syndrome is a unique form of idiopathic ventricular fibrillation (IVF), exhibiting ST segment elevation in right precordial leads of ECG and is also caused by mutations in *SCN5A*. Mutant Na^+ channels found in Brugada syndrome show either no expressed currents or decreased functions mainly due to altered inactivation properties. A single mutation in *SCN5A* is shown to exhibit clinical phenotypes of both LQT3 and Brugada syndrome. We also demonstrated the *SCN5A* mutation, S1710L, in a patient with IVF without showing typical ECG changes of Brugada syndrome. The functional abnormality of S1710L has close similarity to that of other type of mutations in *SCN5A* causing the progressive cardiac conduction disturbances. Thus, Na^+ channelopathy may bring a new disease entity of cardiac pathogenesis. Catecholaminergic polymorphic ventricular tachycardia is caused by mutation in ryanodine receptor gene. (RyR2).

Abstract N° L5

Janice Pfeffer Distinguished Lecture : Cardiac dyssynchrony and resynchronization

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The failing heart reflects the conspiracy of multiple abnormalities of the myocyte, molecular signalling, cellular/chamber remodelling, load, and neurohumoral stimulation. Recently added to this long list is the presence of dyssynchronous contraction from conduction delay. Cardiac dyssynchrony is induced by routine single-site pacing, and in normal hearts results in a modest decline in cardiac function and energetic efficiency. However, its impact in failing hearts is exacerbated, and clinical studies have shown that creating dyssynchrony in patients where none previously existed can worsen heart failure. Importantly, the reverse — *resynchronizing* by of left- or bi-ventricular stimulation improves symptoms, reduces hospitalizations and mortality in patients with severe heart failure. Dyssynchronous ventricles are effectively polarized into two regions ; an early activated territory that contracts against little load and largely pre-stretches the remote myocardium, and a late activated area that generates higher stress and also wastes work by re-stretching the early stimulated region. Dilated failing hearts enhance both geographic and temporal separation of these regions, enhancing the impact of dyssynchrony. Cardiac resynchronization (CRT) improves contractile coordination and thereby systolic function and efficiency, often leading to reverse remodelling.

At the basic level, dyssynchrony alters local and transmural electrical conduction and repolarization in the late activated lateral wall. When combined with failure, more intense changes in the expression of calcium handling proteins, stress kinases, gap junction proteins, and myocyte dysfunction are observed localized to the high stressed lateral endocardium. New studies are assessing the impact of varying site and delivery method to CRT efficacy, and expanding our exploration into molecular and cellular signalling changes and impact that CRT has on these abnormalities.

Abstract N° S1A

Controversies in adult stem cell plasticity

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A large body of evidence now supports the idea that certain adult stem cells, particularly those of bone marrow origin, can engraft alternative locations (e.g. non-haematopoietic organs), particularly when the recipient organ is damaged and transdifferentiate in to phenotypes appropriate to their new location. Hence there is considerable excitement in using HSCs in cell based therapies and as vectors to deliver therapeutic genes. However the field is not without its detractors. The reason for this is twofold, 1) certain instances of so-called plasticity have now been attributed to cell fusion between bone marrow cells (or their myeloid/monocytic descendants) and cells of the recipient organ, and 2) several remarkable claims have not been able to be confirmed, most recently the inability to show that purified HSCs can contribute to the healing of a myocardial infarction. This last observation is particularly noteworthy given the number of Medical Centres embarking upon the injection of autologous bone marrow to assist in myocardial healing after infarction.

Certain criteria should be met before claims that stem cells can cross their apparent lineage barriers are accepted, in particular they should be functional and in continually renewing tissues they should be able to proliferate. In the context of renal injury and in epidermis we have shown proliferation of bone marrow-derived cells with an appropri-

ate epithelial phenotype, and even clonal expansion of such cells to contribute to whole Epidermal Proliferative Units (EPUs). We have also shown that bone marrow contributes to vasculogenesis (endothelium and smooth muscle). We have also shown that intestinal subepithelial myofibroblasts (niche cells of the crypt) are bone marrow derived, as are myofibroblasts in a variety of other organs, moreover bone marrow contributes to the tumour desmoplastic response. Bone marrow cells also make a significant contribution to the hepatic myofibroblast population that is instrumental in causing the hepatic scarring of cirrhosis.

Abstract N^o S1B

The reality of myogenic stem cells

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Transplantation into the heart of autologous muscle precursor cells (myoblasts) routinely derived from primary cultures of mature skeletal muscle appears to alleviate the damage caused by necrosis of cardiomyocytes. The potential clinical use of stem cells as a superior source of myoblasts for transplantation therapy for heart repair and gene replacement in myopathies is an area of intense investigation. The two main sources of such putative (autologous) stem cells are (1) within skeletal muscle tissue as either a sub-population of satellite cells (conventional muscle precursors on the surface of myofibres) or the interstitial connective tissue, and (2) from non-muscle tissue such as bone- marrow, the dermis or thymus. The intrinsic and extrinsic factors controlling commitment of such cells into myoblasts or their conversion into cardiomyocytes and their expansion in vivo are critical issues to consider. The key question is the extent to which such stem cells might be harnessed for clinical purposes and whether any of them represent a superior source of myoblasts for heart repair.

Abstract N^o S1C

Plasticity of adipose tissues : cardiac and vascular potential

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Adipose tissue development and remodelling are closely associated with the growth of vascular network. We hypothesized that adipose tissue may contain progenitor cells with cardiogenic and angiogenic potential and that therapy based on adipose tissue-derived progenitor cells administration may constitute a promising cell therapy. Firstly, we demonstrate that rare beating cells with cardiomyocyte features can be identified after culture of murine adipose stroma cells without addition of 5-azacytidine. The cardiomyocyte phenotype is identified by morphological studies, immunohistological confocal analysis, expression of specific cardiac markers and electrophysiological and functional analyses.

Second, we demonstrate that cultured human SVF cells differentiate into endothelial cells, incorporate into vessels and promote both post-ischemic neovascularization in Nude mice and vessel-like structures formation in Matrigel plug. These cells are as efficient as bone marrow mononuclear cells to reconstitute neo-vessels. *In vitro*, these cells can spontaneously express the endothelial cell markers CD31 and von Willebrand factor when cultured in semi-solid medium. Interestingly, dedifferentiated mature human adipocytes have the potential to rapidly acquire endothelial phenotype *in vitro* and to promote neovascularization in ischemic tissue and vessel-like structures formation in Matrigel plug suggesting that cells of endothelial and adipocyte phenotypes may have a common precursor. This study demonstrates, for the first time, that adipocytes and endothelial cells have a common progenitor.

Taken together, these results highlight the concept that cells from adipose tissue represent a suitable new cell source for therapeutic angiogenesis in ischemic disease and for heart regeneration after heart failure.

Abstract N^o S2B

Cardiac lim proteins and mechanical stress sensing

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Cardiac myocytes respond to mechanical stretch stimuli by triggering downstream signals for cell growth and survival. Inadequate stress sensing may lead to the development of various forms of cardiomyopathies and to trigger the abnormal remodelling of surviving myocardium in the infarcted heart, which is chronically exposed to high wall stress. Although the involvement of stretch activated channels and cell adhesion complex proteins has been propose, the molecular components of the cardiac muscle stretch sensor have remained uncertain. Our group recently identified a selective role for muscle LIM protein (MLP), a Z disc protein, in sensing passive stretch in cardiomyocytes via biological and physiological studies in MLP deficient mouse cardiac muscle. MLP interacts with and co-localizes with telethonin (T-cap), a titin interacting protein. Based on our understanding that other Z disc associated cardiac LIM proteins including ALP and ZASP/Cypher/Oracle are also linked to dilated cardiomyopathy (DCM) in human and rodents, we propose cardiac titin/Z disc structure and associated LIM proteins constitute in vivo cardiomyocyte stretch sensor machinery, and that defects in the complex can lead to human DCM and associated heart failure.

Abstract N^o S2C

A Molecular Switchboard : Communicating with myofilaments through the actin capping protein CapZ

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The cardiac Z-discs have traditionally been viewed as passive structural elements of myocytes. However, recent studies have expanded the function of cardiac Z-discs to include roles in intracellular signalling and regulation of muscle mechanics. Using the Z-disc component actin cap-

ping protein (CapZ), we have investigated its impact on intra-cellular communication pathways thought to be involved in the development of heart failure, as well as its ability to regulate myofilament function. We have exploited two experimental models in our investigations : a transgenic mouse line deficient in cardiac CapZ ; and a procedure whereby CapZ is extracted from wild-type myofilaments with PIP2. Both models have yielded similar results. We have found that a reduction in cardiac CapZ enhances myofilament Ca^{2+} sensitivity and increases isometric force development by 60 %. The downregulation of CapZ attenuates the inhibition of myofilament activation by PKC-o_{II}, a putative messenger in the development of heart failure. Finally, we have found that type 1 protein phosphatase — which normally increases both maximum isometric tension and myofilament Ca^{2+} sensitivity — decreases myofilament activation when CapZ levels are reduced. Together these results strongly support the concept that the Z-discs are active regulators of cardiac function by virtue of their ability to control intracellular signalling systems and myofilament activation. The vital role of CapZ in a signalling pathway though to promote heart failure, coupled with its apparent ability to dampen force development suggests that the therapeutic manipulation of this Z-disc protein may be a viable treatment option for the management of cardiac dysfunction.

Abstract N° S2D

Deciphering the multiple roles of actin filament capping proteins in thin filament length regulation

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Actin (thin) filament length regulation and stability are essential for striated muscle function. To identify the mechanisms required for this process we have taken the approach of deciphering the physiological significance of specific actin filament capping proteins at the pointed and barbed ends (Z-line) with their binding proteins. For example, to determine the role of the actin filament pointed end capping protein, tropomodulin1 (Tmod1) with tropomyosin, we generated monoclonal antibodies against Tmod1 that specifically disrupted its interaction with tropomyosin in vitro. Perturbing this interaction in chick cardiac myocytes caused a dramatic loss of the thin filaments, as revealed by immunofluorescence deconvolution microscopy. Real-time imaging of live myocytes expressing GFP-α-tropomyosin and microinjected with the function-blocking antibodies revealed that the thin filaments depolymerized from their pointed ends. In a thin filament cell permeabilization reconstitution assay, stabilization of the filaments with phalloidin or jasplakinolide, prior to the addition of the function blocking antibodies prevented the loss of thin filaments. As a complementary approach, we also expressed a recombinant N-terminal fragment of Tmod1 containing the tropomyosin binding site. Again, a loss of actin filaments was observed, likely due to a dominant-negative mechanism. These studies indicate that the interaction of Tmod1 with tropomyosin is critical for thin filament stability. These data, together with previous studies, indicate that Tmod1 is a multifunctional protein. Its actin filament capping activity is responsible for maintaining actin length, by preventing elongation from the pointed ends ; the tropomyosin binding activity stabilizes actin-thin filaments by preventing depolymerization of the thin filaments.

Abstract N° S2E

The role of a novel Z-disc protein family, calsarcins, in striated muscle function and disease

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Signaling by the calcium-dependent phosphatase calcineurin profoundly influences growth and gene expression of cardiac and skeletal muscle. Calcineurin binds to a novel family of muscle-specific proteins, calsarcins, which are tethered to the Z-disc of the sarcomere, a focal point for pathological signalling in human cardiomyopathies and muscular dystrophies. We now show that calsarcin-1 negatively modulates the functions of calcineurin, such that calcineurin signaling is enhanced in cardiac and skeletal muscle of calsarcin-1 null mice. As a consequence of inappropriate calcineurin activation, calsarcin-1 mutant mice display an excess of slow skeletal muscle fibers. Moreover, despite the absence of hypertrophy or an overt cardiac phenotype, calsarcin-1 mutant mice exhibit a marked induction of the —fetal gene program » and an enhanced cardiac growth response to biomechanical stress. In contrast, cardiac adaptation to other types of stress signals, such as chronic catecholamine stimulation, is unaffected in calsarcin-1 null mice. These findings reveal important roles for calsarcins as modulators of calcineurin signaling and the transmission of a specific subset of stress signals leading to cardiac remodelling *in vivo*.

Abstract N° S3A

Cell death and cytosolic CA^{2+} overload : cause or consequence of mitochondrial dysfunction ?

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Mitochondria are central to apoptotic Ca^{2+} signalling. A key switch between cell survival and cell death is the mitochondrial permeability transition pore (PTP), a high conductance channel that may open in response to mitochondrial Ca^{2+} uptake. Matrix Ca^{2+} is a permissive factor for pore opening, yet Ca^{2+} alone may not be sufficient. In fact, the PTP open-closed transitions are modulated by additional factors that may critically affect the outcome of an identical Ca^{2+} signal. Addition of A23187 should mimic the Ca^{2+} overload taking place under pathological conditions. Unexpectedly, however, we found that addition of A23187 to MH1C1 cells caused a slow mitochondrial depolarization and death of about 30 % of cells, findings that are inconsistent with the expected consequences of cellular Ca^{2+} over-

load. Here I will show that addition of A23187 causes a fast but only transient rise of $[Ca^{2+}]_c$, which is sequentially followed by a rapid increase of $cPLA_2$ activity and then by PTP opening, the latter event occurring when $[Ca^{2+}]_c$ had already returned to nearly basal levels. We identified the proapoptotic signal triggered by A23187 as free arachidonic acid, whose levels could be further increased by treatment with the lipoxygenase inhibitor MK886 plus the cyclooxygenase inhibitor indomethacin. These findings identify $cPLA_2$ as the mechanistic link between A23187-dependent perturbation of Ca^{2+} homeostasis and the effector mechanisms of cell death. Whether cytosolic Ca^{2+} overload can bypass the otherwise necessary activation of $cPLA_2$ is under active investigation.

Abstract N° S3B

PTP and ischemia-reperfusion injury

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The viability of the ischemic myocardium is jeopardized by alterations, such as ATP decrease and elevation in intracellular $[Ca^{2+}]$, that are related to derangements in mitochondrial function. Besides these established notions, the elucidation of the apoptotic cascade and the availability of novel methodologies for in situ studies prompted a renovated interest in mitochondria. The characterization of mitochondrial channels provided a contribution that is particularly relevant to cardiovascular research.

Here we focus on the role of the permeability transition pore in ischemia reperfusion injury by analyzing (i) the methodological requirements for its characterization in isolated cells and intact organs ; (ii) the consequences of its opening highlighting the derangements in NAD^+ metabolism ; (iii) the contribution to necrosis and apoptosis ; (iv) the possible relationship with the formation or reactive oxygen species.

Abstract N° S3C

The inner mitochondrial membrane proteins altered with preconditioning : complexity and novel proteins revealed by proteomics

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Alteration of the mitochondrial proteome and corresponding changes in mitochondrial function has been implicated in a variety of degenerative diseases, heart disease, aging and cancer. The heart can be protected against severe ischemia either through preconditioning, induced by either brief transient ischemia or pharmacologically using adenosine or diazoxide. Although adenosine and diazoxide are potent PC agents they are known to work via different molecular pathways. Adenosine stimulates a complex signaling cascade that includes activation of PKC while diazoxide is proposed to act specifically on the KATP channels. Our hypothesis is common proteome changes would represent key protein(s) involved in induction of PC. Isolated rabbit myocytes were treated for 60 minutes with either drug and various subproteomes (myofilament, mitochondria, cytoplasmic) were analyzed by 2 dimensional gel electrophoresis (pH 4-7, 6-11 and 3-10).The protein alterations converged on to the mitochondrial proteins involved in TCA or the oxidative phosphorylation pathway. Surprisingly, the inner mitochondrial proteome is not well established, in part, due to the hydrophobic nature and basic isoelectric point of these membrane proteins. Combining 2DE (pH 4-7, 6-11 and 3-10) and 2 dimensional liquid chromagraphy (Beckman, pH 4-8.5) we identified over 200 proteins, 20 of which were post translationally modified, was created of the proteins associated with and spanning the mitochondrial inner membrane. Further subfractionation using Nabicarbonate to enriched for transmembrane mitochondrial inner transmembrane proteins revealed an additional 40 proteins. Several novel channels and associated proteins of unknown function were identified and functional roles are being investigated.

Abstract N° S3D

Limitation of electron flow during ischemia and mitochondrial and myocardial protection

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Mitochondria are sources and targets of oxidative damage during myocardial ischemia. In the isolated, perfused rabbit heart, ischemia damages the distal electron transport chain (ETC), decreasing the contents of cardiolipin and cytochrome *c*, leading to a decrease in the rate of oxidation through cytochrome oxidase. In isolated mitochondria in vitro, limitation of electron flow into complex III by the complex I inhibitor rotenone blunted the production of reactive oxygen species during the oxidation of complex I substrates. We asked if blockade of electron flow immediately before in situ ischemia would limit damage to the distal ETC. Treatment immediately before ischemia with rotenone attenuated ischemic mitochondrial damage, preserving the contents of cardiolipin, cytochrome *c*, and respiration through cytochrome oxidase. Rotenone treatment before ischemia preserved mitochondrial oxidative function in the aged rat heart, a model of enhanced ischemic damage. Treatment of the isolated rat heart before ischemia with amytal, a reversible complex I inhibitor at the rotenone site, also preserved mitochondrial oxidative function at the end of ischemia. Thus, limitation of electron flow during ischemia is a robust mechanism to minimize ischemic damage to the distal ETC. Ischemic damage to the distal ETC augments the production of reactive oxygen species, a likely mechanism for the oxidant burst that occurs at the onset of reperfusion. Limitation of electron flow during ischemia preserves mitochondrial function at the end of ischemia. Preservation of mitochondrial function following ischemia is likely to translate into myocardial protection during ischemia and reperfusion.

Abstract N° S3E

Mitochondrial criticality : role of mitochondrial ion channels and ROS

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Synchronization of mitochondrial function is an important determinant of cell physiology and survival, yet little is known about the mechanism of interorganellar communication. We have recently observed that coordinated cell-wide oscillations in the mitochondrial energy state of heart cells can be induced by a highly localized perturbation of a few elements of the mitochondrial network, indicating that mitochondria represent a complex, self-organized system. The oscillations were associated with bursts of mitochondrial reactive oxygen species (ROS) production, but did not involve the classical permeability transition pore or intracellular Ca2 + overload. Propagated synchronized oscillations were abolished by inhibiting mitochondrial complex III-derived reactive ROS, by increasing ROS scavenger levels, or by inhibiting mitochondrial anion channels, suggesting that an inner membrane, superoxide-permeable, anion channel opens in response to free radicals. To investigate the mechanism of intramitochondrial synchronization, we have applied percolation theory to demonstrate that a global phase transition (mitochondrial depolarization) occurs when a critical density of mitochondria accumulate reactive oxygen species (ROS) above a threshold to form an extended spanning cluster. The scaling and fractal properties of the mitochondrial network at the edge of instability agree remarkably well with the idea that mitochondrial network is organized as a percolation matrix, with ROS as a key messenger. The mechanism of the oscillator is further explored in a newly developed computational model describing how the balance between mitochondrial ROS production and intracellular ROS scavenging determine the dynamics and stability of the mitochondrial energy state of cardiac cells. Our findings indicate that the mitochondria can be viewed as a network of oscillators that may constitute a ROS-dependent signalling system.

Abstract N° S4A

Sodium/bicarbonate cotransport current in the configuration of the cardiac action potential

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The Na^+/HCO_3^- cotransport (NBC) contributes to 40-50 % of total acid extrusion in ventricular myocardium. There are at least two electrogenic isoforms of the NBC in the heart, NBC1 and NBC4. Measuring Na^+-dependent bicarbonate-sensitive currents with perforated-patch we were able to demonstrate the existence in the heart of an electrogenic NBC with a HCO_3^- :Na^+ stoichiometry ratio of 2 :1. This electrogenic NBC produces an anionic current which is outward (hyperpolarizing and repolarizing current) at potentials positive to the reversal potential of the NBC. Since the determined reversal potential for this electrogenic NBC is close to —95 mV, when the myocytes are exposed to a solution containing a physiological concentration of bicarbonate a hyperpolarization of resting membrane potential together with a shortening of action potential duration are observed. Thus, the electrogenic NBC participates in the configuration of the cardiac action potential, being this effect usually masked by the wide use of non-bicarbonate solution in electrophysiological recordings. The role of NBC in ischemic-reperfusion injury, heart failure and generation of arrhythmias remains to be determined. However, there is increasing evidence that involves this transporter as one of the Na^+-loading mechanisms during reperfusion.

Abstract N° S4B

Pathophysiological and protective roles of katp channels in ischemia/reperfusion : re-evaluation using katp channel-knockout mice

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To elucidate the pathophysiological roles of ATP-sensitive (KATP) channels in ischemia/reperfusion, we conducted functional experiments using Kir6.x knockout (KO) mice. The KATP channel current could be recorded in ventricular cells of Kir6.1 KO but not Kir6.2 KO mice. The action potential duration was shortened during no-flow ischemia in coronary-perfused ventricular muscle preparations of wild-type (WT) but not Kir6.2 KO mice. The mitochondrial KATP (mitoKATP) channel function, evaluated by flavoprotein oxidation, was preserved in both Kir6.2 KO and Kir6.1 KO ventricular cells, suggesting that neither Kir6.1 nor Kir6.2 is a component of mitoKATP channel. However, ischemic preconditioning failed to reduce the infarct size in Kir6.2 KO mice, indicating that sarcolemmal KATP (sarcKATP) channels composed of the Kir6.2 pore subunits play an important role in cardioprotection, at least, in mice. Kir6.1 KO mice showed sudden cardiac death resulting from myocardial ischemia associated with spontaneous ST elevation and atrioventricular block in ECG. Functional analysis of vascular smooth muscles of WT and Kir6.1 KO mice revealed that sarcKATP channels having the Kir6.1 pore subunit are important in the regulation of vascular tone. Thus sarcKATP channels having a distinct pore-forming subunit play a crucial role in the cardiovascular system.

Abstract N° S4C

Factors involved in the antiarrhythmic and proarrhythmic effects of thyroid hormones (TH).

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Acute or chronic intercellular channel connexin (Cx) alterations and abnormal Ca handling are crucial in the devel-

opment of lethal arrhythmias (VF). Recently we have shown that TH up-regulate Cx43 and attenuate Ca overload in neonatal heart cells. Further studies were aimed to examine whether acute or prolonged TH-treatment can affect arrhythmogenic factors, susceptibility of the heart to VF and its ability for self-defibrillation (SVD). Results showed that acute administration of TH : a) decreased previously elevated intracellular free Ca^{2+} in aged rat and guinea pig hearts, while higher dosage abolished Ca overload transiently followed by its dramatic increase ; b) prevented electrically-induced VF in guinea pig heart. Prolonged TH treatment : a) increased susceptibility of young but not old rat hearts to VF and facilitated SVD in the latter ; b) decreased of Cx43 expression and its phosphorylation isoforms (P2 and P3) in young rat hearts, while to much lesser extend in old one. The results indicate that TH can modulate both, Ca^{2+} (nongenomic effect) and Cx-43 (genomic effect) in the rat and guinea pig hearts. These effects can be involved in antiarrhythmic or proarrhythmic potentials of TH that are dose- and age-dependent.

Abstract N° S4D

Role of the gap junction in cardiac arrhythmias

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In the cardiac muscle, the gap junctions greatly contribute to electrical cell coupling. Then dysfunction of the gap junction impairs conductivity and can be one of arrhythmogenic factors. The physiological function of the gap junction depends on phosphorylation of the connexins which composes the gap junction channel since the connexin is phosphoprotein. In this study, alterations of phosphorylation of Cx43 in aconitine (alkaloid) - induced atrial and ventricular fibrillation were examined on adult guinea-pig and rat hearts.

Atrial and ventricular fibrillation were induced within about 10 min after application of aconitine (10^{-7}M) on Langendorff perfusion and continued for 30 to 40 min after wash-out of aconitine. At the beginning of the fibrillation, dephosphorylation of Cx43 was observed in Westernblot and it was augmented as the fibrillation was lasing. Confocal-image-analysis of Cx43 in the immuno-histochemistry revealed less immninoreactive particles at the intercalated disk in the fibrillating heart. The pathological hearts, such as ischemic or diabetic in which phosphorylation of Cx43 was impaired and expression of Cx43 was reduced, were susceptible to the fibrillation. Dephosphorylation of Cx43 is possibly one of arrhythmogenic substrata.

Abstract N° S4E

Gap junction remodeling and altered connexin expression in human cardiac diseases

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Central to the organizational pattern of cell-to-cell current transfer are the myocardial tissue architecture and specialized junctions responsible for electrical coupling, the gap junctions. Recent experimental and clinical studies have emphasized that altered distribution of gap junctions and changes in the expression of their constituent connexins may lead to abnormal coupling between cardiomyocytes and likely contribute to arrhythmogenesis. Thus, in the diseased ventricle, disturbed patterns of gap junction distribution have reportedly been noted in myocytes bordering regions of healed myocardial infarcts, small areas of replacement fibrosis and myocardial inflammation. The most consistently observed quantitative alteration in ventricular connexin expression involves down regulation of connexin 43. This reduction of connexin 43 is found irrespective of whether heart failure is due to idiopathic dilated, inflammatory, ischemic or pressure-overload heart disease. In contrast, the compensated stage of left ventricular hypertrophy in patients with aortic stenosis is characterized by increased connexin 43 expression which may constitute an important part of the immediate adaptive response of the heart to increased workload. In the diseased atria, disturbed patterns of gap junction organization and changes in connexin 43 and connexin 40 expression have been implicated in the initiation and maintainance of atrial fibrillation. Taken togther, these data indicate that alterations in gap junctions and connexins are typical features of myocardial remodeling that may play an important role in the development of pro-arrhythmic subtrates in a variety of forms of human heart diseases.

Abstract N° S4F

Ionic and molecular mechanisms of acquired QT prolongation in association with complete atrioventricular block

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Bradyarrhythmias resulting from complete atrioventricular block (AVB) are known to predispose to acquired long-QT syndrome (LQTS) and torsade de pointes (TdP). The underlying mechanisms remain to be clarified. We created a rabbit and a mouse model with chronic AVB (n = 34 and 20), which showed prominent QT prolongation, high incidence of spontaneous TdP (71 % in rabbits), and cardiac hypertrophy. Ventricular myocytes from rabbits at 3 weeks of AVB showed a marked prolongation of action potential duration (by 61 % at 0.5 Hz). Both rapidly- (I_{Kr}) and slowly- (I_{Ks}) activating components of delayed rectifier K^+ current in AVB myocytes were significantly smaller than controls (by 50-55 %). Peak amplitude of L-type Ca^{2+} current ($I_{Ca,L}$) and transient outward current (I_{to}) appeared to be unchanged, whereas the inward rectifier K^+ current (I_{K1}) significantly increased in AVB myocytes compared with controls. KCNH2 mRNA and KCNQ1 mRNA were downregu-

lated in AVB rabbits (by 48-55 %). KCNH2 protein and KCNQ1 protein amounts were reduced in parallel with their mRNAs, whereas KCNE1 protein expression was unchanged. Kir2.1 protein was upregulated (by 45 %). In mice with AVB for 10 days, gene profiling with microarray analysis in ventricular muscle revealed downregulation of mRNA expression for Kv4.2 and Kir 2.1. Complete AVB causes arrhythmogenic modulation of K^+ channel expression probably through a volume overload to the ventricles. Although the modulation is species- and model-dependent, the phenotypes closely resemble the clinical characteristics of congenital LQTS. These results provide new insights into the ionic and molecular mechanisms of chronic bradycardia-induced LQTS.

Abstract N° S18B

Cardiac hypertrophy : role played by the NA + /H + exchanger

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Cardiac hypertrophy has been established as one of the most powerful predictors for cardiovascular morbidity and mortality. In the last years, there have been impressive progress in the understanding of the mechanism(s) of myocardial hypertrophy and good evidence has emerged about the main role played by Na + /H + exchanger (NHE) activity in many, if not all, types of CH. The sarcolemmal NHE, through the electroneutral exchange of intracellular H^+ for extracellular Na^+, constitutes a main cell proton-extruding mechanism. The NHE-1 isoform is the most abundant in myocardium an has a major role in the regulation of cardiac function particularly under pathological conditions. Moreover, its enhanced activity has been linked to the deleterious effects of ischemia/reperfusion. Therefore, NHE inhibition seems to be a potential novel therapeutic strategy to interfere with the hypertrophic process independently of load mechanisms and also beneficial in the treatment of ischemia/reperfusion injury. About the mechanism by which the activation of the NHE-1 can underlie the development of cardiac hypertrophy the hypothesis is that elevated activity of this exchanger will increase Na^+ influx to the cell therefore increasing its cytosolic concentration. This, in turn will favour the reverse mode of operation of the Na^+/Ca^{2+} exchanger leading to a secondary increase in $Ca^{2+}{}_i$. The notion that the increase in $Ca^{2+}{}_i$ is a primary signal for cardiac hypertrophy is supported by numerous studies since it can activate several intracellular signaling pathways like PKC and Ca-calmodulin dependent phosphatase (calcineurin) and kinases (CaMKs) involved in the hypertrophic response. On the other hand, the link between Na^+ influx, activation of PKC-ooand oooand hypertrophy has been also demonstrated.

Abstract N° S18C

Lysophospholipid and AKT signaling pathways in cardiac hypertrophy and protection

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Lysophospholipids including sphingosine-1-phosphate (S1P) and lysophosphatic acid (LPA) are biological mediators that signal through G-protein coupled receptors Cardiomyocytes from rats and mice express multiple subtypes of receptors for both of these ligands i.e. $S1P_{1,2,3,5}$ and $LPA_{1,2,3}$ receptors. LPA and S1P are weak inducers of hypertrophy in neonatal rat ventricular myocytes, perhaps as a result of their relatively limited ability for Gαq-mediated phospholipase C activation. In contrast both are highly efficacious activators of the small G-protein RhoA. While Rho has been implicated in hypertrophy induced by GPCR activation, we have also determined that this small G-protein regulates PI3 kinase dependent Akt activation. In cells from mice in which the $S1P_2$ and $S1P_3$ receptor subtypes are deleted, Rho activation by S1P is inhibited. Infarct size following *in vivo* ischemia-reperfusion (IR) is significantly increased in these S1P receptor knockout mice, coincident with decreased Akt activation. We suggest that S1P released during I-R confers cardioprotection via activation of Rho and/or Akt pathways. The mechanisms underlying Akt-induced protection appear to include mitochondrial PT pore inhibition.

Abstract N° S18D

Distinct components of the Jak/STAT signaling pathway are involved in the onset of myocardial hypertrophy and ischemia and in cardioprotection

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It is well established that binding of the vasoactive peptide angiotensin II (AngII) to its receptors triggers induction of several intracellular signaling pathways, notable among them is the Jak/STAT pathway. We have previously identified the components of the Jak/STAT signaling pathway that are activated in rat heart subjected to hypertrophy associated stimuli an ischemia/reperfusion. The activated STAT proteins cause stimulation of the renin-angiotensin system (RAS) via their role in transcription regulation of the promoter of the pro-hormone angiotensinogen (ANG). To demonstrate the regulatory linkage between the Jak/STAT signaling and activation of localized and systemic RAS in an animal context, we produced transgenic mice harboring ANG promoter containing the wild type or mutant STAT target site (St-domain) fused to the luciferase reporter. We show here that administration of AngII, a positive feedback agonist of RAS, to the mice resulted in prominent expression of luciferase in the liver and the heart of animals containing the wild type St-domain but not in mice harboring mutant

St-domain. AngII induced signaling caused the activation of STAT proteins, the pattern of which was distinct in the heart relative to that of liver. The inducible function of STAT protein appears to be mediated by physical association of p300 with STAT5B in the liver and STAT3 and STAT5A in the heart. In parallel, mice were subjected to transaortic constriction which resulted in development of pressure overload hypertrophy. The hypertrophic response followed the activation of signaling events manifested by prominent activation of Jak/STAT, MAPK and P13 kinase pathways. We observed that the expression of SOCS3, a negative regulator of Jak2, was also increased. Thus, these findings point to the differences in the signaling mechanisms in the circulating and localized RAS reflecting their distinct functional involvement in the cardiovascular system. The activation of Jak/STAT pathway plays a dual role, one that affords the development of pressure overload hypertrophy and perhaps the activation of the negative feedback loop for cell survival.

Abstract N° S18E

Role of metalloporphyrins in therapeutics of cardiovascular complications during hypoxic stress

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Hypoxia challenges the uptake, transfer and delivery of oxygen thus affecting the essential components of the cardiorespiratory system of the body. In addition, pressure overload of the right ventricle, induced experimentally by hypoxia or pulmonary artery banding is known to increase hemoxygenase (HO-1) expression. HO also plays a critical role in cellular homeostasis by regulating the availability of heme. This substantiates the critical role of hemoxygenase in cardiovascular adaptation. A series of metalloprophyrins can be of immense value in exploring the role of haemoxygenase during hypoxia. The main aim of the current study was to study the role of hemoxygenase in cardiovascular adaptation during hypoxia and whether metalloporphyrin treatment can prove beneficial for management of hypoxia. Cardiovascular variables were monitored using DS telemetry technique in conscious rats. Increase in Heart rate, MAP with progressive hypoxia showed a significant decrease following pretreatment with CrMP. Furthermore, we have studied the expression of HO-1, the inducible isozyme in chronic hypoxic rats. It was concluded out of the five metalloporphyrins studied, CrMP, at a concentration of 5 μM, was a selective inhibitor of HO activity and was the most useful metalloporphyrin for the conditions tested. Thus, CrMP would appear to be a valuable chemical probe in modulating HO mediated effects of chronic hypoxia on cardiovascular variables.

Abstract N° S18F

Shift and cleavage of myocardial dystrophin (DYS) is a common pathway to advanced heart failure (AdHF)

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AdHF is the leading cause of death and the progression mechanism of cardiac dysfunction should be clarified to establish its prevention and/or treatment. In TO-2 hamsters with hereditary dilated cardiomyopathy (DCM), we have identified the responsible gene (δ-SG) in Dys-associated proteins. The same gene mutation was detected in human families. Present analyses reveal the age-dependent cleavage and translocation of myocardial Dys from sarcolemma (SL) to myoplasm, increased SL permeability *in situ* and close relation between the loss of Dys and hemodynamic indices. In addition, the amount of Dys was surprisingly correlated with the animal's survival rate. The Dys disruption is not an epiphenomenon but directly precedes the AdHF, because long-lasting transfer of the missing δ-SG gene to the degrading cardiomyocytes *in vivo* ameliorated all of the pathologic features and animal's prognosis. Furthermore, Dys disruption occurs with both acute HF after isoproterenol toxicity, chronic HF after the coronary ligation in rats and AdHF in human DCM that required cardiac transplantation. The Dys cleavage in animal models and humans supports a novel scheme of the SL instability and Dys proteolysis in all 3 types of HF. The hereditary HF is curable with potential gene therapy, when the responsible gene is identified and precisely corrected with biological inert rAAV vector.

Abstract N° S19A

The molecular basis for the atypical o-adrenoceptor pharmacology of CGP12177A

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The aryloxypropanolamine CGP12177A originally developed as a o_1/o_2-adrenoceptor (AR) agonist was later demonstrated to be a o_3-AR agonist and more recently an agonist at the putative o_4-AR. Agonist responses to CGP12177A occur in heart or adipose tissue which express high concentrations of o_1-AR and in cells expressing recombinant o_1-AR. Studies in o_1-AR knockout mice suggest that o_4-AR are a form or state of the o_1-AR although the molecular basis for the actions remains unknown. We have examined whether the o_1-AR interacts with receptor activity modifying proteins (RAMPs) to produce a receptor with an altered phenotype. CHO K1 cells stably expressing o_1-AR were transfected with RAMPs 1,2 or 3 and used 48 hrs later. Immunohistochemistry showed that RAMPs 2 and 3 but not RAMP1 were expressed at the cell surface. [^{125}I] CYP binding was unaffected by expression of RAMPs as was competition for binding by isoprenaline, propranolol or CGP12177A. cAMP production by cells co-expressing RAMP 1,2 or 3 in response to isoprenaline (o_1-AR) or CGP12177A (atypical response) was unchanged. Although there is evidence that RAMPs interact with o_1-AR (Bouvier pers communication) this does not appear to influence the binding characteristics or ability to

generate cAMP. Another possible explanation for altered phenotype is the formation of o_1-AR homo and heterodimers which have been demonstrated by co-immunoprecipitation and bioluminescence resonance energy transfer (BRET). Co-expression of o_1-AR containing GFP2 or Rluc produced a BRET signal which was reduced by co-expression of untagged o_1-AR or a peptide corresponding to transmembrane 6 (TM6) of the o_1-AR. Current studies suggest that co-expression of o_1-AR and TM6 increased the response of cells to CGP12177A and also to the o_1-AR agonist isoprenaline. In conclusion, formation of complexes with RAMPs or receptor dimerisation do not appear to explain the formation of alternative phenotypes of the o_1-AR. However, interactions at an alternative site, production of a ligand specific state or alternative ligand binding of a phosphorylated receptor remain feasible mechanisms.

Abstract N° S19B

Human heart β_1- and β_2-adrenoceptors (AR) ; high and low affinity states

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The identification of 2 β_1-AR states was prompted by the characteristic effects of non-conventional partial agonists (NCPA). NCPA cause cardiostimulant effects at concentrations considerably greater than those that antagonize the effects of catecholamines. The density of both sites is decreased in failing hearts. Twelve clinically used β-blockers have consistently higher affinity for the H-site than the L-site. The Arg389Gly polymorphism can reduce the catecholamine responsiveness but hardly affects the (-)-CGP12177 responsiveness and does not modify the affinity of clinically used β-blockers for the β_1-AR H-site. Mutation of the β_1-AR Asp138 to Glu138 reduces catecholamine agonist potency 10,000-fold but (-)-CGP12177 potency only 6-fold, abolishing the (-)-bupranolol-evoked antagonism of the catecholamine effects but not of the (-)-CGP12177 effects. Thus, β_1-AR binding partners are clearly different for NCPA and catecholamines. Human myocardial β_2-AR mediate increases of contractility and hastening of relaxation, consistent with coupling to G_s protein. Activation of feline and murine β_2-AR, however, does not hasten relaxation although causes increases in contractility. The lack of lusitropic effects is presumably due to dual coupling of β_2-AR to G_s and G_i proteins, G_i preventing the manifestation of the G_s/cyclic AMP-dependent protein kinase (PKA) pathway that leads to phosphorylation of proteins involved in relation. In human β_2-AR overexpressed \\ 126\\200-fold in murine heart (TG4 mice), low adrenaline concentrations increase contractility but 1000-fold higher concentrations cause pertussis toxin-sensitive cardiodepression, effects mirrored by equivalent changes of PKA activity. On the other hand, noradrenaline does not cause cardiodepression and increases contractile force and PKA activity at both low and high concentrations. These results are simulated with a model for 2 β_2-AR states activated by adrenaline, one with high affinity (H) coupled to G_s, the other with low affinity (L) coupled to G_i. Noradrenaline only activates β_2-AR through G_s. These H and L states appear to differ from the high (GTP-sensitive) and low affinity states of β-AR coupled and uncoupled to G proteins, respectively.

Abstract N° S19C

β-adrenoceptor subtype signaling

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Coexisting cardiac β-adrenergic receptor (βAR) subtypes, mainly β_1AR and β_2AR, activate different signaling cascades with β_1AR coupling to G_s and β_2AR to G_s and G_i pathways. As a result, β_1AR activation induces myocyte apoptosis, whereas β_2AR activation protects heart cells from assaulting factors, including enhanced β_1AR stimulation, hypoxia, and reactive oxygen species. Inhibition of β_2AR-activated G_i-Goo-PI3K-Akt pathway converts β_2AR stimulation to apoptotic. Furthermore, we have demonstrated that sustained β_1AR stimulation promotes myocyte apoptosis by activation CaMKII, independently of PKA signaling. Overexpressing a cardiac CaMKII isoform, CaMKII-oC, markedly exaggerates β_1AR apoptotic effect. These findings indicate that CaMKII constitutes a novel PKA-independent linkage of β_1AR stimulation to cardiomyocyte apoptosis that has been implicated in the overall process of chronic heart failure. These studies on βAR subtype signaling elucidate mechanisms underlying the beneficial effects of βAR blockers in patients with chronic heart failure, might also delineate rationale for combining selective β_1AR blockade with β_2AR activation as a potential therapy for chronic heart failure.

Abstract N° S19D

β-adrenergic signaling in the heart — novel insights about mechanisms of cardiac hypertrophy and fibrosis

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Chronic β-adrenergic stimulation leads to cardiac hypertrophy, fibrosis and heart failure in experimental models and contributes to the progression of heart failure in humans. The pathways mediating the detrimental effects of chronic β-adrenergic stimulation are only partly understood. In order to identify these pathways, we are making use of different strategies. We are analyzing the function of known candidate genes *in vivo* in transgenic animal models and thereby identified abnormal calcium handling as a critical step underlying the detrimental effects of chronic β_1-adrenergic signaling.

Alternative approaches aim at identifying novel candidate genes involved in β-adrenergically mediated cardiac hypertrophy and fibrosis. We are making use of gene arrays to search for differentially regulated genes early in the course of cardiac hypertrophy and validate their importance in the β-adrenergic growth response through the generation of transgenic animal models for these candidate genes. In addition, we are trying to elucidate mechanisms underlying car-

diac fibrosis. There is increasing evidence pointing towards communication between cardiomyocytes and fibroblasts mediated through secreted factors. To identify such factors, we undertook a yeast-based genetic screening approach to identify genetically encoded factors by which cardiomyocytes might signal to cardiac fibroblasts.

In conclusion, novel mechanisms involved in β-adrenergically mediated cardiac hypertrophy and fibrosis will be discussed.

Abstract Nº S19E

Place of β3-adrenoceptors among other β-adrenoceptor subtypes in the regulation of the heart function

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The sympathetic system knowledge is a basic element in the understanding of numerous physiological and physiopathological phenomena. In the heart, at least three populations of β-adrenoceptors (β-AR) potentially modulate the cardiac function. The effects of β_1- and β_2-AR are well established both in human and other mammals. Their stimulation produces positive chronotropic and inotropic effects. We have demonstrated the functional presence of β_3-AR in human heart. Stimulation of the β_3-AR produced a negative inotropic effect. The inhibition of contractility involved the inhibitory G protein, $G_{i/o}$ and results from the production of nitric oxide (NO) by the endothelial isoform of NO synthase and an increase in intracellular cGMP level. Compared to β_1- and β_2-AR, the β_3-AR presents a relative *in vitro* and *in vivo* lack of desensitization following activation with agonists. These features suggest that the expression of β_3-AR in heart may have pathophysiological significance. Recently, we have described opposite regulation for β_1-AR and β_3-AR in the human failing heart. Beside the classically observed β_1-AR, an upregulation of β_3-AR was reported. Despite increased β_3-AR expression, the negative inotropic effect was mildly reduced in failing heart tissue compared with responses observed in nonfailing samples because of concurrent alterations in post-receptor coupling mechanisms, especially decreased eNOS expression. Nevertheless, the reduction in β_3-AR response is less than that obtained with β_1-AR stimulation. Therefore, the functional loss of catecholamine positive control of cardiac contractility during heart failure may result from a shift in the balance between β_1-AR-mediated positive and β_3-AR-mediated negative inotropic pathways. The efficiency of some β-blockers used in the treatment of heart failure, could result from on action on β_3-adrenoceptors. As we have descived the presence of β3-AR in vessels, the mechanism responsible for the beneficial effects of blocking β-AR activation could be actually due to the effects of these agents on the vasculature. Thus, β-blockers with vasodilator action (β-blockers of 3rd generation) may be more efficient to treat heart failure than non-vasodilatory ones, because arteriolar and venous dilatation would reduce afterload and preload respectively, in the failing heart.

Abstract Nº S20A

Development of molecular therapy to treat ischemic arterial disease

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Regenerative medicine is emerging as a potential strategy for the treatment of cardiovascular disease such as peripheral arterial disease (PAD). Especially, most fruitful strategy is to stimulate blood vessel formation, so called angiogenesis. Recently, the efficacy of therapeutic angiogenesis using VEGF gene transfer has been reported in human patients with critical limb ischemia and myocardial ischemia. As we reported the potent angiogenic activity of hepatocyte growth factor (HGF) in animal study, we planed a prospective open-labeled clinical trial of gene therapy (TREAT-HGF) by intramuscular injection of naked plasmid DNA in patients with PAD who had failed conventional therapy as phase I/IIa trial. In this trial, gene transfer was performed in 22 patients by intramuscular injection of naked plasmid DNA encoding human HGF. ABI (ankle-brachial pressure index) was significantly increased at 2 months after injection, and increase in ABI over 0.1 was observed in 11 of 17 patients. Reduction of pain scale (over 2cm in visual analog scale) was observed in 8 of 13 patients, while the reduction in ulcer size (> 25 %) was observed in 11 of 18 ulcers. Currently, severe adverse effects related to gene transfer could not be detected in any patient. Of importance, different from VEGF, no edema formation could be observed throughout the gene therapy periods. Overall, the present results indicated that intramuscular injection of naked plasmid DNA encoding human HGF cDNA resulted in a significant improvement of clinical symptom or increase in ABI in 18 patients out of 22 patients. In addition, HGF gene transfer also induced angiogensis in myocardium. Naked plasmid DNA as drug delivery system is useful to treat cardiovascular disease such as PAD. Currently, phase III trial in Japan, and phase II trial in USA to treat PAD using HGF gene are underway.

Abstract Nº S20B

Calreticulin regulates angiogenesis

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The Sarco/endoplasmic reticulum (SR/ER) membrane is one of the most important intracellular organelles. It is involved in the control of virtually every aspect of cellular function including Ca^{2+} transport and storage, protein and lipid synthesis, protein modification and folding. Calreticulin (CRT) is a SR/ER resident protein. It plays an important role in chaperoning the newly synthesized proteins and regulation of intracellular Ca^{2+} homeostasis. Previously we showed that gene targeted deletion of CRT is embryonic lethal with main defect in the cardiovascular development. We have also demonstrated that at early mouse development CRT gene is highly expressed in the heart and blood vessel. CRT gene is down regulated in the cardiomyocytes of newborn mice. To

investigate the *in vivo* role of CRT in the vessel wall we generated transgenic mice overexpressing CRT in the vascular smooth muscle cells. Overexpression of CRT resulted in increased coronary artery angiogenesis, increased vessel wall permeability, coronary aneurysm and formation of vascular tumor. Other phenotypes of these transgenic mice include enlarged heart, congested lungs and defects in the kidney (necrosis in severe cases). Immunohistological analysis of the vascular wall of these mice showed that the observed defects are due to altered extracellular matrix (ECM) composition. We conclude that CRT affects angiogenesis by regulating enzymes affecting ECM degradation.

Supported by the Canadian Institute for Health Research.

Abstract N° S20C

New approaches to treat cardiovascular diseases targeting NFkB

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Nuclear factor-kappa B (NFkB), a multipotential transcriptional factor, has been reported to play a significant role in the coordinated transcription of a variety of inflammatory molecules. Therefore, blocking the activation of NFkB may inhibit gene expression of essential inflammatory molecules. Inflammation is one of the key pathophysiological processes leading to the development of acute cardiac rejection, graft arteriopathy, myocarditis, arterial intimal hyperplasia, ischemia/reperfusion injury, ventricular remodeling, and heart failure. This investigation was attempted to test the hypothesis that NFkB blockade with a « decoy » against the *cis* element or new IkB inhibitors (IMD0354/1041) which blocks NFkB activation could prevent progression of these cardiovascular diseases. Mice hearts were heterotopically transplanted. Scrambled decoy-transfected allografts were acutely rejected, while NFkB decoy prolonged their survival. While severe cell infiltration and intimal thickening with enhancement of inflammatory factors were observed in untreated or scrambled decoy treated allografts at day 28, NFkB decoy attenuated these changes. Experimental autoimmune myocarditis was developed by immunizing rats with purified porcine cardiac myosin. NFkB decoy was infused into the rat coronary artery on day 0 or day 14 and harvested them on day 21. The ratios of myocarditis-affected areas to the ventricular cross sectional area of all treatment groups were significantly lower than that of control group. Also, in a rat model of coronary ischemia/reperfusion, IMD0354 significantly reduced myocardial infarct size at 24 hours in association with reduction in inflammatory cytokines and chemokines. IMD1041 improved survival rate and late left ventricular remodeling at 28 days after occlusion of rat left anterior descending artery in association with reduction in fibrosis and serum levels of BNP. These data indicate that NFkB is critically involved in the development of a variety of cardiovascular diseases. Treatment targeting NFkB is promising in the prevention of these diseases.

Abstract N° S20D

Cardiac adenosine receptors & protein kinase C

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Adenosine is involved in regulation of the coronary circulation. In addition adenosine displays antiarrhythmogenic, angiogenic, antiadrenergic and inotropic effects in the heart. The cardiac antiadrenergic effects of adenosine are brought about by adenosine A1 receptor (A1R) activation that fosters a negative feedback modulation of β1-adrenergic mediated contractile and metabolic responses in the myocardium. On the other hand, adenosine A2a receptor (A2aR) stimulation elicits a positive inotropic response in various *in vitro* and *in vivo* cardiac preparations. The importance of protein kinase C (PKC) in the antiadrenergic and inotropic actions of adenosine were investigated using isolated ventricular myocytes stimulated to contract and changes in sarcolemmal length were determined to assess contractile performance. The A1R agonist chlorocyclopentyladenosine (CCPA) reduced the contractile response caused by the β1-adrenergic agonist isoproterenol. The PKC inhibitor, chelerythrine (CHEL), prevented the CCPA reduction of the isoproterenol contractile response. However, CHEL did not prevent the positive inotropic response elicited by an A2aR agonist CGS-21680. Since PKC epsilon (PKCε) is a major isoform in the rat myocardium subsequent studies were performed where PKCε was over-expressed in ventricular myocytes. Overexpression of PKCε enhanced the CCPA reduction of the isoproterenol elicited contractile response. In addition stimulation of myocytes with CCPA caused a translocation of PKCε to the t-tubular system as evidenced by using a GFP-tagged PKCε. Administration of a specific PKCε inhibitor peptide prevented the antiadrenergic effect of CCPA. In summary protein kinase C epsilon appears to play an important role in the A1R mediated antiadrenergic action of adenosine in the heart. The results also suggest that PKC is not involved in the cardiac contractile response caused by A2aR stimulation.

Abstract N° S20E

Modulation of nuclear protein import in vascular smooth muscle cells by lipids

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Cell proliferation is a key process during organ development and during pathological conditions like cancer and atherosclerosis. The growth of the cells is closely regulated by proteins that are transported into the nucleus to turn genes on or off. RNA exits the nucleus to initiate protein translation. The transportation of molecules in and out of the nucleus, therefore, becomes critical to the process of growth. Factors that regulate nucleocytoplasmic transport become

extremely important modulatory factors in cell growth and proliferation. However, little is currently known of the cytoplasmic factors that have the capacity to alter transport of molecules through the nuclear pore complex in vascular smooth muscle. We have examined the capacity for lipids to influence nuclear protein import in vascular smooth muscle cells using a standard cell permeabilized methodology and a fluorescent marker protein to measure the movement. We have also used the technique of cell micro-injection to examine the capacity of these lipids to affect protein import into the nucleus. Our results will be summarized to show that some of these lipids stimulate import whereas others inhibit it. The MAPK pathway appears to function as a central intracellular effector of these actions. Stimulation of its activity by specific lipids can either stimulate or inhibit import depending upon its activation and its cytoplasmic targets. Nuclear protein import appears to be a complex, multipathway, highly regulated process that may provide mechanistic insight into cell proliferation under conditions of normal development and during conditions of accelerated growth like atherosclerosis.

Supported by CIHR and NSERC.

Abstract N° S21A

The role of MMPS and the plasminogen system in the LV remodeling process after myocardial infarction

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Matrix metalloproteinases (MMPs) are a large family of proteinases with a central role in the degradation of extracellular matrix components, a requirement for cell migration and tissue remodeling. Recent evidence implicates the MMP family as a mediator of cardiac diseases, in particular in left ventricular (LV) dilatation and the development of heart failure in both human and animal studies. To elucidate the role of MMPs in the healing and remodelling process of the heart after myocardial infarction (MI) we have performed 3 different interventions (pharmacological MMP inhibition, TIMP-1 knock-out and plasminogen knock-out mice) in the MI model in mice. In the first study, the broad-spectrum MMP inhibitor GM6001 (100 mg/kg/day) was administered to mice undergoing MI. GM6001 treatment led to a reduction in LV dilatation, less thinning of the infarcted wall, and improved systolic function at 14 days post-MI. In a second study the role of TIMP-1, an endogenous inhibitor of MMPs, was investigated in the post-MI remodelling process. In that study, enhanced MMP activity caused diastolic dysfunction after infarction, as was evidenced by a greater degree of LV dilatation and increased filling pressures. In addition, TIMP deficient mice showed reduced collagen levels in the non-infarcted myocardium and exhibited a greater hypertrophic response. Systolic function was not changed in the TIMP-1 deficient MI group compared to the wild-type MI group. In the third study, the plasminogen system, which is an endogenous activator of MMPs, was identified as a key regulator in the control of infarct healing. In this regard, infarct healing was abolished in plasminogen deficient mice, as inflammatory cells did not migrate into the infarcted myocardium, necrotic cardiomyocytes were not removed and scar tissue was not being formed until at least 5 weeks post-MI. In these scarless infarcted plasminogen deficient hearts, LV dilatation was not affected but systolic function was depressed after stressing the hearts with dobutamine. Gelatinolytic activity of MMP-2 and MMP-9 was reduced the plasminogen deficient hearts, suggesting that the the effect of plasminogen on infarct healing is mediated through the activation of MMP-s.Together, these studies have contributed to the recent view that MMP inhibitors could potentially be used as therapy for patients with both acute and chronic MI, to improve postinfarction remodeling and prevent the development of heart failure.

Abstract N° S21B

Cardiac remodeling in hyperhomocysteinemia and diabetes : a NO and ECM connection

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Accumulation of oxidized-matrix between endothelial and muscle is associated with endocardial endothelial (EE) dysfunction in diabetes mellitus (DM). High levels of circulating homocysteine (Hcy) are associated with oxidative stress and DM. These high levels of Hcy (hyperhomocysteinemia, HHcy) have a negative correlation with peroxisome proliferator activated receptor (PPAR) expression. The hypothesis is that Hcy exacerbates diabetes by antagonizing PPAR. Previous studies have demonstrated that Hcy decreases bioavailability of endothelial nitric oxide (eNO), generates nitrotyrosine, activates latent matrix metalloproteinase (MMP), and instigates EE dysfunction. PPAR* ligands ameliorate endothelial dysfunction by competing with Hcy. Here we have demonstrated that EE dysfunction in DM is due to increased levels of reduced oxygen species (ROS), Hcy, nitrotyrosine, MMP activity, collagenolysis, and decreased levels of eNO in response to antagonizing PPAR*. To determine whether the Hcy induces oxidative stress by antagonizing PPAR* in DM, DM is created by alloxan to wildtype and heterozygous cystathionine * synthase (hyperhomocysteinemic) mice. To induce PPAR*, ciglitazone was administered in drinking water. Cardiac ROS and eNO were measured by dichlorofluorescin, and by estimating nitrate/nitrite, respectively. Total collagen and fragments were measured by Western analysis using anti-collagen antibody. Levels of collagenolytic MMP were measured using collagen-gel zymography. The cardiac inhibitor of metalloproteinase was measured by reverse zymography. The responses to acetylcholine, bradykinin, and nitroprusside in cardiac rings from HHcy-DM mice were measured in tissue myobath. The results elucidate intra- and extracellular mechanism by which Hcy amplifies DM and have therapeutic ramifications for diabetic cardiomyopathy.

Abstract N° S21C

Intracellular action of MMP-2 and TIMP-4 in the oxidatively stressed heart

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Matrix metalloproteinases (MMPs) are conventionally known as proteases which are activated by proteolysis of their zymogen form at the cell membrane. They are then released from cells to degrade extracellular matrix proteins. Their activity is regulated at several levels, including direct inhibition by endogenous tissue inhibitors of metalloproteinases (TIMPs). However, recent evidence shows that the zymogen form of MMPs is activated by peroxynitrite. In the first minutes of reperfusion following myocardial ischemia, a burst of peroxynitrite is generated in the myocardium. Moreover, pro-inflammatory cytokines also increase peroxynitrite levels in the heart. This may activate MMPs without loss of the propeptide domain and inactivate TIMPs. We therefore assessed whether there is an imbalance between MMPs and TIMPs in two models of the oxidatively stressed heart.

In isolated rat hearts subjected to ischemia-reperfusion injury we found that there is activation of MMP-2 and release from the heart as a consequence of the injury. TIMP-4 is also rapidly lost from the heart. In situ zymography, however, reveals an imbalance between MMPs/TIMPs and enhanced proteolytic activity in the reperfused heart. MMP inhibitors functionally protect the heart from either ischemia-reperfusion injury or exposure to pro-inflammatory cytokine stress. We found that MMP-2 and TIMP-4 are localized to the thin myofilaments of the cardiomyocyte. MMP-2 co-localizes with troponin I (TnI), the regulatory element of actin-myosin force generation. MMP-2 readily degrades TnI in vitro and in vivo in both ischemic-reperfused and in cytokine-stressed hearts.

Our working hypothesis is that MMPs are activated inside the cardiomyocyte by peroxynitrite. TnI is a proteolytic target of MMP-2 and is an early effector of peroxynitrite-induced cardiac injury. Inhibition of MMP activity is a novel therapeutic target approach to prevent myocardial injury due to oxidative stress.

Abstract N° S21D

In vivo activation and broad-spectrum inhibition of matrix metalloproteinases

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Activation of matrix metalloproteinases (MMPs) in the heart facilitates cardiac remodeling and progression to failure. Considerable efforts have therefore been devoted towards unraveling the processes by which MMPs are activated. Despite these efforts, surprisingly little is known about the biomechanical mechanisms underlying *in vivo* activation of MMPs in the heart. Our group has employed atrial-ventricular epicardial pacing to induce abnormal wall motion of the ventricular myocardium. We hypothesized that regional abnormal wall motion of the left ventricle would stimulate activation of MMPs. Atrial-ventricular pacing resulted in abnormal motion at a left ventricular (LV) pacing site yielding early shortening and late systolic lengthening.Assessment of global myocardial MMP activity showed a seven-fold increase in substrate cleavage ($p < 0.02$) at the LV pacing site relative to a remote site of the left ventricle distal to the pacing site. Gelatin zymography revealed increases of fifty-fold in 92kDa MMP-9 activity ($p < 0.001$) and ten-fold in 84 kDa MMP-9 activity ($p < 0.01$) at the LV pacing site relative to the remote site, whereas MMP-2 activity was unaffected. Abnormal wall motion was associated with a two-fold increase in myocardial collagen degradation ($p < 0.03$) and with increases in plasmin activity (\\ 126\\two-fold ; $p < 0.05$) and inflammatory infiltrate (\\ 126\\two-fold ; $p < 0.02$) relative to the remote site. Our results indicate that regional dyskinesis induced by epicardial activation is sufficient to stimulate significant MMP activity in the heart, suggesting that abnormal wall motion is a major stimulus for MMP activation. As an adjunct to these studies, we have also explored the molecular mechanisms of MMP inhibition by broad-spectrum MMP inhibitors. We have employed hydrogen/deuterium exchange mass spectrometry to investigate inhibition by the broad-spectrum MMP inhibitor doxycycline. Model studies with the MMP-7 reveal two putative doxycycline-binding sites (residues 145-155 ; residues 231-243) of similar affinity that flank the zinc-binding domains of the enzyme. Examination of the X-ray crystal structure of MMP-7 shows that the doxycycline-binding site at residues 231-243 is positioned within the active site cleft adjacent to the catalytic zinc atom. Inhibitor binding occurs in the absence of major changes in structure. These results suggest a mode of MMP inhibition by doxycycline that could involve interactions with the catalytic zinc atom.

Abstract N° S22A

SERCA2 and CSQ2 transcriptional regulation in cardiomyocytes

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The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA2) and calsequestrin (CSQ2) are two proteins that in cardiocytes play a key role in Ca^{2+} transport, storage and release during the contraction/relaxation cycle. The SERCA2 transcription is up-regulated by thyroid hormone and down-regulated during cardiac hypertrophy and failure. The sequence of the SERCA2 gene proximal promoter is conserved among mammals and is highly G + C-rich, it contains a TATA-box, an E-box/USF sequence and a CAAT-box which are part of the endoplasmic reticulum stress response element (ERSE), four Sp1 binding sites that play a role in the hypertrophic response, and one thyroid hormone receptor site (THR). There are two distal conserved regulatory regions (-410 to -661 bp and from -919 to -1410 bp) that have putative sites for : THR. GATA-4, Nkx-2.5/Csx, OTF-1, USF, MEF-2, SRF, PPAR and AP-2. Transfection experiments

demonstrate that the human proximal promoter region is necessary for high level of expression in cardiac myocytes, response to thyroid hormone, calcium and glucose metabolites, as well to PKC isoforms. he human CSQ2 gene consists of 11 exons that span 78 kb and has very similar organization compared to the rabbit and mouse CSQ2 genes. The proximal 5'-regulatory region contains one Sp1 site, one TATA-box, one CArG-box and 3 CAAT-boxes. Transfection of neonatal cardiocytes with CSQ2 promoter constructs containing the -582 kb to -3.1 kb region increases 3- to 4-fold transcription of the gene. This region contains several MyoD, MEF2, NFAT and AP1 sites suggesting that are important for CSQ2 transcription in cardiocytes.

Abstract N° S22B

CaMKII-dependent phospholamban phosphorylation as a mechanism to limit Ca^{2+} overload

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Phosphorylation of phospholamban (PLN) relieves its inhibitory effects on SERCA2a activity resulting in : a) increased sarcoplasmic reticulum (SR) Ca^{2+} uptake rate, which accelerates myocardial relaxation ; and b) increased SR Ca^{2+} load and release, which modulates myocardial contractility. The physiological significance of the phosphorylation of PLN at Thr^{17} by CaMKII remains unclear. While β-adrenergic agonists consistently increase Ser^{16} phosphorylation (PKA site), an increase in Thr^{17} phosphorylation is not always found by this intervention. We have shown that Thr^{17} phosphorylation, independently of that of Ser^{16}, requires the increase in intracellular Ca^{2+} together with the inhibition of phosphatases, two conditions met by the β-adrenergic stimulation and experimentally, by the perfusion of high Ca^{2+} with phosphatase inhibitors or acidic pH. We studied PLN phosphorylation in the perfused heart under two pathological situations in which increased intracellular Ca^{2+} co-exits with acidic pH : reperfusion after ischemia and recovery from hypercapnic acidosis. PLN was phosphorylated at Thr^{17}, independently of Ser^{16}, at the onset of reflow and immediately after hypercapnia was settled. The mechanical recovery after both, the ischemic and acidic insults, was impaired when the increase in Thr^{17} phosphorylation was abolished by CaMKII inhibition. Studies in transgenic animals with Thr^{17} mutated to Ala confirmed these results : mice with mutated PLN have lower contractile and delayed relaxation recoveries after ischemia than mice with non-mutated PLN. Although phosphorylation of PLN at Thr^{17} may not be sufficient to prevent myocardial injury, it may provide a mechanism to limit the intracellular Ca^{2+} overload in pathological situations.

Abstract N° S22C

Molecular control of SR Ca^{2+} release

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Ca^{2+} sparks are the elementary unit of Ca^{2+} release from the sarcoplasmic reticulum (SR) in heart muscle. The Ca^{2+} sparks are triggered nearly synchronously during the cardiac action potential to produce the $[Ca^{2+}]_i$ transient. New findings related to the triggering of Ca^{2+} sparks and Ca^{2+} spark restitution will be presented. The discussion will re-examine how Ca^{2+} sparks are controlled in normal and diseased myocardium.

Abstract N° S22D

Control of the Ca spark during E-C coupling

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Cardiac E-C coupling has been resolved at the microscopic level as Ca sparks (Cheng et al., 1993) which show that we must consider the local micro-environment surrounding the channels that regulate calcium release in order to explain E-C coupling in the heart. Several fundamental questions about cardiac E-C coupling remain unclear e.g. : (1) How many sarcoplasmic reticulum (SR) ryanodine receptors (RyR) are activated during a Ca spark ? (2) If SR release is regulated entirely via —calcium — induced calcium release » how does this process terminate ? (3) Why is it so hard to model/explain the observed spatial dimensions of the Ca spark ? In order to clarify how E-C coupling works, we combine structural data obtained at the limitS of light microscopy and EM with computer models for movements of calcium that give rise to the Ca spark.

Methods for determining the Ca flux underlying the Ca spark should be able to help answer question 1 —provided we can also estimate the flux underlying the gating of a single RyR. The second question is being addressed by computer modelling to examine the necessary roles of SR depletion and coupled gating of RyRs in the junctional space. Our modelling and data analysis also indicated that during the genesis of Ca spark the region of release appears to extend and this may be due to the development of a microscopic wave of RyR activation within the junction and some recent immunocytochemical results support the idea that the t-SR junction can be spatially extended. Other regions show multiple release sites at close spacing which raises the possibility that, under some circumstances, SR release may involve more than one site although fully regenerative release in the form of a Ca wave does not develop.

Référence

Cheng et al., Science 262, 740-744 (1993).

Abstract N° S22E

Beta-adrenergic modulation of cardiac ryanodine receptors

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β-adrenergic stimulation of ventricular myocytes results in activation of PKA and subsequent phosphorylation of target proteins. Phospholamban (PLB) and the ryanodine receptor (RyR) are the most important substrates for PKA in the sarcoplasmic reticulum (SR). In general, PKA phosphorylates substrates to increase the force (inotropism) or the rate (chronotropism) of contraction. In line with this general role, PLB phosphorylation results in acceleration of Ca^{2+}-ATPase activity and increased SR Ca^{2+} load, which in turn augments Ca^{2+} release and force of contraction. The functional effect of PKA-phosphorylation of RyRs has been more controversial, however, and the apparently contradicting results reported in the literature make it difficult to integrate β-adrenergic modulation of RyRs with a clear inotropic response. Here we used antibodies directed against the most important PKA site of cardiac RyR (RyR2-S2809), single RyR channel recordings, and Ca^{2+} sparks to assess the effect of β-adrenergic stimulation on RyR activity. Western blots of cardiac homogenates using the phospho-specific antibody revealed that RyRs are phosphorylated *in vivo* to a basal level. ^{32}P-o-ATP-phosphorylated SR showed that an endogenous Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) is likely to mediate this basal level of phosphorylation since RyRs appear to be poor substrates for PKA. Furthermore, immunoblot analyses with the phospho-specific antibody showed that stimulation of isolated ventricular myocytes with 300nM isoproterenol (Iso) in the absence of Ca^{2+} produced poor phosphorylation of RyRs, but Iso stimulation with Ca^{2+} resulted in higher phosphorylation. These results indicate that β-adrenergic stimulation of RyRs involves the participation of CaMKII and is conditioned to an $[Ca^{2+}]_i$ elevation. [^{c}H]Ryanodine binding assays, which may be used as an index of the activity of the channel, showed that PKA or the endogenous CaMKII modestly inhibit RyR activity, whereas acid phosphatase (AcPh), a protein phosphatase of broad spectrum that readily dephosphorylates RyRs, increases RyR activity. In line with these results, direct application of PKA or CaMKII to RyRs reconstituted in lipid bilayers also modestly inhibited channel activity, but AcPh caused a dramatic increase of channel activity. Again, the modest effect of PKA and CaMKII and the greater effect of AcPh in binding and lipid bilayer assays suggest that the basal level of RyR phosphorylation is relatively high, leaving a few phosphorylation sites free. In isolated saponin-permeabilized ventricular myocytes loaded with fluo-4, dephosphorylation by the endogenous phosphatase PP1 increased the size of Ca^{2+} sparks in thapsigargin-treated cells, implying that the observed effects resulted from direct activation of RyRs. Taken together, the results indicate that PKA and CaMKII phosphorylation decreases RyR activity. Hence, a potential mechanism whereby RyR phosphorylation contributes to the positive inotropic response of β-adrenergic stimulation is one in which phosphorylation of RyRs decreases Ca^{2+} « leak » from the SR, which in turn increases SR Ca^{2+} load and, like PLB, increases force of contraction.

Abstract N° S22F

Therapeutic potential of sodium-calcium echange inhibitors

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The cardiac Na^+-Ca^{2+} exchanger has been implicated as a major contributor to the cellular toxicity associated with ischemia-reperfusion injury. Specifically, in response to the elevation of intracellular Na^+ occurring during ischemia and reperfusion, there is an impairment of Ca^{2+} efflux and/or an augmentation of Ca^{2+} influx via this transporter. Depending upon severity, the resultant Ca^{2+} overload can trigger functional impairment of cardiac performance, arrhythmias, and cell death. In this scenario, inhibition of Na^+-Ca^{2+} exchange appears to be a promising target for cardioprotection. Here, we describe the inhibitory properties of newer Na^+-Ca^{2+} exchange inhibitors (KB-R7943 and SEA0400) that exhibit remarkable cardioprotective effects against ischemia-reperfusion injury. Both agents functionally interact with the intrinsic ionic regulatory mechanisms of the cardiac Na^+-Ca^{2+} exchanger. Moreover, the inhibitory potency of SEA0400 is increased as intracellular Na^+ is increased. This inhibitory profile leads to an apparent transport mode selectivity for these agents whereby inhibition of the reverse transport mode (i.e. Ca^{2+} influx) is favored. These attributes provide nearly ideal characteristics when considering their potential utility as cardioprotective agents against ischemia-reperfusion injury.

Abstract N° S23A

Chronic chymase inhibition prevented cardiac fibrosis and improved diastolic dysfunction in the progression of heart failure

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Angiotensin-II (Ang-II) which plays a crucial role in the cardiac remodeling process is generated via angiotensin-converting enzyme (ACE), however, an alternative generation pathway, chymase, which is stored in the mast cells also exists in the heart. We have revealed chymase pathway is activated inMsevere stage of heart failure (HF), despite ACE pathway is activated throught the progression of HF. However, the role of chymase in left ventricular (LV) function has not been fully elucidated as yet. We evaluated the effects of a specific chymase inhibitor, (Chy-I), on changes in cardiac structures, Ang-II levels and the expression of several failing

heart related genes in dogs with tachycardia induced HF.MIn HF, the number of chymase-positive cells and cardiac Ang-II concentrations increased in the LV compared with the normals, however, Chy-I significantly decreased them. Despite no significant differences in LV systolic function compared with the HF group, Chy-I significantly decreased LV filling pressure, shortened the time constant of relaxation and upregulated the expression of sarcoplasmic reticulum Ca^{2+}-ATPase mRNA. Chy-I also decreased collagen type I and III mRNA and decreased cardiac collagen deposits compared with theMHF group. Cardiac chymase may be involved in diastolic dysfunction rather than systolic dysfunction, via modification of Ca^{2+} handling as well as suppression of collagen accumulation in HF.

Abstract N° S23B

Angiotensin II-induced oxidative stress as a possible mediator of contractile dysfunction in the failing heart

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The peptide Angiotensin II (Ang II) exerts both positive and negative contractile actions in the heart via distinct mechanisms, and the net effect on contraction is determined by the relative magnitudes of the opposing pathways. The components of Ang II signaling, underlying its positive contractile actions, have been examined in detail and are currently well understood. In contrast, the mechanisms underlying the negative inotropic effect are presently a largely uncharted territory. Because in heart failure the circulating levels of Ang II are increased, the balance between positive and negative inotropic actions could be upset. Thus, under these circumstances the Ang II-induced negative inotropic effect could be enhanced and contribute to the contractile dysfunction observed in these pathological condition. Ang II triggers several mechanisms, any of which could potentially reduce myocardial contractility. In this scenario, p38 MAPK has been recently recognized as a downstream target of the renin-angiotensin pathway and redox-sensitive intermediates have been implicated in Ang II-mediated p38 MAPK activation. Furthermore, coupling of Ang II receptors to NAD(P)H oxidase, resulting in superoxide formation and p38 MAPK activation has been recognized in ventricular myocytes. Moreover, in experiments with transgenic mice, it has been demonstrated that the cardiac-specific activation of p38 MAPK markedly attenuates cardiac contractility. These results suggest a previously unrecognized link between Ang II, NAD(P)H oxidase-reactive oxygen species generation, p38 MAPK-mediated negative inotropic effect and heart failure induced contractile dysfunction.

Abstract N° S23D

Chronic β-adrenoceptor blockade prevents progression of heart failure in ageing SHR

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Although once a contraindication, β-adrenoceptor blockade is now an established treatment to prolong survival in human heart failure. This project has determined changes in cardiac structure and function in 15 month old male normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) treated with metoprolol (M ; 30-80 mg/kg po for 24 weeks ; *p < 0.05vsWKY ; **p < 0.05vsSHR). The increased left ventricular weight in SHR was reduced by metoprolol (WKY 2.37±0.08, SHR 4.53±0.06*, SHR + M 3.69±0.26** mg/g bwt). In ageing SHR, metoprolol prevented the following changes : cardiac dilatation (LV internal diameter WKY 7.90±0.30, SHR 8.96±0.32*, SHR + M 7.48±0.37** mm), decreased fractional shortening (WKY 59.5±2.8, SHR 40.4±2.9*, SHR + M 67.7±3.9** %) and increased mitral valve flow (E/A) ratio (WKY 1.19±0.01, SHR 3.64±0.69*, SHR + M 2.24±0.28**). In isolated hearts, metoprolol reversed both the increased cardiac stiffness (WKY 28.8±1.4, SHR 38.3±4.1*, SHR + M 31.5±1.4**) and the increased interstitial collagen deposition (WKY 4.4±1.3, SHR 15.6±2.0*, SHR + M 5.9±0.9** % of area) and prevented further increases in APD90. Further, metoprolol markedly improved survival at 21 months. Thus, chronic metoprolol treatment attenuated the pathological structural and functional changes during the progression of heart failure in the ageing SHR.

Abstract N° S23E

Pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase is a promising new approach for the therapy of various forms of heart failure

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Acute and chronic myocardial ischemia and ischemic cardiomyopathy are major causes of hospitalization, morbidity and mortality worldwide. The mechanism of myocardial injury has been extensively studied, and there is accumulating evidence for the key role of oxidative and nitrosative stress in this process.

Oxidant induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP), which in turn contributes to cardiac and vascular dysfunction in various pathophysiological conditions including reperfusion injury, heart transplantation, diabetic cardiomyopathy, drug induced and chronic heart failure. Accordingly, neutralization of peroxynitrite or pharmacological inhibition of PARP protect against cardiovascular dysfunction.

A multitude of novel PARP inhibitors are in various stages of preclinical or clinical development, many with potency that greatly exceeds the prototypic agents successfully used in earlier animal studies. The remarkable efficiency of new PARP inhibitors against various forms of myocardial injury in animal models strongly suggests that these agents show

considerable promise and hope in the treatment of cardiovascular disorders in humans.

Abstract N° S23F

Cell transplantation for the treatment of heart failure : active contributor or passive bystander ?

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Immature muscle cells transplanted into the scar formed after myocardial infarction can not only survive, but also develop and grow. Nevertheless, if such cells are to make a useful active contribution to cardiac function, the transplanted tissue must be organized in an appropriate manner. Despite the encouraging results published documenting apparent enhancement of function, this crucial architectural imperative in the transplant process has received little attention. In rat hearts, the bolus injection of neonatal cardiac muscle cells and fibroblasts into an infarct resulted, 6 months later, in a highly disorganized musculature containing large amounts of collagen. Such organization is unlikely to provide active functional improvement and could even, under some circumstances, be detrimental. However, the presence of the transplant tissue did minimize or prevent paradoxical systolic bulging of the infarct, which was consistent with a passive benefit. Thus, while cell transplantation leading to active functional improvement is the ultimate goal, there may also be some advantage to be gained from implantation of elements specifically designed to strengthen and thicken the infarct. The fabrication of polymer nanofibers, including collagen, using the techniques of electro-spinning provides a potential source for such material. In addition, it may be possible to use fabricated nanofibers to serve as guides to train transplanted cells to align in the desired orientation. The application of such tissue engineering approaches to address the architectural problems associated with cell transplant may provide a rational basis for the treatment of heart failure.

Abstract N° S24A

Oxidant signals and cardioprotection : dual role in susceptibility to ischemia/ reperfusion injury

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Myocardial ischemia/reperfusion alters cellular redox status and results in a burst of free radicals (FR) including those derived from O_2 and NO traditionally viewed as cardiotoxic. Over the last decade, however, FR have become recognised as important triggers of cell signaling mechanisms leading to cardioprotection. Treatment with antioxidants and/or inhibition of NO synthesis appears to be cardioprotective only in non-adapted hearts exhibiting myocardial dysfunction or arrhythmias, but it abrogates protective response in the hearts preconditioned by a short-term ischemic stress. Oxidative load is a common feature of a number of chronic processes associated with remodeling, hypertrophy and functional disorders on the one hand, but on the other hand, it contributes to development of long-term adaptation that increases ischemic tolerance. Chronic high altitude hypoxia, despite its negative cardiac effects, leads to adaptation that renders rat hearts more resistant to ischemia, and protection is blunted by antiradical interventions. Similarly, long-term adaptation of rats during chronic L-NAME-induced hypertension associated with an enhanced production of FR resulted in an improved postischemic contractile recovery and suppression of reperfusion-induced arrhythmias, in conjunction with an increased ERK1/2 activity, and the effect was reversed by mito K(ATP) blocker 5-HD. It is concluded that FR may play a dual role in the heart. Being deleterious in the myocardium exposed to ischemia/ reperfusion alone and contributing to low ischemic tolerance in the non-adapted heart, they might be also implicated in cardioprotection conferred by both, short-term and long-term adaptation. Potential downstream mechanisms may involve mito K(ATP) opening and/or activation of certain MAPK cascades.

Abstract N° S24B

Redox regulation of vascular cell function

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Endothelial nitric oxide synthase (eNOS) and heme oxygenase-1 (HO-1) have properties that may protect against vascular disease. Nitric oxide (NO) produced by eNOS is an important molecule required for maintenance of vascular homeostasis. Vascular disease is characterized by impaired NO bioactivity due, in part, to excess production of reactive oxygen species. While the implications of superoxide anion on NO bioactivity have been studied, the effect of hydrogen peroxide (H_2O_2) in this regard is less clear. We observed that H_2O_2 activates eNOS activity in aortic endothelial cells, and this was dependent on the ability of H_2O_2 to induce cell-signaling pathways involving Src, phosphoinositide 3-kinase and Akt kinases that alter eNOS phosphorylation at the regulatory amino acid residues Ser-1177 and Thr-495. Despite promoting eNOS activity H_2O_2 impaired agonist-stimulated endothelial-derived NO bioactivity and relaxation of rabbit arterial rings. This impairment appeared to result from H_2O_2-induced, iron-dependent oxidative inactivation of NO, highlighting an additional mechanism by which oxidative stress may impact on NO bioactivity in vascular diseases. Induction of HO-1 inhibits proliferation of vascular smooth muscle cells (VSMC) and intimal thickening after arterial injury. We observed that inhibition of intimal thickening and VSMC proliferation in balloon-injured rabbit aortas by the antioxidant drug probucol was associated with induction of HO-1 in VSMC. Probucol also induced HO-1 in cultured VSMC, and pharmacologic or molecular inhibition of HO-1 induction resulted in a loss of the drug's ability to inhibit VSMC proliferation. These findings may explain how probucol in-

hibits restenosis and highlight HO-1 as a redox-sensitive target for therapeutic intervention against occlusive vascular disease. Together, these studies provide new information on how oxidants and antioxidants regulate the function of vascular cells relevant to vascular disease.

Abstract N° S24C

Role of cytokines in redox regulation in the ischemic heart

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Circulating and cardiac TNF-a levels are increased during myocardial ischemia in experimental animals and in patients with ischemic heart disease and advanced heart failure. Soluble TNF-a receptor 1 (sTNFR1) is an extracellular domain of TNF-a receptor 1 and an antagonist to TNF-a. We have examined whether or not sTNFR1 improves cardiac function after myocardial infarction in rats. Rat sTNFR1 cDNA was produced by RT-PCR and cloned into the TA expression vector. Male Wistar rats (250-300g) were subjected to left coronary artery (LCA) ligation. Immediately after LCA ligation, a total of 200ug of the sTNFR1 (n = 26) or LacZ plasmid (n = 26) was injected into three different sites in the left ventricular wall. The vector-derived sTNFR1 transcripts and protein expression were detected from 1 day to 21 days after the sTNFR1 plasmid injection. At 1, 7, 14 and 21 days after LCA ligation, TNF-a bioactivity in the heart was higher in rats receiving LacZ plasmid than in sham-operated rats, whereas sTNFR1 plasmid significantly suppressed the increase in TNF-a bioactivity. At 21 days after LCA ligation, the LV diastolic dimension (LVDd) was significantly lower and the fractional shortening (FS) was significantly higher in the rats treated with the sTNFR1 plasmid than in those treated with the LacZ plasmid (LVDd : 9.4 ± 0.4 mm vs. 11.0 ± 0.5, FS : 19.0 ± 1.0 % vs. 6.4 ± 0.6. n = 8 in each group, mean ± SEM). The LV end diastolic pressure was also significantly lower in the rats treated with the sTNFR1 plasmid than in those treated with the LacZ plasmid (10.5 ± 0.6 mmHg vs. 14.4 ± 1.1, n = 8). In addition, the sTNFR1 expression plasmid had significantly reduced the infarct size at 21 days after LCA ligation (16.6 ± 1.8 % vs. 23.5 ± 2.5 (LacZ plasmid), n = 8 in each group). In myocardial infarction, the TNF-a bioactivity in the heart increased from the early stage of infarction and remained elevated. This elevation is thought to be partially responsible for the impairment of LV function and the increase in infarct size.

Abstract N° S24D

Thioredoxin is a negative regulator of cardiac hypertrophy

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Thioredoxin 1 (Trx1) has redox sensitive cysteine residues and acts as an anti-oxidant in cells. However, the extent of Trx1 contribution to overall cellular anti-oxidant mechanisms is unknown in any organs in vivo. We generated transgenic mice with cardiac specific over-expression of a dominant negative (DN) mutant (C32S/C35S) of human Trx1 (Tg-DN-Trx1), where the activity of endogenous Trx was significantly diminished. Markers of lipid peroxidation and oxidative DNA damage were significantly increased in hearts from Tg-DN-Trx1 compared with those from non-transgenic littermates (NTg). Tg-DN-Trx1 exhibited cardiac hypertrophy with maintained cardiac function at baseline. Intraperitoneal injection of N-2 mercaptopropionyl glycine, an antioxidant, normalized cardiac hypertrophy, suggesting that oxidative stress plays an important role in mediating cardiac hypertrophy in Tg-DN-Trx1. Thoracic aortic banding caused significantly greater increases in myocardial oxidative stress and enhanced the extent of cardiac hypertrophy in Tg-DN-Trx1 than in NTg. By contrast, transgenic mice with cardiac specific overexpression of wild type Trx1 did not show cardiac hypertrophy at baseline but exhibited reduced levels of hypertrophy and oxidative stress in response to pressure overload. Studies on the signalling mechanism indicated that Trx1 directly associate with Ras, thereby negatively regulating the Ras-ERK pathway in cardiac myocytes. These results for the first time demonstrate that endogenous Trx1 is an essential component of the cellular anti-oxidant mechanisms and plays a critical role in regulating oxidative stress in the heart in vivo. Furthermore, inhibition of endogenous Trx1 in the heart primarily stimulates cardiac hypertrophy both under basal conditions and in response to pressure overload through redox-sensitive mechanisms.

Abstract N° S24E

Redox signaling in mending the broken hearts

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Reactive oxygen species (ROS) play a crucial role in the pathophysiology of ischemic heart disease by causing cardiac dysfunction and cell death. Several redox-sensitive anti- and pro- apoptotic transcription factors including NFoB and AP-1 progressively and steadily increase in the heart as a function of the duration of ischemia and reperfusion. When the heart is preconditioned to ischemic stress by repeated short-term ischemia and reperfusion, NFoB remains high while AP-1 is lowered to almost baseline value. The anti-apoptotic gene Bcl-2 is downregulated in the ischemic/reperfused heart, while it is upregulated in the adapted myocardium. Cardioprotective abilities of the preconditioning are abolished when heart is pre-perfused with N-acetyl cysteine, a scavenger for ROS, suggesting the role of ROS in redox signaling. Mammalian heart is protected by several lines of defense, which include among others, redox-regulated proteins, thioredoxin and glutaredoxin. Reperfusion of ischemic myocardium results in the downregulation of thioredoxin 1 (Trx 1) expression, which is upregulated in the preconditioned myocardium. The increased expression of

Trx 1 is completely blocked with an inhibitor of Trx 1, CDDP, which also abolishes cardioprotection afforded by ischemic adaptation. The cardioprotective role of Trx 1 is further confirmed with transgenic mouse hearts overexpressing Trx 1. The Trx 1 overexpressed mouse hearts displayed significantly improved post-ischemic ventricular recovery and reduced myocardial infarct size and apoptosis as compared to the corresponding wild-type mouse hearts. Taken together, preconditioning appears to potentiate redox signaling, which converts the —death signal » into —survival signal ».

Abstract N° S25A

From contraindication to general acceptance : the mechanisms of the clinical response to beta-blockers in heart failure

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In the early seventies major opinion leaders in cardiology suggested that long-term inotropic stimulation of the failing heart should be the state of the art treatment. This proposal was based on experimental studies of heart failure models, myocardial biopsies from human failing hearts and plasma samples showing low content of noradrenalin in tissue and high levels of circulating catecholamine. Retrospectively however these data could also be interpreted in the opposite manner concluding that myocardial protection by reducing overload would be a better alternative. For the next two and a half decades unfortunately the first interpretation was prevailing. Our own clinical observations in patients with acute myocardial infarction with heart failure receiving intravenous selective beta-blockade indicated that the metabolic unloading by reduction in heart rate and blood pressure was well tolerated and lead to reduction of ischemia and eventually to smaller infarcts and decreased mortality. The same principle was therefore applied to chronic heart failure with tachycardia. By stepwise slow titration of beta-blockers the sudden withdrawal of sympathetic stimulation was avoided and only small transient depression of heart function was observed during the first month followed by gradually improvement over the next 6 to 12 months. The excellent tolerability for beta-blockade has finally been confirmed by major placebo —controlled trials also showing marked reduction in morbidity and mortality as well as improved wellbeing. The working hypothesis for the beneficial effect of beta-blockers in heart failure from the very beginning was to improve the myocardial energy balance by reducing the metabolic load and energy waste caused by strong sympathetic stimulation. Myocardial efficiency was improved and the ratio between myocardial phosfocreatine and ATP was restored to normal values. Moreover it was noticed that inflammatory cytokines and neurohormones including angiotensin, endothelin-1 and noradrenalin was reduced after beta-blockade. Some of the conditions leading to heart failure are characterized by increased sympathetic tone such as hypertension and type-II diabetes and beta-blockade seems therefore a logical treatment concept already in the early stages of heart failure or left ventricular dysfunction. MR- studies with gadolinium enhancement has shown that the amount of hibernating myocardium is predictive of functional improvement. Polymorphism of the $beta_1$-receptor may also play a role for outcome. Conclusion : There is now a strong rationale for the use of beta-blockade in heart failure treatment in addition to blockade of renin-angiotensin-aldosteron-system.

Abstract N° S25B

Restoration of calcium-handling proteins after β-blocker treatment in heart failure patients

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Beta-adrenergic receptor blockers (BB) are established as highly effective disease modifying drugs for patients with chronic congestive heart failure. Although typical target doses of BB can acutely worsen cardiac performance and exacerbate heart failure, numerous studies have demonstrated that gradual dose-titration and sustained administration of BB usually results in improved cardiac function among patients with both ischemic and nonischemic cardiomyopathies. Though incompletely understood, the mechanisms of improved contractility and reverse remodeling induced by BB seem to involve improvements in beta-adrenergic signal transduction and alterations in the abundance of calcium handling proteins. In particular, numerous studies demonstrate that BB therapy increases sarcoplasmic reticulum ATPase (SERCA) mRNA and protein abundance in failing hearts and several studies have correlated function and/or prognosis with changes in SERCA abundance during BB therapy. While reported changes in other calcium handling proteins have been less consistent, studies to date suggest that changes in phospholamban and ryanodine receptor abundance and/or phosphorylation also contribute to improved myocardial function during sustained BB therapy in heart failure.

Abstract N° S25C

Does β-blocker-mediated stimulation of gi contribute to recovery mechanisms ?

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β-blockers not only prevent the worsening of heart failure but promote recovery in terms of increased cardiac output. We have shown that β_2AR-selective b-blockers can activate the coupling of the receptor to Gi. Acutely, this produces a negative inotropic effect in failing (but not non-failing) human ventricular myocytes. Similar negative effects of b-blockers are seen when conditions in rat myocytes are adjusted to resemble those in failing human heart by overexpression of the β_2AR, Gi or the Na^+/Ca^{2+}-exchanger. Additionally, overexpression of the human β_2AR *per se* has a biphasic time-dependent effect on contraction, increasing basal amplitudes at 24 h but reducing them below control values at 48 h. This implies a switch from constitutive Gs

coupling at early time points to later tonic coupling of the β_2AR through Gi to a negative inotropic mechanism. This sequence recapitulates effects observed in the transgenic mouse overexpressing the human β_2AR. There are several mechanisms by which chronic stimulation of Gi could promote recovery. First, desensitisation of tonic Gi-mediated negative inotropic effects may occur when this pathway is continually stimulated. Application a specific β_2AR-blocker for 48h to rat myocytes overexpressing the β_2AR increased contractility. Second, there are a number of Gi-coupled pathways whose activation may be protective to the myocyte in terms of apoptosis and hypertrophy, including PI3kinase and MAPKinase. Gi-mediated activation by b-blocker stimulation of the β_2AR has the potential to contribute to the recovery process, and may explain the superior effect of the β_2AR-selective carvedilol.

Abstract N° S25D

Metoprolol and carvedilol : pharmacological differences and their impact on the clinical use in patients with chronic heart failure

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β-Adrenoceptor (β-AR) blockers improve ventricular function and survival in patients with chronic heart failure. It is presently debated whether carvedilol, a non-selective β_1-/β_2-AR-blocker with vasodilating properties, is superior to the β_1-AR selective compound metoprolol. Besides their different AR selectivity, carvedilol and metoprolol differ profoundly regarding β-AR binding and β-AR-G-protein interaction. In human myocardium, metoprolol inactivates β-ARs (inverse agonism) to a higher degree than carvedilol. This has important implications for myocardial β-AR regulation in heart failure patients. Furthermore, carvedilol displays much slower on- and off-kinetics of β-AR binding compared to metoprolol in intact human myocardium. Metoprolol induces a monophasic rightward shift of isoproterenol concentration-response curves, whereas carvedilol induces a biphasic one. This suggests that in contrast to metoprolol, carvedilol is not a competitive antagonist and thus may not interact with the orthostheric, but rather an allosteric site of β-ARs. The clinical implications of the slow kinetics and allosteric binding of carvedilol was tested in healthy volunteers that were treated with either carvedilol or metoprolol succinate for 11 days. Dobutamine stress-echocardiography was performed before, during and 44 hours after withdrawal of treatment. While both β-AR blockers reduced dobutamine-responses of various hemodynamic parameters during treatment, after withdrawal of β-blockers, the responses were restored in metoprolol-, but not in carvedilol treated subjects despite complete plasma elimination of β-blockers. These differences in β-AR kinetics have important clinical implications for patients with chronic heart failure that require inotropic support due to cardiac decompensation. Furthermore, the fact that carvedilol provides β-AR blockade far beyond its plasma elimination may contribute to its beneficial effects in the treatment of chronic heart failure.

Abstract N° S25E

Paradoxical pharmacology-the way forward ?

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Asthma is a chronic inflammatory airway disease in which β2-adrenoceptors (β2-AR) act as the major bronchodilating system. β2-AR agonists are current drugs of choice, however, they may be less effective when given chronically and morbidity may be increased. β-blockers are contraindicated due to their deleterious acute effects. These effects of β-AR agonists and antagonists are analogous to what was the paradigm in heart failure before chronic β-blocker use. In our murine model of asthma, we observe that acute (15 min) treatment (TX) with the β2-AR inverse agonist, nadolol, increased airway resistance (R_{aw}) while chronically (28 days) R_{aw} was significantly reduced. In contrast, β-AR agonist TX with salbutamol reduces R_{aw} acutely, but is chronically ineffective. β-AR density measured by radioligand binding correlates with the in vivo R_{aw} data.

Figure 1 : Airway responsiveness in murine model of asthma : 15 min TX with salbutamol or 28 d with nadolol decrease R_{aw} significantly compared to sensitized mice. Values are mean ± s.e.m. of n = 8-25 animals in each group (*$P < 0.05$ comp. to Sensitized, ANOVA. Note Y-axis is different for the 15 min nadolol).

Table 1. – 15 min treatment with salbutamol or alprenolol significantly increased B_{max} (fM mg^{-1} protein) to control values, while 28 d with carvedilol or nadolol increased B_{max} values significantly for ß-AR receptors in lung membranes. Values are mean ± s.e.m. of n = 3-5 animals in each group (*$P < 0.05$ comp. to Sensitized, Student's t-test). Samples were run in triplicate.

Treatment	15 minutes	28 days
Ctrl	286.8 ± 88.02 *	286.8 ± 88.02
Sensitized	109.2 ± 9.72	109.2 ± 9.72
Salbutamol	256.5 ± 29.24 *	97.0 ± 23.02
Alprenolol	299.5 ± 12.19 *	179.2 ± 53.05
Carvedilol	86.3 ± 19.42	904.1 ± 43.46 *
Nadolol	181.9 ± 48.28	785.5 ± 154.8 *

Abstract N° S26A

Vascular tissue engineering : challenges and opportunities

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Tissue engineering will need to move from the repair of tissues that for the most part are structural in nature to a focus on the vital organs. For the heart, where a key component is the epicardial coronary arteries, the replacement of such vessels requires the development of a tissue-engineered

small-diameter blood vessel substitute. This has been a —holy grail » for cardiovascular tissue engineering, and in this, there are a number of critical issues. First is the issue of providing an —endothelial-like », non-thrombogenic inner lining. There are a variety of endothelialization strategies being pursued, with possibilities including the use of (i) autologous cells, either from adipose tissue or circulating progenitor cells, (ii) allogeneic cells, and (iii) embryonic stem cells. A second critical issue is the matrix, including engineering in the appropriate mechanical properties, not just sufficient strength but visco-elastic properties that match those of native vessels. Approaches here range from incorporating intact elastin scaffolds derived from xenogeneic tissues to the genetic engineering of smooth muscle cells in order to enhance elastin production and assembly. Third, when a tissue-engineered blood vessel substitute is implanted into a host, an important issue is the variety of biological responses that will take place. In this one must confront not only such issues as thrombosis and remodeling, but in addition the immunological barrier. In order to address the wide clinical need, it will be important to have off-the-shelf availability. If allogeneic cells are to be employed, this will require the engineering of immune acceptance. This is perhaps the biggest challenge if we are to move vascular tissue engineering from benchtop research to the patient bedside.

Abstract N^o S26B

Myocardial vascular regeneration using human progenitor cells of endothelial and mesenchymal lineage

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Congestive heart failure remains a major public health problem, and is frequently the end result of cardiomyocyte apoptosis and fibrous replacement after myocardial infarction, a process referred to as left ventricular remodelling. In response to ischemic insult, adult cardiomyocytes undergo cellular hypertrophy, nuclear ploidy, and a high degree of apoptosis. Although most myocytes seem to be terminally differentiated, recent studies suggest that a few cardiomyocytes retain the ability to proliferate and regenerate in response to ischemic injury. The dividing myocytes can be identified on the basis of immunohistochemical staining of proliferating nuclear structures such as Ki67 and cell surface expression of specific cardiomyocyte lineage markers. Whether these cells are derived from a resident pool of cardiomyocyte stem cells or are derived from a renewable source of circulating bone marrow-derived stem cells that home to the damaged myocardium remains to be determined. More importantly, the signals required for homing, *in situ* expansion and differentiation of these cells are, at present, unknown. Gaining an understanding of these issues would open the possibility of manipulating the biology of endogenous cardiomyocytes in order to augment the healing process after myocardial ischemia. Bone marrow-derived endothelial precursors resembling embryonic angioblasts can induce infarct bed neovascularization after experimental myocardial infarction, resulting in induction of proliferation and regeneration of endogenous cardiomyocytes, and sustained improvement in cardiac function. Regeneration of functional cardiac muscle after an ischemic insult to the heart could also be achieved by implanting exogenous donor-derived or allogeneic cells such as fetal or embryonic cardiomyocyte precursors or bone marrow-derived mesenchymal stem cells. It is reasonable to anticipate that cell therapy strategies for ischemic heart disease will need to incorporate (1) a renewable source of proliferating, functional cardiomyocytes, and (2) strategies to generate a network of capillaries and larger size blood vessels for supply of oxygen and nutrients. The common mesenchymal origin of cardiomyocytes and supporting vascular structures such as pericytes and smooth muscle cells has spurred efforts to identify mesenchymal lineage precursors in various mammalian species including humans which could serve as substrate for exogenous regeneration of these cells. Recent data have been obtained which suggest that mesenchymal precursors can be precisely identified in vivo on the basis of perivascular anatomical location and expression of specific surface markers, and that these cells can be immunoselected to high purity by monoclonal antibodies directed to these markers. Combining ex vivo expanded mesenchymal lineage cells with cells of endothelial lineage allows for building of capacitance vessels and arteriogenesis, and may result in both effective neovascularization and myogenesis.

Abstract N^o S26C

Differentiation of macrophages into myofibroblasts

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Polyethylene tubing implanted into the peritoneal cavity of mice, rats, rabbits and dogs becomes encapsulated with multiple layers of myofibroblasts and collagen and a single outer layer of mesothelial cells. When the tubular capsule of living cells is transplanted as an autologous vascular graft, the myofibroblasts further differentiate into smooth muscle-like cells and the mesothelial cells function as 'endothelium'. The aim of the study was to determine the cellular origin of the myofibroblasts that form the capsule. Foreign bodies were implanted into the peritoneal cavity of C57BL/6 and *c-fms* EGFP mice (in which only cells of myeloid origin, ie macrophages, fluoresce green), then the tissue capsule that formed around the foreign body harvested at different time points. Cells within the peritoneal cavity and those comprising the capsule were isolated for analysis of EGFP (enhanced green fluorescent protein) transgene and expression of other cell markers using flow cytometry and immunofluorescence microscopy. The recruited cells were mainly rounded macrophages, confirmed by their expression of F4/80 and CD11b, that consisted of two subsets, EGFP-high and EGFP-low. The EGFP-low subset also expressed Ly6C/G (Gr1).

The two subsets of EGFP + ve cells comprised greater than 85 % of the cells of the capsule at day 2, with 75 % of the cells EGFP-high and 10 % EGFP-low. The relative proportion of EGFP-low increased over the next few days such that by day 7, when the cells had become spindle-shaped, there were equal numbers of EGFP-high and -low. Co-expression of EGFP and the myofibroblast marker α-smooth muscle actin was observed in the spindle-shaped cells by day 10.

In conclusion, cells that encapsulate a foreign body in the peritoneal cavity and differentiate into myofibroblasts are of myeloid (monocyte/ macrophage) origin. This suggests that peritoneal monocyte/ macrophages are capable of trans-differentiation into smooth muscle-like cells in the development of an artificial artery.

Abstract N^o S26D

Tissue engineering heart valves : from concepts to constructs

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Replacement tissue valves continue to have limitations due to their inability to grow, limited durability and susceptibility to infection. Creation of a tissue-engineered cardiac valve populated with viable cells, thus having the potential to grow and repair offers a promising alternative. The successful tissue-engineering of a valve will rely on the production of a construct that resembles the unique cellular and load bearing characteristics, exhibit similar dynamic properties and replicate as closely as possible the structure and biological properties of the native valve. The choice of cell type to populate the scaffold, the composition of the scaffold and the dynamic conditions used during development will all play a vital role in its success.

Valve interstitial cells are a heterogeneous and dynamic population of specific cell types. Many express smooth muscle α-actin, genes encoding structural components of the cardiac and skeletal contractile apparatus and the skeletal muscle-specific regulatory gene myogenin. When seeded in collagen gel, valve interstitial cells display a characteristic force generation profile, where the force generated is inversely related to cell density and they do not display tensional homeostasis. These characteristics may be vital for a fully functioning valve and may also provide a template for identifying potential sources of cells for tissue-engineered valve constructs. We are currently studying the phenotype, functional responses and the effect of mechanical force on cells from other sources in order to identify and/or direct suitable candidate cells for the constructs. Ideally the cells should not be immunogenic and studies investigating the immunogenicity of candidate cells will also be discussed.

The scaffolds are produced from type I collagen using a technique known as rapid prototyping, whereby a negative mould of a computer program design is printed out in microbeads to create a 3-D structure. The cells populating the construct should respond to the haemodynamic environment in a manner that will promote valve function. Mechanical forces can induce changes in gene expression, cell morphology and cell metabolism. Using a novel bioreactor, we can accurately reproduce a wide range of haemodynamic pressures and flow rates, enabling us to determine the optimal conditions for construct production. Preliminary experiments are underway to evaluate the mechanical strength of seeded constructs and to determine how cells affect the mechanical properties of the scaffold and how haemodynamic forces affect the integrity of the construct.

The desired goal of any successful tissue-engineered heart valve will be to produce a construct that closely replicates the structure and function of the native valve. Knowledge of the complex mechanisms of cell-cell interaction and cell-matrix interaction is essential and is now beginning to be investigated and understood at the molecular level.

Abstract N^o S26E

Grafting of engineered heart tissue to repair infarcted myocardium

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Cardiac tissue engineering may yield surrogate cardiac tissue for the repair of diseased myocardium. Recently, we developed a technology to construct Engineered Heart Tissue (EHT) with structural and functional properties of native myocardium from neonatal rat heart cells, collagen type I, matrigel, and serum containing culture medium. EHTs can be engineered in different sizes and geometries according to in vivo demands. Here, we utilized large star-shaped EHTs (diameter : \\ 126\\20 mm ; thickness : 1-4 mm) to test whether EHTs can reconstitute heart muscle in rats with transmural infarcts. Infarcts were generated by ligation of the left descending coronary artery and EHTs were implanted 2 weeks thereafter. We assessed structural and functional integration 4 weeks after EHT-grafting. Sham operated rats served as controls. Hematoxylin&Eosin staining of paraffin sections and confocal laser scanning microscopy of DAPI labeled EHT-grafts revealed survival, differentiation, and structural integration of EHT grafts into the native myocardium. Multi-electrode epicardial mapping indicated electrical coupling of EHT grafts to native myocardium. Echocardiography and nuclear magnetic resonance imaging demonstrated that EHT grafts improved left ventricular wall kinetics and systolic anterior wall thickening in rats with myocardial infarcts. Thus, we conclude that EHT may be utilized to repair infarcted and possibly otherwise damaged myocardium in vivo.

Abstract N^o S26F

Myocardial tissue reconstruction by cell sheet technology

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Regeneration therapy has currently emerged as one of the most promising treatments for the patients suffering from

severe heart failure. Direct transplantation of isolated myoblasts or bone marrow mononuclear cells, and recruitment of stem cells from bone marrow by G-CSF administration have been already clinically performed. As further advanced therapy, research on fabricating three-dimensional (3-D) cardiac grafts by tissue engineering technology has also now begun. For creating functional myocardial tissue, critical points are how to connect whole cardiomyocytes electrically and how to grow enough vascularization within the construct. We have exploited novel cell manipulation technique to construct 3-D tissue by layering cell sheets without any biodegradable scaffolds. Confluent neonatal rat cardiomyocytes on temperature-responsive culture surfaces can be harvested as an electrically-communicated contiguous cell sheet only by lowering temperature without any enzymatic digestions. Both electrical and morphological communications are developed between layered cardiomyocyte sheets, leading simultaneous beating 3-D myocardial tissues. Layered cardiomyocyte sheets in vivo demonstrated synchronized beating, 1-year survival and characteristic structures of native heart tissue including well-differentiated sarcomeres, gap junctions and microvasculars. To overcome thickness limitation due to primary insufficient oxygen permeation, multi-step transplantation of triple-layered cell sheets were performed. The latter graft synchronized with the former. No necrotic tissue was observed within the overlaid grafts, because neovascularization occurred step-by-step. Finally, 10-time transplantations of triple-layer grafts have realized about 1 mm-thick functional myocardial tissue. In conclusion, cell sheet technology should have enormous potential in myocardial tissue engineering.

Abstract N° S27A
Regulation of fatty acid oxidation in perfused hearts from diabetic mice
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Altered fatty acid metabolism in diabetic hearts may contribute to cardiac dysfunction (lipotoxicity). We have examined the metabolism of palmitate complexed to albumin in the perfusate of isolated perfused working hearts from both type 1 (streptozotocin-induced insulin-deficient) and type 2 (monogenic db/db) mice. Rates of palmitate oxidation and esterification (to triacylglycerol) were both markedly elevated in both models of diabetic hearts. Similar results were obtained when diabetic hearts were perfusion with chylomicrons ; lipoprotein lipase-derived fatty acid oxidation and esterification was also increased. Thus, fatty acid metabolism was enhanced in diabetic hearts, irrespective of the fatty acid source. Rates of cardiac fatty acid oxidation were normalized in transgenic db/db-hGLUT4 mice with global overexpression of the insulin-regulatable glucose (GLUT4) transporter. In addition, fatty acid oxidation was normalized in hearts from db/db mice after chronic oral administration of a PPARgamma ligand (COOH, from Merck). COOH treatment also enhanced insulin-stimulated glucose uptake in isolated cardiomyocytes, providing evidence for insulin sensitization in db/db hearts. The mechanism(s) responsible for elevation of fatty acid oxidation rates in diabetic hearts is(are) under investigation.

Abstract N° S27B
Diabetes and cardiac carbohydrate metabolism
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It is commonly believed that diabetes decreases cardiac glucose oxidation and increases lipid oxidation. This shift away from glucose towards lipids for energy metabolism has been attributed to the development of contractile dysfunction and increased sensitivity to ischemic injury. Recently we have focused on the impact of Type-2 diabetes on the regulation of cardiac metabolism using substrate mixtures that include lactate and pyruvate at physiologically realistic concentrations as well as glucose and palmitate. In isolated hearts from Zucker diabetic fatty rats (ZDF) perfused under normoxic conditions, total carbohydrate oxidation was decreased by \\ 126\\50 % compared to non-diabetic controls ; however, this was due entirely to \\ 126\\4-fold decrease in lactate oxidation rates with no change in glucose oxidation. On the other hand glycolytic lactate production was significantly lower in the ZDF group. Even during low flow ischemia (30min at 0.3mls/min) and subsequent reperfusion, there were no differences in glucose oxidation between the two groups ; whereas, lactate oxidation remained depressed in the ZDF group under all conditions. Interestingly, the decrease in glycolysis observed during normoxia was not present during ischemia or reperfusion. Furthermore, despite the alterations in metabolism functional recovery follow ischemia was not depressed in the ZDF group. These data suggest that in the presence of physiological substrate mixtures the shift from carbohydrates to fatty acids for oxidative energy production may not be important in mediating the adverse effects of diabetes on the heart.

Abstract N° S27C
Alterations in malonyl coa control of fatty acid oxidation in the diabetic heart
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Alterations in energy substrate preference in the heart can compromise cardiac function and decrease the ability of the myocardium to withstand an ischemic episode. In uncontrolled Type I diabetes, myocardial fatty acid oxidation rates markedly increase, resulting in a dramatic inhibition of glucose oxidation. This increase in fatty acid oxidation is due both to an increase in circulating fatty acid levels, as well as alterations in malonyl CoA control of mitochondrial fatty acid uptake. If mice are fed a high fat diet for a 10 week period, they become obese and insulin-resistant. Hearts from these mice show an energy substrate use profile that parallels what is seen in the diabetic heart (i.e a switch towards to fatty acid oxidation and a decrease in the contribution of glucose oxidation to overall energy production). In both diabetic rat

and obese mice hearts, the activity of malonyl CoA decarboxylase (MCD) increases. This results in a decrease in malonyl CoA levels, and a resultant increase in fatty acid oxidation rates. The increase in cardiac MCD in diabetes and obesity appears to occur, in part, due to an increase in peroxisome proliferators-activated receptor α (PPARα) activity, an important transcriptional regulator of fatty acid oxidative enzymes in the heart. In support of this, overexpression of PPARα in the mouse heart will increase fatty acid oxidation and decrease glucose oxidation, similar to what is observed in hearts from diabetic or obese mice. Part of this effect of PPARα is mediated by an increase in MCD expression. This suggests that inhibition of MCD may be a potential approach to overcoming the alterations in fatty acid and glucose oxidation that occur in the myocardium of diabetic and/or obese individuals.

Abstract N^{o} S27D

Ketone bodies affect fatty acid utilization and gene expression in cardiomyocytes from diabetic rats

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It was explored whether chronic exposure of the heart to both elevated extracellular ketone body and fatty acid (FA) levels, as readily seen in STZ-induced type 1 diabetes, alters FA utilization and the expression of FA handling genes in cardiac muscle cells (CMC). CMC isolated from adult diabetic rat hearts showed increased uptake and oxidation rates of extracellular FA. The ketone body acetoacetate, but not beta-hydroxybyturate, nullified the diabetes-induced increase in FA oxidation. The sensitivity towards the inhibitory effect of acetoacetate, however, was similar in diabetic and control cells. Chronic exposure of neonatal CMC to elevated extracellular FA significantly induced the expression of a panel of FA-handling genes, including FAT/CD36, FABP, ACS, mCPT1 and LCAD. In the intact diabetic heart, however, mRNA levels of these proteins only showed a tendency to increase, suggesting that other factors are apparently mitigating FA-induced gene expression. Beta-hydroxybutyrate may play a role as this ketone body partly blocked the FA-induced mCPT1 promoter activity in transfected neonatal CMC.

Abstract N^{o} S27E

Cardiac and skeletal muscle metabolism after chronic PPARγ activation in type 2 diabetes mellitus

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Despite normal cardiac function, patients with type 2 diabetes mellitus (DM) have impaired cardiac and skeletal muscle high-energy phosphate metabolism. It is unknown whether the energetic abnormalities can be rectified by improving insulin sensitivity via activation of peroxisome proliferator-activated receptor γ (PPARγ). To study this, 25 patients with DM, with normal cardiac function and without cardiovascular disease, were randomised in a double-blind fashion to receive either placebo or the PPARγ agonist Rosiglitazone (RSG, 8 mg od) for three months. Before and after this treatment, fasting blood metabolites and cytokines were measured, and cardiac function was assessed using echocardiography. Cardiac energetics (phosphocreatine to ATP ratio, PCr/ATP) were measured using ^{31}P magnetic resonance spectroscopy (MRS) at rest. Skeletal muscle energetics (right gastrocnemius muscle) and tissue oxygenation were measured at rest and during and after dynamic exercise using MRS and near-infrared spectrophotometry, respectively. Treatment with RSG for three months decreased insulin resistance and significantly lowered plasma free fatty acids (FFA, 0.3 ±0.1 vs. 0.5 ±0.1 with placebo, $p < 0.01$) and highly-sensitive C-reactive protein levels (hs-CRP, 1.3 ±0.3 vs. 3.0 ±0.6 with placebo, $p < 0.05$). Whereas placebo did not change cardiac PCr/ATP ratios, treatment with RSG increased PCr/ATP by 0.41 ±0.18 (vs. —0.04 ±0.01 with placebo, $p < 0.05$), the increase in PCr/ATP correlating with the decrease in hs-CRP ($r^b = 0.29$, $p < 0.05$). Neither treatment with placebo nor with RSG changed exercise tolerance and skeletal muscle energetics. However, a correlation between PCr recovery and FFA levels became apparent after treatment with RSG, which was not evident prior to treatment.In summary, treatment with RSG improved cardiac energetics by lowering plasma FFA and hs-CRP. Whereas skeletal muscle energetics were unchanged, a new negative correlation between PCr recovery and FFA was revealed. Thus, treatment with RSG may have altered cardiac and skeletal muscle substrate utilisation and improved inflammatory status with a beneficial effect on cardiac energetics.

Abstract N^{o} S28A

Pathways regulating cellular mobilization in cardiac hypertrophy

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Cardiac remodeling is a common feature in patients suffering from chronic hypertension or following myocardial infarction. Recent evidence suggests the presence of undifferentiated progenitors in many adult tissues. These cells could be activated in injured organs and participate to the healing process. In the present study, we investigated whether the cardiac non-myocyte population contained a subset of precursor cells with a capacity to differentiate into functional cardiomyocytes. We isolated proliferative non-myocyte cells from the neonatal heart, and demonstrated that, when cultured under the appropriate conditions, these cells could give rise to spontaneously beating cardiomyocytes. Interestingly, a significant percentage of neonatal undifferentiated non-myocyte cells express the stem cell markers Sca-1 and c-kit. In a second part, we search for humoral factors that might regulate the differentiation process. The

Fibroblast growth factor-2 (FGF-2) is known as a differentiation factor. In additon, we have shown previously that mice deficient for FGF-2 expression suffer from dilated cardiomyopyathy. We then tested whether the absence of FGF-2 could affect cardiogenesis. The capacity of non-myocyte cells lacking FGF-2 to differentiate into cardiomyocytes was significantly reduced as compared to wild type cells. Moreover, the subset of Sca-1 positive cells in the undifferentiated non-myocyte population markedly increased in FGF-2 knockouts, suggesting that the absence of FGF-2 interfered at an early time point with cardiogenesis. Together, these results indicate the presence in the heart of a population of undifferentiated precursors that might be recruited for tissue repair. In addition, the mobilization and differentiation process of this particular cell subset appeared to require the presence of FGF-2.

Abstract N° S28B

Growth induction and contractile dysfunction in the heart — a variable phenotype

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Significant progress has been made recently in the development of genetic models of cardiac hypertrophy in which intrinsic enlargement of the heart is observed in a normotensive setting. In this context, non-load dependent aspects of excitation-contraction coupling dysfunction in hypertrophy may be identified. We have investigated and compared polygenic in-bred and uni-gene targeted transgenic models of hypertrophy, where cardiac growth is linked with various metabolic and endocrinologic manipulations. Whilst disturbances in cardiomyocyte Ca and pH homeostasis are usually evident, the nature of the alterations in these ionic fluxes and effect on contractility is model-specific. We have recently reported the development of a novel polygenic rat strain of primary cardiac hypertrophy derived from a cross of Fisher (F344) and SHR (Physiol Genomics 9 :43-48, 2002). Our new Hypertrophic Heart Rat (HHR) strain exhibits cardiac and cardiomyocyte hypertrophy in the absence of hypertension. In parallel we have co-developed a Normal Heart Rat (NHR) strain with small hearts and low blood pressure as a control strain. We have investigated transgenic and gene-knockout models to explore the role of trophic and metabolic factors in inducing cardiac hypertrophy. Our studies of a transgenic cardiac-specific angiotensinogen-over expressing mouse and a cardiac-specific glucose Glut4 transporter Cre-Lox KO mouse have revealed that similar functional adaptations can be linked with quite different alterations in myocyte calcium handling in hypertrophy. Our gene expression profiling studies indicate that in contrasting models of cardiac hypertrophy, altered mitochondrial metabolic function emerges as a major 'common denominator' associated with cellular dysfunction.

Abstract N° S28C

Regulation of PKA targeting by AKAPs in failing hearts

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Impaired signaling of the β-adrenergic pathway is closely linked to development of heart failure. Many studies have focused on altered signaling at the level of the o-adrenergic receptor (o-AR), but there is now increasing evidence for defects distal to the o-AR. We investigated regulation of PKA-dependent substrate phosphorylation by protein kinase A (PKA) targeting to PKA substrates via A-kinase anchoring proteins (AKAPs). Using surface plasmon resonance, we showed that affinity of different cardiac AKAPs for PKA can increase up to 250 fold upon phosphorylation of serine 96 of the regulatory subunit of PKA, RII, by the catalytic subunit, C. In failing vs non-failing human hearts, we showed decreased phosphorylation of RII by C, implying decreased targeting of PKA near PKA substrates by AKAPs. In separate experiments, we investigated the effect of altered affinity of PKA binding to AKAPs by expressing either muscle-specific AKAP (mAKAP) or its inactive prolinated derivative (mAKAP- P) in CHO cells expressing the type I ryanodine receptor (RyRI). We activated adenynlyl cyclase by forskolin and determined the effect of disruption of PKA targeting to the RyR by mAKAP-P expression. Results showed that when PKA targeting to AKAPs is abrogated, both RyR phosphorylation and the size of the caffeine releasable Ca^{2+} pool, are decreased. These findings, together with our observation of decreased RII phosphorylation in failing vs non-failing human hearts, and decreased phosphorylaton of other PKA substrates (troponin-I and myosin binding protein C) imply reduced affinity of RII for mAKAP during heart failure. We conclude that decreased PKA targeting by AKAPs contributes to decreased phosphorylation of PKA substrates and thus impaired contractile function of failing hearts.

Abstract N° S28D

Ca homeostasis in the hypertrophied and failing heart

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Progression from hypertrophy to heart failure (HF) is accompanied by contractile dysfunction and increased propensity for life-threatening arrhythmias. Both effects may be related directly to altered cellular Ca handling in ventricular myocytes. In HF the twitch Ca transient ($\Delta[Ca]_i$) which is a main cause of systolic dysfunction. The lower $\Delta[Ca]_i$ can be attributed largely to reduced SR Ca content ($[Ca]_{SRT}$) and 3 factors may contribute. First, there is reduced SR Ca-ATPase function in HF which reduces net SR Ca uptake during the cardiac cycle (consequently reducing SR Ca availability and also contributing to diastolic dysfunction). Sec-

ond, increased expression and function of Na/Ca exchange (NCX) favors Ca extrusion from the cell during relaxation (competing with SR Ca uptake, but also limiting the extent of diastolic dysfunction). Third, there may be enhanced diastolic Ca leak from the SR in HF, which would also reduce SR Ca availability (& this could be mediated by either CaMKII or PKA effects on the RyR). Relative contributions of these systems may vary with HF etiology. Triggered arrhythmias including early and delayed after depolarizations (EADs & DADs) are prominent initiators of arrhythmias in HF. DADs are due to spontaneous SR Ca release and consequent activation of Ca-dependent transient inward currents (I_{ti}). DADs are normally associated with SR Ca overload, so at first glance they would seem unlikely in HF. However, we have shown that I_{ti} is directly attributable to NCX current, and the higher NCX expression means that there will be a greater I_{ti} for a given SR Ca release. In addition, there is a down-regulation of the inwardly rectifying K current (I_{K1}), which normally stabilizes the resting membrane potential. Thus any I_{ti} will lead to a greater DAD amplitude, making it much more likely to induce a propagating arrhythmogenic action potential. We also show that preserved β-adrenergic responsiveness in HF, allows the SR Ca content to reach levels required to induce spontaneous SR Ca release, I_{ti}s, DADs and action potentials. Thus, altered Ca handling contributes to both contractile dysfunction and arrhythmogenesis in HF.

Abstract N^o S28E
Anti-arrhythmic actions of calmodulin kinase inhibition

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Disturbances in intracellular Ca^{2+} are associated with cardiac arrhythmias and the Ca^{2+} and calmodulin-dependent protein kinase II (CaMK) is a central molecular signal for regulating cellular Ca^{2+} homeostasis. CaMK activity is also up-regulated in patients and in diverse animal models of structural heart disease, while transgenic over-expression of CaMK causes cardiac hypertrophy, dilation and premature death. Taken together, these findings lead us to hypothesize that CaMK is a pro-arrhythmic signaling molecule and a potential target for anti-arrhythmic therapy. Here we present data from isolated cardiomyocytes showing that CaMK increases L-type Ca^{2+} channel openings and that increasing cellular Ca^{2+} can activate Na^+/Ca^{2+} exchanger current by a CaMK-dependent mechanism. CaMK inhibition can suppress afterdepolarizations, cellular arrhythmia mechanisms linked to L-type Ca^{2+} channels and Na^+/Ca^{2+} exchanger currents. Furthermore, pharmacological and genetic CaMK inhibition successfully reduces arrhythmias in two murine models of structural heart disease (one due to CaMKIV over-expression and the other due to calcineurin over-expression) and significantly reduces mortality in mice with severe cardiomyopathy, due to calcineurin over-expression. These findings support the concept that CaMK inhibition is a pro-arrhythmic signal and serve as a proof of concept that CaMKII inhibition can suppress cardiac arrhythmia

Abstract N^o S29A
Fat as an endocrine organ

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Adipose tissue is an important endocrine organ, producing, or regulating production of, numerous hormones involved in energy metabolism and the cardiovascular system. Collectively, these hormones have been termed adipokines. Included in the former group are leptin, adiponectin, TNFo and steroid hormones, all of which influence energy metabolism and insulin sensitivity and which are involved in the pathogenesis of the Metabolic Syndrome The latter group of hormones includes PAI-1, TGF and members of the renin-angiotensin system. These hormones have more direct effects on the cardiovascular homeostasis, including BP regulation, myocardial structure/function, endothelial function and thrombotic diathesis. The role of these hormones in the pathogenesis of the metabolic syndrome will be discussed, with particular emphasis on cardiovascular disease. A number pharmaceutical therapies based on regulation or manipulation of adipokine levels or production are currently under trial. It is hoped that these strategies will efficacious in targeting the multiple manifistations of the metabolic sydrome.

Abstract N^o S29B
Obesity and coronary artery disease

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Although a relationship between obesity and coronary artery disease (CAD) has long been recognised, the precise nature of this relationship remains unclear. Central obesity has been shown to correlate more closely with CAD than excess total fat mass. There is a strong association between central obesity and many of the traditional risk factors for CAD such as type 2 diabetes mellitus, hypertension and dyslipidaemia. It remains debatable whether obesity *per se* is an independent risk factor for CAD, however evidence suggests that obesity confers risk for CAD over and above that due to its association with the traditional risk factors. It is likely that other emerging risk factors associated with obesity and the metabolic syndrome are contributing. These factors include hyperinsulinism/insulin resistance, and a prothrombotic and proinflammatory state, as well as other hormones secreted by adipose tissue.

The recognition that adipose tissue is a highly active endocrine organ has led to interest in the role of adipose tissue-derived hormones and cytokines in CAD in obesity. Obesity is associated with increased levels of TNF-alpha, IL-6, and PAI-1. CRP is also elevated in obesity and has been

shown to strongly correlate with CAD. More recently it has been shown that —adipokines » such as adiponectin and leptin not only play a role in mediating insulin sensitivity and energy balance, but also contribute to the regulation of cardiovascular function. Adiponectin appears to be anti-inflammatory and anti-atherogenic and levels are low in obese and insulin resistant individuals. Leptin is elevated in obese individuals and may be an independent risk factor for the development of cardiovascular disease.

Thus, the relationship between obesity and CAD is also influenced by both traditional and several less traditional risk factors.

Abstract N° S29C
Obesity cardiomyopathy : pathogenesis, clinical recognition and management
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Obesity, particularly morbid obesity is associated with a variety of alterations in cardiac performance and morphology. Excessive adipose accumulation produces augmentation of circulating blood volume. This in association with a decrease in systemic vascular resistance leads to an increase in cardiac output. Since heart rate changes little with increasing fat accumulation, the rise in cardiac output is due primarily to augmented stroke volume. These hemodynamic alterations produce dilatation of the left ventricular (LV) which predisposes to an increase in left ventricular wall stress. Eccentric LVH (LVH) occurs in an attempt to normalize wall stress. Eccentric LVH predisposes to LV diastolic dysfunction. If LV wall stress remains high (inadequate hypertrophy), LV systolic function may ensue. Concurrent systemic hypertension and morbid obesity produces a hybrid form of eccentric-concentric hypertrophy. Obesity cardiomyopathy is a term used to describe congestive heart failure that occurs due to the aforementioned alterations in cardiac structure and function. In such individuals LV failure predominates. Right ventricular failure occurs primarily due to LV failure. Pulmonary arterial hypertension due to obesity/hypoventilation may contribute to the development of right ventricular failure. Many of the hemodynamic, morphologic and clinical manifestations of obesity cardiomyopathy are reversible with substantial weight loss.

Abstract N° S29D
Fat, salt and hypertension : the leptin-cardiorenal axis
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Leptin is a recently isolated circulating peptide hormone that is primarily synthesized and secreted by adipocytes. A major function of this hormone is the control of energy balance by binding to receptors in the hypothalamus, leading to reduction in food intake, as well as elevation in temperature and energy expenditure. In addition, increasing pharmacological evidence suggests that Leptin, through its direct and indirect actions, may play an important role in cardiovascular and renal functions.

In the systemic vasculature, leptin appears to produce both vasoconstriction indirectly via central nervous system activation, as well as vasodilation ; this latter effect mediated by an endothelial mechanism consistent with NO. In chronic hyperleptinemic conditions, however, these potential balanced effects of leptin on peripheral vascular resistance may not remain, and there is information to suggest that in these situations leptin may be hypertensinogenic.

The Kidney is among the tissues that express the leptin receptor and numerous studies have recently reported that leptin produces a robust natriuresis and diuresis in the normal rat. These findings acquire additional relevance when it is considered that feeding (and consequent intravascular sodium-volume expansion) is associated with an increased secretion of leptin into the circulation, and this hormone, in turn, may be an important natriuretic factor facilitating postprandial sodium-volume regulation. Moreover, the concept of a physiologically relevant natriuretic action of leptin is further emphasized by more recent studies, indicating that blockade of endogenous leptin significantly attenuates sodium and volume excretion in the rat.

Thus, while the relevance of endogenous leptin needs further clarification, it appears to be a potential pressure and volume regulating factor, and may function pathophysiologically as a common link to obesity and hypertension.

Abstract N° S29E
Obesity hypertension and the heart
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In Addition to a genetic pre-disposition, several endocrinic, metabolic mechanisms have been linked to the development of obesity hypertension. These include : insulin resistance and hyper-insulinemia, increased serum aldosterone levels, salt sensitivity and possibly increased leptin levels. Hemodynamic studies have shown expanded plasma volume and cardiac output in the presence of increased peripheral vascular resistance, which induces structural changes in the heart characterized by concentric-eccentric left ventricular hypertrophy. The hemodynamic alteration may also cause increased renal blood flow and reduced renal vascular resistance, expansion of the extracellular matrix, increased interstitial hydrostatic pressure and increased compression of the tubules. Weight reduction effectively controls blood pressure, improves left ventricular hypertrophy and cardiac output and may also benefit kidney function and renal damage.

Angiotensin-converting enzyme inhibitors and AΠ receptor blockers may offer an efficient antihypertensive approach in obese hypertensive patients.

Abstract N° S30A
Adenoviral targeting of vascular nox isoforms : therapies to attenuate hypertrophy and estenosis
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Numerous studies report the existence of vascular NAD(P)H oxidase components including p22-phox, p67-phox, rac and gp91-phox and its homologues nox1 and nox4. Early induction of a combination of these components in different cell types suggests a complex interplay of vascular reactive oxygen species (ROS) leading to medial hypertrophy and neointimal hyperplasia. Recently we showed that systemic delivery of specific peptide inhibitor pf p47-phox :gp91-phox interaction, gp91ds, decreases vascular superoxide anion and medial hypertrophy in hypertension and neointimal hyperplasia in response to balloon injury. We have begun developing adenoviral strategies targeting this oxidase inhibitor to the vasculature. In one study, we compared the ability of an adventitially applied virus containing a fibroblast-active promoter driving expression of the gp91ds peptide inhibitor with an adenovirus expressing dominant negative p67-phox (p67dn) to prevent neointima formation. Rat common carotid arteries were transfected with Ad-CMV-p67dn, gp91ds-expressing virus or control virus in pluronic gel and injured using standard techniques by balloon angioplasty catheter. Fourteen days after injury, CCAs were perfusion-fixed and analyzed by digital morphometry. Neointimal hyperplasia (as measured by neointimal area) was significantly lower with the gp91ds-expressing virus could attenuate medial hypertrophy of the mouse CCA in response to a 7-day infusion of angiotensin II *in vivo*. Immounohistochemical staining for maker protein eGFP showed that expression was limited to the adventitia (primary with fibroblast) in CCAs from the gp91ds group. As expected, angiotensinII induced a medial hypertrophic response and increased ROS levels. Both effects were inhibited by the gp91ds-expressing virus but not by the control virus. Thus our data seem to suggest that gp91-phox-based oxidaise in adventitial fibroblasts plays a modulatory role in angiotensin II indused medial hypertrophy. Taken together, these data may also suggest an important fundamental role vascular gp91-phox-based oxidases in vessel wall homeostasis and vascular angiogenesis. Current studies are aimed at testing novel inhibitors of the gp91-phox homologues nox1 and nox4 to investigate the unique contribution of these isoforms in various vascular cell types to both hypertrophy and hyperplasia.

Abstract N^{o} S30B

NAD(P)H oxidase-derived superoxide-a source of oxidative stress or signaling in coronary artery and myocardium ?

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Oxygen-derived free radicals (ODFR) are known to play a role in modulating myocardial and vascular function. Although the sources of ODFR in vasculature and myocardium are uncertain, NAD(P)H oxidases in vessel wall and cardiac myocytes appear to be a major site of superoxide (O2-) generation. We have previously demonstrated that in the heart, O2- inactivates NO, increases vasoconstrictive prostanoids, activates PKC-dependent signaling, and elicits coronary dysfunction. In addition, O2- damages mitochondria and induces systolic and diastolic dysfunction. Lactate and pyruvate modulates NAD(P)H oxidase-derived O2- in the presence of inhibition of SOD causing vascular dysfunction via suppression of guanylate cyclase activity and NO-elicited relaxation. In contrast, H2O2-a product of O2- and SOD interaction, activates vascular relaxation and protects the heart from O2- and ischemia-reperfusion induced injury. Our recent studies indicate NOX-2 and NOX-4, which have distinct localization patterns, are present in the bovine coronary artery, and activation of NAD(P)H oxidase by PKC is mediated by Src. Furthermore, H2O2 and not O2- derived from NAD(P)H oxidase mediate PDBu-induced contraction. Thus, we believe NAD(P)H oxidase-derived free radicals activate both signaling and stress depending on their levels, balances between endogenous antioxidant defense, and their localization in the cell.

Abstract N^{o} S30C

Role of NADPH oxidase-derived $O_2^{\cdot-}$ in cardiac hypertrophy and failure

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Oxidative stress is involved in the pathophysiology of cardiac hypertrophy and heart failure through (a) direct deleterious effects, (b) modulation of redox-sensitive signalling pathways, (c) inactivation of nitric oxide (NO). Although there are several potential ROS sources in the cardiovasculature, a family of NADPH oxidases (Noxs) has been recognised as especially important in vascular pathologies. We have studied the roles of Noxs in the heart.

All the major components of the classic NADPH oxidase are found in cardiomyocytes and endothelial cells (EC), which co-express both the gp91phox (Nox2) and Nox4 catalytic subunits of the oxidase. In pressure-overload LVH in guinea-pigs, we found a progressive increase in LV NADPH oxidase subunit expression and activity in parallel with MAPK activation and evidence of ROS-mediated endothelial and diastolic dysfunction. Myocardial NADPH oxidase activity is also increased in human heart failure. To *specifically* address the role of the Nox2-containing oxidase, we undertook studies in gp91$^{phox-/-}$ mice. In a model of AngII-induced *in vivo* cardiac hypertrophy (by subpressor infusion), increases in LV NADPH oxidase activity were inhibited in gp91$^{phox-/-}$ mice. In parallel, *in vivo* myocyte hypertrophy, increases in ANF mRNA, and AngII-induced interstitial fibrosis were all markedly inhibited. These data provide the first definitive evidence for an essential role of the Nox2 oxidase in AngII-induced cardiac hypertrophy and interstitial fibrosis. By contrast, LV hypertrophy induced by aortic banding was similar in wild-type and gp91$^{phox-/-}$ mice. Unexpectedly, NADPH oxidase activity was increased by aortic banding in gp91$^{phox-/-}$ mice, which was attributable to increased expression of Nox4. In addition, LVH was inhibited

by antioxidant treatment in both wild-type and knockout mice. However, gp91$^{phox-/-}$ mice had preserved contractile function and reduced interstitial fibrosis after banding as compared to banded wild-types. Taken together, the above data suggest distinct roles for Nox2 versus Nox4 in different components of the overall cardiac hypertrophic response.

Abstract N^{o} S30D

Regulation of non-phagocytic nadph oxidases

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Reactive oxygen species (ROS) have been shown to play an important role in the regulation of blood pressure and in the formation of atherosclerotic plaques. However, the source of ROS in the vascular system remains to be elucidated.

In the last few years, six homologues of the ROS-generating subunit of the phagocyte NADPH oxidase (gp91^{phox}) have been discovered. They all belong to the same family of enzymes, called NADPH oxidases (NOXes). Interestingly, four members of that family have been detected in the vasculature including NOX1, NOX2 (alias gp91^{phox}), NOX4 and NOX5. The expression of the individual NOX enzymes is unequal throughout the vascular wall : endothelial cells express NOX4, vascular smooth muscle cells express NOX1, NOX4, and NOX5, while NOX2 is found mainly in macrophages and in the fibroblasts of the adventitia. In order to unravel the *in vivo* function and the relative importance of individual NOX enzymes in vascular- and other tissues, we investigated their regulation. Our results demonstrate that NOX1, similar to NOX2, is not a stand-alone enzyme, but part of a multisubunit complex consisting of a NOX Organizer (NOXO1) and a NOX Activator (NOXA1) protein beside NOX1. NOX5 functions apparently independently of any additional subunits. Indeed, NOX5 itself contains an in-built —activator subunit » : its unusually long N-terminus possesses four EF-hands, Ca^{2+} binding domains. Upon Ca^{2+} influx, Ca^{2+} is bound to the N-terminus of NOX5, which, as a result, becomes able to interact with the catalytic C-terminus and triggers superoxide generation. In contrast, the regulation of NOX4 is largely unknown. Currently it is regarded as a constitutively active enzyme, which may require additional subunits to function.

Abstract N^{o} S30E

NAD(P)H oxidase-derived superoxide signaling - from mouse to man

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Free radicals and radical-derived, nonradical reactive species, originally thought to be cellular metabolic byproducts are now considered as regulatory mediators in signaling processes playing important roles in cell function. In physiological conditions low, but measurable, levels of intracellular reactive oxygen species (ROS) are maintained by the balance between pro-oxidant and anti-oxidant systems. In vascular cells ROS are typically generated by tightly regulated NAD(P)H oxidase, which is activated both constitutively and in response to multiple stimuli, such as Ang II, EGF, PDGF, thrombin and stretch. From studies in 47phox-/- mice and humans, it is evident that phosphorylation of p47phox, which initiates translocation of cytoplasmic subunits, is essential for assembly and activation of fully functional NAD(P)H oxidase in endothelial and vascular smooth muscle cells. PLD, PLA_2, PKC, Rho and c-Src are upstream regulators of the vascular oxidase and influence activation by stimulating phosphorylation and translocation of NAD(P)H oxidase subunits and by increasing gene and protein expression of p22phox, gp91phox/nox1/nox4, p47phox and p67phox. Furthermore regulation by NOXO1 (Nox organizing protein 1) and NOXA1 (Nox Activating protein 1) may be important. ROS function as important intracellular second messengers to activate many downstream signaling molecules, such as MAP kinases, protein tyrosine phosphatases, protein tyrosine kinases and redox-sensitive transcription factors. Cell responses to redox signaling depends on the specific species of free radical generated, the kinetics of ROS formation and the cell compartment in which ROS are localized. In the vasculature activation of redox-sensitive signaling cascades leads to VSMC growth and migration, modulation of endothelial function, expression of pro-inflammatory mediators, modification of extracellular matrix, apoptosis and anoikis. Furthermore, ROS increase cytoplasmic free Ca^{2+} concentrations, decrease cytoplasmic free Mg^{2+} levels, and alter intracellular pH_i, major determinants of vascular reactivity and growth. Exact molecular mechanisms whereby ROS influence signaling molecules remain elusive, but oxidative modification of proteins, particularly protein tyrosine phosphatases, is important. In addition, redox-dependent processes are critically involved in vascular injury associated with hypertension, as evidenced by findings in mice deficient in gp91-/- or p47phox, experimental rat models of hypertension and in human essential hypertension. Accordingly, ROS and the signaling pathways that they modulate, provide putative targets to regress vascular remodeling, reduce peripheral resistance and prevent hypertensive end organ damage. Here, we will discuss the role of NAD(P)H oxidase-derived ROS as second messengers and will focus on implications of these events in processes contributing to arterial remodeling and vascular dysfunction in hypertension.

Abstract N^{o} S30F

Role of oxygen free radicals in myocardial ischemia-reperfusion

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The oxygen-centered radical, superoxide (O_2^-), is generated during reperfusion and dismutated to hydrogen peroxide (H_2O_2) which is converted to hydroxyl radical (OH^-). In addition, O_2^- reacts with NO and forms peroxinitrite

($ONOO^-$). These reactive species inhibit enzymes such as creatine kinase, aconitase, glyceraldehydes dehydrogenase, SERCA, etc. Moreover, OH^- and $ONOO^-$ damages mitochondria and open transition pores, which elicit apoptosis or even necrosis resulting in myocardial dysfunction and injury. Our data indicates *in vitro* reperfusion of the heart with O_2^- induces systolic and diastolic dysfunction, which is mediated by $ONOO^-$ and prostaglandins. O_2^- decreases Ca^{2+} sensitivity to the myofilament, fractures mitochondria, and induces DNA fragmentation. Although high concentrations (0.7-1.0 mM) of H_2O_2 causes myocardial injury, in contrast H_2O_2 prevents the myocardial dysfunction induced by O_2^-, and perfusion of the hearts with low concentrations (0.010-0.20 mM) of hydrogen peroxide, which activates PKC and mitochondrial K_{ATP} channels, protects the heart from reperfusion injury and induces pre-conditioning. Nevertheless, the mechanisms that mediate the protective effects of hydrogen peroxide are still elusive, since in our hands opening of mitochondrial K_{ATP} channels appears to mediate O_2^--induced myocardial dysfunction and injury. Thus depending upon the type of radical species, their concentration, and site of their generation and action determines the role of free radicals such as signaling or stress in the myocardium.

Abstract N° S31A

Aldosterone and remodeling in the cardiovascular system

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Clinical and experimental studies have established that aldosterone plays a major role in the pathophysiology of cardiovascular and renal diseases. The aldosterone receptor antagonists spironolactone and eplerenone have demonstrated specific effects, not related to their hypotensive properties, in hypertension or cardiac diseases. It appears that a key action of these molecules is related to prevention or treatment of end-organ damage. This, and the evidence of aldosterone escape on long-term treatment with ACE inhibitors in heart failure, in diabetic nephropathy and in some forms of hypertension, will likely increase the clinical use of aldosterone receptor antagonists.

The mechanisms of aldosterone actions in cardiovascular system are not totally understood, but recent experimental results allow us to draw a tentative scheme. It is now well established that high levels of aldosterone induce fibrosis in heart and vessels. Cardiac fibrosis in heart appears to be triggered by activation of pericoronary inflammation, with a possible involvement of oxidative mechanisms. All these deleterious phenomena may be due to initial aldosterone-induced changes in intracellular ionic homeostasis. On the other hand, transgenic mice with moderately increased aldosterone specifically in heart display a major coronary dysfunction but no myocardiac structural or functional alterations. Together, these observations indicate that coronary vessels are a target of aldosterone in heart. Despite not being demonstrated, it is possible that similar mechanisms may occur in other target organs of aldosterone.

Abstract N° S31B

Physiopathological role of the mineralocorticoid receptor in the heart : use of conditional transgenic model

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To assess the role of the mineralocorticoid receptor (MR) in the heart independently of the kidney, we generate two transgenic mouse models that allow spatio-temporal control of MR expression in vivo. A first model allows conditional expression of a 320bp murine MR antisense mRNA restricted to cardiomyocytes. These mice develop dilated hypokinetic cardiomyopathy. Heart/Body weight ratio is increased, cardiac remodelling with extensive interstitial fibrosis is observed. Neither inflammation nor apoptosis are observed. Administration of spironolactone, an MR antagonist, to one mo old animal has a synergistic effect with MR antisense expression. Most interestingly, the cardiopathy is reversible if transgene expression is turned off in 2 mo old animals. A second model allows conditional over-expression of the human MR. These mice experience sudden death with normal histology suggesting fatal arrhythmia. Administration of spironolactone prevents premature death. Electrophysiological analyses performed on isolated cardiomyocytes reveal an increase in the length of action potential associated with an increase in whole Ca current. ECG analyses indicated an increase in PR and QT intervals. These two mirror models should help to understand the physiopathological role of the MR in the heart.

Abstract N° S31C

Eplerenone, but not steroid withdrawal, reverses cardiac fibrosis in doc/salt rats

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Effects of nonepithelial MR activation have been demonstrated, in the context of inappropriate mineralocorticoid (MC) for salt status, including vascular inflammation and cardiac fibrosis. Eearly inflammatory responses in the coronary vasculature of rats treated with MC plus salt is now a well-characterised precursor of the appearance of fibrosis at these sites. The enzyme inhibitor carbenoxolone (CBX) plus salt has also been shown to produce an MR-mediated inflammatory response identical to MC administration. Oxidative stress, via expression of NADPH oxidases, may account for

these inflammatory and fibrogenic responses. We hypothesised that when 11βHSD2 is inhibited, endogenous glucocorticoids (GC) bound to unprotected VSMC MR will similarly increase the early NADPH oxidase expression. When endogenous GC were allowed to activate VSMC following administration of CBX, inflammatory and fibrotic responses were identical, in terms of time course and extent, as those seen in rats given DOC. DOC or CBX administration for 4 days saw a 2-fold increase in expression of the VSMC specific subunits of NADPH oxidase ; macrophage infiltration was elevated at 4 and 16 days following either DOC or CBX ; cardiac collagen deposition was also increased. All responses induced by administration of CBX were inhibited by the MR antagonist eplerenone. Secondly, we tested whether established fibrosis and vascular damage could be reversed by MR blockade or by steroid withdrawal. DOC raised cardiac collagen accumulation at 4 wk, and higher at 8 wk. Rats given DOC for 4 wk and killed at 8 wk showed levels of fibrosis identical to those killed at 4 wk, i.e. persistently elevated above control. Rats given DOC for 8 wk, and eplerenone for the second half of this period, showed cardiac collagen levels indistinguishable from control. ED-1, osteopontin and COX-2 in coronary vessels showed similar patterns of expression. These findings show (i) that MR antagonists can not only prevent cardiac fibrosis when given concomitantly, but can reverse established cardiac fibrosis (ii) that the continued vascular inflammatory response and fibrosis post DOC withdrawal is further evidence for a role for GC activation of vascular MR under conditions of tissue damage. Our findings provide further evidence that exogenous MC or endogenous GC can activate VSMC MR and influence intracellular redox state which may account for the early vascular inflammation that precedes onset of fibrosis.

Abstract N° S31D

Nongenomic cardiovascular effects of aldosterone

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Aldosterone has rapid nongenomic effects in a variety of tissues and it has been postulated that these actions are mediated via a membrane receptor, distinct from the classical mineralocorticoid receptor (MR) since they are neither blocked by spironolactone nor mimicked by cortisol. Recent studies in human vascular smooth muscle cells (vsmc) have implicated the classical mineralocorticoid receptor (MR) in at least some of the rapid nongenomic effects of aldosterone (Alzamora et al., 2000). In these studies while spironolactone did not block the acute effects of aldosterone on Na^+/H^+ exchange, the water soluble, open E-ring MR antagonist RU28318 did. Cortisol alone was without effect, but when the enzyme 11ohydroxysteroid dehydrogenase (11oHSD2) was blocked by carbenoxolone, cortisol mimicked the agonist effect of aldosterone.

Similarly, we have shown that the rapid nongenomic effects of aldosterone on cardiomyocyte $Na^+/K^+/2Cl^-$ and/or Na^+/K^+ pump activity cannot be blocked by canrenone, but are blocked by canrenoate (and spironolactone in vivo). These effects of aldosterone can be mimicked by oPKC agonist peptides, and blocked by oPKC blocking peptides, but not by oPKC, oPKC or scrambled o agonist peptide. The rapid effects of aldosterone on Na^+/K^+ pump activity are maintained over 7 days aldosterone infusion in vivo, and over the time of the cell isolation, but are reversed within 15 min by oPKC blockade. Since mineralocorticoid receptors in cardiomyocytes are always occupied (but not activated) by much higher concentrations of free glucocorticoids, we also examined the effect of cortisol. Cortisol alone was without effect, but cosuperfused with aldosterone, blocked the acute nongenomic effect of aldosterone. Further studies are in progress to define this complex action of aldosterone in the heart.

Alzamora et al. (2000). Hypertension 35 : 1099-1104.

Abstract N° S31E

Primary aldosteronism : a common cause of hypertension with a genetic basis

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At the Greenslopes Hospital Hypertension Unit (GHHU), adoption of the policy in 1991 to screen all hypertensives (and not just those with hypokalemia or resistant hypertension) by aldosterone/renin ratio testing led to a tenfold increase in detection rate of PAL (only 22 % hypokalemic) and a fourfold increase in rate of removal of aldosterone-producing adenomas (APAs ; 44 % hypokalemic) leading to cure of hypertension in 60 % and improvement in all remaining operated patients. The GHHU reported a prevalence of PAL of 8.5 % among 199 consecutively referred normokalemic hypertensives and 12.5 % among 52 antihypertensive drug trial volunteers. The combined GHHU/Princess Alexandra Hospital Hypertension Unit PAL series stands at over 1000 patients diagnosed, and over 260 aldosterone-producing tumors removed. Reliable detection requires that (1) the diagnosis is considered in all hypertensives, (2) samples are collected under standardised conditions of diet, posture and time of day, (3) medications known to alter the ratio are avoided or their effects taken into account, and (4) reliable methods (such as fludrocortisone suppression testing) are used to confirm PAL. Adrenal venous sampling is the only dependable way to differentiate APA from bilateral adrenal hyperplasia. The availability of laparoscopic adrenalectomy and a new selective aldosterone receptor antagonist (eplerenone) represent important advances in management. Elucidation of the genetic mutation responsible for a familial, glucocorticoid-remediable form of PAL (an ACTH-regulated « hybrid » 11beta-hydroxylase/aldosterone synthase gene) and development by the GHHU of a PCR-based diagnostic test has greatly facilitated detection and led to a fuller appreciation of phenotypic diversity and aldosterone regulation in that subtype. The identification of mutations causing another, more common familial variety of PAL described by the GHHU in 1991 should further aid in the detection of this specifically treatable condition.

Abstract N° S31F

Impact of aldosterone on cardiovascular disease

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There are compelling new studies in animals and humans suggesting aldosterone has adverse cardiovascular effects that are in addition to its well-known renal actions on sodium and potassium balance, plasma volume and blood pressure. Two studies of patients with congestive heart failure, Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), demonstrated marked benefits on mortality when mineralocorticoid receptor blockade was added to standard therapy. In studies of patients with essential hypertension, mineralocorticoid receptor blockade reduced left ventricular hypertrophy, proteinuria and blood pressure. Beneficial effects of MR antagonists on myocardial necrosis, cardiac fibrosis, stroke, proteinuria, and vascular remodeling have been demonstrated in different animal models. In a rodent model of low nitric oxide availability and high angiotensin II, mineralocorticoid receptor blockade reduced vascular inflammation, myocardial necrosis and proteinuria. This protection by mineralocorticoid receptor antagonists occurred in the absence of elevated plasma aldosterone levels, was independent of changes in blood pressure, and did not appear to be mediated by plasminogen activator inhibitor-1 or by the potassium sparing effects of mineralocorticoid receptor blockade. Vascular inflammation and dysfunction appears to be one early event in aldosterone-mediated injury, and may be an important mechanism underlying aldosterone's widespread contributions to cardiac damage, cerebrovascular disease and nephropathy.

Abstract n° S32A

Adenosine receptor agonism and nhe inhibition limit post-ischaemic coronary dysfunction

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We studied effects of ischaemia-reperfusion (I/R) on coronary function in mouse hearts. After 20 min ischaemia and 30 min reperfusion endothelial function was selectively impaired : I/R inhibited coronary sensitivity to the endothelial-dependent/independent 2-chloroadenosine (pEC_{50} = 7.5 ± 0.1 *vs.* 8.4 ± 0.1 in non-ischaemic) and endothelial-dependent ADP (pEC_{50} = 6.8 ± 0.1 *vs.* 7.6 ± 0.1 in non-ischaemic) without altering responses to endothelial-independent nitroprusside. Reactive hyperaemia, NO/EDHF and K_{ATP} channel dependent in control hearts, was also attenuated by I/R. Coronary dysfunction was unaltered by anti-oxidant therapy (300 μM MPG + 150 U/ml SOD + 600 U/ml catalase) or endothelin antagonism (200 nM PD142893), but was effectively reduced by agonism of A_3 adenosine receptors (100 nM Cl-IB-MECA) and inhibition of Na^+/H^+ exchange (10 or 50 μM BIIB-513). Effects of A_3 agonism and NHE inhibition wer enot additive. Antagonism of A1 adenosine receptors also worsened vascular dysfunction. These data indicate post-ischaemic coronary dysfunction is reduced by intrinsic activation of A_1 receptors, exogenous A_3 agonism and NHE inhibition. NHE inhibition and A3 agonism may share common signalling.

Abstract N° S32B

Comprehensive *in vivo* assessment of cardiac function in mice by pressure-volume analysis

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The explosion of research utilizing murine models that involve genetically engineered protein changes has highlighted the need for rigorous and comprehensive tools to assess the impact they have on cardiac function. To this aim, we first developed a miniaturized pressure-volume conductance catheter to assess function in these small hearts. Combined with physiological anesthesia, proper ventilation, and rapid instrumentation methods, *in vivo* murine heart function can be fully assessed at heart rates (500-600 min^{-1}) and contractility (dP/dt_{max} of often 14,000 mmHg/s or higher) typical of intact conscious animals.

The method involves use of a 1.8F multi-electrode catheter with a single (or dual) pressure sensor. It is often placed via an apical stab under direct visualization as this facilitates proper and rapid placement. The catheter volume signal is based on the local introduction of high frequency low amplitude current. It is calibrated to a directly assessed aortic flow (Doppler or ultrasound probe) and by a saline-injection or dual-frequency method to derive absolute volume. The mouse heart is well suited to this method as the current field is locally distributed, so there is minimal effect of conductance from structures external to the heart.

From simultaneous pressure-volume data, one can accurately assess systolic and diastolic function parameters, relaxation, the impact of varying preload and afterload on these parameters, force frequency dependencies, pharmacologic interventions, and many other features of cardiac function. The method is not suitable for chronic repeated analysis, although ongoing efforts to miniaturize telemetry systems recently developed for rats may make this feasible in the future.

Abstract N° S32C

Cardiac metabolism, function and efficiency recordings in ex vivo mouse hearts

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The use of genetically engineered mouse models in cardiovascular research has led to the establishment of miniaturized (in vivo and ex vivo) techniques for assessment cardiac physiology. Experimental conditions (pre- and afterload, heart rate, substrate availability etc.) can be easily controlled in ex vivo systems, and several parameters are therefore still best measured on isolated hearts. Thus, the isolated perfused mouse heart model (both isovolumetric and working heart preparations) is now in use in several labs, despite technical challenges related to the small size of the heart.

We have established a working isolated mouse heart model, where measurements of cardiac mechanics (left ventricular (LV) pressure, cardiac output) can be combined with measurements of cardiac metabolism (radiolabelled substrates) and myocardial oxygen consumption (fibre-optic probe). Using this approach, the association between cardiac function and metabolism can be studied in various mouse models.

Cardiac work in the above model is, however, limited to external work. The development of the new 1.4 French pressure-volume catheter provides a direct high-fidelity measurement of LV pressure and volume and, from them, estimates of pressure-volume area (PVA), which is representative of the total (internal + external) work of the heart. Moreover, cardiac efficiency is the relationship between cardiac work (PVA) and oxygen consumption (MVO_2). We have demonstrated that the substrate supply significantly influences the PVA-MVO_2 relationship in murine hearts, and believe that this methodology can be applied to various models requiring phenotypic assessment of the relationship between metabolism, contractile performance and cardiac efficiency.

Abstract N^o S32D

Echo-doppler evaluation of heart function in small animals

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Echo-Doppler assessment of heart function is in small animals (rat, mice) is a major progress in experimental projects. The advantages of this procedure are 1) the wide availability of the equipment and of competence for performing the examination, 2) the non invasive character of the procedure allowing to assess in vivo cardiac function and to repeat assessment over time thus allowing to quantify progression or regression of cardiac disease.

The disadvantages of the procedure are 1) the cost of equipment, 2) the operator-dependent character of the results obtained, 3) time consuming examination. Echo-Doppler can be conducted in a way similar to that in human with the assessment of cardiac size and myocardial function (systolic and diastolic) including new development of echocardiography such as tissue Doppler. Valvular disease can also be studied. In all cases, technical limitations due to the size of the animal and the high heart rate must be taken into account. Needs are of new devices and developments in order to improve reproducibility and sensitivity and to develop new parameters.

Abstract N^o S33A

Coronary microembolization – signal transduction of contractile dysfunction

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Coronary microembolization in patients with spontaneous or peri-interventional atherosclerotic plaque rupture has recently been identified as a potential cause of arrhythmias, contractile dysfunction and microinfarction. In anesthetized dogs with intracoronary embolization of 3.000 microspheres (diameter 42 μm) per ml and min coronary inflow, there is a rapid decrease in coronary blood flow with subsequent reactive hyperemia. Regional contractile function is also decreased and then recovers over several minutes almost back to normal. Subsequently a secondary profound progressive contractile dysfunction develops over several hours, in the absence of changes in myocardial blood flow, i.e. a characteristic perfusion-contraction mismatch. The total amount of microinfarction is about 2 % of the area at risk, and apoptotic cardiomyocytes are less than 0.1 %. However, there is a marked inflammatory response with leukocyte infiltration and TNFα accumulation. TNFα gene expression is enhanced in cardiomyocytes surrounding the microinfarcts. TNFα is causal for the observed contractile dysfunction, as evidenced by the prevention of progressive dysfunction by intravenous TNFα-antibodies before microembolization. The TNFα-initiated signal transduction cascade further involves NO and – as a more distal effector – sphingosine. In chronically instrumented dogs the progressive contractile dysfunction following microembolization recovers back to control over 6-7 days. Methylprednisolone, even when given 30 min after coronary microembolization, fully prevents the progressive contractile dysfunction. Microembolized myocardium is characterized by reduced coronary and inotropic reserves. Early clinical studies in patients with coronary interventions confirm the reduction of coronary reserve with microembolization and the causal.

Abstract N^o S33B

Heart failure by coronary microembolization

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Embolization of plaque contents of platelet-thrombus into the microvasculature has been reported in patients with acute coronary syndrome. Mechanical crushing and fragmentation of culprit coronary lesions during PCI has emerged as a major cause of coronary microembolization. Until recently, however, the consequences of coronary microembolizations on LV function and on the development of ventricular arrhythmias have been seriously underappreciated. In anesthetized dogs, repetitive injections of 42 μm spheres into the left circumflex coronary artery was shown to markedly decrease LV systolic posterior wall thickening and to reduce both coronary flow reserve as well as LV inotropic reserve. Studies in our laboratory showed that repetitive coronary embolizations with \\ 126\\90 μm polystyrene latex microspheres in both the left anterior descending and left circumflex coronary artery over the course of several weeks can lead to the formation of transmural micro-infarcts and to the gradual development of LV systolic and diastolic dysfunction and to the emergence of malignant ventricular arrhythmias. We also showed that discontinuation of coronary microembolizations once LV dysfunction is established invariably leads to pro-

gressive worsening of LV function and remodelling that ultimately culminates in the syndrome of heart failure (HF). At the global level, development of HF in these animals is characterized by progressive decline in LV ejection fraction, cardiomegaly, LV hypertrophy, exercise intolerance, functional mitral regurgitation and increased and sustained activation of the sympathetic and renin-angiotensin systems. At the cellular level, dogs with HF manifest interstitial fibrosis, cardiomyocyte apoptosis and abnormal cardiac sarcoplasmic reticulum calcium cycling. At the molecular level, dogs with HF manifest most if not all of the molecular maladaptations described in patients with HF including induction of the fetal gene program. Conclusions : While these studies represent an extreme consequence of coronary microembolizations, they nonetheless point to the serious adverse outcomes that can develop as a result of microembolization-mediated coronary microvascular obstructions.

Abstract N^{o} S33C

No-reflow and coronary microvascular dysfunction in acute myocardial infarction

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No-reflow after coronary reperfusion is often observed in patients with anterior acute myocardial infarction (AMI). No-reflow is detected by myocardial contrast echocardiography (MCE) and frequently associated with early systolic retrograde flow and accelerated deceleration of flow velocity during diastole. Approximately 20 % of the pts with TIMI grade 2 flow after coronary angioplasty showed no-reflow on MCE. The patientts without no-reflow showed better outcome and less complications. Multivariate analysis demonstrated that pre-infarction angina within 48 hrs before symptom onset (ischemic preconditioning), number of Q waves on ECG, wall motion score and TIMI flow grade 0 at initial CAG are related to the no-reflow phenomenon. The causes of the no-reflow may be multiple. After PCI we aspirate more atheromatous plaque from patients with no-reflow than patients without no-reflow, and aspiration of the gruel derived from atheroma improved the no-reflow. Intracoronary administration of verapamil and nicorandil attenuated no-reflow and improved regional wall motion, indicating that microvascular function was improved by these pharmacological interventions. We conclude that no-reflow often observed in AMI represents the coronary microvascular dysfunction partially induced by microvascular embolization and functional dysintegrity which should be prevented or treated for better outcome of this disease.

Abstract N^{o} S33D

Role of NO and EDHF during ischemia reperfusion injury in coronary microcirculation

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Background : (1) Recent studies in vitro have demonstrated that endothelium-derived hydrogen peroxide (H2O2) is an endothelium-derived hyperpolarizing factor (EDHF) in animals and humans. We tested our hypothesis that endothelium-derived H2O2 plays an important role in coronary autoregulation. (2) Recent studies have demonstrated that Rho-kinase is substantially involved in the pathogenesis of cardiovascular diseases, however, it remains to be examined whether it is also involved in ischemia/reperfusion (I/R) injury. We examined whether Rho-kinase is involved in myocardial ischemia/reperfusion (I/R) injury and if so, whether NO is involved. Methods and Results : (1) We evaluated vasodilator responses of canine (n = 41) subepicardial small coronary arteries (> 100fÊm) and arterioles (< 100fÊm) with an intravital microscope in response to acetylcholine and to a stepwise reduction in coronary perfusion pressure (from 100 to 30 mmHg) before and after inhibition of NO synthesis (L-NMMA 200fÊM,IC) under cyclooxygenase blockade (ibuprofen, 12.5mg/kg,IV). After L-NMMA, the coronary vasodilator responses were attenuated mainly in small arteries ($P < 0.05$), whereas combined infusion of L-NMMA plus catalase (400,000U/kg IC) inhibited the vasodilator responses of both-sized arteries ($P < 0.05$). The arteriolar responses were also comparable after catalase plus L-NMMA. Coronary venous adenosine concentrations were increased in response to decreasing perfusion pressure after L-NMMA alone, L-NMMA plus catalase ($P < 0.05$), but were not further increased after catalase alone. (2) Canine subepicardial small arteries and arterioles were observed by microscope during myocardial I/R. Myocardial I/R significantly impaired coronary vasodilation to acetylcholine, as did L-NMMA, whereas hydroxyfasudil, a specific Rho-kinase inhibitor, preserved the response. Vasoconstriction by L-NMMA was significantly improved by hydroxyfasudil. Hydroxyfasudil significantly reduced the size of myocardial infarction induced by I/R. Conclusions : (1) H2O2 is an endogenous EDHF in vivo and plays an important role in coronary autoregulation as a compensatory mechanism for NO and adenosine. (2) Hydroxyfasudil exerts cardioprotective effects on I/R injury in vivo, for which NO-mediated mechanism may be involved.

Abstract N^{o} S34A

Lessons from the toad pacemaker cells ; role of intracellular calcium

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Conventionally pacemaker function is attributed to a series of voltage- and time-dependent inward currents. In the last decade evidence that intracellular calcium $[Ca^{2+}]_i$ modifies the firing rate has accumulated rapidly. We showed that interventions which increased $[Ca^{2+}]_i$ accelerated the firing rate and vice-versa. A possible mechanism is provided by the Na^{+}/Ca^{2+} exchanger which removes Ca^{2+} and generates an inward current ($I_{Na/Ca}$)which contributes to the pacemaker currents. For instance ryanodine, a drug which prevents sar-

coplasmic reticulum (SR) Ca^{2+} release, can slow or stop the spontaneous firing. In this type of model, the acceleration due to sympathetic stimulation is because the increased $[Ca^{2+}]_i$ which drives a larger $I_{Na/Ca}$. Further interest arises from the discovery that pacemaker cells exhibit spontaneous brief Ca^{2+} releases (Ca^{2+} sparks) which precede the rise of the action potential. Where these sparks occur close to surface membrane they may drive a local $I_{Na/Ca}$ which further contributes to pacemaker currents.

These new results suggest that Ca^{2+}-driven currents are at least as important as the voltage-sensitive currents in determining pacemaker activity. Many interventions which affect pacemaker function e.g. ischaemia, ATP probably do so at least in part by altering Ca^{2+} handling.

Abstract N° S34B

Calcium cycling in the heart is a general mechanism of chronotropy and inotropy

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Excitation induced Ca^{2+} cycling into and out of the cytosol via the sarcoplasmic reticulum (SR) Ca^{2+} pump, ryanodine receptor (RYR) and Na^{+}-Ca^{2+} exchanger (NCX) proteins, and modulation of this Ca^{2+} cycling by β-Adrenergic Receptor stimulation (βARs), governs the strength of ventricular myocyte contraction and the cardiac contractile reserve. Recent evidence indicates that heart rate modulation and chronotropic reserve via βARs also involve intracellular Ca^{2+} cycling by these very same molecules. Specifically, sinoatrial nodal pacemaker cells (SANC), even in the absence of surface membrane depolarization, generate localized, rhythmic, submembrane Ca^{2+} oscillations via SR Ca^{2+} pumping-RYR Ca^{2+} release. During spontaneous SANC beating, these rhythmic, spontaneous Ca^{2+} oscillations are interrupted by the occurrence of an Action Potential (AP), which activates L-type Ca^{2+} channels to trigger SR Ca^{2+} release, unloading the SR Ca^{2+} content and inactivating RYRs. During the later part of the subsequent diastolic depolarization (DD), when Ca^{2+} pumped back into the SR sufficiently replenishes the SR Ca^{2+} content, and Ca^{2+}-dependent RYR inactivation wanes, the spontaneous release of Ca^{2+} via RYRs again begins to occur. The local increase in submembrane $[Ca^{2+}]$ generates an inward current via NCX, enhancing the DD slope, modulating the occurrence of the next AP, and thus the beating rate. βARs increases the submembrane Ca^{2+} oscillation amplitude and reduces their period (the time from the prior AP triggered SR Ca^{2+} release to the onset of the local Ca^{2+} release during the subsequent DD). This phase shift in spontaneous sub-membrane Ca^{2+} release by âARs causes the DD modulation by NCX current to occur at earlier times following a prior beat, promoting the sooner arrival of the next beat and thus, an increase in the spontaneous firing rate. Ca^{2+} cycling via the SR Ca^{2+} pump, RyR and NCX, and its modulation by βARs is, therefore, a general mechanism of cardiac chronotropy and inotropy.

Abstract N° S34C

I_F and sino-atrial node pacemaking

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Normal cardiac pacing is driven by the spontaneous activity of sino-atrial node (SAN) cells, chracterized by the presence of a slow diastolic (« pacemaker ») depolarization phase of the action potential. Several independent results, including I_f inhibition by specific heart-rate reducing agents such as ivabradine, indicate that activation of the I_f current plays a key role in the generation and autonomic control of the pacemaker depolarization, operated through a cAMP-dependent, Ca^{2+} independent mechanism of rate regulation.

Disruption of SR Ca^{2+} release in SAN myocytes slows spontaneous rate and strongly reduces βAR-induced rate acceleration, which has been taken to indicate that the target of βAR-modulation of pacemaking is the intracellular Ca^{2+} regulatory process. However, abolishement of SR Ca^{2+} transients (by incubation of SAN cells with ryanodine) does not impair cAMP-dependent rate acceleration mediated by I_f; also, reduction of Ca^{2+} transients slows rate by depolarizing the action potential threshold, while βAR-stimulation accelerates rate by increasing the rate of diastolic depolarization, in agreement with the hypothesis that it is caused by an increased degree of I_f activation.

These and other data suggest that rather than being directly involved in pacemaking, normal Ca^{2+} homeostasis is necessary for the βAR-cAMP-I_f rate-controlling mechanism to function properly.

Supported by MIUR- FISR 02.00652.ST97 and COFIN 2001055324 to DD.

Abstract N° S34D

Sinoatrial node heterogeneity and pacemaking

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The pacemaker activity of the sinoatrial (SA) node is a function of the ion channels expressed in the SA node and we are using immunohistochemistry, real-time PCR and in situ hybridisation to map the distribution of ion channel mRNAs and proteins in and around the SA node of the rabbit and rat. Results are reported below for mRNA abundance in the SA node measured using real-time PCR. All results reported are in relation to atrial muscle and are significant ($P < 0.05$; $n = 7$ rabbits). The large and medium conductance connexin isoforms (Cx40 and Cx43) were less abundant in the SA node, whereas the small conductance Cx45 was of equal abundance. The pattern of expression of connexins in the SA node explains the weak electrical coupling in the SA node. The cardiac Na^{+} channel isoform, $Na_v1.5$, was less abundant in the SA node. The lack of $Na_v1.5$ explains the slow upstroke of the SA node action potential. Interestingly, the

neuronal Na^+ channel isoform, $Na_v1.1$ (protein detected by immunohistochemistry), was of equal abundance in the SA node. We have shown that block of $Na_v1.1$ slows pacemaking (S.K.G. Maier et al. *Proc.Natl.Acad.Sci.U.S.A.* 100 :3507-3512, 2003). We have obtained evidence of a L-type Ca^{2+} channel isoform switch : whereas $Ca_v1.2$ was less abundant in the SA node, $Ca_v1.3$ was more abundant. We have also obtained evidence of a switch in the isoform responsible for the transient outward current : whereas Kv1.4 was less abundant in the SA node, Kv4.2 was more abundant. Two other K^+ channels (ERG and KvLQT1) were more abundant in the SA node. The funny current, I_f, plays an important role in the pacemaker potential in the SA node and, as expected, two ion channels responsible for I_f (HCN1 and HCN4) were more abundant in the SA node. The ryanodine receptor (RYR2) has been implicated in pacemaking and RYR2 was less abundant in the SA node.

Abstract N° S34E

Future directions : gene therapy to enhance pacemaking

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Electrophysiological and pharmacological studies over past decades have provided insight into the ionic basis of cardiac pacemaker function. Recent molecular and genetic advances have raised the possibility for targeted gene therapeutic approaches to rhythm disorders. We recently have explored the feasibility of developing a biological pacemaker, either by introducing pacemaker channel (HCN) genes into myocardial cells in the intact canine heart or by genetically engineering adult human mesenchymal stem cells (hMSCs) to express these same genes and then integrating the hMSCs into the cardiac syncytium. In the former studies, the HCN2 pacemaker channel gene isoform was placed in an adenovirus (Adv) and injected directly into left atrial tissue or, via catheter, into the left bundle branch of the ventricular conducting system. Approximately 1 week later we recorded ECGs during vagal stimulation to suppress normal sinus rhythm and AV conduction. Both Adv studies resulted in pacemaker activity in HCN2 injected animals that differed significantly from that of control animals (injected with a GFP Adv or saline). More recent studies have non-virally transfected hMSCs with the HCN2 gene. The hMSCs normally express connexins 40 and 43 and can functionally couple to each other and to myocytes in culture. HCN2-transfected hMSCs express a large I_f-like current but are not otherwise excitable. HCN2-expressing hMSCs, but not GFP-expressing hMSCs, increase the spontaneous rate of co-cultured neonatal myocytes, and when injected into the in situ canine heart result in a stable escape rhythm during transient AV block. Thus, both adenoviral and cell based over-expression of HCN2 channels create a biological pacemaker in the in situ heart.

Abstract N° S35A

New paradigms in estrogen signaling with particular reference to cardiovascular function

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The discovery that estrogen signaling is mediated by two partially antagonistic principles, estrogen receptors α and β, is gradually changing our understanding of estrogen action in different organs and systems in the body. It has since long been appreciated that estrogens have an impact on cardiovascular function but great controversies exist regarding the details of estrogen regulation of heart and vessels. This lecture will address some of these issues and attempt to offer some explanations for unresolved inconsistencies.

Abstract N° S35B

Novel selective agonists as tools to dissect biological functions of eralpha and erbeta

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Estrogens exhibit physiological effects mainly through two different nuclear estrogen receptors (ERs). ERβ is predominatly expressed in ovarian granulosa cells, prostate, vascular tissue, intestine and specific brain regions, whereas pituitary, uterus and liver express ERα at high levels. Data from various lines of ERβ knockout mice showing different degrees of female subfertility or infertility due to reduced follicular maturation and ovulation rate imply that ERβ plays an important role in the control of ovarian function and folliculogenesis. The stimulatory activity of estrogens on granulosa cell growth was demonstrated in diverse studies in rodents. Less information is available on direct effects of estrogen on the human ovary. However, a number of primate studies indicate that estrogen-free or reduced intraovarian estrogen levels are associated with reduced rates of meiotic maturation and fertilization. Additional information on the function of ERα and ERβ will be provided by the application of subtype-selective-ER agonists. Based on the crystal structure of the ERα ligand-binding domain steroidal ligands were designed to bind preferentially to either ERα or ERβ and were tested in vitro using transactivation assays. This approach directly led to highly ER isotype-selective (\\ 126\\200 fold) and potent ligands (50 % of E2). To unravel physiological effects, in vivo experiments were performed using the ERα- and ERβ-selective ligands. In ovariectomized rats the ERα ligand induced uterine growth and caused bone-protective effects, while the ERβ ligand exhibited estrogen-like effects on these parameters only at high doses. Therefore estrogen effects on the uterus, pituitary, bone and liver may be primarily mediated via ERα. In hypophysectomized animals, the ERβ agonist caused stimulation of early folliculogenesis and a decrease in follicular atresia, accompanied by an increase in the number of ovulated oocytes. In contrast, the ERα agonist had little or no effect on these parameters implying that direct estrogen effects on follicular development are mediated primarily by ERβ.

Abstract N° S35C

Modulation of cardiac hypertrophy by selective estrogen receptor agonists and serms

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The biological effects of estrogens are transmitted by two different estrogen receptor subtypes, ERα and ERβ, which posses redundant, divergent or even opposing functions in several organ systems. Thus, the function of estrogens in cardiovascular disease cant be understood and preventive treatment strategies cant be developed without understanding the biological function of both receptor subtypes, which is still largely unknown. The recent synthesis of subtype-selective ERα and ERβ agonists has provided an additional tool to dissect the biological function of both estrogen receptors which complements already existing genetic mouse models to study functional differences between ERα and ERβ. The task ahead is to apply these tools to animal models of human heart disease. Cardiac hypertrophy, which increases with age and with declining estradiol serum levels, is effectively attenuated by non-selective ERα and ERβ ligand such as 17β-estradiol. Therefore, the development of cardiac hypertrophy appears as an established model to evaluate existing and innovative ER ligands. This presentation will provide insight into the effects of subtype-selective estrogen agonists and selective estrogen receptor modulators (SERMs) on cardiac hypertrophy, function and gene expression via an integrative approach of small animal physiology, functional cardiac imaging (MRI, PET) and cardiac gene expression and includes very recent and unpublished data on novel ER ligands.

Abstract N° S35D

Estrogens block cardiac hypertrophy

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Important gender related differences have been observed, when comparing cardiac mass and hypertrophy. In female cardiac mass increases after the menopause, suggesting an important role for sex hormones in hypertrophy suppression. In female mice we assessed the hypertrophic response upon transverse aortic banding in the presence or absence of estrogen (E2) replacement. In this model a marked reduction of the hypertrophic response was observed in the E2 substituted animals. Hypertrophy reduction was p38 and ANF dependent. The role of ANF, ANF receptor (guanylyl cyclase A receptor), cGMP and cGMP dependent protein kinase in blocking hypertrophy was further substantiated in *in vitro* studies using isolated neonatal rat ventricular myocytes (NRVM). In NRVM induced cGMP activity was observed upon E2 treatment. In addition, the hypertrophic response induced by endothelin and phenylephrine treatment was blocked as shown by smaller cell surface and protein content. The hypertrophy suppressive effect of E2 was inhibited by adding ANF antibody to the medium, reducing the bioavailability of ANF and by blocking of the cGMP dependent protein kinase by KT-5823. In the next series of studies in either estrogen receptor (ER) a or b deficient animals banding was performed showing the requirement of functional ERb for E2 to block hypertrophy. The analysis of the molecular pathways is ongoing using also microarray technology to unravel changes in gene expression, comparing the various experimental groups.

Conclusion : E2 blocks cardiac hypertrophy *in vivo* and *in vitro* by the induction of ANF expression and activation of cGMP. Blocking hypertrophy is dependent on the presence of functional ER.

Abstract N° S36A

Oxidant stress and vascular function : insights from the human heart

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Reactive oxygen species (ROS) play an important role in modulating vascular tone. Most prominent in this regard is the marked reduction in functional activity of nitric oxide during conditions associated with increased superoxide formation such as atherosclerosis or its risk factors. In these conditions alternative mechanisms of dilation involving an endothelial derived hyperpolarization factor (EDHF) often compensate for loss of nitric oxide. In the human coronary microcirculation, EHDF may actually be a ROS derived from superoxide, hydrogen peroxide. Other ROS derived from superoxide (i.e. peroxynitrite) impair EDHF-mediated dilation by inhibiting activity of the effector potassium channels present on underlying vascular smooth muscle. This presentation will highlight the complex influence of ROS on vasomotor function in the human coronary microcirculation, focusing on the role of hydrogen peroxide as an EDHF, and elucidating the mechanism of ROS-mediated inhibition of hyperpolarization-mediated vasodilation in diabetes and other risk factors for atherosclerosis.

Abstract N° S36B

Endothelial dysfunction and vascular disease

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The endothelium is the source of a number of important vasoactive factors and plays a critical role in the regulation of cardiovascular function. The most important endothelium-derived factor in the macrovasculature is nitric oxide (N0) whereas in the microvasculature another factor, or cellular process, termed EDHF (Endothelium-Derived Hyperpolarizing Factor) appears to be of at least equivalent importance to NO. Endothelial dysfunction, defined as a reduced endothelium-dependent vasorelaxation to acetylcholine, is a common feature and early indicator of cardiovascular disease in both humans and animals. Endothelial dysfunction is seen both in the macro- and microvasculature and may be

linked to the reduced contribution of both NO and EDHF. We have studied endothelial function in blood vessels from humans and mice with type II diabetes. A common feature of endothelial dysfunction in type II diabetes is that the bioavailability of NO is reduced and that endothelial function can be at least partially restored by acutely providing tetrahydrobiopterin, a key co-factor for eNOS. In our studies of blood vessels from the db/db mouse [which develops insulin resistance, hyperinsulinemia and severe hyperglycaemia] we have found that elevated oxidative stress, most likely secondary to the hyperglycaemia, is the major contributor to endothelial dysfunction and that oral treatment with sepiapterin, a precursor of tetrahydrobiopterin, restores endothelial function without affecting the metabolic abnormalities. In addition, treatment of db/db mice with a PPARγ agonist also restores endothelial function as well decreases blood glucose levels. These data suggest that dietary supplements designed to improve NO bioavailability may prove to be a valuable adjunct for the treatment of type II diabetics and improving cardiovascular function.

Abstract N° S36C

Infection and atherosclerosis – what role for antibiotics ?

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Interest in human antibiotic trials in the context of *C pneumoniae* infection and atherosclerosis was triggered by publication of the first two pilot clinical studies in 1997 – one from UK ; the other from Argentina. These small studies suggested a potential benefit of antimicrobial therapy in patients with coronary heart disease (CHD). A series of studies examining the effects of antibiotic prescribing and cardiovascular events ensued. Results have been mixed. More recently, exposure to antichlamydial antibiotics during the 3 months after acute MI was associated with a better survival. ACADEMIC, CLARICOR, ANTIBIO and ISAR-3 studies have been inconsistent in showing a positive effect with antibiotics – but it is possible that a sub-group of patients may be deriving benefit. The anti-*chlamydial* agents, particularly the macrolides used in early antibiotic intervention studies could be acting through non-antimicrobial effects (such as anti-inflammatory responses) thereby halting the progression of atherogenesis or atherothrombosis. Interestingly, other broad-spectrum antibiotics with anti-*chlamydial* activities such as tetracyclines inhibit macrophage matrix metalloproteinases and may also hypothetically stabilise the atherosclerotic plaque. Several large-scale trials of anti-*chlamydial* antibiotic therapy in various subsets of patients with CHD are currently underway. These include WIZARD, ACES, AZACS, PROVE-IT and CLAINF. Some 25,000 patients in total have now been recruited and randomised to receive antibiotic or placebo and are currently being followed up for adverse cardiovascular events. Results from some these trials will be discussed.

Abstract N° S36D

The sphingosine kinase / sphingosine-1-phosphate phosphohydrolase system as an endogenous regulator of microvas-cular function – focus on the modulation of rhoa signalling

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Exogenously applied sphingosine-1-phosphate (S1P) elicits a RhoA/Rho kinase-dependent vasoconstriction in resistance arteries (RA), and hence may play a role in the development of hypertension. *In vivo*, production and degradation of S1P is controlled by two enzymes, respectively, sphingosine kinase (Sphk1) and S1P phosphohydrolase (SPP1). To study the role of S1P as an *endogenous* modulator of microvascular tone, we employed a genetic approach to alter the expression/activity of these enzymes in the smooth muscles cells of isolated RA.

Overexpresssion of Sphk1 augmented resting tone in RA. When Sphk1 was co-expressed with dom. – neg. mutants of RhoA or Rho kinase (N19RhoA ; KD1A), resting tone was abolished, indicating a RhoA/Rho kinase-dependent mechanism. Expression of a dom.-act. mutant of RhoA (L63RhoA) also increased tone, further implicating a RhoA/Rho kinase-dependent mechanism. Pressure-induced vasoconstriction (myogenic response, MR) was augmented by Sphk1, but was inhibited by the expresssion of the inactive Sphk1 mutant (hSk^{G82D}) or by coexpression of Sphk1 with N19RhoA or KD1A. Overexpression of L63RhoA moderately enhanced the MR.

Expression of dom.-act. SPP1 reduced basal tone, the MR, calcium sensitivity of the contractile apparatus and vasoconstriction induced by exogenous S1P. Conversely, antisense-mediated reduction in SPP1 expression enhanced basal tone, the MR, calcium sensitivity and exogenous S1P-mediated vasoconstriction.

The pronounced, opposing effects of altering Sphk1 or SPP1 activity highlight their importance as regulators of microvascular tone, presumably through control of S1P bioavailability. Therefore, these enzymes represent novel targets for the pharmacological treatment of hypertension.

Abstract N° S36E

Anti-inflammation therapy targeting monocyte chemoattractant protein-1,as novel strategy to treat cardiovascular disease

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Clinical challenges for cardiovascular disease, which need new therapeutic options, include restenosis, atherosclerotic events resulting from plaque rupture, post-transplantation arteriosclerosis, ischemia-reperfusion injury and so on. Emerging evidence suggests that an inflammatory process is involved in the pathogenesis of such intractable diseases. In particular, inflammatory responses to arterial injury, which cause continuous recruitment and activation of monocytes

mainly through activation of the monocyte chemoattractant protein-1 (MCP-1) pathway, have a central role in restenosis and atherogenesis. We recently devised a new anti-inflammation (MCP-1) therapy by transfecting an N-terminal deletion mutant of the MCP-1 gene into skeletal muscles. This mutant MCP-1 lacks the N-terminal amino acid 2 to 8, called 7ND, and works as a dominant-negative inhibitor of MCP-1. We demonstrated that 7ND gene transfer suppressed monocyte infiltration/activation after arterial injury and attenuated restenotic changes after balloon injury or stent placement. Stent-based or adenovirus-mediated local transfection of 7ND gene reduced in-stent neointimal formation but did not affect process of endothelial regeneration or tissue repair, suggesting that local transfection strategy is a practical and promising means for prevention of in-stent restenosis in animals including monkeys. Furthermore, 7ND gene transfer not only attenuated the initiation of atherosclerotic lesions, but also limited progression of pre-existing atherosclerotic lesions and changed the lesion composition into a more stable phenotype, i.e., containing fewer macrophages, less lipid, more smooth muscle cells and collagen in hypercholesterolemic mice and monkeys. Vascular inflammation mediated by MCP-1 might create a positive feedback loop to enhance restenotic and atherosclerotic changes through activating lesional monocytes. We also reported that 7ND gene transfer attenuated ischemia-reperfusion injury, post-transplantation arteriosclerosis, and left ventricular remodeling and failure after myocardial infarction. In conclusion, blockade of MCP-1 with 7ND gene transfer is effective not only in reducing experimental restenosis, atherosclerosis, and plaque destabilization leading to acute coronary syndrome, but also in attenuating other forms of cardiovascular diseases. Our finding in nonhuman primates has significant clinical significance, implying that this anti-inflammation strategy targeting MCP-1 might be a promising therapy against human restenosis and atherosclerotic complications.

Abstract N^o S37A

Pathophysiological significance of adrenomedullin in the cardiovascular system

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Adrenomedullin (AM) has a wide range of biological actions including vasodilatation, natriuresis, diuresis, inhibition of aldosterone secretion and inhibition of cardiac hypertrophy and fibrosis. Ten years has passed since AM was discovered as a novel vasodilator peptide. During this decade, much research, basic and clinical, has been done to clarify its role in the homeostasis of cardiovascular functions, and a substantial amount of data has been accumulated in this field. Both the organs and tissues belonging to the cardiovascular system, such as the myocardium and vascular wall, were found to produce AM. AM is present in the bloodstream in picomolar concentrations and plasma AM levels were found to be progressively elevated in patients with hypertension, myocardial infarction and heart failure in association with severities of the diseases. In addition, its biological actions are closely related to the homeostasis of cardiac and vascular functions. Based upon these findings, AM is considered a humoral or locally acting factor modulating the development and progression of various cardiovascular diseases. For example, AM likely functions to counteract the elevation in blood pressure and progression of target organ damage in hypertension. AM also appears to be acting against progression of heart failure through its natriuretic action and inhibition of the renin-angiotensin-aldosterone system. Our recent studies showed that AM administration during an early period of acute myocardial infarction ameliorated chronic progression of cardiac remodeling and heart failure. Research on AM now seems to be entering a new phase, with clinical benefits to be examined and specified. It appears certain that further data will be provided as to the clinical application of AM in diagnosing or treating patients with cardiovascular diseases.

Abstract N^o S37B

Adrenomedullin and adrenomedullin receptor-ramp interactions modulate cardiac function

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Co-expression of the potent hypotensive peptide adrenomedullin (AM) with its receptor in the heart leads to localized actions on cardiac function. Modulation of cardiac AM signaling may come about as a consequence of altered expression or function of either AM, AM receptor or one or more of the receptor activity modifying proteins (RAMPs) that are required for AM receptor activity.

In cultured rat neonatal cardiac myocytes (MC), hypertrophy was associated with down-regulation of AM gene expression and peptide secretion, however, addition of exogenous AM inhibited the hypertrophic response as determined by protein :DNA ratio and the transcriptional activation of ANP and MLC-2 reporter gene expression.

In whole rat heart and in MC, RAMP2 expression predominates over RAMP1 with little or no detectable RAMP3 expression. Using a CRE-Luc reporter to determine AM receptor activity, we showed that AM signaling in MC is dependent upon Calcitonin Receptor-Like Receptor (CRLR) and RAMP2 expression. RAMP3 mRNA was shown to be rapidly induced in MC by serum and phorbol esters and adenovirus-mediated expression of RAMP3 in MC led to enhanced AM-mediated signaling.

Thus AM signaling in the heart is potentially regulated by modulating both AM and RAMP expression.

Abstract N^o S37C

Autocrine and paracrine functions of adrenomedullin in myocardium

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A possible direct role for adrenomedullin (AM), produced locally within the heart, in the attenuation of cardiac growth has been implied. Increased cardiac mass can be attributed either to growth of cardiomyocytes or proliferation of non-

myocytes. On account of the documented attenuation of the proliferation of cardiac fibroblasts by AM, utilisation of isolated cardiomyocytes is essential to dissect out the effects of the peptide that are intrinsic to the myocytes themselves. Increased plasma levels of AM correlate positively with left ventricular hypertrophy (LVH) and the severity of heart failure. The hypothesis that AM may constitute an endogenous defence mechanism to attenuate ventricular hypertrophy and cardiac decompensation was tested by investigating the effects of AM on growth (protein turnover, phenotypic gene expression) of healthy rat ventricular cardiomyocytes *in vitro* both under basal conditions and in response to hypertrophic growth stimuli. Complete attenuation of phorbol-12-myristate-13-acetate (PMA)-stimulated protein synthesis was observed at concentrations of AM $\geq 10^{-8}$M. Secretion of endogenous AM was increased in these cells in response to growth stimuli. In experimental models of hypertension-induced LVH, gene expression of AM in myocytes increased (x3) after nitric oxide synthase inhibition in the rat and this was accompanied by a modest increase in expression of the receptor activity modifying protein (RAMP)-3, which was observed also in the spontaneously hypertensive rat (SHR). It appears that the cardiac AM system at the level of the myocyte is upregulated in the hypertrophic state with possible consequence for anti-remodelling actions in myocardium.

Abstract N^o S37D

Adrenomedullin gene delivery protects against cardiovascular remodeling and apoptosis

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We investigated the potential roles of adrenomedullin (AM) in cardiovascular remodeling and apoptosis by somatic gene delivery. We showed that a single intravenously injection of the human AM gene induces a prolonged delay in blood pressure rise and reduction of left ventricular mass, cardiomyocyte diameter and interstitial fibrosis in several hypertenisve animal models. Local AM gene delivery significantly inhibited arterial thickening, promoted re-endothelialization in rat artery after balloon angioplasty. In a rat model of myocardial ischemia/reperfusion injury, local AM gene delivery significantly reduced myocardial infarction, occurrence of sustained ventricular fibrillation and apoptosis without affecting hemodynamics, and these protective effects were abolished by an AM antagonist, calcitonin gene-related peptide, CGRP(8-37). These AM's effects were accompanied by increased phospho-Akt and Bad and Bcl-2 levels, but reduced NAD(P)H oxidase activities, superoxide levels, p38 MAPK activation, Bax levels and caspase activities in the ischemic heart. In cultured cardiomyocytes, AM also attenuated apoptosis induced by hypoxia/reoxygenation, which was accompanied by increased phospho-GSK-3b, but reduced GSK-3 and caspase-3 activities. AM's effects on anti-apoptosis and increased cell viability were blocked by dominant-negative Akt but not by inhibitor of GSK-3b and caspase-3. These results indicate that AM attenuates cardiovascular remodeling and apoptosis by suppression of oxidative stress and activation of Akt signaling pathways. These findings provide new insights into the role of AM as an anti-oxidant in protection against cardiovascular dysfunction.

Abstract N^o S37E

Potential for targeting adrenomedullin mechanisms in heart failure

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Evidence suggests that adrenomedullin plays a role in pathophysiology of heart failure. Circulating concentrations of adrenomedullin are elevated in cardiovascular disease in proportion to severity of cardiac and hemodynamic impairment. Raised plasma adrenomedullin levels following acute cardiac injury and in heart failure provide prognostic information on adverse outcomes. Administration of adrenomedullin in experimental and human heart failure induces reductions in arterial pressure and cardiac filling pressures, and improves cardiac output, in association with inhibition of plasma aldosterone (despite increased renin release) and augments renal glomerular filtration and sodium excretion. Furthermore, adrenomedullin in combination with other therapies (angiotensin-converting enzyme inhibition and augmentation of the natriuretic peptides) results in hemodynamic and renal benefits greater than those achieved by the agents separately. Manipulation of the adrenomedullin system holds promise as a therapeutic strategy in cardiac disease.

Abstract N^o S38A

How to assemble myofibrils in the developing vertebrate heart and how to deal with them during cell division

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Development of contractile force in the heart depends on the assembly of sarcomeric proteins to para-crystalline structures, the myofibrils. This process was investigated in developing embryonic chick and mouse hearts by confocal microscopy of triple-stained whole mount preparations. All sarcomeric proteins investigated so far are already expressed long before the first periodic contractions occur and the assembly of the myofibrils happens within hours in the embryonic heart. No stress fibre-like structures, typically seen in cultured cardiomyocytes, have been observed during myofibrillogenesis in the embryonic heart. The first organised complexes that can be observed involve Z-disk components and occur in close association with the plasma membrane. A cytoskeletal framework seems to exist that consists of the Z-disk protein alpha-actinin, the M-band protein myomesin and the elastic titin filaments stretching in-between and is

essential for the integration of thin and thick filaments, respectively.

Embryonic heart growth is achieved by cell division. This is not performed by a stem-cell like population, but fully differentiated cardiomyocytes can enter the cell cycle, disassemble their myofibrils and go through mitosis including cytokinesis. Interestingly, disassembly of myofibrils happens in a biphasic fashion, with Z-disks and thin filaments being disassembled before M-bands and thick filaments. After cytokinesis rapid reassembly can be seen. This costly process might provide an additional explanation, why cardiomyocytes cease to divide after birth in vertebrates.

Abstract N° S38B

Myofibril inducing RNA (MIR) rescues mutant salamander heart by promoting myofibrillogenesis

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Recessive cardiac mutant gene c in axolotls (Ambystoma mexicanum) is a natural occurring genetic mutation that provides an excellent model for studying heart development and muscle gene regulation. Mutant embryonic hearts that lack tropomyosin and organized myofibrils and fail to beat can be rescued by organ-culturing with normal anterior endoderm, a known heart muscle inductor tissue, or the medium conditioned by normal anterior endoderm, or RNA molecules from a MIR (Myofibril Inducing RNA) gene originally deduced from the active RNA in endoderm-conditioned medium. The RNA from this gene is capable of promoting tropomyosin synthesis and myofibrillogenesis in mutant hearts. RT-PCR shows that MIR RNA is present in embryos at early stages. Two proteins (30 and 12 kDa) are known to interact with MIR RNA. We found a point mutation (G-- > T) in mutant RNA resulting in an altered protein binding pattern and a failure to promote tropomyosin synthesis and myofibril formation in mutant hearts. The genomic sequence was identified and indicates a putative promoter at the 5' end of the gene which contains a well conserved TATA box, Oct-binding sites, etc. on the positive strand and some tissue specific transcription factors' (MyoD, TEF-1) binding sites on the negative strand. These suggest there may exist a mechanism to control tissue-specific expression during embryogenesis in skeletal and cardiac myocytes. Testing the promoter using red fluorescence protein as a reporter gene in organ-cultured hearts has confirmed the driving ability of the promoter. Double stranded MIR RNA has been applied to the cultured normal whole embryonic hearts and proved to be effective in inhibiting a heart beat and myofibril formation, indicating the MIR gene is essential for myofibrillogenesis and heart development. (NIH HL58435, HL61246 and AHA Grants to LFL).

Abstract N° S38C

Role of a novel tropomyosin in vertebrate heart development

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Our long term objective is to understand the mechanisms of myofibrillogenesis in relation to cardiogenesis as well as to various cardiovascular disease such as Familial Hypertrophic Cardiomyopathy (FHC) and Dilated Cardiomyopathy (DCM). Tropomyosin (TM) is one of the five proteins comprising the thin filaments of sarcomeres. Various missense mutations in TM have been implicated in FHC & DCM. One of the limitations in TM research is the complexity of its isoform diversity. We have recently discovered a novel TM isoform, TPM1k, expressed predominantly in cardiac tissues. Due to the occurence of multiple isoforms of TM, it is not clearly understood how various missense mutations in *TPM1*, one of the four TM genes in vertebrates, affect cardiac but not skeletal muscles. We have found that the new isoform TPM1k of the *TPM1* gene is expressed mainly in cardiac tissues via alternate splicing. TPM1k contains exons 1a,2a (instead of 2b as in TPM1a, the known striated muscle specific isoform), 3,4,5,6b,7,8,& 9a/b. The Mexican axolotl (*Ambystoma mexicanum*) is very useful for studying the role of TPM1k in myofibrillogenesis in situ and in vivo. Some Mexican axolotl carry a genetic mutation in gene 'c'. Homozygous embryos (c/c) form hearts that are deficient in TM, lack organized myofibril, and fail to beat. The mutant hearts can be rescued with exogenous TM. We are exploring whether both TPM1a and TPM1k take part in cardiac myofibrillogenesis *in vivo* and play a role in cardiac disease such as FHC.

This work was supported by the American Heart Association.

Abstract N° S38D

Troponin I gene regulation during heart development

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Troponin I (TnI) gene family has been used as a model to study the regulation of gene expression during the differentiation and maturation of cardiac muscle tissues. Two major TnI genes are expressed in the mammalian heart under the control of a developmentally regulated program. Fetal TnI, which is identical to slow skeletal TnI (ssTnI), is expressed first and predominates throughout embryonic and fetal development. After birth, ssTnI is downregualted and eventually disappears in the adult heart. Meanwhile cardiac TnI (cTnI) is upregulated and becomes predominant in the adult heart.

TnI may become depleted in ischemic, infarct and failing hearts. TnI deficiency can result in diastolic dysfunction. We have investigated TnI isoform switching during heart development in normal mouse hearts and in cardiac TnI gene knockout hearts. The effects of thyroid hormone on TnI gene regulation have also been studied both in cultured cells and in whole animals. Very recently, the up-stream part of mouse ssTnI has been identified. Analysis of the sequence with a Genomatix software package has revealed several potential regulatory domains and binding sites. The results from transfection assays have indicated that conserved GA-rich sequences, an Oct binding site and a CCAAT box within the first 300 bp upstream of the transcription start site are critical for the gene expression. An inhibitory domain has been revealed within the sequence between – 1700 to – 1780. The inhibitory effect seems more significant in C2C12 Myoblast cells than that in CHO cells, suggesting that some inhibitory factors existed inside of muscle type cells. Our experimental results have enhanced our understanding of the mechanism underlying TnI gene regulation during heart development.

Abstract N° S38E

C-protein and cofilin in myofibril formation and maintenance

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Assembly of actin and myosin into filaments and their precise alignment into sarcomeric structures is a major process of myofibril formation. Myosin- and actin-binding proteins are deeply involved in this process. Cardiac myosin-binding protein C (MyBP-C), also known as C-protein, is one of the major myosin-binding proteins localizing at A-bands of myofibrils. MyBP-C has myosin- and connectin (titin)-binding domains in the C-terminal side of the molecule and actin- and myosin-binding domains in the N-terminal side. We found that two MyBP-C variants are generated from a single gene in both chicken and mice. An alternative spliced form of mouse cardiac MyBP-C, MyBP-C (+), includes extra 10 amino acids in the C-terminal connectin/titin-binding domain. This spliced form has a decreased binding affinity to myosin filaments and connectin/titin in vitro and does not localize to A-bands in cardiac myocytes. When MyBP-C (+) was expressed in chicken cardiac myocytes, sarcomere structures were disorganized, suggesting that it exerts dominant negative effects on sarcomeric organization. Of particular interest is that expression of MyBP-C (+) is scarcely detectable in ventricle through cardiac development, but its expression gradually increases in atria and becomes the dominant form after 6 m after birth. The age dependent MyBP-C (+) expression may lead to sarcomere disorganization partly at least and decreased heart activity in the aged animal. Cofilin is an actin-binding protein that plays a critical role in actin filament dynamics in a variety of cells. We observed that excess of cofilin in cardiac myocytes or myotubes leads to disruption of actin filaments followed by actin-cofilin rod formation in the cytoplasm. On the other hand, cofilin expression in cultured muscle cells was suppressed by applying an anti-sense method, bundles of actin filaments were formed and ordered assembly of actin into sarcomeric structures was significantly suppressed. These results indicate that cofilin plays a critical role for the regulated assembly of actin in the process of myofibril formation.

Abstract N° S39A

Group B coxsackievirus persistence in cardiac cells and heart via a novel deletional mechanism

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The group B coxsackieviruses are among the primary causes of acute inflammatory cardiomyopathies, conditions than often lead to dilated cardiomyopathy and heart failure. Enteroviral RNA can be detected in 20-25 % of cardiomyopathic hearts but infectious virus is isolable only from pediatric cases. In humans and in mouse models of cardiomyopathy, enteroviral RNA can persist for weeks to months in the absence of detectable cytopathic virus. We demonstrate that coxsackievirus B3 (CVB3) -inoculated primary mouse cardiomyocyte lysates or homogenized heart tissue from CVB3-inoculated mice do not induce cytopathic effects (CPE) in tissue culture, yet viral RNA is readily detected by RT-PCR in the cultures. Sequence analysis of cDNA derived from this RNA demonstrated variable deletions at the 5' end ranging from 7-49 nucleotides. These deletions have been cloned into an infectious CVB3 cDNA ; these produce progeny infectious virus that replicated very slowly and induced no detectable cytopathic effect in culture. Although normal enterovirus replication produces ≥ 40 fold more positive strand RNA than minus strand, these viruses display a positive/negative strand ratio of only 1.5/1. Inoculation of mice with the terminally deleted virus strains show viral RNA detectable in hearts days after inoculation, demonstrating that the deletions do not ablate infectivity in vivo. This evolution of a novel defective enterovirus quasispecies in vivo provides a mechanism by which to explain long-term viral persistence in human cardiomyopathies in the absence of cytopathic virus.

Abstract N° S39B

Receptors for enteroviruses : their role in pathogenesis and their normal function in the heart

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Viruses initiate infection by attaching to specific receptor molecules on the cell surface. Coxsackie B viruses and adenoviruses— the major agents of viral heart disease— both interact with a single protein, the Coxsackievirus and Adenovirus Receptor (CAR) ; attachment to CAR may explain viral tropism for cardiac muscle. I will discuss the role of CAR and other receptors in the pathogenesis of viral

infection, and present new evidence indicating that CAR is essential for normal cardiac development.

Abstract N° S39C

From expression profiling to biological validation in coxsackievirus infections : how far to leap, when, and where ?

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Coxsackievirus B3 (CVB3) is the primary causative agent of viral myocarditis, an inflammatory disease of heart muscle. We have utilized mRNA differential display, cDNA array and *Affymetrix* oligonucleotide arrays, in an integrative manner, to identify differential transcriptional events at viremic, inflammatory and reclamative stages in CVB3-infected hearts. Gene expression changes were investigated in the context of histological evidence of injury and dysfunction, the latter measured with 2D echocardiography. Gene expression profiles were selected for further validation at both mRNA and protein levels using semi-quantitative RT-PCR and immunohistochemistry, respectively. We observed acute increases in complement-related genes C2, C3, C4 and factor B (fB), and further that mice null for fB and C2, deficient in both classical and alternative complement cascades, have increased myocardial injury, inflammation and infectious virus particles. These investigations into the pathobiological role of complement pathways in viral myocarditis illustrate our strategy to gain biological understanding from high throughput transcriptional studies. Other notable differentially-regulated genes include host survival genes, peripheral-type benzodiazepine receptor and S100 proteins, and remodeling genes like muscle LIM protein, cathepsins and serine protease inhibitors serpins, just to name a few. We also utilized *Affymetrix* oligonucleotide arrays to identify genomic changes in cultured cells post-CVB3 infection. Such studies have led to a better understanding of the balance between mitochondria-mediated apoptosis signalling and pro-survival PI3K/Akt signalling triggered by virus infection. Thus, our expression profiling studies have guided us in novel directions, allowing us to better understand viral pathogenesis, organ injury and dysfunction in enteroviral heart disease.

Abstract N° S39D

Inflammation and therapeutic strategies for viral heart diseases

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Recent studies suggest that hepatitis C virus is involved in development of myocarditis and cardiomyopathies. Our preliminary study showed that interferon therapy was beneficial for the treatment of myocardial diseases associated with hepatitis C virus infection. Elevated levels of circulating cytokines have been reported in patients with myocarditis and cardiomyopathies, and various cytokines have been shown to depress myocardial contractility in vitro and in vivo. A transcription factor, NF-kB is activated by viral infections, and this activation, leads to the coordinated expression of cytokines, and the further amplification and perpetuation of the inflammatory response. NF-kB is therefore an obvious target for new types of anti-inflammatory treatment. We found that pimobendan and a new NF-kB inhibitor suppressed cytokine production, and prevented development of encephalomyocarditis virus (EMCV) myocarditis. We have shown that angiotensin II is increased, and NF-kB is activated in EMCV myocarditis, and that inflammatory responses and NF-kB activation are attenuated in angiotensin II type I receptor (AT1) knock-out mice, and in mice treated with an AT1 antagonist. We have also shown that mast cells play an important role in the pathogenesis of viral myocarditis, and an anti-allergic drug that stabilizes mast cells is useful for the treatment of viral myocarditis. Cytokine gene therapy which inhibits inflammatory response by viral IL-10 and IL-1ra using recently developed method of electroporation has been shown to be effective in EMCV myocartidis.

Abstract N° S39E

The Role of Ubiquitination in Viral Pathogenesis : A Therapeutic Opportunity ?

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Viral myocarditis and its main sequela, dilated cardiomyopathy, are the most prevalent causes of morbidity and mortality worldwide, particularly in children. Coxsackievirus B3 (CVB3) is the primary causative agent of viral myocarditis. The ubiquitin/proteasome pathway is a major intracellular protein degradation pathway in mammalian cells. In addition, this pathway has also been found to be involved in a variety of intracellular functions, including cell proliferation, cell death, and inflammatory responses, processes important in the progression of many diseases. We recently demonstrated that the ubiquitin/proteasome pathway plays a critical role in CVB3 replication. Cell culture experiments using HeLa cells and murine cardiomyocytes showed that inhibition of the ubiquitin/proteasome pathway decreased CVB3 viral RNA and protein levels, and inhibited CVB3 progeny release. These results strongly suggest that inhibition of the ubiquitin/proteasome pathway may represent a novel therapeutic approach against viral myocarditis. To explore the potential mechanisms by which the ubiquitin-proteasome pathway regulates viral replication, we further studied the impact of proteasome inhibitors on mitogen-activated protein kinase phosphatase (MKP-1) protein expression and extracellular signal-regulated kinase (ERK) phosphorylation. We have previously reported that the ERK signaling pathway is activated during CVB3 replication and activation

of ERK is required for CVB3 replication and contributes to virus-medicated pathogenesis. Recent studies have suggested that MKP-1, which dephosphorylates and inactivates ERK signaling, can be regulated post-translationally via the ubiquitin-proteasome pathway. We found that proteasome inhibition led to a loss of ERK phosphorylation in a dose-dependent manner, which is correlated with an induction of the MKP-1. Blockade of MKP induction using either MKP-1 anti-sense or MKP-1 short-interfering RNA attenuated the loss of ERK phosphorylation, and subsequently increased viral replication. Our results suggest that inhibition of the ERK signaling pathway contributes, as least in part, to proteasome inhibitor reduction of coxsackievirus replication. Data will also be presented on the role of proteasome inhibition in viral replication, in host protein degradation and in virus-mediated myocardial damages in a well-established murine myocarditis model. Taken together, our data ? ? ?

Abstract N^o S40A

Plasma potassium regulation by skeletal muscle

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The skeletal muscles contain the largest single pool of K^+ in the body (2600 mmol), 45x the K^+ content of the extracellular phase and 185x the K^+ contained in blood plasma. The action potentials eliciting muscle contractions cause a rapid release of K^+ from the muscle cells. During intense exercise, the K^+ leaking out of the working muscles is sufficient to double the K^+ concentration of arterial blood plasma within one min and to limit further contractile performance (Clausen, Physiol. Rev., 2003). The hyperkalemia is often followed by hypokalemia, and both events carry a risk for cardiac arrest. The net uptake of K^+ into skeletal muscle is mediated by the Na^+,K^+-pump. It can be calculated that if all Na^+,K^+-pumps in the skeletal muscles run full speed, all K^+ will be cleared from the extracellular phase in 28 s. Several factors promote Na^+,K^+-pump mediated uptake of K^+ into skeletal muscle (excitation, insulin, catecholamines, theophylline, calcitonin gene related peptide, amylin and IGF-I). Therapeutic use of insulin or β_2 agonists carries the risk of inducing hypokalemia. Hypokalemia may also arise during acute myocardial infarction, intense pain, hypoglycemia or sepsis, reflecting Na^+,K^+-pump stimulation induced by elevated plasma catecholamines. Conversely, inhibition of the Na^+,K^+-pumps as induced by digitalis intoxication leads to hyperkalemia. The clearance of K^+ from plasma depends on the content of functional Na^+,K^+-pumps in skeletal muscle. When the content of Na^+,K^+-pumps is upregulated by training, exercise-induced hyperkalemia is diminished. Conversely, when the Na^+,K^+-pump content is downregulated as in cardiac insufficiency, myotonic dystrophy and McArdle disease, exercise-induced hyperkalemia is more pronounced and may cause fatigue.

Abstract N^o S40B

Potassium handling during exercise

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Potassium is released from contracting skeletal muscle cells. As a consequence, muscle intracellular $[K^+]$ is reduced, accompanied by large $[K^+]$ increases in muscle interstitial fluid, and in venous and arterial blood. These changes in muscle are likely to be important factors in muscle fatigue. During heavy exercise, muscle interstitial $[K^+]$, measured by microdialysis, may rise more than 2-fold ; muscle-venous plasma $[K^+]$ may also double, whilst arterial plasma $[K^+]$ may reach 6 - 7 mM or even higher in some chronic diseases. These increases in circulating $[K^+]$ are regulated by numerous factors including muscle Na^+,K^+ pump content and activity, muscle blood flow, type of contraction (isometric versus dynamic), upper versus lower limb exercise, fibre composition of muscles recruited, hormonal responses, training status, health status and numerous medications. During exercise K^+ is continually released from contracting muscle, but cleared by non-contracting muscle. Hence total muscle mass may affect circulating $[K^+]$ during exercise. Thus in chronic disease, including heart failure, a loss of muscle mass may diminish the capacity to clear plasma K^+. Furthermore, muscle Na^+,K^+ pump content declines with chronic inactivity, and may exaggerate K^+ loss from contracting muscles. Together these result in an exaggerated rise in plasma $[K^+]$ during exercise, which is also magnified by digoxin or beta-blockers. The Na^+,K^+ pump maximal activity in muscle is also impaired with fatigue, which may further exacerbate K^+ loss from contracting muscles. This appears to be independent of training status, even though muscle Na^+,K^+ pump content is enhanced and plasma $[K^+]$ reduced by training. Thus K^+ disturbances in muscle are an important factor in fatigue and exercise limitation, especially in patients with chronic disease.

Abstract N^o S40C

Potassium handling during treatment of heart disease

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Total body K^+, and hence presumably intracellular K^+, are reduced in congestive heart failure (HF) while intracellular Na^+ levels are raised, at least in the heart. Since cellular ion regulation is pivotal in HF it is of interest how treatment affects membrane Na^+ and K^+ transport. Trials have addressed neurohumoral abnormalities in HF -abnormal angiotensin II, aldosterone, catecholamine, endothelin and natriuretic peptide levels. We examined how pharmacological intervention directed at neurohumoral abnormalities affects the sarcolemmal Na^+-K^+ pump by measuring electrogenic pump current (I_p) in myocytes isolated from rabbits. In vivo treatment of rabbits with the angiotensin converting enzyme (ACE) inhibitor captopril or the angiotensin receptor antago-

nist losartan caused an increase in I_p. The increase was abolished by *in vitro* exposure to angiotensin II. Infusion of aldosterone *via* osmotic minipumps to achieve serum levels similar to those seen in HF induced a decrease in I_p and an increase in intracellular Na^+ concentration. Abnormalities were abolished by co-treatment with an aldosterone antagonist. We examined the effect of exposing myocytes to atrial natriuretic peptide (ANP) *in vitro*. ANP in concentrations near the estimated normal interstitial concentration caused pump stimulation. Stimulation was lost with a higher concentration of ANP expected to be « seen » by myocytes in HF, particularly during treatment with neutral endopeptidase inhibitors. The loss of stimulation was mediated by activation of protein kinase A (PKA). We have not directly examined the effect of catecholamines on the pump. However, we have found that activation of PKA (a key messenger activated by beta adrenergic receptors) with cAMP causes pump inhibition. Finally, exposure of myocytes to endothelin causes pump stimulation. ACE inhibitors/angiotensin receptor antagonists, beta blockers and aldosterone antagonists have proven efficacy in HF while neutral endopeptidase inhibitors and endothelin antagonists have no effect or are harmful. Thus, there is a perfect correlation between clinical efficacy of treatment and effects on Na^+-K^+ pump activity.

Abstract N° S40D

In vivo assessment of the k homeostasis in hypo- and hyperkalemia in animals

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Myocardial and skeletal muscle Na,K-pump regulations have extensively been elucidated in various diseases and conditions. The physiological impact of these regulations is not necessarily obvious as a number of other - attenuating or enhancing - regulations may take place in parallel. An important effect of changes in Na,K-pump concentration and/or activity is alterations of the potassium (K) homeostasis. A straight forward interpretation of for example a reduction in skeletal muscle Na,K-ATPase concentration would be a reduction in skeletal muscle K uptake and a reduced tolerance to K exposure. However, in vivo this may not be the case. On this basis we developed an animal model to assess the K homeostasis in vivo using intravenous infusions of large KCl doses. During infusions plasma K is measured and immediately after the infusions are ceased skeletal muscle or myocardial tissues are harvested for ion measurements. Selective K depletion reduces skeletal muscle Na,K-ATPase and K and increases Na and Mg. K supplementation has the opposite effects. In the heart K depletion causes a minor decrease in K, and increases Na,K-ATPase and Mg concentration and show a tendency to an increase in Na. Again, K supplementation has the opposite effects. K depletion increases the tolerated K dose 2-4 fold, whereas K supplementation only induced a change after fasting, which reduces skeletal muscle K considerably in K supplemented animals. The increased K tolerance in K depleted animals was not the outcome of increased renal K excretion. Net skeletal muscle K uptake showed a significant positive correlation with pre-infusion skeletal muscle Na levels, whereas no significant correlation was observed between net K uptake and skeletal muscle Na,K-ATPase concentration. In the heart there was a negative correlation between net myocardial K uptake and pre-infusion K content as well as between K uptake and myocardial Na,K-ATPase concentration. In conclusion, physiological impact of changes in Na,K-ATPase concentration and/or activity is difficult to predict, and integrated assessments in *in vivo* studies – that takes parallel changes into account – seem warranted.

Abstract N° S40E

Potassium and sudden death

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Nihilism has prevailed regarding potassium (K) in clinical cardiology for the last decades. Moreover, whereas diuretics are prescribed to a large fraction of heart failure patients, K-supplementation is in some clinics given only relatively rarely. Recent research however throws new light on K-homeostasis. The myocardial Na,K-ATPase is of importance for K handling in the extracellular space (ECV) of the myocardium - moreover myocardial ECV-K is influenced by K coming by the blood stream. Here skeletal muscle Na,K-ATPase is of importance. Thus, maintaining K in myocardial ECV within safe limits requires optimum regulation of the Na,K-ATPase. Accordingly its dysregulation may cause severe local hyper- or hypokalemia. Moreover, during exercise K leaks out of muscular cells and is then pumped back by the Na,K-ATPase. Thus, plasma-K may within minutes increase to around 8 mmol/l during activity and decrease even below resting level during rest. Furthermore, these fluctuations are modified by Na,K-ATPase regulation by e.g. physical conditioning, disease and medicine. In diuretic treatment of human subjects plasma-K is maintained for some time by mobilising K from intracellular compartments - skeletal muscle Na,K-ATPase and K-content is reduced. Similarly muscle magnesium (Mg) may be reduced. In heart failure patients myocardial Na,K-ATPase is reduced. Thus, dysfunction of extrarenal K-homeostasis may *per se* cause arrhythmia. Moreover such dysfunction may disclose conditions that are usually well tolerated but becomes dangerous when exposed to hyper- or hypokalemia. Thus, in e.g. long QT-syndrome caused by mutations in genes coding for potassium-channels, exercise and hypokalemia may elicit syncope and sudden death. Recently, aldosterone-antagonist has been added to heart failure treatment showing significant reductions in mortality. Furthermore, it has been given to patients with long QT-syndrome. A part of the effect may be the outcome of improved extrarenal K-homeostasis. In conclusion, plasma-K and plasma-Mg may be normal despite K and Mg depletion. Resting plasma-K should be kept high. Plasma-K

is a dynamic parameter that should be assessed not only during rest but probably also during e.g. exercise. Major plasma-K shifts and dysfunction of the K- homeostasis *per se* as well as in combination with preexisting disease may cause arrhythmia, syncope and sudden death. Especially in situations with syncope or sudden death during exercise, a thorough evaluation of the K-homeostasis seems in demand. In the area of aldosterone-antagonist trails, a trial comparing the protective effect of optimum K-supplementation with that of aldosterone-antagonism is in demand. Time has come to fend off K nihilism in clinical practice.

Abstract N^o S40F

Potassium, ion channelopathies and genes in heart diseases

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The course of the action potential (AP) of the cardiomyocyte is the result of a well orchestrated combination of several ion channels conducting potassium, sodium and calcium currents. Deviations from normal function of these channels is the pathophysiological basis for diseases such as long – and short - QT syndromes, Brugada syndrome, progressive conduction disease, idiopathic ventricular fibrillation and other genetic arrhythmic syndromes as well as many cases of drug induced arrhythmia and AP changes seen in other cardiac diseases. The sequencing of the human genome has made it possible to identify the genes coding for the proteins constituting the ion channels and, thus, to associate some arrhythmias with specific genetic variants. Such arrhythmias are characterised by variable expressivity and penetrance and cardiac events may be rare. Potassium is an important regulator of ion channel function, as hypokalemia may cause both an increase in the electrochemical potassium gradient but also a paradoxical reduction in the funtion of – among others – the HERG channel conducting the rapid repolarising potassium current (Ikr) and thus a reduction of the repolarisation reserve. This mechanism may result in the clinical precipitation of arrhythmia in genetic arrhythmic syndromes as well as the occurrence of arrhythmia during drug intake. This calls for a careful control of extracellular potassium and potassium reserves in conditions prone to arrhythmia. There seems to be a link between the phenotypic presentation of genetic arrhythmic syndromes and potassium status and the recent finding of frequent genetic variants and polymorphisms in the ion channel genes *SCN5A* and *KCNE2* suggests that hypokalemia may also be of quantitative importance in sudden infant (and adult) death syndrome.

A table may be set in 8 point text, as follows :

Item	Size	Type	Remark(s)
Title	12pt	Bold	Capitals

Abstract N^o S42A

Metabolic remodelling in cardiac hypertrophy - a factor in heart failure ?

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Cardiac hypertrophy is an important yet independent risk factor in heart failure. The adaptations that occur in hypertrophy result in a cellular remodelling of the heart, and, in particular, in alterations in the profile of metabolism. Our hypothesis is that these changes in fuel selection and utilisation predispose the hypertrophied heart to injury and subsequent failure.

Using experimental models of hypertrophy (hypertension, uraemia and pressure overload), we have investigated the relative contribution of ^{13}C labelled substrates to oxidative fluxes in the intact heart with ^{13}C NMR spectroscopy. Hearts were perfused in the isovolumic mode, with a physiological mixture of substrates, including 0.3mM palmitate, 5mM glucose, 1mM lactate and 0.1mM pyruvate, and 100μU/ml insulin. In all cases, we observed a reduction in the myocardial energy reserve (phosphocreatine to ATP ratio). Furthermore, as the extent of hypertrophy increased, the heart demonstrated a growing reliance on glucose and carbohydrate metabolism, and a marked reduction in fatty acid oxidation. In addition, a depletion of essential cofactors (such as carnitine and creatine) and alterations in expression of metabolic enzymes and proteins were observed. Taken together, these factors may contribute to the impaired functioning of key energy generating steps. Our recent studies on isolated cardiomyocytes, using confocal microscopy, have indicated that exposure to high palmitate concentrations can modify the mitochondrial membrane potential, rendering the hypertrophied heart more susceptible to apoptosis and functional deterioration. Currently we are investigating strategies (such as carnitine supplementation) which can reverse or limit the metabolic remodelling in cardiac hypertrophy and may have the potential to delay or prevent the onset of heart failure.

Abstract N^o S42B

Metabolic phenotype of the hypertrophied heart

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Myocardium hypertrophies in response to a prolonged elevation in workload. The type of workload determines if the hypertrophy is adaptive or maladaptive, as demonstrated by functional outcome from a subsequent acute stress, such as ischemia-reperfusion. Hypertrophy due to exercise (physiologic hypertrophy) is cardioprotective, while that due to pathologic stimuli, such as hypertension (pathologic hypertrophy), is not. The pattern of substrate utilization (or metabolic phenotype) of the heart is recognized as a determinant of functional outcome after ischemia, so it is of interest to know if the metabolic phenotype differs between Pathologic and Physiologic Hypertrophy, both of which have dramatically different post-ischemic outcomes. Fatty acid oxidation

is impaired, glycolysis is accelerated, and the extent of glycolytically-derived pyruvate oxidation is reduced in Pathologic Hypertrophy. In contrast, fatty acid oxidation is increased, glycolysis reduced, and the extent of glycolytically derived pyruvate oxidation enhanced in Physiologic Hypertrophy. That the metabolic phenotype of Physiologic Hypertrophy resembles that produced by metabolic modulators, such as dichloroacetate or trimetazidine, both of which are cardioprotective in normal and pathologically hypertrophied hearts, supports that concept that alterations in metabolic phenotype contribute, at least in part, to the differing post-ischemic outcomes of Physiologic and Pathologic hypertrophy. Differences in expression of key proteins or enzymes that control myocardial substrate utilization do not account for the differing metabolic phenotypes of Pathologic and Physiologic Hypertrophy, indicating that other factors, such as differences in protein phosphorylation, metabolite control, or subcellular distribution, are responsible. A corollary of this latter finding is that change in the content of potentially relevant proteins or enzymes in a metabolic pathway is insufficient by itself to draw meaningful conclusions about metabolic flux in the intact organ and how it is controlled.

Abstract N^o S42C

Myocardial insulin resistance impairs mitochondrial function and the metabolic adaptation of the heart to pressure overload hypertrophy

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Individuals with type 2 diabetes are at greater risk of developing heart failure particularly when there is coexistent cardiac hypertrophy and myocardial ischemia. We have shown that impaired myocardial insulin signaling develops in the hearts of various mouse models of obesity, type 2 diabetes and the metabolic syndrome. Myocardial insulin resistance in these mice is associated with mitochondrial abnormalities that include reduced mitochondrial respiratory capacity and uncoupling of oxygen consumption and oxidative phosphorylation. To determine if myocardial insulin resistance was sufficient to account for the mitochondrial phenotypes observed in diabetic hearts, we generated transgenic mice with cardiomyocyte-restricted deletion of insulin receptors (CIRKO). These mice revealed an age-related decline in mitochondrial function. In young < 8-week-old CIRKO mice state 3 respiration with pyruvate as a substrate was reduced but respirations with a long-chain fatty acid (FA) substrate were increased. This change in substrate preference was associated with increased generation of reactive oxygen species, and mitochondrial uncoupling. Mitochondrial uncoupling and diminished ATP generation worsened with age and the ability of the mitochondria to metabolize fatty acids ultimately declined. Thus CIRKO mitochondria eventually develop reduced oxidative capacity for glucose and fatty acid substrates. When CIRKO mice were subjected to pressure overload hypertrophy by transverse aortic banding (TAB), CIRKO hearts developed an accelerated decline in cardiac function. Hypertrophied wild type (control) hearts maintained their function up to 4-weeks following TAB and this was associated with an increase in rates of glycolysis and glucose oxidation and increased expression of the GLUT1 glucose transporter. In contrast, glucose oxidation and glycolytic rates and GLUT1 expression declined in banded CIRKO hearts, and expression of uncoupling protein 2 increased. These results indicate that an intact myocardial insulin signaling pathway is required for the maintenance of mitochondrial integrity and for the metabolic adaptations that preserve myocardial function in the face of pressure overload hypertrophy.

Abstract N^o S42D

Potential role of the circadian clock in metabolic adaptation of the heart

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Circadian clocks are defined as a set of proteins that generate self-sustained transcriptional positive and negative feedback loops with a free-running period of 24 hours. These circadian clocks are intrinsic to the cell, and exist even when cells are isolated and cultured in vitro. These intracellular molecular mechanisms confer the selective advantage of anticipation, allowing the cell to synchronize responsiveness with diurnal variations in its environment. We have characterized the circadian clock within the rat heart, exposing an array of transcription factors whose expression fluctuates as much as 60-fold over the course of the day. The heart is exposed to dramatic diurnal variations in multiple environmental influences, including workload, sympathetic activity, hormonal stimulation, and substrate availability. In the latter case, fatty acids, the primary fuel utilized by the heart, oscillate approximately 2-fold within a 24 hour period. Previous studies show that a loss of synchronization between fatty acid availability and fatty acid oxidative (FAO) capacity results in intramyocellular accumulation of detrimental fatty acid derivatives, and subsequent contractile dysfunction (i.e. lipotoxicity). We therefore hypothesized that the circadian clock within the heart allows anticipation of periods of increased fatty acid availability by increasing FAO capacity. Our data suggest that the circadian clock allows the heart to anticipate periods of prolonged fasting, when the animal in the wild is unsuccessful in foraging for food.

Abstract N^o S42E

Inhibition of myocardial fatty acid oxidation for the treatment of heart failure

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The chronically failing heart is metabolically abnormal in both animal models and in patients. Little data are available on the rate of myocardial glucose, lactate and fatty acid metabolism and oxidation in heart failure patients, thus at present, it is not possible to draw definitive conclusions about cardiac substrate preference in the various stages and mani-

festations of heart failure. Normal cardiac function is dependent on a constant resynthesis of ATP by oxidative phosphorylation in the mitochondria. The healthy heart gets 60 – 90 % of its energy for oxidative phosphorylation from fatty acid oxidation, with the balance from lactate and glucose. There is some indication that in early stage compensated heart failure there is normal or accelerated lipid oxidation, and decreased glucose uptake and carbohydrate oxidation compared to healthy age-matched individuals, and that therapies that acutely switch the substrate of the heart away from fatty acids result in improvement in left ventricular function. On the other hand, in advanced late stage heart failure there is a clear downregulation of myocardial fatty acid oxidation and accelerated glucose oxidation. We recently found that that chronic treatment with inhibitors of carnitine palmitoyltransferase I (oxfencine) or long chain 3-ketoacylthiolase (trimetazidine) improves cardiac function and slow the progression of heart failure in dog and rat models of heart failure, respectively. Thus manipulation of myocardial substrate oxidation toward greater carbohydrate oxidation and less fatty acid oxidation may improve ventricular performance and slow the progression of clinical heart failure. At present, this intriguing hypothesis requires further evaluation.

Abstract N^{o} S43A

The effect of p38 MAP kinase on cardiac remodeling and dysfunction - upper or downer ?

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Development of heart failure involves pathological remodeling in myocardium, including changes in gene expression, interstitial fibrosis and loss of cardiac function. Activation of stress-activated protein kinase p38 has been implicated in the onset of heart failure. To investigate the functional role of p38 in cardiac remodeling, we established transgenic animals with targeted and temporally regulated activation p38 and knockout of p38a isoform in heart. Induction of p38 activity led to lethal restrictive cardiomyopathy in transgenic heart, associated with loss of contractility, induction of extracellular matrix content and regulatory genes. Treating the transgenic animals with p38 inhibitor SB239068 reversed remodeling process and improved contractile function and survival rate. At cellular level, activation of p38 leads to repressed contractility without affecting SR calcium cycling. Both in vitro and in vivo, p38 activity is both sufficient and necessary to induce inflammatory gene expression in myocytes. These data suggest that p38 activity is a critical signaling component in stress induced cardiac pathologies, including extracellular matrix remodeling and contractile dysfunction. The underlying mechanisms may involve inflammatory gene regulation and sarcomere modulation. Our observation implicates p38 MAP kinase as a potentially important therapeutic target for treating heart failure.

Abstract N^{o} S43B

P38 MAPK, a kind or kallous kinase (symposium) role in maliganant hypertension : appeaser, pleaser or bystander

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Chronic inflammatory processes contribute to the pathogenesis of atherosclerosis and represent a pathophysiologic link to its major risk factors ; diabetes, obesity, hypertension and dyslipidemia. Pro-inflammatory cytokines have been implicated in a variety of important cellular processes associated with vascular inflammation, i.e. cellular activation, adhesion, infiltration, dedifferentiation, apoptosis and dysfunction. Thus, suppression of pro-inflammatory cytokines in the vasculature has emerged as an attractive approach to the treatment of cardiovascular disease. In this regard, the inhibition p38 MAPK has been implicated as a compelling target because of its role in the elaboration of pro-inflammatory mediators as well as in the signal transduction of initiated by pro-inflammatory cytokines, mechanical stress, and reactive oxygen species (ROS). Numerous studies performed in a variety of pre-clinical cardiovascular models (hypertension, heart failure, restenosis and atherosclerosis) suggest that activation of vascular p38 MAPK is associated with endothelial dysfunction, ROS generation, adhesion, macrophage activation, hypertension and vascular remodeling. Chronic treatment with p38 MAPK inhibitors a elicit dose-related improvement in survival, endothelial function, renal function, blood pressure, stroke incidence, indices of vascular ROS and inflammation in hypertension induced by salt-sensitivity and NOS inhibition. In models of atherosclerosis, p38 MAPK inhibitors reduced the formation of atheroma and macrophage activity. Finally, p38 MAPK inhibitors reduce the enhanced cytokine and matrix metalloproteinase production observed in human carotid endarterectomy cultures. The results support the role of vascular inflammation in the pathogeneisis of atherosclerosis and attendant cardiovascular disease and strongly suggest that inhibition of the p38 MAPK signaling pathway is a valid pharmacological approach to treatment.

Abstract N^{o} S43C

p38-MAPK activation during myocardial ischaemia. slayer or redeemer

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The activation of p38-MAPK has been implicated in the injury that follows ischaemia. Furthermore in recent studies its activation has been associated with contractile dysfunc-

tion. However under other circumstances p38-MAPK activation has been associated with protection. We have investigated p38-MAPK under a variety of circumstances. During ischaemia our findings suggest that the phosphorylation of the T-G-Y activating motif is not by upstream kinases (MKK3/6) but is sensitive to the p38-MAPK catalytic-site inhibitor SB203580. This, together with other findings, is consistent with the notion that activation during ischaemia is by an autophosphorylation mechanism. These results contrast directly with the activation profile in response to TNF, which is dependent on MKK3. During low flow in the isolated perfused heart contractile dysfunction is accompanied by p38-MAPK activation. However, despite a contractile reserve, SB203580 does not improve contractile performance. Once again these findings differ to those seen with TNF. In the isolated heart perfused under constant pressure conditions TNF reduces coronary flow and contractile performance. These effects are attenuated by SB203580 or the absence of MKK3. Similar, though less marked, findings are seen under constant flow conditions and in isolated cardiac myocytes. In combination these results suggest that both the pattern and consequence of p38-MAPK activation may differ by circumstance. If understood this allows the possibility of circumstance specific inhibition of p38-MAPK to prevent the detrimental, but perhaps not the beneficial, consequences of p38-MAPK activation.

Abstract N^o S43D

P38MAPK – Role in myocardial mechanical and metabolic function : modulation or mirage ?

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The p38MAPK cascade is activated by various stresses, including ATP depletion, hypothermia-rewarming and ischemia-reperfusion. In order to assess the role of p38MAPK in stress-induced alterations in myocardial mechanical and metabolic function, we have studied the consequences of its activation in isolated working rat hearts perfused with Krebs solution containing palmitate (1.2 mM), glucose (11 mM) and insulin (100 mU/L).

Activation of p38MAPK (assessed by phospho-specific immunoblot analysis and MAPKAPK2 activity) in hearts that are subjected to cardioplegia (St Thomas' II, 3 ^oC), hypothermic storage (8 hr, 3 ^oC) and rewarming (10 min, 37 ^oC) is associated with an impaired recovery of mechanical function during a subsequent period of normothermic reperfusion. The p38MAPK inhibitor, SB202190 (10 μM), when present during normothermic reperfusion, greatly improves recovery of mechanical function. Inhibition of p38MAPK by SB202190 in reperfused hearts was confirmed by inhibition of MAPKAPK2 activation. Thus, p38MAPK activation worsens recovery of mechanical function of hearts following prolonged hypothermia.

As p38MAPK is activated by ischemia and ATP depletion, it may also act as an energy sensor that regulates substrate metabolism. Activation of p38MAPK by adenosine in hearts perfused aerobically following transient (2 x 10 min) ischemia is accompanied by a stimulation of AMPK phosphorylation and AMPK activity as well as an acceleration of glycolysis and H^+ production from glucose metabolism. This stimulation of p38MAPK and AMPK, which occurs in the absence of ischemia, did not affect rates of fatty acid oxidation or glucose uptake. The ability of either SB202190 (10 μM) or SB203580 (10 μM) to prevent the adenosine-induced increase in AMPK phosphorylation, as well as the downstream acceleration of glycolysis, suggests that p38MAPK lies upstream of AMPK in a signaling cascade that regulates myocardial glucose metabolism.

Abstract N^o S43E

Therapeutic potential of p38 mapk inhibition in cardiovascular disease

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p38 mitogen-activated protein kinase (MAPK) is activated in cardiac cells by a range of extracellular stimuli including ischemia, hemodynamic stress and neurohormonal factors such as angiotensin II, endothelin and phenylephrine. *In vitro,* p38 MAPK may regulate myocyte apoptosis and hypertrophy, inflammation and fibroblast proliferation.

Sustained p38 MAPK activation in the heart has been associated with LV remodeling and dysfunction arising from various etiologies in both humans and in animal models. Cardiac myocyte-specific activation of p38 in transgenic mice results in LV remodeling marked by interstitial fibrosis, myocyte hypertrophy, systolic and diastolic dysfunction and ultimately premature death. p38 MAPK activation is also implicated in hypertensive cardiac hypertrophy and end-organ damage in spontaneously-hypertensive stroke-prone rats maintained on a high salt/high fat diet.

Our group has demonstrated highly significant beneficial effects of the specific p38 MAPK a/b inhibitor RWJ 67657 on cardiac hemodynamics and echocardiographic parameters of LV remodeling following MI in the rat. These benefits on LV structure and function were associated with increased hypertrophy of myocytes in the peri-infarct and the non-infarct zones, and reduced myocardial collagen and a-smooth muscle actin (αSMA) immunoreactivity. In addition, RWJ 67657 protected cultured myocytes from apoptosis induced by hydrogen peroxide.

Taken together, these data support a key role for p38 MAPK in pathological cell signaling in these processes and its inhibition as a potential novel therapeutic strategy in these settings.

Abstract N^o S44A

Molecular mechanism of myocardial angiogenesis

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Angiogenic therapy for the human heart is currently being vigorously pursued. In the past ten years, alternative

revascularization/ angiogenesis strategies have, progressed from bench to bedside, focusing on the capillary sprouting and/or growth of new vessels to replace the old. The results of our study documented that ischemic preconditioning (IP) can possess angiogenic potential and can improve myocardial blood flow and cardiac function followed by severe ischemic myocardial injury. Therefore, myocardial adaptation to intermittent ischemia appears to be a highly promising approach to induce angiogenesis in a rat model of myocardial infarction as evidenced by increased capillary and arteriolar density. This increased micro vascular growth was found to be associated with a reduced infarct size and significant preservation of contractile functional reserve. Pharmacological cardiac stress testing with dobutamine revealed differences in the extent of cardiac contractile reserve between ischemic preconditioned (IPMI) and non-preconditioned (CMI) myocardium subjected to myocardial infarction (MI). The IPMI displayed significantly elevated contractile reserve compared to CMI. In the present study IP triggered significant expression of VEGF and increased perfused capillary density along with increased blood flow. In summary, we observed significant improvements in regional myocardial function along with increased capillary and arteriolar density following induction of survival factors VEGF, Bcl_2 and survivin in the setting of fully established chronic rat myocardial infarction model subjected to ischemic preconditioning. Again, transient suppression of Src activity within several days following MI might reduce ischemia induced heart injury and prevent long-term myocardial damage without disrupting VEGF-mediated revascularization. Thus, co-administration of or sequential gene therapy with VEGF, Bcl_2 and survivin might prove beneficial to enhance myocardial collateral blood vessel function and may represent a new approach to the treatment of cardiovascular disease.

Abstract N° S44B

Endothelial progenitor cells for vascular regeneration

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The regenerative potential of stem cells has recently been under intense investigation. In vitro, stem and progenitor cells possess the capability of self-renewal and differentiation into organ-specific cell types. In vivo, transplantation of these cells may reconstitute organ systems, as shown in animal models of diseases. In contrast, differentiated cells do not exhibit such characteristics. Human endothelial progenitor cells (EPCs) have been isolated from the peripheral blood of adult individuals, expanded in-vitro and committed into an endothelial lineage in culture. The transplantation of these human EPCs has been shown to facilitate successful salvage of limb vasculature and perfusion in athymic nude mice with severe hindlimb ischemia, while differentiated endothelial cells (human microvascular endothelial cells) failed to accomplish limb-saving neovascularization.

Future studies will clarify the mechanisms and circumstances that may be responsible for modulating the contribution of vasculogenesis to postnatal neovascularization. Specifically in this regard, it is intriguing to consider the possibility that certain angiogenic growth factors which are acknowledged to promote both angiogenesis and vasculogenesis in the embryo, but have been assumed to promote neovascularization exclusively by angiogenesis in the adult, may in fact promote migration, proliferation, and mobilization of EPCs from BM. The possibility that modulation of vasculogenesis can be used therapeutically to augment as well as inhibit neovascularization deserves further investigation.

Abstract N° S44C

Cell transplantation to improve heart function : cells or matrix ?

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Current attempts to regenerate the damaged myocardium after myocardial infarction have focused on therapies directed at increasing regional perfusion and salvaging viable cardiomyocytes. Accumulating evidence suggests that implantation of healthy muscle cells into the damaged myocardium can prevent infarct thinning and chamber dilatation. Cell transplantation has been suggested to encourage the recruitment of stem cells from the bone marrow or from the heart to repopulate the infarct region. These neo-myogenic cells in the myocardial scar tissue prevent ventricular dilatation and delay the onset of cardiac dysfunction. Early clinical trials suggest encouraging results for cellular cardiomyoplasty. Although the beneficial effects of cell therapy for myocardial regeneration after an infarction have led to phase I clinical trials, the mechanism of benefit of this novel therapy has not been elucidated. Stimulating neo-vessel formation and engraftment of muscle cells within the scar may contribute to the enhanced regional and global function. However, the number of the cells, which survived implantation, is too small to account for the functional benefit. The number of surviving cells may not be enough to replace the cells lost during the infarction. An alternate explanation for the benefit of cell transplantation may be the effect of the engrafted cells on matrix remodeling. The extracellular matrix plays an important role in the response to the infarction. Disruption of the matrix network may contribute to the apoptosis of cardiomyocytes leading to chamber dilation. Cell transplantation may prevent infarct thinning and cardiac dilatation by altering the matrix response to infarction. We implanted smooth muscle cells into the heart and found that the cells survived and altered matrix remodeling both within and remote from the region of implantation. Matrix metalloproteinase activity decreased in the transplanted group as compared to a control group. The matrix structure was maintained and ventricular dilatation was prevented. These data suggest that implanted cells prevented ventricular dilatation through an alteration of matrix metabolism, which is a possible mechanism by which cell transplantation improves heart function.

Abstract N° S44D

Arteriogenesis – Haemodynamic and Cellular Aspects

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Growth of collateral blood vessels is potentially able to preserve structure and a variable degree of function in subtended tissues in the presence of arterial occlusions. The process of transformation of a small arteriole into a much larger conductance artery is called arteriogenesis. Physical forces are important triggers of arteriogenesis : Fluid shear stress on the collateral endothelium markedly increases when due to arterial occlusion a pressure gradient develops between the proximal and the distal end of a pre-existing interconnecting anastomosis. This potentially activates endothelial cells, reflected in the release of cytokines and expression of adhesion molecules, and leads to monocyte adhesion and infiltration. We could demonstrate that arteriogenesis is enhanced by local delivery of the monocyte chemoattractant MCP-1 and the MCP-1-CCR2-pathway plays an essential role during the early phase of arteriogenesis. Within the perivascular space, monocytes subsequently differentiate into macrophages with the production of growth factors and proteases. Thereby, the second phase of arteriogenesis is initiated which is stamped by induction of cell proliferation and remodelling processes.The role of stem or progenitor cells for arteriogenesis is currently under intensive investigation. Increasing evidence is gained showing that the paracrine function of bone marrow-derived cells like release of arteriogenic growth factors, chemokines and proteases is a pre-requisite for arteriogensis rather than their incorporation into structures of the growing collateral vessel.

Abstract N° S44E

Regulation of coronary vascular branching : role of FGF2 and synectin

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Fibroblast growth factors (FGF) have long been considered as key regulators of vascular branching. However, the molecular mechanism of FGF1 and FGF2-dependent regulation of branching are not well understood. Previously we have demonstrated that syndecan-4 plays a major role in FGf2 signaling. Synectin was isolated as a cytoplasmic partner of syndecan-4 using yeast two hybrid screening. To elucidate function of synectin (PDZ2 scaffold protein) in vivo, we have generated synectin$^{-/-}$ mice. The synectin$^{-/-}$ mice are viable, but are significantly smaller than wild type controls (Table). Coronary angiography demonstrated reduced branching of epicardial coronary arteries in the synectin$^{-/-}$ mice compared to wild type (WT). To further address the issues of intramural arterial branching, ventricular cross-sections from the WT and synectin$^{-/-}$ mice were stained with anti-alpha-smooth muscle actin antibody. The volume (V_v) and surface (S_v) density of total alpha smooth muscle actin positive profiles were measured per whole cross sections of left ventricle. In agreement with angiography, quantification of vascular density parameters demonstrated a significant reduction in vascular density in synectin$^{-/-}$ mice. To assess the impact of this reduced vascularity on myocardial function, left ventricular (LV) fractional shortening was examined using 2-D echocardiography. We observed a significant reduction in LV function at rest. In conclusion, synectin gene disruption results in reduced formation of myocardial coronary arteries, decreased heart size and impaired left ventricular function. These results suggest that synectin could play an important role in arterial branching.

	synectin$^{-/-}$	WT	Pvalue
Body weight (g)	16.7 ± 1.3	19.9 ± 0.5	0.03
Heart/body wt ratio	0.41 ± 0.05	0.53 ± 0.02	0.03
V_v (%)	0.57 ± 0.02	0.87 ± 0.06	0.01
S_v (%)	0.13 ± 0.01	0.17 ± 0.003	0.01
Fractional shortening (%)	56.8 ± 4.2	66.9 ± 1.8	0.04

Abstract N° S45A

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Abstract N° S45B

The importance of metabolic substrates and antioxidants in myocardial protection during cardiac surgery

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Myocardial energy production is a major determinant of the outcome of cardiac surgery. Cardiac surgery involves multiple stresses to the myocardium that impair energy production : ischaemia/reperfusion, hypoxia/re-oxygenation (oxidative stress) and aerobic (oxygen-demanding) stress. These are commonly exacerbated by preoperative myocardial impairment due to heart failure, infarction and old age. Myocardial energy metabolism is enhanced by substrates given at the time of surgery, aspartate, glutamate, TCA cycle intermediates etc. A promising form of energy enhancement is myocardial metabolic conditioning given preoperatively. We and others have shown that energy production can be enhanced and oxidative stress reduced, particularly at a mitochondrial level and in the elderly myocardium, by preoperative use of coenzyme Q_{10}, orotic acid and glucose insulin potassium. Further reductions in oxidative stress can be achieved by intraoperative leukocyte depletion. We have recently combined in the preoperative period, metabolic and antioxidant-mediated conditioning with holistic therapy using physical exercise and mental stress reduction. These measures can be augmented by postoperative use of glucose-insulin-potassium. Such simple and inexpensive multimodality approaches can markedly enhance myocardial energy metabolism and improve postoperative recovery particularly in the elderly and energy- depleted myocardium.

Abstract N° S45C

The Vascular endothelium as a therapeutic target for surgical myocardial protection

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The coronary vascular endothelium is a major player in regulation of blood flow, blood pressure, coagulation and the inflammatory response. The pathogenesis of myocardial infarction involves a well-scripted response by inflammatory cells interacting with endothelium « primed » by ischemia and activated by reperfusion, via the various mediators generated by this interaction, such as oxidants, pro-inflammatory cytokines and proteases. This inflammatory response to myocardial ischemia and reperfusion involving the endothelium is exacerbated by extracorporeal circulation used in coronary artery bypass surgery. Both regional ischemia and global ischemia during cardioplegic arrest damage the endothelium ; this damage is not avoided by use of cardioplegia solutions without specific vasculoprotective additives. Endothelium-specific, as well as broader spectrum anti-inflammatory, therapeutic strategies that attenuate this endothelial damage have been developed for cardiac surgery. Therapy such as adenosine, nitric oxide and its precursors, inhibitors of adhesion molecules, and inhibitors of endothelin-1 have proven of benefit in reducing the generalized inflammatory response to on-pump cardiac surgery and the associated pathogenesis of myocardial infarction and apoptosis after acute ischemia. Promising therapies to attenuate endothelial dysfunction include insulin, cytokine inhibitors (TNFα inhibition), inhibitors of transcription factors, neutrophil inhibitors, and protease inhibitors. These agents can be delivered either systemically or selectively to the heart in the cardioplegic solution used to arrest the heart. Therefore, the endothelium is far from being a pedestrian « cellophane » wrapper for the myocardial vasculature, but its central role in the pathogenesis of ischemia-reperfusion injury during cardiac surgery offers the opportunity to target a central mechanism of injury. Therefore, the cardioprotective armamentarium and cardioplegic solution formulation should incorporate strategies to specifically protect the vascular endothelium and inhibit its contributions to the inflammatory/oxidant response to surgical ischemia-reperfusion injury.

Abstract N° S45D

Mitochondria as a target for myocardial protection

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Reperfusion of the ischemic heart causes opening of a non-specific pore in the inner mitochondrial membrane, known as the permeability transition pore (MPTP). This causes mitochondria to become uncoupled and capable of hydrolysing rather than synthesising ATP. Unrestrained, this will lead to loss of ionic homeostasis and ultimately necrotic cell death. Thus MPTP opening probably represents a critical phase in the transition between reversible and irreversible reperfusion injury. In support of this, we have demomstrated that functional recovery of the Langendorff-perfused heart from ischaemia inversely correlates with the extent of MPTP opening. Furthermore, hearts are protected from reperfusion injury by inhibiting the MPTP, either directly using the specific inhibitors Cyclosporin A and Sanglifehrin A, or indirectly by decreasing reactive oxygen species (ROS) and calcium loading (key inducers of pore opening) or by lowering pH_i. Pyruvate and propofol protect in this way and have been shown to be effective in both the Langendorff and working rat heart and in a pig model of reperfusion injury that closely parallels routine open heart surgery. We have shown that ischemic preconditioning (IPC) also leads to inhibition of the MPTP, but this effect is indirect and prob-

ably secondary to changes in calcium loading and ROS production. Mitochondrial K_{ATP} channels (mitoK_{ATP}) have been implicated in IPC, but our data suggest that the channel openers and blockers routinelu used to support this hypothesis, such as diazoxide and 5-hyroxy-decanoate, act through other mechanisms independent of the putative mitoK_{ATP}.

Acknowledgements. – The numerous contributions of many colleagues and financial support from the British Heart Foundation are gratefully acknowledged.

Abstract N° S45E

The endogenous cell defense : its possible role in myocardial protection during cardiac surgery

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In experimental studies ischemic preconditioning inhibits all aspects of ischemia-reperfusion injury : necrosis, apoptosis, cardiomyocyte and endothelial stunning, and arrhythmias. In cardiac surgery preconditioning by short cycles of clamping and declamping the aorta before cardioplegic arrest increase cardiac index and reduce release of biochemical markers of cardiomyocyte injury during the first 24 postoperative hours. Postoperative arrhythmias were also significantly reduced. Unfortunately repeated clamping and declamping of the ascending aorta is not acceptable in routine cardiac surgery due to the risk of atherosclerotic embolization.

We have recently presented data that isolated heart from rats and mice were preconditioned by the animals breathing 100 % oxygen for 30-60 minutes before heart harvesting. The precondition was caused by oxidative stress and was as powerful as ischemic preconditioning. Oxygen-induced myocardial protection was dependent on the transcription factor Nuclear factor kappa B, and it may easily be applied to patients, but so far there are no such data.

A year ago the first paper appeared showing that short episodes of ischemia and reperfusion in the first minutes of reperfusion *after* a sustained ischemic injury, postconditioning, was able to reduce the injury caused by ischemia and reperfusion. Postconditioning reduced infarct size probably by reducing oxidative injury. We have recently shown that postconditioning is able to convert persistent ventricular fibrillation during reperfusion into regular beating.

If a pharmacological increase of the endogenous celle defense is used, this is expected not only to protect the heart, but may also cause a « whole body protection ». It is highly unlikely that the exploitation of the endogenous cell defense will not be a routine part of organ protection in the not so far future.

Abstract N° S46A

New insights into NHE regulation and function

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The cardiac Na^+/H^+ exchanger (NHE) is a sarcolemmal protein encoded by the NHE1 isoform of the multi-gene NHE family. Based largely on experiments with selective pharmacological inhibitors, NHE1 activity has been identified as a causal or permissive factor in the inotropic and growth responses of myocardium to neurohormonal and mechanical stimuli, and in the development of myocardial injury during ischemia and reperfusion and myocardial hypertrophy and remodeling during hemodynamic overload. Data from recent clinical trials with cariporide, a selective NHE1 inhibitor, support an important role for NHE1 activity in myocardial injury during ischemia and reperfusion in humans ; however, serious adverse effects preclude therapeutic application of the treatment modality that was tested. Improved understanding of the molecular signaling mechanisms that regulate NHE1 activity in healthy and diseased myocardium may lead to the development of new approaches to its therapeutic manipulation. Recent studies in isolated myocytes indicate that extracellular signal regulated kinases 1 and 2 (ERK1/2) and their downstream effector, the 90 kDa ribosomal S6 kinase ($p90^{RSK}$), play key roles in mediating increased sarcolemmal NHE activity in response to diverse stimuli, such as prolonged intracellular acidosis, stimulation of G protein-coupled receptors, and oxidative stress. Investigations are ongoing to determine the molecular mechanism(s) of this regulation, including the roles of direct phosphorylation of the NHE1 regulatory domain at multiple sites and altered interaction with accessory proteins.

Abstract N° S46B

NHE in diabetes

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The cardiac sarcolemmal Na^+-H^+ exchanger (isoform-1 of NHE) has recently gained considerable interest in the context of myocardial damage. Previous studies have shown that the streptozotocin-induced model of type-1 diabetes in rats is associated with a decrease in NHE activity. However, type-2 diabetes comprises the largest group of diabetic patients since it accounts for > 90 % of all cases of diabetes. This led us to examine the activity of NHE in ventricular myocytes from hearts of GK (Goto-Kakizaki) rats which phenotype exhibits several typical features of metabolic, hormonal and vascular disorders usually described in human type-2 diabetes. Doppler echocardiography was used to assess GK-diabetes alterations in cardiac phenotype in vivo. The main results were a significantly increased LV mass and LV dilation. Myocytes isolated from GK rat left ventricles (LV), but not from right ventricles, exhibited significantly greater sarcolemmal NHE activity (+ 100 %) than LV myocytes isolated from control rats. This was not associated with increased NHE1 protein expression but with upregulation of the exchanger. In particular, this appeared to be mediated via the MAPK(ERK)-dependent pathway since ERK phosphorylation was markedly increased in GK LV myocytes with greater sarcolemmal NHE activity, an effect that was abolished by the ERK inhibitor U0126, as was abolished the

increase in NHE activity. However, an increase in basal $[Ca^{2+}]i$, as assessed by INDO measurements, may also contribute to enhanced NHE activity. Altogether, our results suggest a relationship between NHE activity and the development of the hypertrophic process in LV myocardium of type-2 diabetic GK rats.

Abstract N° S46C

Role of NHE in myocardial remodelling and heart failure

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Increasing evidence implicates NHE-1 as a candidate for targeted intervention to attenuate the remodelling and hypertrophic processes contributing to heart failure (HF). NHE-1 inhibitors including cariporide and EMD 87580 reduce cardiomyocyte hypertrophy induced by various factors and attenuate HF in vivo. With respect to the latter, this effect has been shown to occur in various models of heart failure including experimental myocardial infarction, hypertension as well as in heart failure-prone transgenic mice overexpressing Beta1 adrenergic receptors. Moreover, inhibiting NHE-1 reverses the myocardial remodelling and HF processes when treatment has been delayed by up to 6 weeks. The beneficial effects of NHE-1 inhibition occurs independently of either blood pressure lowering or infarct size reduction. The cellular mechanisms for NHE-1 involvement remain to be elucidated but it appears that NHE-1 is a downstream mediator for numerous hypertrophic factors including angiotensin II, endothelin-1, aldosterone and alpha1 adrenoceptor agonists and indeed NHE-1 blockade attenuates the hypertrophic effect of these agents. Thus, NHE-1 inhibition represents a potentially effective approach for reducing remodelling and treating HF.

Abstract N° S46D

Clinical trials with nhe inhibitors : where are we now and where are we going ?

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Since there is extensive preclinical evidence that sodium-hydrogen exchange (NHE) inhibition is cardioprotective, the purpose of this review is to examine the clinical findings to date and develop an understanding regarding future directions. In an early study, Rupprecht et al randomized 100 patients to receive placebo or an NHE inhibitor (cariporide) prior to reperfusion therapy for acute myocardial infarction (AMI). The findings suggested that NHE inhibition might result in enhanced functional recovery. In the first stage of the ESCAMI trial, 433 patients were randomized to placebo or low, intermediate, or high doses of eniporide prior to reperfusion therapy for AMI. In the second stage, 978 additional patients were enrolled. While stage one results were encouraging, the stage two results failed to demonstrate a reduction in infarct size or improved clinical outcomes. In the GUARDIAN trial, 11,590 patients undergoing high-risk percutaneous coronary intervention and coronary artery bypass grafting (CABG) surgery were randomized to receive a placebo or one of three doses of cariporide. While GUARDIAN failed to demonstrate an overall reduction in MI/death, a subgroup analysis showed a 25 % relative risk reduction (RRR) at 36 days in patients undergoing CABG surgery ($P = 0.027$). In the EXPEDITION trial, 5761 patients were randomized to receive cariporide or placebo. At 5 days, the results showed an 18.3 % RRR in MI/death ($P = 0.0003$) and a 23.8 % RRR in nonfatal MI ($P = 0.000005$). This finding was offset by the observation of an imbalance in mortality that favored cariporide (2.2 % versus 1.5 %, $P = 0.028$). Future studies need to be directed towards whether it is possible to dissociate the adverse effects from the beneficial effects of cariporide and whether the adverse effects associated with cariporide treatment is dose related, unique to cariporide, or a manifestation of NHE inhibitors in general.

Abstract N° S47B

The context of cvd among indigenous australians

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CVD is the major cause of death of adults in Australia and the biggest cause of deaths and of excess deaths for the Aboriginal population of Australia. Age-adjusted CVD death rates in Aboriginal people are almost 3 times as high as in the non-Aboriginal population and age specific mortality differentials are much greater between the ages of 25-54, where Aboriginal deaths rates are 7-12 times that of non-Aboriginals. Most developed countries have witnessed dramatic declines in ASDR's from CVD. Yet these improvements have not been witnessed equally among all population groups. Transition from traditional to 'Western' lifestyles are an important antecedent to an increase in prevalence of conventional risk factors, and subsequent high rates of CVD, renal impairment, impaired glucose tolerance and diabetes among a number of Indigenous groups globally. Conventional risk factors have an important role to play in explaining differentials in CVD burdens. There is also interest in a growing list of 'novel' risk factors (including homocysteine and C-reactive protein) which have been shown to be at high levels in a range of Aboriginal communities. The contribution of these factors to the burden of CVD in Indigenous Australians is yet to be demonstrated. There are many barriers to care for Aboriginal clients with established CVD. Access to specialists, diagnostics and acute care is limited in remote/regional areas where large Aboriginal groups reside. Even when facilities are available, Aboriginal people are less likely than non-Aboriginal people to receive cardiac procedures. Aboriginal Australians utilise a range of health care services differently, dominated by publicly provided services, with under-utilization of Pharmaceutical, Medicare, specialist and GP consultations. Numerous studies have also confirmed that 'psychosocial stress' has important associations with CHD. The determinants of health among Aboriginal Australians include poor housing, income inequality,

poverty, low levels of education, poor environmental conditions, high levels of stress and social dysfunction. Further, the loss of land, culture, language and identity, racism, premature death, grieving, pain and loss have long been highlighted as fundamental causes of ill health. Through what bio-psycho-social pathways these factors incur risk of CHD and whether they contribute or account for the excess burden of CVD among Indigenous population groups remains to be qualified or quantified. Understanding the complex interactive hierarchy of CVD aetiology and the large and expanding CVD differentials experienced by Aboriginal Australians stands as a critical target for future research, from the cell to the community, and for clinical, public health, and health service policy development.

Abstract N° S47C
Cardiomyopathies in the tropics
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Cardiovascular diseases (CVDs) prevalent commonly in the tropics are no different from those in the non-tropical regions. Nevertheless, there are CVDs, which are almost exclusively seen in the tropical countries. Cardiomyopathies among them offer diagnostic and therapeutic challenges to the inexperienced. Their pathogenic mechanisms are elusive and for several of them causal factors are enigmatic. Differences in the diet, lack of nutritional essential elements, traditionally used local medicines, poisonous plants, parasites and viruses endemic in tropical environs, have all been incriminated as causes for the difference in the epidemiologic pattern of cardiomyopathies. Technological advances and availability of powerful diagnostic techniques in the last five decades have led to a better understanding of the clinical features and natural history of the tropical cardiomyopathies. Causes and pathogenic mechanisms of some of them have been identified. Much is now known about the immunopathogenesis and genetic susceptibility in Chaga's cardiomyopathy secondary to Trypanasoma Cruzi infection. Endomyocardial fibrosis (EMF) has been clinically well characterized and extensively investigated. Investigators in Kerala have linked its causation to geochemical factors, specifically higher content of cerium, a lanthanide in the monazite sands of coastal regions in Kerala. While divergent views on causation of EMF persist, a common mechanism has been proposed for pathogenesis of the lesions in the disease ; endocardial endothelial injury leads to subendocardial fibroblast activation and collagen accumulation. In the next decade, it is hoped that specific genetic and molecular basis of cardiomyopathies in the tropics would be defined leading to definitive information on causation and mechanisms.

Abstract N° S47D
Alternative treatments for ventricular tachyarrhythmia in developing countries
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It is estimated than 50 % of the cardiac deaths are sudden, and most result from ventricular tachyarrhythmia (VT). Implantable cardioverter defibrillator (ICD) has improved in both primary and secondary prevention trails ; it still remains a costly and unaffordable modality for most of the needy patients in developing countries. Optimized drug therapy and radiofrequency catheter ablation could offer cost effective alternative to ICD in developing countries. Currently the conventional catheter ablation technique is mostly adjunctive to drugs and ICD. Refinements in ablation techniques have extended the indications of catheter-based ablation of VT, especially with the use of CARTO and ENSITE. These techniques are still evolving, and a combination of drugs and radiofrequency ablation may prove to be an affordable alternative. The short-term experience of combined RFA using CARTO and drugs in 10 such cases will be presented

Abstract N° S47E
TTK-Chitra prosthetic heart valve for mitral replacement
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Rheumatic heart disease is still a major cause for mitral valve replacement in India and other developing countries. In 1981, it was estimated that 1.2 million children were at risk of acquiring rheumatic disease in India – mostly from the low socio-economic groups.

A project was initiated in the late 70's for the development of a low cost Indian valve ; at a time when little expertise and facilities for biomedical research existed in the country. Just like others, this project group had to go through a series of 3 failures, before a successful valve could be put into clinical use. The tilting disc TTK-Chitra Heart Valve is made of an integrally machined cobalt alloy cage, an ultra-high molecular weight polyethylene disc occluder and a polyester sewing ring. The development included extensive in-vitro and in-vivo preclinical evaluation in a sheep model. Between December 1990 and January 1995, 205 patients underwent isolated mitral valve replacement in 6 institutions. 191 patients were followed till September 1998 for a total of 767 patient years. Twelve patients (1.6 %/py) developed valve thrombosis, while embolic episodes occurred in 18 cases (2.4 %/py). Other complications like bleeding, infective endocarditis, paravalvular leak or structural dysfunction were negligible or absent. Actuarial survival rates at 7 years and freedom from the valve related complications were comparable to other tilting disc models. Current clinical results indicate favourable performance even in poorly anti-coagulated patient groups ; possibly as a result of it's unique features like low impact forces and absence of cavitation.

Abstract N° S47F
Cardiovascular system in a problem-based curriculum at the arabian gulf university
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The College of Medicine and Medical Sciences at the Arabian Gulf University (AGU) adopted a problem-based learning (PBL), student-centered curriculum since its establishment in 1982. The cardiovascular system (CVS) is studied, together with the respiratory system (Unit II) during the pre-clerkship phase. Teaching and learning are carried out through an integrated organ-system process with the faculty members acting as facilitators to small group students. The health problems cover the general themes such as function of the heart as a pump including valves, development of the heart and great vessels, coronary circulation, cardiac electrical impulse generation and conduction and hemodynamics. The Unit committee chooses the appropriate problems that address not only the above themes/objectives but also provide the basis for important pathological processes and pharmacological principles of treatment. The students learn laboratory and professional skills as well as community medicine such as burden of illness, risk factors, screening and health education. Emphasis is given in students' assessment to critical thinking, reasoning and problem solving. Since monitoring and evaluation of a curriculum is considered integral to fine-tuning and improvement of an educational system, students' perception of teaching cardiovascular system is continuously sought in order to identify where improvements can be made. While some gaps are already identified, it is generally believed that problem-based approach practised at AGU is considered an interesting method to study cardiovascular system.

Abstract N° S48A

Exercise training for heart failure patients : factors affecting mortality and morbidity and predicted response to exercise training

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Exercise training improves functional capacity and quality of life in CHF patients. Uncertainty about a survival benefit, safety and availability of resources have prevented optimal use of exercise therapy in this patients group.

Aims. – 1. Identify optimal form of delivery ; 2. Define survival benefit. 3. Examine safety and efficacy. 4. Identify sub-groups that benefit most. 5. Predict, from baseline data, which patients will benefit, thus preventing unnecessary patient risk/discomfort and wasted resources.

Methods. – 1. A systematic review of 81 CHF exercise training studies up to August 2003 was conducted. 2. Data added from Piepoli, BMJ Dec 2003 of individual patient data from 9 RCT studies. 3. A 16 week mechanistic study of exercise training in CHF patients (EF < 35 %) was conducted.

Results. – The review demonstrated mean increment in Peak VO_2 of 17 %. Both the review and analysis reported > 80 % male subjects, predominantly middle aged \\ 126\\60 yrs, LVEF\\ 126\\27 % and approximately 60 % ischemic. Only the meta-analysis was sufficiently precise to produce a significant survival effect and a reduction in composite end-points of deaths (p = 0.015, 95 % CI 0.46-0.92) and hospitalizations (p = 0.011, 95 % CI 0.56-0.93), although the review also suggests a survival benefit. There was a hint that sub-groups such as age > 60yrs, LVEF < 27 %, ischemic, Peak VO2 < 15ml/kg/min, NYHA Class III/IV and exercise training duration > 28 weeks were more likely to respond to training, although composite analyses were not significant. No deaths were directly attributable to exercise in over 60,000 pt/hours of exercise and one non-fatal, adverse event can be expected every 3,300 pt/hours of training. In the mechanistic study at 8 weeks, peak VO_2 increased by 8.8 % (12.4 ± 4.6 vs 13.5 ± 4.2, $p = 0.26$), and at 16 weeks, peak VO_2 increased by 26 % (12.4 ± 4.6 vs 15.0 ± 4.9, $p < 0.001$). Change in peak VO_2 at 16 weeks was associated with baseline myocardial strain and strain rate. Change in 16 week peak VO_2 was predicted by baseline strain, baseline peak VO_2 and the absence of diabetes ($r^2 = 0.56$; $p < 0.001$), and prediction was improved by inclusion of change of peak VO_2 at 8 weeks in the model ($r^2 = 0.76$, $p < 0.001$). Moreover, myocardial parameters rather than LVEF and volumes were altered by training in this study ; change in 16-week peak systolic strain was predicted by baseline diastolic tissue velocity and strain.

Conclusions. – Exercise training is safe and effective, there is good evidence of a survival and morbidity benefit. Sub-group analysis provided only limited evidence of who will benefit most. Changes in functional capacity following 16 weeks exercise training may be predicted by baseline and 8-week functional capacity, and myocardial strain.

Abstract N° S48B

Exercise, antioxidant supplementation and cardioprotection

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During exercise, skeletal muscle increases oxygen utilisation resulting in a potential source of oxidative stress. Elevated oxidative stress has been implicated in the pathogenesis of cardiovascular disease with epidemiological data reporting that individuals involved in large amounts of physical activity have higher rates of mortality. Antioxidant systems protect against exercise-induced oxidative stress however it is currently unclear whether exercising individuals may benefit from supplementing the diet with additional antioxidants. This presentation will summarise literature concerning the production of reactive intermediates during exercise and the adaptations this may cause. Although the health benefits of regular exercise are well-documented, evidence supporting the notion that high levels of physical activity may have a detrimental effect on health will be evaluated. Finally, the effects of antioxidant supplementation on the cardioprotection and performance of exercising individuals will be discussed.

Abstract N° S48C

Exercise training and endothelium-derived vasoactive factors, endothelin and no

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Vascular endothelial cells play an important role in the regulation of vascular activity by producing vasoactive factors, e.g., endothelin-1 (ET-1) and nitric oxide (NO). ET-1 has a potent vasoconstrictor effect and a potent proliferating activity on vascular smooth muscle cells ; therefore, ET-1 has been implicated in the progression of hypertension and/or atherosclerosis. On the other hand, NO has a potent vasodilative effect and has been proposed as having antiatherosclerotic property ; therefore, NO has been implicated in the prevention of progression of hypertension and/or atherosclerosis. We investigated the effects of exercise training on the production of ET-1 and NO in humans and animals. We revealed that aerobic exercise training causes a decrease in plasma ET-1 concentration and an increase in plasma NO concentration (measured as the stable end product of NO, i.e., nitrite/nitrate [NOx]) in young and older humans. The plasma ET-1 concentration increased with age, and exercise training reduced aging-induced increase in plasma ET-1 concentration in older humans. In older humans, aerobic exercise training also reduced their blood pressure. Thus, these changes in endogenous ET-1 and NO production by exercise training may contribute to the exercise training-induced decrease in risk of developing hypertension and atherosclerosis. Furthermore, we revealed that the production of endothelial NO synthase (eNOS) in the aorta is increased by exercise training in aged rats. In conclusion, exercise training has favorable effects on the production of endothelium-derived vasoactive factors, i.e., ET-1 and NO, to decrease cardiovascular risk (i.e., prevention of progression of hypertension and/or atherosclerosis).

Abstract N° S48E

Functional genomics of the remodeling heart : profiling the cardio-protective effect of prior exercise training

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We have previously shown that prior swimming exercise training improves the outcome of acute MI as manifested at the remodeling phase by better heart function, reduced scar size and increased arteriole density. To elucidate molecular mechanisms underlying this outcome we conducted hight-hroughput expression analysis. Rats underwent a 7-week exercise training (Ex) or remained sedentary (Sed). At 7 weeks, animals were subjected to acute MI and were sacrificed 4h, 2d or 4w thereafter (ExMI4h, ExMI2d, ExMI4w, SedMI4h, SedMI2d, SedMI4w). RNA of the viable left ventricle was analyzed by DNA-microarrays. Out of 3,686 detected transcripts, 1542 were regulated ± 1.5-fold or more in at least one group. Global analysis of the expression profiles indicated that early after MI the impact of infarction on the genes expressed is stronger than that of training. At 4 weeks, however, the prior-exercised hearts differed markedly from their non-exercised counterparts. Twelve clusters of co-regulated genes were identified. Fibrosis-related genes peaked earlier in ExMI than in SedMI hearts. The tensile force-regulated collagen XII clustered with the load-dependent atrial natriuretic peptide, both being markedly enhanced in SedMI hearts during remodeling. Expression of genes involved in aerobic energy metabolism remained higher in the exercised hearts. It is concluded that exercise training conducted prior to acute MI reprograms the heart for better tolerance of MI injury, diminished remodeling and ameliorated energy metabolism, all contributing to the improved heart function.

Abstract N° S49A

Dilated cardiomyopathy in alpha-1-adrenergic receptor knockout mice

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In the ALLHAT clinical trial in hypertension, an a1-adrenergic receptor (AR) antagonist increased heart failure, but the mechanisms are uncertain. Knockout (KO) of the two main a1-AR subtypes, the a1A and the a1B (the ABKO) causes reduced heart growth during post-natal development, without changing resting blood pressure (*J Clin Invest* 111 : 1783, 2003). Here we tested the response of the ABKO to the stress of pressure overload. Transverse aortic constriction (TAC) caused \\ 126\\50 % mortality in ABKO mice vs 0 % in wild type (WT) mice. Death in the ABKO was due to heart failure. By echo in awake mice, the ABKO had worse dilated cardiomyopathy than WT, with larger LV volume and lower ejection fraction. The ABKO cardiomyopathy was not caused by impaired hypertrophy per se, since heart and myocyte size increased after TAC the same as in WT mice. However, the ABKO heart after TAC had (1) failure of fetal gene induction ; (2) increased fibrosis ; and (3) increased apoptosis. Interestingly, ABKO myocytes were predisposed to apoptosis even before TAC. We conclude that the a1A and a1B ARs are required for an adaptive response to cardiac stress. These results provide a plausible biological basis for the adverse effects of a1-AR antagonists in clinical trials.

Abstract N° S49B

Cardiovascular and metabolic changes in mice lacking the alpha1b-adrenergic receptor subtype

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Three α1-adrenergic receptors (AR) subtypes (α1a, α1b and α1d) are present in different mammalian organs in which

they mediate a variety of effects such as vasoconstriction, glycogenolysis and a number of behavioural responses. To investigate the role of different α1-AR subtypes *in vivo*, we created a knock out mouse model lacking the α1b-AR (α1b-/-). In the α1b-/- mice the number of α1-AR was decreased by 74 %, 98 % and 42 % in the heart, liver, and cerebral cortex, respectively. The α1b-/- mice displayed a 45 % reduction in blood pressure response to phenylephrine, suggesting that the α1b-AR mediates a portion of the vasopressor effect of catecholamines. This effect might, at least in part, involve its control on the vascular tone. To investigate the potential role of the α1b-AR on glucose homeostasis, several parameters related to whole body glucose metabolism were measured in the α1b-/- mice. The α1b-/- mice displayed high hepatic glycogen stores in fed and fasted states, hyperinsulinemia in the fasted state, hyperleptinemia, insulin-resistance as well as higher sensitivity to high fat diet-induced obesity. Interestingly, treatment with atropine or methyl-atropine could revert the hyperinsulinemia of the α1b-/- mice to levels similar to those of control mice. This was also associated with increased levels of hypothalamic NPY mRNA. Finally, the results of the glucose turnover experiments indicated that the α1b-/- mice were insulin resistant. Altogether these findings indicate that, in the absence of the α1b-AR, the parasympathetic system and the expression of the hypothalamic NPY are increased resulting in hyperinsulinemia and insulin resistance as well as favouring obesity and glucose intolerance during the high fat feeding.

Abstract N° S49C

Hypertension and α_1-adrenergic receptor subtype

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To study the functional role of individual α_1-AR subtypes in blood pressure regulation and the development of hypertension, we created mice lacking the α_{1B}-AR and/or α_{1D}-AR, and studied hemodynamic and vasocontractile responses in these mutant mice. Both the α_{1D}-AR knockout and α_{1B}-/α_{1D}-AR double knockout, but not the α_{1B}-AR knockout mice, had a significantly lower level of basal blood pressure than wild type mice by monitoring blood pressure. All mutants showed reduced catecholamine-induced pressor and vasoconstriction responses. We further examined blood pressure changes in wild type and the mutant mice, which were subjected to subtotal nephrectomy and dietary salt loading. After 35 days of salt loading, blood pressure of the α_{1B}-AR knockout mice increased to a comparable level to wild type mice, while blood pressure changes of α_{1D}-AR knockout and α_{1B}-/α_{1D}-AR double knockout mice were significantly attenuated, which is attributable to a combination of decreased vascular responsiveness and lower sympathetic nerve activity. Our data indicated that deletion of the functional α_{1B}-AR gene did not lead to an anti-hypertensive effect in this model and that α_{1D}-AR played a critical role in the development of salt induced hypertension, showing differential contributions of α_{1B}- and α_{1D}-ARs to development of hypertension.

Abstract N° S49D

The role of alpha 1-adrenergic receptor subtypes in hypertropy, inotropy and ischemic preconditioning

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Systemic transgenic expression of the a_1-Adrenergic Receptor (AR) subtypes was used to determine subtype-specific roles in cardiovascular functions. Transgenic mice overexpressing a constitutively active mutant (CAM) a_{1B}-AR had enlarged hearts and diastolic dysfunction but did not transition to failure. Microarray analysis indicated increased growth-related genes involving Src-related signaling pathways and a large inflammatory response. Cardiac gene expression was decreased for common failure-associated proteins. In isolated hearts, a_{1B}-AR overexpression did not increase inotropy, suggesting that this subtype is not a major factor in contractility but caused downregulation of the a_{1a}-AR, which suppressed phenylephrine-induced inotropy. Hearts isolated from normal, CAM a_{1A}-ARs or CAM a_{1B}-ARs, were preconditioned followed by 30 min ischemia and reperfusion. Ischemic preconditioning significantly enhanced the post-ischemic recovery of normal hearts. Hearts expressing CAM a_{1A}-ARs recovered completely from 30 min ischemia without undergoing the preconditioning protocol. Thus, the a_{1A}-AR transgenic hearts were inherently preconditioned. However, expression of CAM a_{1B}-ARs provided no protection against ischemic injury. These data suggests that a_{1A}-AR, but not the a_{1B}-AR, mediate ischemic preconditioning.

Abstract N° S49E

Genetic enhancenment of ventricular contractility protects against pressure – overload-induced cardiac dysfunction

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Background. – In response to pressure-overload cardiac function deteriorates and may either progress to fulminant heart failure and death, or may be restored to normal by the development of hypertrophy. Here we questioned if genetic enhancement of left ventricular (LV) contractility protects against pathological overload.

Methods and Results. – Transgenic (TG) mice with cardiac-restricted overexpression (66-fold) of the a_{1A}-adrenergic receptor (AR) and their non-TG littermates, were subjected to transverse aorta constriction (TAC)-induced pressure-overload for 12 weeks. TAC-induced hypertrophy was similar in the non-TG and TG mice but the TG mice with TAC were less likely to die of heart failure compared to the non-TG animals ($P < 0.05$). The hypercontractile phenotype

of the TG mice was maintained over the 12-week period following TAC with LV fractional shortening being better preserved than in the non-TG mice (42 ± 2 vs 29 ± 1 %, $P < 0.01$). In the TG animals, b-AR-blockade with atenolol for 11 weeks neither induced hypertrophy nor suppressed the hypercontractile phenotype.

Conclusions. – The hypertrophic response to pressure-overload is not altered by cardiac a_{1A}-AR overexpression. Moreover, a_{1A}-AR-mediated enhancement of LV contractility protects against pressure-overload-induced cardiac decompensation and death.

Abstract N° S5A

Signaling Mechanisms of Preconditioning

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Current evidence indicates that ischemic preconditioning's protection is triggered by receptor-dependent opening of mitochondrial ATP-sensitive potassium channels (mK_{ATP}) that cause the mitochondria to generate reactive oxygen species (ROS). The ROS then act as second messengers to activate downstream protein kinases, including PKC, that mediate the protection during the index ischemia. The ROS hypothesis explains why a period of reperfusion must follow a preconditioning ischemia because that is when the ROS are produced. Timing studies indeed show that the critical time for mK_{ATP} opening is prior to rather than during the index ischemia. By utilizing an isolated cardiomyocyte model in which we monitor ROS production in response to receptor stimulation we have been able to map out the signal transduction pathway between the muscarinic receptor and the mK_{ATP}. Using the preconditioning mimetic acetylcholine we find that population of the muscarinic receptors causes activation of a metalloprotienase that liberates heparin-binding epidermal growth factor (HB-EGF) from a membrane-bound pro-HB-EGF. Occupation of the EGF receptor then activates PI3-kinase. The phospholipid product causes activation of Akt. Akt then activates eNOS through phosphorylation. The eNOS makes nitric oxide that causes guanylyl cyclase to make cGMP that in turn activates protein kinase G (PKG) resulting in mK_{ATP} opening and ROS production. While it appears that any Gi- or Gq- coupled receptor in the heart muscle cell can trigger the preconditioned state, only agonists for the adenosine A1 and A3, the bradykinin B2 and the delta opioid receptors are released during a preconditioning ischemia. There is even diversity among these 3 receptors as adenosine receptors bypass the mK_{ATP}/ROS pathway and only the opioids utilize the EGF receptor. These multiple parallel pathways provide many opportunities for pharmacological preconditioning.

Abstract N° S5B

G-protein coupled receptor cross-talk : implications for preconditioning

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Ischemic preconditioning (IPC) is multifactorial with many potential triggers and mediators. Activation of G protein-coupled receptors (GPCRs) such as adenosine, bradykinin and opioid receptors appear to be essential to triggering IPC. Growing evidence suggests that GPCR cross-talk between beta-adrenoceptors, adenosine, opioid and endothelin receptors regulates cardiovascular function under both normal and pathophysiological conditions. GPCR cross-talk is well documented, however, its role in IPC is not known. We have previously demonstrated that adenosine and opioid receptors exhibit cross-talk in a rat model of myocardial infarction whereby infarct abrogation elicited by opioid and adenosine receptor agonists is reversed by adenosine and opioid antagonists, respectively. Furthermore, simultaneous activation of both opioid and adenosine receptors failed to provide an additive effect suggesting a converging pathway. Interestingly, in an isolated murine heart model of stunning ìcross-talkî appears to be incomplete. That is, while opioid receptor-mediated protection can be abolished via adenosine receptor antagonism, opioid receptor blockade has little effect upon adenosinergic cardioprotection. Furthermore, preliminary data obtained from western blot analysis indicate that increased activation of phosphoERK following morphine exposure is abolished with concomitant blockade by the A1 adenosine receptor antagonist, DPCPX. These results suggest that an intact CNS maybe required for receptor cross-talk between adenosine and opioids.In addition, receptor cross-talk may be explained by receptor dimerization. Dimerization can occur among identical GPCRs and those from distinct families. GPCR dimerization may effect receptor activation, ligand binding and signaling. Understanding the consequences of cross-talk and dimerization may provide novel approaches to limiting the detrimental effects of cardiac ischemia and reperfusion.

Abstract N° S5C

K_{ATP} channel deficit and cardiac maladaptation

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ATP-sensitive potassium (K_{ATP}) channels are evolutionarily conserved plasma-membrane protein complexes, widely represented in tissue beds with high metabolic activity. There, they are formed through the physical association of the inwardly rectifying potassium channel pore, most typically Kir6.2, and the regulatory sulfonylurea receptor subunit, an ATP-binding cassette protein. Energetic signals, received via tight integration with cellular metabolic pathways, are processed by the sulfonylurea receptor subunit that in turn gates the nucleotide sensitivity of the channel pore thereby controlling membrane potential dependent cellular functions. Recent findings, elicited from genetic disruption of channel proteins, have established *in vivo* the requirement of intact K_{ATP} channels in the proper function of cardiac muscle under stress. Indeed, in the heart where K_{ATP} channels were originally discovered, ablation of the channel complex compromises cardioprotection under ischemic insult.

New data further implicate the requirement of intact K_{ATP} channels for the cardiac adaptive response to acute stress. Moreover, K_{ATP} channels have been implicated in the adaptive cardiac response to chronic physiologic and pathophysiologic hemodynamic stress, with K_{ATP} channel deficiency affecting the structural remodeling response, rendering the heart vulnerable to calcium-dependent maladaptation predisposing to heart failure. These findings are underscored by the identification in humans that defective K_{ATP} channels induced by mutations in *ABCC9*, the gene encoding the cardiac sulfonylurea receptor subunit, confer susceptibility to dilated cardiomyopathy. Thus, these results expand our understanding of the requirement for intact, functioning sarcolemmal K_{ATP} channels in the cardiac stress response.

Abstract N° S5D

K_{ATP} channels and long-lasting cardioprotection

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Adaptation to chronic hypoxia (CH) confers long-lasting myocardial protection against all major end-points of acute ischemia and reperfusion injury but its mechanism remains obscure. We examined roles of mitochondrial K_{ATP} (mK_{ATP}) channels, reactive oxygen species (ROS) and protein kinase C (PKC) in myocardial infarct size-limiting effect of CH (20-min coronary artery occlusion and 3-h reperfusion in an open-chest model). Adult male Wistar rats exposed to intermittent (8 h/day) hypobaric hypoxia of 5000 m or 7000 m for 5-6 weeks exhibited infarct size reduction by 15 % or 30 %, respectively, compared to normoxic controls. This protective effect persisted and was still significant 5 weeks after the last hypoxic exposure (10 % reduction). Chronic antioxidant treatment (N-acetylcysteine, 100 mg/kg daily before the hypoxic exposure) attenuated infarct size limitation by CH. Similar blunting effect occurred in rats exposed to CH combined with hypercapnia (4.1 % CO_2 in the air) which lowers oxidative stress. MK_{ATP} blocker (5-hydroxydecanoate, 5 mg/kg), administered before ischemia, completely abolished the protective effect of CH, while mK_{ATP} openers (diazoxide or BMS-191095, 10 mg/kg) limited infarct size in normoxic but not in CH rats. Neither antioxidants (tempol, 100 mg/kg or melatonin, 10 mg/kg) nor PKC inhibitor (chelerythrine, 1 mg/kg), given before ischemia, affected CH-induced cardioprotection. It is concluded that oxidative stress associated with CH contributes to the development of increased cardiac ischemic tolerance. The protective mechanism involves the activation of mK_{ATP} channels but presumably not ROS- and PKC-dependent pathways during acute ischemia.

Supported by GACR 305/04/0465.

Abstract N° S5E

The importance of the reperfusion phase in ischaemic preconditioning

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The actual mechanism through which *ischaemic preconditioning* (IPC) protects the heart against ischaemia-reperfusion injury is unclear. Our recent studies have demonstrated that IPC can protect the heart against ischaemia-reperfusion injury by modifying crucial events, which take place during the first few minutes of reperfusion.

The opening of the *mitochondrial permeability transition pore* (mPTP), during the first few minutes of reperfusion, which occurs in response to the prevailing conditions of oxidative stress, a high mitochondrial $[Ca^{2+}]$, and relative ATP depletion, is a critical determinant of lethal reperfusion injury. Our previous studies have demonstrated that pharmacologically inhibiting mPTP opening at the time of reperfusion is cardio-protective, in both the animal and human heart. We have demonstrated that IPC protects the heart by inhibiting the opening of the mPTP, at the time of reperfusion. IPC may inhibit mPTP opening : (1) by reducing oxidative stress at the time of reperfusion ; (2) by rendering mitochondria more resistant to mPTP opening at the time of reperfusion ; or (3) through the activation of pro-survival kinases, such as, PI3K-Akt and MEK1/2-Erk1/2, which we have termed the *Reperfusion Injury Salvage Kinase (RISK)-Pathway*.

We have demonstrated that the pharmacological activation of this RISK-Pathway, during the first few minutes of reperfusion, is cardio-protective. Interestingly, we have recently found that IPC induces the activation of the RISK-Pathway, at the time of reperfusion, and that the activation of the pro-survival kinases, PI3K-Akt and MEK1/2-Erk1/2, at this time, is essential for IPC-induced protection. We postulate that these kinases mediate protection by inhibiting mPTP opening at the time of reperfusion. Interestingly, we have also demonstrated that the recently described phenomenon of *ischaemic postconditioning,* also protects the heart by activating components of the RISK pathway, at the time of reperfusion.

In conclusion, our recent data suggests that IPC protects the heart by modifying crucial events, which occur during the first few minutes of reperfusion, and demonstrates the importance of the reperfusion phase in IPC.

Abstract N° S5F

K_{ATP} channels and preconditioning in the neonatal heart : are they up to the job ?

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We have previously described a developmental change in the ability of the heart to be protected by ischemic preconditioning (IPC). Protection is absent in 4-day old neonatal rats but appears gradually over the first 3 weeks of life. In the human myocardium, the same maturational response occurs over the first post-partum year. In adult heart, the signalling pathway underlying protection has been defined and there is general agreement that it involves - G-protein coupled receptors > ?PKC ? > K_{ATP} channels > mitochondrial perturbation > oxidant stress > an elusive end-effector. In the

adult heart, K_{ATP} channel activation has been shown to mimic IPC-induced cardioprotection. However, we have shown while nicorandil can dose-dependently shorten the action potential in adult human myocardium, it is ineffective in the neonate. Thus, we have investigated whether (i) K_{ATP} channel openers (KCOs) can protect neonatal hearts ? (ii) K_{ATP} channels are *expressed* in neonatal hearts ?, (iii) K_{ATP} channels are *functional* in neonatal hearts, and (iv) whether bypassing K_{ATP} channels (either at the level of the mitochondria or down-stream) can invoke protection ? The KCOs cromakalim (10^{-6}-10^{-4}) nicorandil (100μM) and diazoxide (30-600μM) all failed to protect the immature heart while conferring protection in the adult. Western blotting for Kir 6.1, Kir 6.2 and SUR2A, shows K_{ATP} channels are expressed in neonatal heart and voltage-clamp data shows that they are functional and their sensitivity to KCOs is, in fact, increased. Bypassing the $^{mito}K_{ATP}$ channel by opening Ca-activated ^{mito}K channels with NS1619 (30μM) protected adult human heart (protection was blocked by the antioxidant NAC (4mM)) but failed to protect the neonate. Preliminary studies with H_2O_2 (1-10μM) suggest that transient oxidant stress may protect the neonatal heart. These results suggest that, in neonatal heart, the lesion in the protective signalling pathway is down-stream from the K_{ATP} channel and may be related to its inability to generate an oxidant stress.

Abstract N° S6A

Myocardial gene profiling during human cardiac hypertrophy and failure

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Right ventricular hypertrophy and failure are prominent features in cyanotic congenital heart disease, tetralogy of Fallot (TF). Patients with TF require primary cardiac surgery at a very young age. To get an insight into the underlying molecular mechanisms of right ventricular hypertrophy and to identify gene(s) involved in TF, differential gene expression profile was assessed using high density DNA microarray chip analysis on right ventricular biopsies from TF patients who underwent primary correction (TF-1, mean age 0.8 yr, n = 12). Employing quantitative immunohistochemistry, expression of VEGF, flk-1 and extracellular matrix (ECM) proteins (collagens and fibronectin) as well as vessels counts and myocyte cell size were evaluated in TF-1 and TF-2 (mean age 30 yrs, n = 12) patients in relation to age matched controls (n = 8). Among 236 genes showing altered expression pattern in TF patients, VEGF (1.8 fold) and ECM markers were clearly up-regulated (fibronectin, 2.4 ; collagen Ia, 7.5 and collagen III, 4.4 folds), flk-1 and most MMPs remained unchanged except the levels of MMP-13 and 17 were declined. TIMPS showed down regulated pattern. Staining of VEGF in cardiomyocytes and of ECM proteins (fibronectin, collagen I and III) in interstitial as well as in peri-vascular area were increased ($p < 0.01$) in TF patients. Morphometric analysis revealed enhanced vascular density (65 ± 9 vs. 33 ± 3 vessels/mm2 ; $p < 0.05$) with unchanged wall thickness and enlarged myocyte cross sectional areas (262 ± 21 vs. 81 ± 7mm2 ; $p < 0.01$) with linear correlation ($r = 0.92$, $p < 0.01$) with the age in TF-1 patients. We conclude that the up-regulation of genes encoding VEGF and ECM proteins are the key events contributing to right ventricular hypertrophy and stunted angiogenesis in patients with TF.

Abstract N° S6B

Molecular regulators of cardiac cell growth and cell death

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One of the most compelling issues to impact on contemporary cardiology, is arguably the phenomenon of programmed cell death or apoptosis. Studies in the nematode *C. elegans* provided the first indication that determinants of cell fate crucial for normal worm development were under genetic influences of the *ced-3* and *ced-9* genes- which promote or prevent cell death, respectively. Extrapolation of these seminal findings led to the discovery of the mammalian ced-3 and ced-9 homologues which broadly encompass a family of cellular cysteine proteases known collectively as caspases and the Bcl-2 proteins. In quiescent cells, caspases exist as inactive zymogens that are readily activated by autocatalytic processes or by other caspases following a death signal. The caspase dependent cleavage of intracellular substrates results in the biochemical dismantling of the cell and morphological features characteristic of apoptosis. Recently, a mitochondrial death pathway for apoptosis has been proposed. Perturbations to mitochondria resulting in the loss of mitochondrial membrane potential $\Delta\Psi m$, permeability transition pore opening and the release of pro-apoptotic factors by mitochondria including cytochrome c, Smac/Diablo, AIF, and others are considered terminal events in the apoptotic pathway. Bcl-2 and related family members are characterized by their ability to promote or prevent cell death. These proteins exert their pro- or anti-apoptosis function by impinging on components of cell death pathway that underlie caspase activation, mitochondrial dysfunction or both. The limited regenerative potential of the adult cardiac muscle itself, together with the heightened and exciting possibility of regenerating cardiac muscle with cardiac progenitor cells, acknowledges the need for new strategies to suppress and/or prevent inappropriate cardiac cell death in patients with ischemic heart disease or heart patients failure as a therapeutic means of preserving cardiac pump function after injury.

Abstract N° S6C

Mechanical stretch activates angiotensin II type 1 receptor without involvement of angiotensin II

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Angiotensin II (AII) type 1 (AT1) receptor plays a critical role in the load-induced cardiac hypertrophy. We here demonstrate an AII-independent mechanism of AT1 receptor activation by mechanical stress. Without the involvement of AII, mechanical stress not only activated extracellular signal-regulated kinases (ERKs) *in vitro* but also induced cardiac hypertrophy *in vivo*. Mechanical stretch induced association of AT1 receptor with Jak2, and translocation of G proteins into the cytosol. All of these responses were inhibited by the treatment with an AT1 receptor blocker, candesartan. Therefore, mechanical stress activates AT1 receptor independently of AII, which is inhibited by an inverse agonist candesartan.

Abstract N° S6D

Hormonal regulation of the Na^+/H^+ exchanger in the heart

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The Na^+/H^+ exchanger (NHE) is a pH-regulatory protein that is present in many different cell types including cardiac myocytes. It removes one intracellular proton in exchange for one extracellular sodium in response to intracellular acidosis, but hormones and growth factors can also activate it, possibly through protein kinases and other regulatory proteins. In the myocardium, angiotensin II (AngII), endothelin (ET), thrombin and a_1-adrenergic stimulation were shown to activate the NHE. Experiments performed by us suggest that NHE activation is the central step for the positive inotropic response to low concentrations of AngII. The enhanced activity of the NHE by increasing the intracellular Na^+ concentration favors the reverse mode of the Na^+/Ca^{2+} exchanger, with the consequent increase in the Ca^{2+}T that determines the increase in force. ET is an autocrine/paracrine factor that mediates this effect.

Abstract N° S6E

Computer simulation of failing heart

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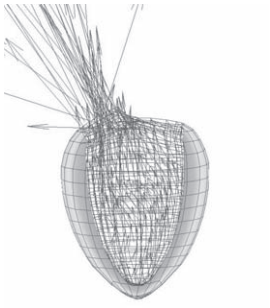
To integrate the vast amount of information acquired at multiple levels of biological system, computational science is now recognized as an indispensable tool. We have developed a fluid-structure interaction finite element model of the human left ventricle contraction and relaxation of each structural element is driven by the molecular mechanism of excitation contraction coupling of cardiac myocyte. Because the structure (ventricular wall) and the fluid (blood) were formulated by strong coupling strategy, the model could successfully reproduce not only the pressure-volume relation but also the instantaneous flow distribution during the ejection and filling of the ventricle. Furthermore, by introducing molecular abnormalities idenitfied in heart failure we could also reproduce the hemodynamics as well as echocardiographic finding of the failing heart. Other diseased conditions will also be discussed in the presentation.



Abstract N° S7A

Stemming myocardial damage with stem cells : an overview

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Patients with heart failure remain a therapeutic challenge. The contemporary therapeutic modalities including heart transplant, assist devices and medical therapy have their shortcomings and limitations. Cell transplantation and angiogenesis are novel and promising strategies at molecular and cellular levels.

Transplantation of multiple adult stem cell types, especially skeletal myoblast and bone marrow derived stem cells, into ischemic and infarction animal models has demonstrated graft survival, cell fusion and improved myocardial function. An interesting aspect of the studies has been the use of transient immunosuppression or even no immunosuppression in some cases, leading to longer term survival of the cellular graft.Human phase-1 studies have proven safety of the procedure with encouraging results.

Another interesting aspect of stem cell research is cellular angiogenesis. Transplantation of cells with inherent ability to express angiogenic growth factors or genetic modulation of donor cells to over-express angiogenic growth factors has shown increased collateral formation and enhanced regional blood flow in ischemically damaged myocardium. Combining cell transplantation and angiogenesis, it has been shown that the donor cells survive and integrate with the host tissue and may carry exogenous gene for cardiac muscle cells. This combined strategy of cell transplantation with gene therapy may be cardioprotective for the ischemic myocardium. Myocardial regeneration with stem-cell transplantation and its combination with angiogenesis potentially reverse the depleted functional, hemodynamic and neurohormonal effects that occur after myocardial infarction.

Abstract N° S7B

Development of the cardiac conduction system

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The conduction system is generally held responsible for the rhythmic and coordinated electrical activity of the heart. Because an adult type of ECG can already be derived from very early embryonic hearts, it is often assumed that these hearts have a conduction system, which, in turn, proofed difficult to identify and led to much controversy. If we accept that the most conspicuous features of the electrocardiogram are caused by the electrical activity associated with the rapid depolarisation of the atria and ventricles it is equally reasonable to assume that the adult type of electrocardiogram in the early heart finds its origin in the formation of the fast-conducting working myocardium of the chambers rather than in the formation of a conduction system.

Why certain areas of the heart tube do not develop into the working myocardium of the chambers and contribute to the formation of the cardiac conduction system is one of the key questions of cardiac embryology. By our recent findings that the transcriptional repressors Tbx2 and Tbx3 repress the chamber-specific program of gene expression and chamber formation in transgenic animals over-expressing these factors, we are beginning to understand these morphogenetic processes. Detailed reconstructions of the developmental patterns of expression of Tbx3 during development have revealed, that Tbx3 is expressed in those areas of the heart tube that do not become chamber, i.e. in the sinu-nodal region, internodal region, atrioventricular junction, atrioventricular bundle and bundle branches. These areas comprise not only the conventional conduction system, but also the highly controversial areas of the internodal region and the entire atrioventricular junction. The pulmonary veins drain directly upon the developing left atrium in a myocardial region that has a « chamber » molecular phenotype (Connexin40 positive) rather than an embryonic or nodal phenotype not supporting the notion that the embryonic (nodal) origin of the pulmonary myocardium explains arrhythmias originating from this area.

Abstract N° S7E

Fasciclins induce differentiation of cardiac cushion mesenchymal cells into valvular fibrous tissues

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Cushion cells are endocardially derived cells that form within primitive segments located at the inlet and outlet of the ventricles. Cushion cells have potential to differentiate into cardiac muscle, bone, bone marrow and cartilage but normally give origin to valvular fibroblasts. Based on microarray analysis and studies with cultured MC3T3 cells, we have proposed that fasciclin genes promote differentiation of cushion cells into fibroblasts and/or inhibit their differentiation into calcifying lineages. In vivo, cushion cells and their fibroblastic progeny express 3 fasciclin proteins, periostin, βigH3 and stabilin 2 during embryonic and fetal life while periostin and βigH3 continue to be expressed into adult life. Fasciclins are cell surface secreted proteins that modify cell-:cell or cell :matrix interactions in invertebrates. Based on antibody, in situ hybridization and promoter-reporter studies, periostin expression in chick and mice embryos correlates spatially and temporally with the Histogenic pattern of fibrous tissue (dense vs. loose) in future valve leaflets and with the formation of tendinous suspensory cords. We used gene targeting strategies to create two lines of periostin knockout mice. We found that valve leaflets in newborn null mice were variably affected, some (aortic leaflet) were totally hypoplastic while others (mitral, tricuspid) exhibited a mixed phenotype of normal dense fibrous tissue interspersed with expansions of undifferentiated mesenchyme. Additionally, tendinous cords were absent and leaflets failed to delaminate from the associated ventricular myocardium. Approximately 20 % of newborns died at day 12. To further assess function, we developed adenoviral vectors to over express or inhibit periostin expression. Infection of micromass cultures with full length antisense virus prevented formation of 3-dimensional, dense, fibrous rings that normally develop in this culture system. Instead, chondrogenic tissue developed. To simulate a more in vivo like setting, we engineered tubular scaffolds of aligned collagen which were seeded with fetal cardiomyocytes and connected to a circulating bioreactor. Embryonic chick cushions were attached to the luminal surface of these pulsatile and contractile linear « hearts ». Over time, they elongated into preleaflet-like structures which expressed fasciclins. Infection with virus carrying full length sense periostin cDNA engendered premature dense fibrous tissue formation within virally infected cells whereas infection with full length, antisense vectors resulted in chondrogenesis. These findings indicate fasciclins regulate valvulogenesis by promoting and sustaining differentiation of cushion stem cells into a fibrogenic lineage. Supported by NIH Grants : HL 33756, HL 53128.

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