AngII infusion caused two-fold increase in ROS production of WT hearts ($p<0.05$) (but not $p47^{phox}$ KO mice), which was inhibited significantly by diphenyleneiodonium (DPI, a flavoprotein inhibitor) or superoxide dismutase, significantly but slightly by NG-nitro-l-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor), but not by rotenone (mitochondrial respiratory chain inhibitor) or oxypurinol (xanthine oxidase inhibitor). Increased ROS production in WT AngII-infused hearts was accompanied by significant phosphorylation of ERK1/2. In conclusion, $p47^{phox}$ and $p47^{phox}$ signalling through ERK1/2 play an important role in AngII-induced cardiac hypertrophy.

219 GALANGIN, A DIETARY FLAVONOID REDUCES MITOCHONDRIAL DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Khalid S Alnumair*, Chinnadurai Veeramani, Chandramohan Govindasamy, Mohammed A Alsaif. *Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh*

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Introduction Hyperglycemia-induced ROS generation within mitochondria plays a major role in the development of diabetic complications. Mitochondria are one of the most important cell organelles in diabetes research because of its crucial role as a regulator of energy balance. The present study was aimed to evaluate the effect galangin, a flavonoid, on oxidative mitochondrial damage in in streptozotocin (STZ)-induced diabetic rats.

Materials and methods Diabetes was induced by intraperitoneal administration of low dose of STZ (40 mg/kg body weight (BW)) into male albino Wistar rats. Galangin (8 mg/kg BW) or glibenclamide (600 µg/kg BW) was given orally daily once for 45 days to normal and STZ-induced diabetic rats.

Results Diabetic rats showed a significant ($p<0.05$) increase in kidney and heart mitochondrial oxidant (Thiobarbituric acid reactive substance) levels and a significant decrease in enzymatic (superoxide dismutase, glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidants levels as compared to control rats. The activities of mitochondrial enzymes such as isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase and mitochondrial respiratory chain enzymes such as NADH dehydrogenase and Cytochrome c-oxidase were decreased significantly ($p<0.05$) in diabetic rats as compared to control rats. Administration of galangin to diabetic rats resulted in the following findings as compared to diabetic control rats: the oxidant levels decreased significantly ($p<0.05$); the enzymatic and non-enzymatic antioxidants levels increased significantly ($p<0.05$); and the function of mitochondrial enzymes and the mitochondrial respiratory chain enzymes increased significantly ($p<0.05$).

Conclusion From the results, we conclude that galangin could maintain kidney and heart mitochondrial function in diabetic rats.

220 DAPHNIA MAGNA AS A MODEL FOR QUANTIFYING CHAOS IN CARDIAC ARRHYTHMIA

[1]Andrew M. Whiteoak*, [2]Peter E. Penson. *[1]Pharmacy and Biomolecular Sciences, Liverpool John Moores University; [2]School of Pharmacy and Bimolecular Sciences, Liverpool John Moores University*

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Introduction *Daphnia magna* are an established model in ecology for the investigation of toxins in freshwater systems, as well as an emerging model in medical science. *Daphnia* have a myogenic heart, exhibiting responses comparable to that of the human heart to a range of established therapeutics, and displaying varying arrhythmias on exposure to pro-arrhythmic agents. Given the multitude of mathematical methods put forward to predict arrhythmia, it is surprising as yet none are in clinical use. This study aims to rectify this issue.

Methods *D. magna* cardiac action was captured on HD film for periods of 24 s (120+ heart contractions) both prior to and following chemical induction of cardiac arrhythmia. A novel semi-automatic process gave heart area values over the full 1440 frames per film. Along with time domain data, this gave parameters for heart rate and cardiac output after parabolic peak interpolation. Data were analysed in linear terms, including ellipse fitting[1] and standard deviation of successive differences;[2] and in non-linear terms including complex correlation,[3] multi-scale ratio analysis,[4] median stepping increment[5] and finite time growth.[6]

Results Results demonstrate that non-linear analysis methods are superior to linear methods in differentiating cardiac arrhythmias from both one another and from normal rhythm. While most published methods do not differentiate arrhythmic heart conditions with significance, finite time growth, by contrast, may offer some headway toward a robust method of quantifying cardiac arrhythmia.

Implications The *Daphnia* filmatographic model presents an opportunity to examine heart action *in vivo*; offering highly accessible means of assessing both current and developing models for the prediction of arrhythmias.

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221 ALTERED BIOPHYSICAL PROPERTIES OF THE VOLTAGE-GATED SODIUM CHANNELS IN MOUSE ATRIAL AND VENTRICULAR CARDIOMYOCYTES

Sian-Marie O'Brien*, Andrew Holmes, Geroge Parnell, Clara Apicella, Larissa Fabritz, Paulus Kirchhof, Davor Pavlovic. *The University of Birmingham*

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Introduction Several antiarrhythmic drugs target the cardiac sodium current I_{NA}. There is an increasing interest in atrial-

specific ion channel inhibition, e.g. to allow selective antiarrhythmic drug development for the treatment of atrial fibrillation. Here, we sought to compare voltage-gated sodium currents (I_{NA}) in atrial and ventricular cardiomyocytes in mice, as Na^+ channel subunits have been found to differ between atria and ventricles in rat and man.

Aim The aim of this study is to examine whether biophysical properties of I_{NA} are altered in mouse atrial cardiomyocytes compared to left ventricular cardiomyocytes.

Methods Na+ channel currents were measured using whole-cell voltage clamp in left atrial and left ventricular cardiomyocytes. Expression of Nav1.5 proteins and their regulatory ?-subunits was measured by western blotting in left atrial, right atrial and left ventricular tissue of wild-type 129/sv mice (15-20 weeks). Protein levels were normalised against calnexin.

Results Mean peak INA was significantly increased in left atrial myocytes compared to left ventricular (LA=−28.63±1.856pA/pF; n=15/4 cells/mice; LV =-19.83±4.186pA/pF; n=5/2 cells/mice; *p<0.05) and V50 for INA inactivation was significantly more negative in left atrial compared to left ventricular myocytes (LA=−92.4±1.877mV; n=16/4 cells/mice; LV=−81.77 ±2.413mV; n=5/2 cells/mice; *p<0.01). No difference in Nav1.5 expression was detected between chambers, however, expression of ÃÂ²2 and ÃÂ²4 subunits was significantly reduced in atrial tissue compared to left ventricular (LA=0.189 ±0.02014; RA=0.3023±0.0333; LV=0.736±0.0718; *p<0.01; n=4) and (LA=0.00145±0.00033; RA=0.00204±0.00102; LV=0.0214±0.000613; *p<0.01; n=4) respectively.

Conclusion Mouse atrial cardiomyocytes display increased INA compared to cardiomyocytes isolated from the ventricles. Alterations in biophysical properties of INA in mouse atrial myocytes may be attributable to reduced expression of the Nav1.5 ÃÂ²2 and Nav1.5 ÃÂ²4 subunits. Considering the interaction between Nav1.5 and its ÃÂ² subunits may provide novel targets for antiarrhythmic drug therapy.

222 AUGMENTATION OF CREATINE KINASE IN VITRO PROTECTS AGAINST SIMULATED ISCHAEMIA REPERFUSION INJURY

Sevasti Zervou*, Hannah J Whittington, Philip J Ostrowski, Fang Cao, Jack Tyler, Hannah A Lake, Stefan Neubauer, Craig A Lygate. *University of Oxford*

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Creatine kinase (CK) catalyses the interchange of high energy phosphates to buffer ATP levels and maintains cellular energy homeostasis. The heart expresses three isoforms: sarcomeric mitochondrial CK (CKMT2), and the cytoplasmic CKM and CKB isoforms which form homo (MM/BB)- and hetero (MB)-dimers. Impaired CK activity is associated with heart failure and increases susceptibility to ischaemia/reperfusion injury.

We hypothesised that augmentation of CK isoenzymes *in vitro* would improve cell viability following exposure to hypoxia/reoxygenation. For this purpose we created CK overexpression systems by cloning the open reading frame of the different CK isoform sequences into pcDNA3.1 expression vector and stably selected and characterised overexpressing HEK293 cell lines.

The generated cell lines displayed increased CK activity in addition to individual CK isoenzyme activities. CKMT2, CKM and CKB cells had elevated total CK activity (p<0.001; p<0.001; p<0.01 One-way ANOVA, Dunnetts post-test vs HEK293). Furthermore immunocytochemistry showed that CKMT2 co-localises with mitochondrial marker COXIV in the intermembrane space following transient transfection in HL1 atrial cell line.

Both stable and untransfected HEK293 cells were exposed to simulated ischaemia/reperfusion by incubating at 1% O_2 for 18 hour, followed by re-oxygenation at 95% O_2 for 2 hour. The positive control rapamycin was supplemented into the cell media 4 hours prior to hypoxia. Viability analysis by propidium iodide detection using a CyAN flow cytometer at 488 nm, showed increased cell survival by 33% in CKMT2, 47% in CKM and 58% in CKB cells when compared to untransfected HEK293 controls (in all cases p<0.05, One-Way ANOVA Dunnetts post-test vs HEK293).

To determine whether protection was due to changes in antioxidant capacity, cells were loaded with the reactive oxygen species indicator dye, $DCFH_2$-DA, and exposed to H_2O_2-induced oxidative stress. Overexpression of CK isoenzymes failed to attenuate fluorescence from oxidised dye in contrast to the known antioxidant, Trolox. Transient expression of CK constructs in the HL1 cell line was used to test the effects of anthracycline exposure on cell viability (48 hour doxorubicin). Pre-treatment with Trolox increased cell survival by 12.4% (79.4%±2.0 vs. 67%±1.5 in empty-vector control cells; p<0.01) whereas overexpression of CK isoenzymes did not alter cell death rates.

In conclusion, overexpression of any one (of three) cardiac creatine kinase isoenzymes protects against ischemia/reperfusion *in vitro*. This most likely reflects enhanced energy reserve due to elevated CK activity, since response to oxidative challenge was unaltered. Further mechanistic studies and *in vivo* confirmation of these findings are merited.

223 INVESTIGATING THE ROLE OF AEROBIC GLYCOLYSIS IN ARTERIAL CALCIFICATION

Nabil Rashdan, Vicky MacRae*. *University of Edinburgh*

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Objective The process of arterial calcification shares many similarities to skeletal mineralisation, and involves the deposition of hydroxyapatite in the arteries. However, the cellular mechanisms responsible have yet to be fully elucidated. Accumulating evidence suggests that aerobic glycolysis (the Warburg effect), plays a critical role in meeting the demand for energy and biosynthetic precursors during proliferation and differentiation in numerous cell types. Therefore we addressed the hypothesis that vascular smooth muscle cell (VSMC) calcification requires aerobic glycolysis to produce energy and the necessary biosynthetic precursors.

Methods Calcification of murine aortic VSMCs was induced by 3 mM Pi for 7 days. Calcium deposition was determined using alizarin red staining and a modified o-cresolphthalein method. VSMCs were cultured with the fluorescent glucose analogue 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)−2-Deoxyglucose (2-NBDG) to determine changes in glucose uptake. Gene expression was analysed by qRT-PCR.

Results Calcium deposition was significantly increased in VSMCs cultured in 3 mM Pi versus control conditions (124%, p<0.001). Calcified VSMCs also showed increased mRNA expression of Runx2, Phospho1, Ocn and PiT-1 (p<0.001), recognised osteogenic markers of arterial calcification.