oxidative stress model. SR Ca^{2+} release was measured by treating cells loaded with fluorescent dye, fluo-4-AM, with caffeine. Cardioprotection was tested by exposing ARVC to metabolic ischaemia-reperfusion. Ned-19 was found to significantly delay the time to mPTP opening by 76%±16%, 55%±20%, 47%±19% and 44%±17% (all $p<0.05$) at concentrations of 100 μmol/l, 10 μmol/l, 1 μmol/l and 0.1 μmol/l, respectively, compared with the control group. Concentrations of Ned-19 at 100 μmol/l, 10 μmol/l and 1 μmol/l, but not 0.1 μmol/l, significantly inhibited caffeine-stimulated SR Ca^{2+} release (71.6%±2.0%, 34.2%±1.9%, 55.6%±5.5% and −14%± 21%, respectively) indicating non-specific effects at higher concentrations. A low dose of 0.1 μmol/l Ned-19 increased the survival of cells following metabolic ischaemia-reperfusion to 46%±19% from 29% (control).

In conclusion, we have shown the involvement of NAADP in SR Ca^{2+} release and mPTP opening, and that by inhibiting NAADP signalling at reperfusion with Ned-19, cardiomyocytes may be protected against ischaemia-reperfusion injury.

BAS/BSCR33 CARDIOPROTECTION BY HYPOXIA-INDUCIBLE FACTOR-1α: UNDERLYING BENEFICIAL EFFECTS ON MITOCHONDRIAL FUNCTION

doi:10.1136/hrt.2010.205781.44

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Introduction Activation of hypoxia-inducible factor-1α (HIF-1α) protects the heart from ischaemia-reperfusion injury, although the underlying mechanisms are unclear. We hypothesised that HIF-1α-induced cardioprotection is mediated by beneficial effects on mitochondrial function.

Methods and results Two different experimental models of HIF-1α activation were used: (1) pharmacological inhibition of proline hydroxylase (PHD) and (2) genetic inactivation of von Hippel–Lindau (VHL), proteins responsible for HIF-1α degradation under normoxic conditions. A single dose (3 mg/kg) of the PHD inhibitor (GSK0360A or PHDi), administered by oral gavage 4 h before ex vivo myocardial infarction, reduced myocardial infarct size (percentage of the area at risk) in male Sprague–Dawley rats (30.6%±2.9% PHDi vs 44.2%±2.9%; $p<0.5$; $N>5$). Next, conditional cardiac-specific VHL knockout mice (VHL-KO) that express an inducible Cre-recombinase transgene to delete the VHL-floxed gene within the heart following tamoxifen induction, expressed higher levels of HIF-1 in the heart as assessed by immunostaining. The activation of myocardial HIF-1 resulted in a smaller myocardial infarct size in comparison with the littermate control (29.1%±4.7% in VHL-KO vs 52.5%±3.3% in control; $p<0.05$; $N>5$/group). In VHL-KO cardiomyocytes subjected to simulated ischaemia-reperfusion injury (SIRI) (120 min ischaemia and 15 min reperfusion, the production of reactive oxygen species (ROS) (measured by reduced Mitotracker Red fluorescence)(1.0%±0.1-fold increase in VHL-KO vs 1.3%±0.2-fold increase in control; $p<0.05$; $N>3$ experiments each with 40 cells) and mitochondrial permeability transition pore (mPTP) opening sensitivity was reduced (measured by TMRM fluorescence) (1.1%±0.1 fold increase in VHL-KO vs 1.4%±0.1 fold increase in control; $p<0.05$; $N>3$ experiments each with 40 cells).

Conclusions HIF-1α activation by genetic deletion of VHL or pharmacological inhibition of PHD, is cardioprotective and this protective effect can be attributed in part to beneficial effects on the mitochondria.

BAS/BSCR34 IS AN INCREASED AMPK ACTIVATION DURING ISCHAEMIA ESSENTIAL FOR THE PROTECTION OF THE HEART AGAINST INFARCTION?

doi:10.1136/hrt.2010.205781.45

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Background During ischaemia, AMPK activation occurs in order to provide energy from alternative resources. However, AMPK activity is known to be impaired in diabetes. We hypothesised that enhancing AMPK activation above physiological levels during ischaemia would protect both the normoglycaemic and the diabetic heart.

Methodology Hearts from Wistar and Goto Kakizaki rats (GK, a mildly diabetic rat strain) were subjected to 35 min coronary artery occlusion in the presence of 10, 20 or 40 μM A-769662 (an activator of AMPK), followed by 120 min of reperfusion with normal buffer ($n \geq 6$). Risk zone and myocardial infarction were assessed using Evans blue and 2,3,5-triphenyltetrazolium chloride (TTC) staining, respectively and expressed as percentage of the area at risk (I/R%). The effect of A-769662 on mitochondrial permeability transition (opening of the mPT pore is associated with reperfusion injury) was also investigated by exposing rat cardiomyocytes loaded with the fluorophore TMRM to a laser oxidative insult; the time to mitochondrial membrane depolarisation and rigour contracture were measured (n=6, 80–100 cells/assay).

Results A-769662 reduced the infarct size in both the normoglycaemic and diabetic hearts in comparison with control hearts at 20 μM (31.8%±3.1 vs 51.4%±1.5 normoglycaemic heart; 22.7%±3.0 vs 37.6%±2.7 for the GK heart; $p<0.05$) and at 40 μM (35.6%±1.9 vs 51.4%±1.5 for the normoglycaemic heart; 18.6%±1.6 vs 37.6%±2.7, for the GK heart; $p<0.05$) In addition, A-769662 also significantly delayed the mPTP opening (147.71%±10.2% at 20 μM, 146.7%± 15.6% at 40 μM, vs control 100%, $p<0.05$).

Conclusions Our data suggest that the enhancement of AMPK activity during ischaemia may lead to infarct reduction and delayed opening of the mPTP in the ischaemic reperfused rat heart.

BAS/BSCR35 EFFECTS OF ALDOSTERONE AND OBESITY ON THE ANTICONTRACTILE PROPERTIES OF PERIVASCULAR ADIPOSE TISSUE IN RAT AORTIC RINGS

doi:10.1136/hrt.2010.205781.46

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The mechanisms by which perivascular adipose tissue (PVAT) can reduce vascular contractility remain to be elucidated and may underlie the associations of obesity with hypertension, insulin resistance and cardiovascular disease. This study investigates the effects of aldosterone and obesity in isolated rat aorta. Healthy and obese male rats were killed by stunning and cervical dislocation. The mesenteric bed was removed and arteries dissected with and without PVAT. Arteries were mounted on a wire myograph and were constricted with 60 mM KPSS. Cumulative concentration responses (10^{-9}–10^{-5} M) to norepinephrine (NE) were performed before and after 10 min incubation with aldosterone (5 nM). Endothelial integrity was confirmed by relaxation to 10^{-5} M acetylcholine. Responses are expressed as mean (±SEM) percentage of KPSS constriction and analysed using two-way ANOVA. PVAT (n=10) significantly ($p<0.05$) reduced constriction in healthy vessels