showed no structural defects compared to control littermates. Additionally, examination of these hearts by immunofluorescence microscopy revealed normal myofibrillar structure and localization of the transgenic protein to intercalated disks, as normally seen with the endogenous protein. Protein markers for cardiomyopathy were examined by qPCR and revealed no difference between non-transgenic and transgenic animals. Echocardiography and magnetic resonance imaging of the N-RAP transgenic animals revealed no significant structural or functional differences when compared to control littermates at 12 weeks of age. Based on these data, it does not appear that overexpression of N-RAP directly leads to an observable cardiac phenotype. The alternative hypothesis that upregulation of N-RAP in dilated cardiomyopathy is compensatory remains to explored.

3737-Pos

Extracellular Matrix Remodelling in an Ovine Model of Ageing and Heart Failure

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We sought to establish a model of heart failure (HF) encompassing young and aged animals to determine if alterations in the amount of cardiac extracellular matrix (ECM) occur in ageing and whether these changes are similar to those in HF.

HF was induced in sheep aged 18 months (young) and those over 8 years (old) by 4 weeks rapid right ventricular pacing (3.5Hz). Paraffin-embedded left ventricular (LV) samples were stained with picro-sirius red. Interstitial collagen was visualised by polarised light microscopy. LV protein extracts were assessed for MMP activity using gelatin zymography. Statistical significance was calculated using the mean ± SEM and a t-test or by 2-way ANOVA.

LV diameter increased with age (OC) compared to young controls (YC) (3.04 ± 0.2cm *vs*.2.44 ± 0.14cm, n=4-13, $P<0.05$), in young heart failure (YF) compared pre-pacing (3.82 ± 0.1cm *vs.* 2.44 ± 0.1cm, n=13, $P<0.001$) and in old heart failure (OF) compared to pre-pacing (3.87 ± 0.1cm *vs*. 3.04 ± 0.2cm, n=4, $P<0.05$). Collagen content increased in OC compared to YC ($2.27 \pm 0.4\%$ *vs*. $0.96 \pm 0.1\%$, n=5, $P<0.05$) and in YF compared to YC ($2.62 \pm 0.3\%$ *vs*. $0.96 \pm 0.1\%$, n=5-8, $P<0.001$), and decreased in OF compared to YF ($1.26 \pm 0.4\%$ *vs*. $2.62 \pm 0.3\%$, n=4-8, $P<0.01$). Normalised MMP-2 activity increased in OC (1.52 ± 0.08), YF (1.66 ± 0.14) and OF (1.74 ± 0.17) compared to YC (0.98 ± 0.22, $P<0.05$, n=6-8).

The commonality of these changes seen in ageing and HF may indicate that cardiac ECM remodelling is important in the predisposition of ageing to the development of HF.

All procedures accord to The UK Animals (Scientific Procedures) Act, 1986.

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3738-Pos

Involvement of Calcineurin/STAT3 Pathways in Heart Hypertrophy during Pregnancy

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Previously we have characterized the physiological heart hypertrophy which occurs during pregnancy. However, the underlying molecular mechanisms of pregnancy-induced hypertrophy are still not elucidated. Here we performed Western Blot analysis together with high resolution confocal microscopy to identify the key signaling molecules involved in the pregnancy-induced heart hypertrophy in non-pregnant in diestrus stage (NP), late pregnant (LP), 24 hours post partum (PP1) and 7 days post partum (PP7) mice. Western Blot analysis of heart lysates showed that phospho-AKT protein levels were decreased ~ 7 fold at the end of pregnancy (N=4 NP and N=4 LP mice). Interestingly, AKT activity was restored one day post-partum to levels comparable to NP. There were no significant changes in total or phosphorylated levels of ERK1/2 with pregnancy. The calcium/calmodulin-dependent serine-threonine phosphatase Calcineurin, which has been shown to be upregulated in pathological cardiac hypertrophy, was significantly downregulated at the end of pregnancy, and this downregulation was reversed 1 day after partum. The phosphorylation of the signal transducer and activator of transcription 3 (STAT3), but not its total protein levels, was also significantly lower at the end of pregnancy and was restored completely one day post-partum. Although there was a tendency in reduction of phospho-GSK protein levels in the LP group, this reduction was not statistically significant. High resolution confocal microscopy demonstrated that pregnancy is associated with relocalization of AKT and pAKT to the nucleus, which is partially reversed 24 hours post-partum. While the subcellular distribution of ERK1 and pERK was not regulated by pregnancy, the nuclear labeling of P38, JNK1 and pJNK was significantly upregulated at the end of pregnancy. Although pJNK localization disappeared completely from the nucleus in the PP1 group, P38 and JNK1 nuclear labeling remained high 24 hours PP.

3739-Pos

Gper Activation Inhibits Mitochondria Permeability Transition Pore Opening Via Erk Phosphorylation and Provides Cardioprotection after Ischemia-Reperfusion

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Recently, several studies have demonstrated G protein coupled receptor 30 (GPER) can directly bind to estrogen and mediate its action. We investigated the role and the mechanism of estrogen-induced cardioprotection after ischemia-reperfusion using a specific GPER agonist G1. Isolated hearts from male mice were perfused using Langendorff technique with oxygenated (95% O2 and 5% CO2) Krebs Henseleit buffer (control), with addition of G1 (1μM), and G1 (1μM)+PD98059 (10μM) to investigate the involvement of Erk pathway. After 20 min of perfusion, hearts were subjected to 20 min global normothermic (37°C) ischemia followed by 40min reperfusion. During the course of experiment cardiac function was measured and myocardial necrosis was evaluated by triphenyltetrazolium chloride (TTC) staining at the end of the reperfusion. Mitochondria were isolated after 10 minutes of reperfusion to assess the calcium load required to induce mPTP opening. G1 treated hearts developed better functional recovery with higher rate pressure product (RPP, 6140 ± 264 *vs*. 2640 ± 334 mmHgxbeats/min, $p<0.05$). The infarct size decreased significantly in G1 treated hearts ($21 \pm 2\%$ *vs.* $46 \pm 3\%$ $p<0.001$) and the Ca^{2+} load required to induce mPTP opening increased (2.4 ± 0.06 *vs.* 1.6 ± 0.11 μM/mg mitochondrial protein, $p<0.05$) as compared to the controls. The addition of PD 98059 significantly prevents G1 effect on heart function RPP (4120 ± 46 mmHgxbeats/min, $p<0.05$), infarct size ($53 \pm 2\%$) and calcium retention capacity (1.4 ± 0.11μM/mg mitochondrial protein $p<0.05$) These results suggest that GPER activation inhibits the mPTP opening and provide a cardioprotective effect after ischemia-reperfusion and this effect is mediated by Erk pathway. Supported by NIH and AHA.

3740-Pos

Opposite Production of Reactive Oxygen Species by Complexes I and III during Heart Ischemia/Reperfusion

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In mitochondria, the main reactive oxygen species (ROS) generators are complexes I and III. Mitochondrial ROS generation has been implicated in cellular damage occurring in a variety of pathologies including ischemia/reperfusion (I/R). Most of the studies have observed an increase of ROS production after I/R. However, there is evidence of an increase of ROS production after cardioprotection by preconditioning interventions.

We investigated the differential production of ROS by complex I and III in I/R and sham isolated mitochondria heart mice, and the action of pro-apoptotic drugs (rotenone and antimycin A) on ROS production by these complexes in sham animals.

Mitochondrial ROS generation by both complexes was measured using amplex red in the presence of horseradish-peroxidase. Specific substrates for complex I (glutmate/malate) and complex II (succinate), and the inhibitors (rotenone and antimycin-A) were used. Mitochondria from I/R mice produced more ROS than mitochondria from sham when the substrate for complex I was used. In contrast, with the substrate of complex II, mitochondria from I/R mice produced less ROS than sham. Application of rotenone and antimycin-A in mitochondria from sham heart significantly increased ROS production when the substrate for the complex I was used. Surprisingly, these inhibitors decreased the ROS production when the substrate for the complex II was used. This data indicate the ambivalent production of ROS by the respiratory chain complexes I and III, and suggest opposite role of ROS depending on the complex being cardio-deleterious for complex I and cardio-protective for complex III. Supported by NIH and AHA.

3741-Pos

Sub-Proteomic Fractionation of Rat Cardiac Tissue: Comparing Ischemic Vs Normal Remote Region with In-Solution Based Proteomics

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