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

doi:10.1136/hrt.2009.191064c

M Dodd, H Atherton, M Schroeder, L Heather, J Griffin, K Clarke, G Radda, D Tyler. *Cardiac Metabolism Research Group, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK*

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

doi:10.1136/hrt.2009.191064d

D Goldoni, A Collins. *Centre for Vision and Vascular Science, School of Medicine and Dentistry, Queens University Belfast, Belfast, UK*

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%±4.2% (mean±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 complimentary 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.

Acknowledgements The authors would like to thank Professor Barbara J McDermott for HL-1 cells and Dr You You Zhao for HL-1 cDNA.

Funding D Goldoni is supported by a studentship from the Harold McCauley Fund for Cardiovascular Research.

REFERENCE

1. **Claycomb**, *et al*. *Proc Natl Acad Sci U S A* 1998;**95**:2979–84.

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

doi:10.1136/hrt.2009.191064e

W H Y Cheng, A P Halestrap, E J Griffiths. *Department of Biochemistry and Bristol Heart Institute, University of Bristol, Bristol, UK*

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

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

Funding This study was supported by the BBSRC and NiCox.

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

doi:10.1136/hrt.2009.191064f

C A Lygate, D Dawson, D Medway, L Sebag-Montefiore, S Neubauer. *Department of Cardiovascular Medicine, University of Oxford, Oxford, UK*

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

doi:10.1136/hrt.2009.191064g

C Mansfield, T West, D Bickham, M Ferenczi. *National Heart and Lung Institute, Imperial College London, SAF Building, London, UK*

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

doi:10.1136/hrt.2009.191064h

[1]S B Marston, [1]A E Messer, [1]C E Gallon, [2]R Sancho-Sollis, [2]J W Walker. *[1]Imperial College, London, UK; [2]University of Arizona, Arizona, USA*

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

doi:10.1136/hrt.2009.191064i

[1]W Song, [2]D J Stuckey, [1]E Dyer, [1]D Wells, [1]S E Harding, [2]C A Carr, [2]K Clarke, [1]S B Marston. *[1]Imperial College, London, UK; [2]Oxford University, Oxford, UK*

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