

involvement. We recently demonstrated inhibition of endogenous catalase, using 3-Amino-1,2,4-triazole (3-AT), significantly increases the rate of ROS production in isolated ventricular cardiomyocytes [1]. This study investigated the effect of catalase inhibition on I/R injury in whole hearts.

Langendorff-perfused rat hearts underwent 25mins stabilization then 25mins global normothermic ischemia (37 °C) and 120mins reperfusion. Hearts were assigned to the groups control or treatment (n = 4/group). The treatment group was perfused with 15 mM 3-AT for 15mins during stabilization. Perfusate was collected pre-ischemia and at intervals throughout reperfusion to determine creatine kinase (CK) activity. Hearts were also stained with Triphenyl tetrazolium chloride at the end of reperfusion to estimate the percentage infarct size. Statistical analysis was performed using a mixed model ANOVA (for CK analysis) and two-tailed unpaired t-test (for percentage infarct). Values are expressed $\pm$ SEM.

The results showed that catalase inhibition with 3-AT significantly increased both markers of myocardial damage. The mean CK level was significantly greater in 3-AT treated hearts than in control hearts ($P < 0.01$). Mean percentage infarct size was significantly greater in 3-AT treated hearts than in control hearts (36.7 ± 4.2 in vs. 17.8 ± 5.4 respectively; $P < 0.05$).

These results provide evidence that the catalase inhibitor 3-AT augments I/R injury. This is suggestive of a role for catalase in cardioprotection, attenuating ROS production.

[1] Catalase Inhibition Augments Reactive Oxygen Species Production in Isolated Ventricular Myocytes. S Boles, A Polak, B Littlejohns, H Lin, M S Suleiman. *Heart* 2014;100:Suppl 1 A3 doi:10.1136/heartjnl-2013-305297.5

Abstract n° P-15-17

The anti-ischaemic effects of Perhexiline in the murine heart

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Background: Perhexiline, originally prescribed as an anti-anginal agent, has been shown to have cardioprotective effects against various cardiovascular disorders such as chronic heart failure and hypertrophic cardiomyopathy. Clinical and animal studies have demonstrated that perhexiline has the ability to inhibit fatty acid oxidation by blocking the carnitine palmitoyltransferase I mitochondrial enzyme, thus acting as a metabolic modulator [1]. However, the protective mechanism(s) of perhexiline remains unclear. Herein, we investigated the anti-ischaemic effects of perhexiline during myocardial ischaemia and explored the effects on glucose metabolism in the form of pyruvate dehydrogenase (PDH).

Method and results: Using the isolated heart Langendorff model, murine hearts were perfused with a modified Krebs Henseleit buffer consisting of 1.2 mM palmitate. Hearts were perfused under normoxic conditions for 40 minutes, followed by 30 minutes no-flow ischaemia. Control or 2 μ M Perhexiline were perfused prior to the ischaemic insult. Heart samples for each group were collected after stabilisation (T1) and after ischaemia (T2), with the cardiac haemodynamics recorded throughout stabilisation. Haemodynamic data revealed that 2 μ M Perhexiline improves left ventricular pressure (LVP), LVdP/dt and coronary flow when compared to control. Western blot analysis on the collected heart samples demonstrated that 2 μ M Perhexiline decreased PDH phosphorylation at ser232, ser293 and ser300 during ischaemia

(T2), thus suggesting an increase in PDH activity when compared to control.

Conclusion: Our results demonstrate that 2 μ M Perhexiline protects the heart against ischaemia by increasing glucose metabolism through PDH activation via the dephosphorylation of ser232, ser293 and ser300.

References:

[1] Ashrafiyani, H.; Horowitz, J.D.; Frenneaux, M.P. Perhexiline. *Cardiovascular Drug Reviews* 2007, 25; 76-97.

Abstract n° P-15-18

Ouabain Induces Metabolic Changes in the Absence of Contractile Work in the Blebbistatin-perfused Rat Heart

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Cardiac hypertrophy is associated with elevated $[Na]_i$, altered workload and impaired cardiac energetics. While increased $[Na]_i$ has been shown to cause metabolic alterations in isolated cardiomyocytes, in the perfused heart it is questionable whether these metabolic changes are driven by $[Na]_i$ elevation alone or by the associated increase in inotropy and ATP demand. The aim of this study was to elucidate the effect of $[Na]_i$ elevation due to Na/K ATPase inhibition on the metabolism of control and blebbistatin (Bleb)-perfused, electromechanically-uncoupled hearts. Rat hearts (n = 6/group) were perfused at constant-pressure for 20 min equilibration followed by a further 10 min with KH buffer ('Control') or 'Bleb' (5 μ M). In the absence or presence of Bleb, hearts were then perfused for a further 20 min with or without ouabain (50 μ M), freeze-clamped and aqueous metabolites extracted and quantified using ¹H Nuclear Magnetic Resonance Spectroscopy. LVDP, HR and QT90 interval were monitored throughout the protocols using a left-ventricular balloon and ECG. Coronary lactate efflux (as a marker of anaerobic glycolysis) was monitored. In control hearts, ouabain increased LVDP by $25 \pm 5\%$ and lactate efflux from 228 ± 49 to 504 ± 37 nmol/min/g wt* as well as the concentrations of acetylcarnitine by $91 \pm 7\%$ and fumarate by $92 \pm 15\%$. In Bleb-treated hearts, LVDP was reduced from 133 ± 5 mmHg to 1 ± 0.8 mmHg and subsequent exposure to ouabain caused no inotropy. In these hearts, LVDP, heart rate, QT90 and lactate efflux all remained unaffected by ouabain suggesting that ATP demand was not increased. However, glutamate was depleted by $22 \pm 8\%$ and glutamine by $20 \pm 12\%$ while all other detectable metabolites were unchanged. Na elevation in isolated hearts is associated with an altered metabolic profile. This profile remains altered when hearts are mechanically uncoupled which suggests that some components of the Na-induced remodeling of energy metabolism are independent of the inotropic state of the heart and the associated increase in ATP demand. (* $P < 0.05$).

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Cardiac Effects of Pharmacological Soluble Guanylate Cyclase Activation in Type-1 Diabetes Mellitus

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