NO donor, possibly by formation of RNS. In addition, the optimum concentration for cardioprotection was different for each donor.

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018 GUANIDINOACETATE N-METHYLTRANSFERASE KNOCKOUT MICE EXHIBIT NORMAL LEFT VENTRICULAR REMODELLING, HAEMODYNAMICS AND SURVIVAL AFTER MYOCARDIAL INFARCTION DESPITE LACK OF PHOSPHOCREATINE

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Guanidinoacetate N-methyltransferase (GAMT) catalyses the final step of creatine biosynthesis such that $GAMT^{-/-}$ mice have undetectable levels of phosphocreatine and creatine and accumulation of the precursor (phospho-)guanidinoacetate (PGA). Like phosphocreatine, PGA acts as an energy reservoir, but energy transfer via creatine kinase is 100 times slower. We hypothesised that reduced energy transfer would be detrimental following myocardial infarction (MI).

Methods $GAMT^{-/-}$ and wild-type controls received coronary artery ligation or sham operation (n=104), with 3D echocardiography and left ventricular haemodynamics after 6 weeks.

Results Sham $GAMT^{-/-}$ mice had reduced pressure-generating capacity compared with wild-type (wt), with left ventricular systolic pressure and dP/dt_{max} both significantly lower and impaired contractile reserve. Despite this, there was no significant difference in post-MI survival between $GAMT^{-/-}$ and wt. Both $GAMT^{-/-}$ and wt infarct groups exhibited left ventricular dilatation compared with sham controls, and systolic and diastolic function was also severely impaired. However, there was no significant difference between $GAMT^{-/-}$ and wt infarct groups for left ventricular systolic pressure, left ventricular end-diastolic pressure, dP/dt_{max}, or Tau, nor for end-diastolic and end-systolic volumes or ejection fraction. Left ventricular/body weight increased by 30% in $GAMT^{-/-}$ and 27% in wt, indicating a similar degree of left ventricular hypertrophy in response to MI.

Conclusions Loss of energy transfer in $GAMT^{-/-}$ mice was not detrimental to left ventricular remodelling, haemodynamics and survival post-MI. As acute reduction of energy transfer in the rat infarct model dramatically reduces survival, this strongly suggests that significant compensatory processes occur in $GAMT^{-/-}$ mice as a result of creatine loss during early life.

019 THE TIME COURSE OF INORGANIC PHOSPHATE RELEASE AND ACTOMYOSIN ATPASE RATE IN PERMEABILISED CARDIAC TRABECULAE

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The rate of inorganic phosphate (P_i) release, and therefore the crossbridge ATPase rate, was determined in permeabilised rat trabeculae. Contraction was elicited by laser-flash photolysis of NPE-caged ATP, and time-resolved P_i release was monitored using MDCC-PBP, a coumarin-labelled phosphate binding protein, which fluoresces upon P_i binding. The ATPase rate during the first turnover of the total crossbridges (assuming 150 μM myosin heads) was 23/s. The rate decreased to a steady state of 4/s after the eighth turnover (0.5–0.6 s after activation). This rate is comparable to published values of 3–10/s, made ~15 s after activation using a NADH-linked enzyme assay of ADP release. The advantage of using MDCC-PBP is that the control of mechanochemical coupling can be examined from the onset of force production. Force production and P_i release were simulated using a seven-step scheme. Force was attributed to the states in the sequence $A.M.ADP.P_i \leftrightarrows A.M.ADP1 \leftrightarrows A.M.ADP2$, with strain sensitivity incorporated into the isomerisation of A.M.ADP. The $A.M.ADP.P_i$ and A.M.ADP2 states populated rapidly as force was increasing. In contrast, the preisomerisation A.M.ADP1 accumulated slowly after the force plateau was reached and became the dominant force-bearing state at the time of the eighth crossbridge turnover. Experiments are ongoing to examine how the distribution of A.M states changes in response to rapid length changes.

020 DETERMINATION OF TROPONIN I PHOSPHORYLATION SITES IN HUMAN HEART MUSCLE

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It is well established that troponin I is a phosphoprotein. Phosphorylation alters its functional properties and this modulation of function through the action of kinases and phosphatases plays a role in tuning the contractile apparatus. The prime example of this is phosphorylation by protein kinase A (PKA) as part of inotropic and lusitropic responses to β-adrenergic stimulation. What is considerably less certain is the `where' and `when' of these phosphorylation processes in the human heart. Recent research has addressed this question with new techniques and the results have been surprising and somewhat disconcerting. Quantitative measurements of total phosphorylation by phosphate affinity sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) indicate that 1.6 mol of Pi are incorporated per mole of troponin I in the donor heart. According to current literature, troponin I is phosphorylated in vitro by PKA at Ser22 and 23, by protein kinase C (PKC) at Ser41, Ser43 and Thr142 and by PAK1 and AMPK at Ser149. Both phosphate affinity SDS–PAGE and Fourier transform mass spectrometry plus ECD show that troponin I is mostly bis-phosphorylated and that three-quarters of the bis-phosphorylated species is phosphorylated at Ser22 and 23, the PKA-specific sites. Somewhat surprisingly, there is no evidence of phosphorylation at Ser41, Ser43, Thr142 or Ser149 in human or rat heart muscle and the remaining phosphorylation is at Ser76 or Thr77 (the mass spectrometry techniques do not yet distinguish between the two). In end-stage failing heart muscle the level of phosphorylation is reduced to one-sixth of the donor level, therefore hypotheses that invoke PKC phosphorylation of troponin I need to be revised.

021 PHENOTYPE OF THE ACTC E99K TRANSGENIC MOUSE REPRODUCES HYPERTROPHIC CARDIOMYOPATHY IN PATIENTS

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The mutation Gly99lys (E99K) in the cardiac actin (ACTC) gene has been found to cause hypertrophic cardiomyopathy (HCM) in 75 hypertrophic obstructive cardiomyopathy (HOCM) or LVNC patients. Transgenic mice expressing 50% E99K mutant cardiac actin in their hearts were generated and studied. Over 30% male and 70% female E99K mice died between 28 and 45 days. Anaesthetised 7-month-old male transgenic mice and their non-transgenic littermates were studied using in-vivo cine magnetic resonance imaging. Abnormal cardiac morphology and significantly lower ejection fractions and reduced stroke volumes were observed in the transgenic mice. Peak left ventricular ejection rates were reduced. Left ventricle function of 9-month-old female non-transgenic and transgenic mice were studied with an in-vivo conductance catheter. The transgenic mice had significantly reduced ejection fraction, increased end-diastolic pressure and impaired relaxation. Left ventricular dilation has been observed

in these mice. We studied mutant actin by an in-vitro motility assay. Thin filaments were reconstituted with purified mouse f-actin and human heart tropomyosin and troponin. The E99K thin filaments had higher Ca^{2+} sensitivity than non-transgenic thin filaments. E99K actin thin filaments did not respond to troponin dephosphorylation. The ACTC E99K mouse reproduces many features of HCM, as observed in patients. The basic effect of the ACTC E99K mutation is increased Ca^{2+} sensitivity together with a blunted response to troponin I phosphorylation. The increased myofibrilar Ca^{2+} sensitivity may be sufficient to provoke arrhythmia and account for the high mortality at early ages. Hypertrophy may be a chronic response to Ca^{2+} overloading or due to energy depletion.

022 HYPOXIC PRECONDITIONING OF CARDIOSPHERE-DERIVED CELLS TO INCREASE RETENTION IN THE INFARCTED HEART

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Myocardial infarction results in the formation of a hypoxic scar region. Resident stem cells have been discovered in the adult heart that may be expanded in vitro via the formation of cardiospheres. Administration of these cardiosphere-derived cells (CDC) to the infarcted heart has been shown to improve cardiac function; however, levels of stem cell retention are low. Preconditioning of CDC to a hypoxic environment may increase cell retention, promote proliferation within the scar and further improve cardiac function. CDC were cultured under 2% oxygen for 1 week. Proliferation rates were calculated and hypoxic inducible factor (HIF1α) protein expression and oxygen consumption were measured in intact cells over 1 week. CDC culture under hypoxia for 24 h increased HIF1α by 214% compared with control cells cultured under normoxia. After 1 week in hypoxia, however, there was no difference in HIF1α levels compared with controls. CDC proliferation was increased fivefold under hypoxia. CDC cultured under hypoxia had decreased oxygen consumption compared with control cells cultured under normoxia, with oxygen consumption decreased by 22% with both ADP and FCCP after 24 h. After 1 week of hypoxia, oxygen consumption was decreased by 92% with ADP and 94% with FCCP. Culture under hypoxia generated sufficient CDC for therapy more rapidly than under normoxia. The resulting CDC had reduced oxygen consumption and thus may be better adapted to survive within the hypoxic scar.

023 TRANSCRIPTIONAL REGULATION OF P40PHOX AND P47PHOX EXPRESSION VIA HBP1 IN ENDOTHELIAL CELLS

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Transcription factor HMG-box protein 1 (HBP1) is a member of the HMG-box family of transcription factors and has been found to play an important role in the transcriptional repression of the p47phox gene. The promoter region of p40phox also has a HBP1 binding site, which makes p40phox a possible candidate for HBP1. In this study, we examined the role of HBP1 in the regulation of p47phox and p40phox in endothelial cells. Knockdown of p47phox in a mouse lymphoid endothelial cell line (SVEC4-10) resulted an ~50% increase of HBP1 protein expression, and this was accompanied with a significant increase in p40phox protein expression as detected by Western blot. The levels of HBP1 expression were significantly higher (~2.3-fold) in coronary microvascular endothelial cells isolated from p47phox knockout mice compared with cells isolated from wild-type mice. The role of HBP1 in the transcriptional regulation of p40phox and p47phox expression was further examined by transient in-vitro knockdown of HBP1 using shRNA in human microvascular endothelial cell (HMEC1). Knockdown of HBP1, as shown by Western blot, resulted in a significant increase in p47phox expression and this was accompanied with a significant reduction in p40phox expression. In conclusion, HBP1 plays dual roles in the regulation of NADPH oxidase: it represses p47phox expression and in the mean time promotes p40phox expression. HBP1 may represent an important transcriptional mechanism involved in the regulation of endothelial reactive oxygen species production by NADPH oxidase.

024 ENDOTHELIAL NOX4 NADPH OXIDASE ENHANCES VASODILATATION VIA HYDROGEN PEROXIDE-INDUCED HYPERPOLARISATION AND REDUCES BLOOD PRESSURE

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Introduction NADPH oxidases (Noxs) are reactive oxygen species-generating enzymes implicated in cardiovascular disease. Nox4 is the most abundantly expressed isoform in endothelial cells but its function remains unknown. We investigated the role of endothelial Nox4 on vascular function and blood pressure (BP) in vivo.
Methods and Results We generated transgenic mice with endothelium-specific overexpression of Nox4 (Nox4TG) and studied the effects on endothelial function (aortic rings ex vivo) and blood pressure (telemetry). Nox4 protein levels were twofold higher in Nox4TG aorta compared with wild-type (wt) littermates, with no changes in the expression of other Nox isoforms. Nox4TG had enhanced relaxation to acetylcholine (ACh) compared with wt mice ($-\log EC50$ 7.76Â±0.07 vs 7.20Â±0.05; n=12; $p<0.001$) but similar relaxation to sodium nitroprusside. The ACh response in Nox4TG and wt mice was dentical in the presence of catalase (1500 U/ml) or with high extracellular potassium (30 mM) pre-contraction, but remained greater in Nox4TG in the presence of inhibitors of nitric oxide synthesis (L-NMMA, 100Â μM), soluble guanylate cyclase (ODQ, 5Â μM) or protein kinase G (KT5823, 2Â μM). Ox4TG also had significantly lower BP than wt mice (mean BP 102.5Â±1.8 vs 109.5Â±2.0 mm Hg; n=10; p=0.05), which was abolished after chronic treatment with N-acetylcysteine or an OD/catalase imetic, EUK-8. Plasma nitrite/nitrate levels and aortic levels of phosphorylated VASP were identical and acute intravenous treatment with L-NMMA (10 mg/kg) increased BP to a similar extent in Nox4TG and wt mice. The hypertensive response to chronic 14-day angiotensin II infusion (1.1 mg/kg per day) was lower in ox4TG compared with wt mice (mean BP 116.7Â±4.7 vs 129.4Â±3.5 mm Hg; n=10; $p<0.05$).
Conclusions Nox4TG had significantly enhanced ACh-induced vasodilatation compared with wt mice as a result of hydrogen peroxide-induced hyperpolarisation. Nox4TG also had a lower BP, which was not attributable to altered nitric oxide bioactivity but was normalised by chronic antioxidant treatment. These results suggest that endothelial Nox4 has potentially beneficial effects on vascular tone and BP.

025 IS DEPRESSED MYOCYTE CONTRACTILITY AN EARLY EVENT IN THE NATURAL HISTORY OF HEART FAILURE?

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It is generally accepted that abnormal intracellular Ca^{2+} handling accounts for the depressed left ventricular (LV) systolic and diastolic