

P5 ACUTE β -ADRENERGIC STIMULATION OR PERFUSION WITH A cAMP analogue results in immediate and sustained inhibition of mitochondrial permeability transition pore opening

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Background Ischaemia/reperfusion (I/R) injury is mediated by opening of the mitochondrial permeability transition pore (MPTP). Experimental studies have shown that a variety of interventions, including ischaemic preconditioning (IP), protect the heart by inhibiting MPTP during reperfusion. Interestingly, we and others have shown that IP has no inhibitory effect on MPTP prior to ischaemia. We have recently shown that acute and transient perfusion of hearts with cell-permeable cAMP analogue confers marked protection against I/R. However, whether this treatment affects MPTP opening is not presently known. The aim of this work was to address this issue.

Methods Isolated Langendorff-perfused rat hearts were perfused with either 0.2 μ M of the β -adrenergic receptor agonist isoprenaline (to increase endogenous level of cAMP) for 3 min, or with 7.5 μ M of the cell-permeable cAMP analogue 8-Br-cAMP-AM (8-Br) for 5 min. Mitochondria were isolated immediately after the treatment. Additional hearts were treated with either intervention and exposed to global ischaemia followed by reperfusion. Mitochondria were isolated from these hearts after 5 min of reperfusion. MPTP opening was assessed using Ca²⁺-induced mitochondria swelling assay or by evaluation of Ca²⁺ retention by mitochondria.

Results Both Iso and 8-Br inhibited MPTP opening immediately after the treatment. This inhibitory effect of MPTP opening was also observed at the beginning of reperfusion.

Conclusion In contrast to IP, acute elevation of cAMP level in myocardium either by Iso or 8-Br is associated with desensitisation of MPTP to Ca²⁺ retention this effect is maintained during I/R.

P6 THE ROLE OF PLASMA MEMBRANE CALCIUM ATPASE 1 IN ANGIOGENESIS

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Angiogenesis, the process of forming new blood vessels, is an important process in both cardiovascular health and disease. A recent student has identified a novel role for plasma membrane calcium ATPase (PMCA) 4 in angiogenesis through mediating endothelial cell migration and tubule formation. However, the role of PMCA1 has yet to be investigated. Here, we aim to establish the role of PMCA1 in angiogenesis and endothelial cell behaviours. We hypothesise that PMCA1 modulates key endothelial cell processes which are associated with the development of new blood vessels.

Knockdown of PMCA1 was achieved in human umbilical vein endothelial cells (HUVECs) using siRNA (si-PMCA1) and confirmed with qPCR. Following PMCA1 knockdown, HUVEC viability and migration was assessed using the MTT assay and wound healing assay respectively. Additionally, the rate of HUVEC proliferation was evaluated by Ki-67

immunofluorescence staining. Finally, apoptosis of HUVECs was investigated using a caspase-Glo3/7 assay.

Transient knockdown of PMCA1 in HUVECs resulted in an 85% reduction in PMCA1 gene expression. Phenotypically, si-PMCA1 HUVECs display decreased HUVEC cell viability but also reduced apoptosis. Staining for Ki-67 revealed that si-PMCA1 HUVECs had a larger percentage of cells active in the cell cycle. Furthermore, loss of PMCA1 impairs migration of HUVECs into the 'wound', 24 hours after the scratch assay was performed.

Overall it appears loss of PMCA1 is detrimental for HUVEC viability and migration which may result in a reduction in angiogenesis, although further work is required to establish the pumps direct role in vessel formation.

P7 PROFILE OF CIRCADIANLY REGULATED METABOLIC GENES IN DYSTROPHIC HEART

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Duchenne muscular dystrophy (DMD) is a monogenic disorder caused by the lack of the integral structural protein, dystrophin, which results in severe muscle wasting and cardiomyopathy in affected boys. Indeed, cardiorespiratory complications are the predominant cause for mortality in DMD patients. We have recently shown that circadian rhythm is disrupted in dystrophic mice as a direct result of the lack of dystrophin protein. It is well reported that disruption of circadian rhythmicity leads to perturbed metabolism and an array of disorders including obesity, diabetes and cardiovascular disease. Disturbed cardiac metabolism in DMD patients and dystrophic mice is also well described, and thus it would be interesting to learn whether pertinent metabolic genes which are known to be circadianly regulated, are disrupted in dystrophic mice. Here we show for the first time, significant changes in the differential expression patterns of multiple genes involved in free fatty acid and glucose metabolism, in 2 mouse models of DMD compared to control mice. These findings provide the foundation for further research to better understand the metabolic/circadian milieu and its effect on dystrophic heart, so that we may devise strategies to augment cardiac metabolism, in an effort to halt the deterioration in cardiac phenotype.

P8 PROPIONATE ANIONS ACCUMULATED IN PROPIONIC ACIDAEMIA INFLUENCES THE CARDIAC GENE EXPRESSION LANDSCAPE

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Propionic acidaemia (PA) is an autosomal recessive disorder characterised by malfunctional mitochondrial propionyl-CoA carboxylase. Consequences of this inborn error of metabolism (IEM) is defective catabolism of propiogenic substrates (branched-chain amino acids, odd-number fatty acids, etc.) leading to mitochondrial accumulation of propionyl CoA and