infarct size was similar ($38.3 \pm 2.0\%$ vs $39.9 \pm 2.8\%$). Transgenic mice also exhibited less apoptosis and interstitial cardiac fibrosis ($0.52 \pm 0.03\%$ vs $0.65 \pm 0.05\%$, $p < 0.05$), and lower increases in the expression of fibronectin and procollagen III mRNA. Both echocardiography and cardiac catheterisation demonstrated less left ventricular cavity dilatation and a preservation of cardiac function in transgenic than wt mice.

Conclusions In contrast to Nox2, which contributes to cardiac dilation, contractile dysfunction and fibrosis, cardiomyocyte Nox4 was found to exert protective effects against adverse remodelling post-MI.

011 A NEW MOLECULAR MECHANISM FOR FAMILIAL DILATED CARDIOMYOPATHY BASED ON STUDIES WITH INTACT MUTANT TRANSGENIC MOUSE AND HUMAN EXPLANTED HEART MUSCLE

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We created a transgenic mouse that expressed the dilated cardiomyopathy (DCM) mutation *ACTC* E361G at 50% of total cardiac actin. We isolated F-actin from transgenic and non-transgenic (NTG) mice and reconstituted thin filaments using native human cardiac troponin and tropomyosin (from donor heart). In in-vitro motility assays we could observe no differences between E361G and non-transgenic mouse thin filaments; however, when troponin was fully dephosphorylated with acid phosphatase we observed that E361G Ca^{2+} sensitivity was lower than non-transgenic, as previously observed with the recombinant proteins (EC_{50} E361G/NTG 2.2 ± 0.1). When we compared natively phosphorylated and dephosphorylated thin filaments we observed that Ca^{2+} sensitivity did not change in E361G mouse thin filaments (EC_{50} P/unP = 1.0 ± 0.1) but the Ca^{2+} sensitivity increased 3.0 ± 0.3 -fold on dephosphorylation of non-transgenic mice as expected. Thus the only functional change induced by the E361G mutation in cardiac actin was a blunted response to troponin I phosphorylation. We also studied troponin extracted from the explanted heart of a DCM patient carrying the cardiac TnC G159D mutation. In-vitro motility assay investigation of reconstituted thin filaments showed that the cTnC G159D mutation also showed little change in Ca^{2+} sensitivity when TnI was dephosphorylated (EC_{50} P/unP = 1.2 ± 0.2). In contrast, with donor heart control troponin, Ca^{2+} sensitivity was increased (EC_{50} P/unP = 4.7 ± 1.9). We conclude that Ca^{2+} sensitivity per se is not the prime determinant of familial DCM. The causative property shared by mutations in contractile proteins that cause DCM is a blunted response to changes in troponin I phosphorylation that could impair the normal response to adrenergic stimulation.

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012 IS NITRIC OXIDE SYNTHASE PRESENT IN MITOCHONDRIA?

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In addition to the three known forms of nitric oxide synthase (NOS) in the heart, it has recently been proposed that NOS is also present in mitochondria. However, studies are controversial due to the possibility of contamination by non-mitochondrial NOS, and because none of the known forms of NOS contain a mitochondrial targeting sequence. We investigated whether NOS was present in isolated mitochondria using antibodies against all three forms of NOS (endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS)). Crude fractions of heart and liver mito-

chondria were obtained by differential centrifugation, and 35% Percoll was used to obtain highly purified mitochondria, as tested using antibodies against subcellular marker proteins: cyclophilin D, mitochondrial marker; monocarboxylate transporter-1, plasma membrane marker; ryanodine receptor, sarcoplasmic reticulum marker (heart mitochondria only) and catalase, peroxisomal marker (liver only). Western blotting using antibodies against eNOS and iNOS revealed that these isoforms were not present in either heart or liver purified mitochondria (whereas whole heart or liver lysate tested positive). We used five different antibodies against nNOS, and again failed to detect anything in purified heart mitochondria. However, in purified liver mitochondria one of the nNOS antibodies revealed the presence of a band at the correct molecular weight. We are currently determining whether this is indeed nNOS. In addition, we will assay for NOS activity in the purified mitochondria. Nitric oxide can inhibit mitochondrial respiration, so the existence of mitochondrial NOS may provide an important modulatory mechanism for respiration under either physiological or pathological conditions.

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013 REGULATION OF GENE TRANSCRIPTION BY HYDROGEN PEROXIDE IN CARDIOMYOCYTES

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Introduction Reactive oxygen species (ROS) levels rise during ischaemia and reperfusion and may contribute to myocardial injury, remodelling and progression to heart failure. ROS (hydrogen peroxide; H_2O_2) activates both apoptotic and pro-survival signalling pathways, although at higher concentrations cardiomyocyte death invariably results. Here, transcriptional responses of cardiomyocytes to varying concentrations and duration of H_2O_2 were investigated.

Methods Neonatal rat ventricular myocytes were exposed to 0.05–1.0 mM H_2O_2 for up to 6 h. Messenger RNA expression of selected genes was analysed by quantitative PCR. Cycloheximide was used to define immediate early genes (IEG) and second-phase genes.

Results Transcription factors of activating protein 1 (AP-1) and early growth response (EGR) families were upregulated rapidly and transiently by 0.1–1 mM H_2O_2 , concentrations, which induce cardiomyocyte apoptosis and are IEG. In contrast, upregulation of transcripts for antioxidant proteins (second-phase genes) was slower and more sustained, occurring at a lower concentration of H_2O_2 (0.05–0.5 mM).

Conclusions AP-1, EGR and antioxidant transcripts were regulated by H_2O_2 in a time and concentration-dependent manner. Induction of antioxidant expression at lower concentrations of H_2O_2 could represent enhancement of ROS-scavenging capacity to prevent apoptosis. At higher H_2O_2 concentrations, the AP-1 and EGR transcription factor IEG may mediate apoptosis. These studies increase the understanding of transcriptional responses in cardiomyocytes to ROS.

014 THIN FILAMENTS RECONSTITUTED WITH TROPONIN EXTRACTED FROM PATIENTS WITH HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY ARE FUNCTIONALLY ABNORMAL

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Tissue obtained from a septal myectomy represented an opportunity to characterise the molecular phenotype of hypertrophic

obstructive cardiomyopathy (HOCM). We have isolated troponin from HOCM muscle and studied its function using the in-vitro motility assay (IVMA). The level of troponin I (TnI) phosphorylation has been measured as 0.29 ± 0.04 mol Pi/mol TnI compared with 1.62 ± 0.06 mol Pi/mol TnI in donor heart samples. The Ca^{2+} -sensitivity of reconstituted thin filaments containing donor and HOCM troponin were measured. The HOCM troponin conferred a Ca^{2+} -sensitivity that was not significantly different to that of the donor troponin. EC_{50} HOCM/donor = 0.88 ± 0.22 (n=8). HOCM troponin was treated with protein kinase A (PKA) (catalytic subunit) to increase the level of TnI phosphorylation to a similar level as found in donor hearts. There was no significant difference in the Ca^{2+} sensitivities of the thin filaments reconstituted with untreated or PKA-treated HOCM troponin. EC_{50} HOCM/PKA treated HOCM = 0.93 ± 0.32 (n=4). Finally, donor troponin was dephosphorylated by treatment with acid phosphatase. In preliminary experiments this was compared with HOCM troponin, which has an intrinsically low level of phosphorylation. Thin filaments reconstituted with dephosphorylated donor troponin had a higher Ca^{2+} sensitivity than thin filaments containing HOCM troponin. Therefore, we conclude that HOCM troponin is modified in a manner independent of the causative mutation. HOCM troponin confers a Ca^{2+} sensitivity similar to troponin from donor hearts, which is independent of the phosphorylation status of TnI. This molecular phenotype is different from both acquired heart failure and genetic dilated cardiomyopathy.

015 INVESTIGATING METABOLIC FLUX IN THE HYPERTHYROID HEART USING HYPERPOLARISED MAGNETIC RESONANCE

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An increase in circulating thyroid hormone (TH) causes hypertrophy, which is accompanied by an increase in contractility and cardiac output. However, the mechanisms behind these observations are yet to be fully elucidated. TH is an important regulator of energy metabolism and has been shown to control the expression of many important lipolytic and glycolytic enzymes. One such enzyme is pyruvate dehydrogenase kinase, which, via phosphorylation, reduces the activity of pyruvate dehydrogenase (PDH) and thus decreases the conversion of pyruvate to acetyl CoA, a key substrate in ATP synthesis. However, it is not known whether PDH activity is inhibited in the hyperthyroid rat heart. Hyperpolarised substrates increase the sensitivity of magnetic resonance spectroscopy (MRS) so that it is possible to investigate the flux of metabolites through specific enzymes in vivo. In this study, hyperpolarised pyruvate has been used in conjunction with MRS localised to the heart to monitor real time metabolic flux through PDH in hearts of control rats and rats injected with TH (triiodothyronine; T3) for 7 days (n=8 per group). PDH activity measurements were made at baseline and after 7 days of injections. Hyperpolarised pyruvate was injected over 10 s into the anaesthetised rat via the tail vein. The conversion of pyruvate to alanine, lactate and bicarbonate was monitored every 1 s for 1 minute. The bicarbonate/pyruvate ratio was used as a measure of flux through PDH. After 7 days administration of T3, flux through cardiac PDH was reduced by 76% ($p < 0.01$). Thus, hyperpolarised pyruvate has revealed PDH inhibition to contribute to the pathology of the hyperthyroid heart.

016 EVIDENCE FOR INWARD RECTIFIER POTASSIUM CHANNELS IN HL-1 CELLS

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Cardiac inward rectifier potassium current (IK1) plays a paramount role in repolarisation and stabilisation of the membrane potential in cardiomyocytes. Its downregulation contributes to arrhythmogenesis in heart failure and post-myocardial infarction. We have undertaken the molecular and functional characterisation of IK1 in the HL-1 cell line, which is derived from a mouse atrial tumour. HL-1 cells express many genes typical of differentiated adult cardiomyocytes and so far they are the only cardiac cell line able to divide continuously while maintaining a differentiated cardiac phenotype.¹ IK1 has not been described in HL-1 cells. Whole-cell recording was performed under conditions that have been shown to preserve IK1 in excised membrane patches; the pipette solution contained 10 mM pyrophosphate, 5 mM fluoride and 0.1 mM vanadate. Extracellular barium at an IK1-selective concentration (0.1 mM) inhibited inward current by $44 \pm 4.2\%$ (mean $\pm$ SEM, n=13 cells), with little or no effect on outward current. The apparent reversal potential of the barium-sensitive current was -58 ± 6.0 mV in 20 mM and -10 ± 3.9 mV in 100 mM extracellular potassium with 160 mM potassium in the pipette (n=7). Reverse transcriptase PCR was performed with HL-1 complementary DNA and primers specific for Kir2.1 (KCNJ2), the dominant IK1 isoform in cardiomyocytes. A PCR fragment of the expected size and sequence was produced. These results support the conclusion that HL-1 cells have an inwardly rectifying potassium current and express Kir2.1.

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017 CONCENTRATION-DEPENDENT PROTECTION BY NO DONORS AGAINST ISCHAEMIA-REPERFUSION INJURY IN PERFUSED RAT HEARTS

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Nitric oxide (NO) has the potential to be beneficial in attenuating ischaemia-reperfusion (IR) injury, or deleterious due to its tendency to form reactive nitrogen species (RNS). In addition, different donors release varying amounts of NO, making them difficult to compare directly. We studied the effects of two NO donors, SNAP (S-nitroso-N-acetyl-DL-penicillamine) and DEA/NO (diethylamine NONOate). Isolated rat hearts were perfused with or without SNAP at 2–100 μM or DEA/NO at 2–20 μM . The NO donors were added 20 minutes before the onset of ischaemia (30 minutes) and remained present throughout reperfusion (60 minutes). Injury was assessed by measuring left ventricular developed pressure (LVDP) and lactate dehydrogenase (LDH) release. SNAP significantly protected hearts from IR injury at 20 and 40 μM , but not 2 μM or 100 μM , compared with controls, as indicated by improved recovery of LVDP and reduced LDH release (n=6 for each condition). Protection with DEA/NO was seen at 2 μM but at 20 μM protection was lost. In conclusion, NO donors protected hearts, but the effect was concentration dependent; protection being lost at higher levels of the