

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 be 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 \pm SEM and a t-test or by 2-way ANOVA.

LV diameter increased with age (OC) compared to young controls (YC) (3.04 ± 0.2 cm vs. 2.44 ± 0.14 cm, $n=4-13$, $P<0.05$), in young heart failure (YF) compared pre-pacing (3.82 ± 0.1 cm vs. 2.44 ± 0.1 cm, $n=13$, $P<0.001$) and in old heart failure (OF) compared to pre-pacing (3.87 ± 0.1 cm vs. 3.04 ± 0.2 cm, $n=4$, $P<0.05$). 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. This work was supported by the British Heart Foundation and EU "Normacor".

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 (1 μ M), and G1 (1 μ M)+PD98059 (10 μ M) 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 mmHg \times beats/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 Ca²⁺ load required to induce mPTP opening increased (2.4 ± 0.06 vs. 1.6 ± 0.11 μ M/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 mmHg \times beats/min, $p<0.05$), infarct size ($53 \pm 2\%$) and calcium retention capacity (1.4 ± 0.11 μ M/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 (glutamate/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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Subcellular fractionation of a complex proteome (cardiac tissue) allows for enrichment of a subset of low abundant proteins. This permits more in depth analysis of the proteome by reducing the complexity. Adult rat cardiac tissue was made ischemic by ligating the left anterior descending coronary artery in vivo for 1 hour with no reperfusion, and the healthy remote area was differentiated from the ischemic tissue by staining with Evans Blue dye after harvesting the heart. A series of differential centrifugation steps produced nuclear, mitochondrial, cytoplasmic, microsomal, and sarcomeric fractions of rat ischemic and remote healthy tissue. The sarcomeric fractions of the remote vs ischemic cardiac tissue were digested with trypsin, and the peptides were labeled with isobaric tags for relative quantitation (iTRAQ). The labeled peptides were then fractionated with an Agilent 3100 OFFGEL fractionator, which separated the peptides in 12 fractions based on their isoelectric point from pH 3-10. The 12 fractions from the OFFGEL were run on a Dionex U-3000 nano LC coupled to a ThermoFinnigan LTQ running in PQD (pulsed Q dissociation) mode to detect the low mass reporter ions of the isobaric tags on the peptides. The peptides were analyzed with MASCOT (MatrixScience), and Scaffold v2.5.2 Q+ with a minimum of two unique peptides and a level of confidence set at 95%. Five-fold more proteins were identified when the labeled digests were fractionated with OFFGEL compared to no fractionation prior to LC-MS/MS. With a one hour ischemic event, we found approximately 11% of the detected proteins in the sarcomeric fraction had changed at least 1.5 fold. Therefore, this in-solution method incorporating sub-proteomic fractionation in conjunction with OFFGEL separation may be an approach for discovery of relative protein changes in cardiac tissue.

3742-Pos

Contractile Function is Altered by Regulation of HO-1 Activity in Single Adult Rat Cardiomyocytes

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Heme oxygenase-1 (HO-1) is a membrane protein upregulated in response to oxidative stress that confers cardioprotection. The therapeutic potential of HO-1 can be assessed by examining cardiomyocytes survival and function using cobalt protoporphyrin (CoPP) or tin protoporphyrin (SnPP) to increase or inhibit HO-1 activity, respectively. Whether altered HO-1 function affects cardiomyocyte contractility, however, is unknown. Thus, we determined the effects of CoPP (n=11), SnPP (n=15), or PBS (control, n=15) on *in vitro* single intact adult cardiomyocyte contraction and relaxation using video microscopy and calcium imaging (IonOptix) at 0.5, 1 and 2 Hz. At 0.5 Hz there was no difference in the rate or magnitude of shortening between groups, and all cells had similar fractional shortening (FS) normalized to peak calcium (FS/Ca²⁺). FS/Ca²⁺ was, however, greater at 1 Hz and 2 Hz in SnPP-treated cells, while CoPP- and PBS-treated cells maintained similar FS/Ca²⁺ relationships across frequencies (p<0.05). Intriguingly, while SnPP-treated cells increased contraction at faster pacing, only 53% of those cells contracting at 0.5 Hz beat synchronously at 1 Hz, compared to 100% of CoPP-treated cells and 80% of PBS-treated cells. This difference became more pronounced at 2 Hz, as only 26% of SnPP-treated cells and 60% of PBS treated cells able to follow stimulation, while 100% of CoPP-treated cells continued to beat synchronously. Similarly, although time to 90% relaxation was not different between groups at 0.5 Hz, it was significantly faster in CoPP- vs. SnPP-treated cells at 2 Hz (p<0.05). These results suggest that increasing HO-1 activity via CoPP treatment maintains cell viability under stress, while SnPP- induced HO-1 inhibition reduces survivability at higher pacing frequencies. The mechanisms behind this action are unknown, but may be partially due to HO-1-induced calcium desensitization of the myofilament. Supported by NSF GRFP (SGN) and NIH HL086709 (MA, MR).

3743-Pos

Study of O-GlcNacylation of Contractile Proteins in Cardiac Myofibrils by Enzymatic Labelling

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Phosphorylation occurs on serine and threonine residues and plays important roles in the regulation of contractile proteins. In heart failure changes in levels of phosphorylation are reported in a number of cardiac sarcomeric proteins. O-linked-N-acetylglucosamine (O-GlcNAc) modification is another possible posttranslational modification on serine and threonine residues and recent publications reported mapping of O-GlcNAc modification sites in some rat contractile proteins, including myosin heavy chain (MHC), actin, cardiac troponin I and myosin light chains (MLC1 and MLC2). O-GlcNAc modification on normal donor hearts (49yr F and unknown) and hypertrophic obstructive cardio-

myopathy myectomy samples (33yr M and 42yr M) were studied. Cardiac myofibrils were isolated and the O-GlcNAc groups labelled using an enzymatic labelling system in the presence of PUGNAc (inhibits O-GlcNAc removal enzyme O-GlcNAcase) and protease inhibitors. This method allows coupling of an azido modified N-acetylgalactosamine (UDP-GalNAz) to O-GlcNAc using the mutant enzyme Y289L β 1,4-galactosyltransferase (Y289L GalT). The labelled groups were detected by reacting the azide group with an alkyne bearing the tetramethylrhodamine (TAMRA) fluorescent tag for direct imaging following SDS-PAGE. The gel was post-stained with a total protein stain for analysis with densitometry. The labelling process showed no impact on myofibril protein profiles when native and labelled myofibrils were compared. Preliminary results showed that O-GlcNAcylation profiles vary between samples with a total of 7 proteins identified. Strong TAMRA signals from α -actinin and MLC1 were observed in all the four myofibrils samples. In three of the samples the proteins actin, tropomyosin (Tm) and myosin binding protein-C (MyBP-C) were positively labelled. MHC and desmin O-GlcNAcylation were observed in one of the four subjects. This enzymatic labelling method will be investigated further for possibility of quantification and methods for mapping sites of these modifications with mass spectrometry will be explored.

3744-Pos

Cannabinoid (CB) Receptors are not Involved in A-955840 Induced Negative Inotropic Effects in Isolated Cardiac Myocytes

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A-955840, a selective CB₂ agonist, has been shown to elicit concentration-dependent decreases in cardiac contractility in the anesthetized dog (decreased maximal velocity of left ventricular pressure development [LV dP/dt max]). However, it is unknown whether this represents a direct effect or a response dependent on other factors (such as autonomic tone and neurohumoral factors) present in vivo. This study examined if A-955840 had a direct effect on contractility of isolated cardiac myocytes, and if so to determine the potential involvement of CB₁ and CB₂ receptors. Contractility was assessed in vitro using percent changes in maximal shortening velocity of sarcomeres (dL/dt max) and fractional shortening of sarcomere length (FS) in rabbit left ventricular myocytes. A-955840 reduced dL/dt max and FS in a reversible and concentration-dependent manner with an IC₅₀ of 11.4 μ g/mL (based on dL/dt max) which is similar to the IC₅₀ value of 5.5 μ g/mL based on the effects of A-955840 on LV dP/dt max in anesthetized dogs. A-955840 (4.0 μ g/mL) reduced myocyte contractility (%FS) to a similar extent in the absence and presence of a CB₂ antagonist, SR-2 (24.0 \pm 3.4 vs. 23.1 \pm 3.0 %, n=5) or a CB₁ antagonist, rimonabant (18.8 \pm 2.3 vs. 19.8 \pm 2.7 %, n=5). A-955840 (4.0 μ g/mL) also reduced L-type calcium current of rabbit ventricular myocytes (1.05 \pm 0.11 vs. 0.70 \pm 0.12 nA, n=5, P<0.01). These results suggest that A-955840 exerts direct negative inotropic effects on isolated rabbit ventricular myocytes, which is mediated by neither CB₁ nor CB₂ receptors, and consistent with off-target negative inotropy mediated by inhibition of the cardiac L-type calcium current.

3745-Pos

Effects of Development of Compensatory Hypertrophy on Force Frequency and Beta-Adrenergic Responses

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Background: The progression to heart failure involves two steps: compensatory hypertrophy, a process where contractile function is enhanced and myocyte size is enlarged, is followed by decompensatory hypertrophy and myocardial weakening. In our current study we aim to understand the temporal resolution of the development of compensatory hypertrophy through the analysis of functional and molecular changes. At present, we are assessing the impact of these changes on contractile modulators through analysis of effects on frequency and beta-adrenergic regulation.

Methods: Trabeculae are excised from the right ventricular free wall of New Zealand White rabbits. Thin linear muscle preparations are then suspended in culture media at high preload and stimulated to contract for up to 24 hours at 1 Hz. At 0-2, 6-8, 12, 18, or 24 hours the effects on either frequency-dependent or beta-adrenergic regulation are measured. Data is analyzed in real-time through customized Labview software.

Results: We have shown that during the progression of compensatory hypertrophy the beta-adrenergic response shifts to the left indicating a higher sensitivity with increasing culture time. Changes in frequency from 1 to 4 Hz of trabeculae cultured from 0-24 hours lead to an increase in developed force of 37% at 0-2 hours (n=5), 53% at 6-8 hours (n=7), 20% at 12 hours (n=6), 30% at 18 hours (n=6) and a decrease of 69% at 24 hours (n=4). This suggests that the