infarct size was similar (38.3±2.0% vs 39.9±2.8%). Transgenic mice also exhibited less apoptosis and interstitial cardiac fibrosis (0.52±0.03% vs 0.65±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 mitochondria 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