size was markedly smaller in ILP (19 ± 3%,n=8) compared to CTRL (55.6 ± 3.4%,n=10). The inhibition of mitochondria permeability transition pore (mPTP) opening during reperfusion has been shown to induce cardioprotection. To investigate whether intralipid-induced cardioprotection occurs by inhibition of the mPTP opening, we compared the viability of isolated mitochondria by calcium overload. Postischemic administration of Intralipid inhibited the opening of the mPTP as calcium retention capacity was higher in the ILP group compared to control (2.7 ± 0.06 *vs.* 1.5 ± 0.11 μM/mg-mitochondrial protein, $p<0.05$). To identifiy the key signaling molecules involved in regulating mPTP opening, Western Blot analyis of heart lysates was performed. The activity of AKT/ERK1/GSK were respectively 2.3, 5 and 2.7 fold higher in ILP compared to CTRL. The involvement of PI3K/AKT pathway was further investigated by LY294002, a specific inhibitor of PI3K. The Intralipid-induced cardioprotection was fully abolished in the presence of LY294002, indicating the cardioprotective action of Intralipid is mediated via PI3K/AKT pathway.

3728-Pos

Proteasome Activity is Reduced at the end of Pregnancy and Fully Restored to Non-Pregnant Levels One Week Postpartum in the Murine Hear

Andrea Ciobotaru[1], Shannamar Dewey[2], Soban Umar[1], Aldrin V. Gomes[2], Mansoureh Eghbali[1].
[1]UCLA, Los angeles, CA, USA, [2]UC DAVIS, DAVIS, CA, USA.

The proteasome is the major protein degradation system in the heart, and its activity has been shown to be affected during pathological cardiac diseases. Proteasome dysfunction in the hypertrophic heart leads to accumulation of abnormal proteins and has been proposed to contribute to the transition to heart failure. Pregnancy places an increased demand on the healthy female's heart resulting in ventricular hypertrophy and diastolic dysfunction as a result of volume overload and increased stretch and force demand. Since the molecular signature of pregnancy-related heart hypertrophy differs significantly from that of pathological hypertrophy, we investigated if the proteasome proteolytic pathway is affected by pregnancy in the mouse heart. We measured the transcripts and protein levels of proteasome subunits as well as proteasome activity in four groups of female mouse hearts: i) non pregnant (NP) at diestrus stage, ii) late pregnant (LP), iii) one day post-partum (PP1) and iv) 7 days post-partum (PP7). Real Time PCR showed that the transcript levels of RPN2 and RPT4 (subunits of 19S) as well as β2 and α7 (subunits of 20S) did not change with pregnancy. Western blot analysis of heart lysates also revealed no significant differences in the expression levels of α7 (a subunit of 20S), RPN2 and RPT4 (subunits of 19S) subunits in the four groups mentioned above. The β1 (caspase-like) and β2 (trypsin-like) activities of the proteasome were significantly decreased in LP. The β5 (chymotrypsin-like) activity was significantly decreased 1 day post-partum. Interestingly, all three proteolytic activities of the proteasome were restored to normal levels 7 days post-partum. These results suggest that the proteasome proteolytic pathway is affected by pregnancy and is restored to NP levels soon after delivery.

3729-Pos

Sepsis Related C5a Peptide Causes Calcium Overload in Adult Cardiac Myocytes

Todd J. Herron, Gelareh Atefi, Jose Jalife, Peter A. Ward.
University of Michigan, Ann Arbor, MI, USA.

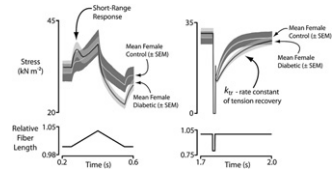
Septic cardiomyopathy is an acute cardiac syndrome that occurs after the onset of sepsis due to infectious agents such as bacteria, viruses and fungi. During septic cardiomyopathy cardiac output falls due to waning contractile function of the heart. However, very little is known about the precise cause of cardiac failure in cases of sepsis. One factor induced during sepsis is the complement activation product C5a. C5a is a peptide that acts through a G-protein coupled receptor (C5aR) and affects cardiac myocyte contractility by unknown mechanisms. Here we have tested the effect of C5a peptide on single adult cardiac myocyte calcium homeostasis. Cardiac myocytes were isolated from healthy rats and intracellular calcium transients were monitored (fluo-4AM) before and after C5a peptide treatment. Intracellular calcium was monitored by two different methods: 1) using a conventional photomultiplier tube and, 2) using a high speed digital CCD camera (200frames/s) to image whole cell calcium transients and waves. Recombinant C5a was applied to cardiac myocytes during electrical pacing (0.5Hz, 40V). After application of C5a (82ng/mL) intracellular calcium concentrations and calcium transient amplitudes initially rose (from F/Fo=1.43 ± 0.12 to 1.86 ± 0.4, n=4). Calcium transient duration was also prolonged after C5a addition (half width=260.2 ± 29.0 ms to 318.7 ± 47.1ms) and spontaneous calcium transients and waves were observed in the diastolic period between electrical stimuli. Consequently the amplitude of calcium transients and contractions varied from stimulated beat to beat after C5a addition. Paradoxically at higher pacing frequencies (3Hz) calcium transient amplitude was smaller after C5a application (F/Fo=1.46 ± 0.2 vs. 1.32 ± 0.04, n=4) and prolonged (half width=127.0 ± 1.15 vs. 167.0 ± 15.6 ms). Spontaneous calcium transients were also observed in the absence of electrical stimulation following C5a treatment. These data suggest that C5a peptide acts through its receptor C5aR to cause cardiac myocyte intracellular calcium overload.

3730-Pos

Short-Range Mechanical Properties of Myocardium from Diabetic Rats

Mihail I. Mitov[1], Leigh Ann Callahan[2], Kenneth S. Campbell[1].
[1]Department of Physiology and Center for Muscle Biology, University of Kentucky, Lexington, KY, USA, [2]Division of Pulmonary, Critical Care and Sleep Medicine; Department of Internal Medicine and Center for Muscle Biology, University of Kentucky, Lexington, KY, USA.

Diabetes mellitus is often associated with abnormalities in active relaxation and passive stiffness of the left ventricle but the molecular mechanisms responsible for the dysfunction are not yet clear. This study was designed to identify the molecular components that are responsible for the increased myocardial stiffness associated with diabetes. Multicelluar myocardial preparations were isolated from control Sprague-Dawley rats and an experimental group of rats injected 4 weeks previously with streptozotocin (model of Type I Diabetes). Preparations were subjected to paired ramp stretches/releases imposed under fiber length control in a series of calcium activations (pCa 4.5 - 9.0). The relative short-range force and elastic limits were substantially higher in the diabetic groups. The rate of tension recovery (k_{tr}) was considerably lower in the diabetic groups. Short range stiffness values did not differ in the control and diabetic animals. Gel electrophoresis showed that the relative content of slower beta Myosin heavy chain increased from 34 ± 15% in control hearts to 100% in the diabetic rat hearts. These results support the hypothesis that pathological changes in the mechanical properties of diabetic rat myocardium are mostly due to alterations in the active component (cycling cross-bridges) of ventricular stiffness.



3731-Pos

Increased Phosphorylation of Myofilament Proteins after Stretch in Rabbit Ventricular Myocardium

Michelle M. Monasky, Paul M. L. Janssen.
The Ohio State University, Columbus, OH, USA.

After a change in muscle length, there is an immediate intrinsic response in the amount of developed force, followed by a slower response. Although it has been well documented that the slow force response is at least in part generated by modification of calcium handling, it is unclear whether regulation at the level of the myofilaments occurs during the slow force response. We set out to investigate myofilament calcium sensitivity and phosphorylation status of myofilament proteins after a step-wise change in cardiac muscle length. Ultra-thin right ventricular intact trabeculae were isolated from New Zealand White rabbit hearts and iontophoretically loaded with the calcium indicator bis-fura-2. Twitch force-calcium relationships and steady state force-$[Ca^{2+}]_i$ relationships were measured at slack and optimal muscle lengths at 37°C using potassium induced contractures. The EC_{50} significantly decreased with increase in muscle length, from 1467 ± 271 nM at the shortest muscle length to 653 ± 121 nM at the longest muscle length. Maximal active force development significantly increased from 19.7 ± 2.7 mN/mm^2 at the shortest muscle length to 51.8 ± 5.0 mN/mm^2 at the longest muscle length. No significant change in the myofilament cooperativity coefficient was found. Phosphoprotein analysis using ratiometric analysis of Pro-Q diamond staining and Sypro-Ruby staining of the same gel, revealed increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 at longer muscle lengths. Since the immediate response is seen virtually instantaneously, and post-translational modifications cannot occur within that timeframe, we hypothesize that these increases in phosphorylation occur during the slow response. Future studies will aim to elucidate the individual effects of the immediate response verses post-translational modifications during the slow response, and also determine to what extent increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 each play a role.

3732-Pos

A Systems Biology Approach to Restrictive Cardiomyopathy in Drosophila

Anthony Cammarato[1,2], Nakissa N. Alayari[1,2], Marjan Gucek[3], Mary C. Reedy[4], Jasma Rucker[5], Jennifer E. Van Eyk[5], Robert N. Cole[5], Brian O'Rourke[5], Rolf Bodmer[2], Sanford I. Bernstein[1], **D. Brian Foster[5]**.
[1]San Diego State University, San Diego, CA, USA, [2]Burnham Institute for Medical Research, La Jolla, CA, USA, [3]National Institutes of Health, Bethesda, MD, USA, [4]Duke University Medical Center, Durham, NC, USA, [5]Johns Hopkins School of Medicine, Baltimore, MD, USA.

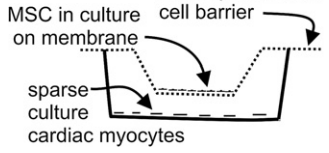
Drosophila melanogaster possess a simple linear heart tube and constitute an excellent genetic model system with which to investigate the effects of cardiomyopathic mutation. The Mhc5 myosin heavy chain mutation is located in the 'transducer'domain and elicits hypercontractile function at the molecular level characterized by high ATPase activity and enhanced in vitro motility properties. Additionally, its expression impairs diastolic relaxation of the cardiac tube reminiscent of restrictive cardiomyopathy in humans. We have investigated the effect of the Mhc5 mutation on cardiac structure/function by quantitative proteomics using isobaric tags for relative quantification (iTRAQ). Excised fly hearts from yw (control) and Mhc5 strains were digested with trypsin, reduced, alkylated and labeled with ITRAQ reagent. Peptides from each pool were mixed together prior to fractionation by strong cation exchange chromatography and subsequent reversed-phase HPLC coupled to tandem mass spectrometry. This approach identified approximately 600 proteins, of which 94 were upregulated and 86 were downregulated in Mhc5 hearts relative to yw hearts ($p<0.05$). Ontological cluster analysis of the genes encoding the regulated proteins revealed that myofibrillar disarray in Mhc5 hearts likely stems from overexpression of actin with concomitant reduction of myofibrillar assembly proteins such as spectrin, and other actin-binding proteins. Structural remodeling was also characterized by increased expression of extracellular matrix proteins. Upregulation of proteins involved in mitochondrial oxidative phosphorylation and fatty acid catabolism suggests further bioenergetic remodeling. The proteomic, structural and ultrastructural data are consistent with a model whereby the elevated ATPase activity caused by Mhc5 mutation increases energetic demand, thereby stimulating a concerted compensatory metabolic response to maintain energetic homeostasis. Ongoing protein-network/interactome analysis will help to further refine the model.

3733-Pos

Mesenchymal Stem Cells Protect Cardiomyocytes

Dushon DeVere Riley[1,2], Terry B. Rogers[1], Aarif Y. Khakoo[3], Cecilia A. Frederick[2], Shibani Pati[3], Shirley A. Gaa[1], W. Jonathan Lederer[1,2].
[1]University of Maryland School of Medicine, University of Maryland, Baltimore, Baltimore, MD, USA, [2]University of Maryland Biotechnology Institute, Baltimore, MD, USA, [3]University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Possible therapeutic benefits of stem cell treatments have been widely investigated recently. We have presented initial reports that co-culturing mesenchymal stem cells (MSC, Lonza) with rat heart cells in primary culture can prevent the consequences of the treatment with a inflammatory bacterial endotoxin (LPS, Lipopolysaccharide-A). We now investigate how the MSC produce their beneficial actions. Using sparse primary cultures of neonatal rat ventricular or adult rat ventricular myocytes with either MSC or control cells (fibroblasts), we examine cardiac Ca^{2+} signaling. LPS causes Ca^{2+} signaling anomalies which include delayed afterdepolarizations (DADs) and Ca^{2+}-enhanced early afterdepolarizations (EADs). We find that co-cultures with cells co-mingled can prevent the untowards actions of LPS on the cardiac myocytes. The negative consequences of LPS are alterations in the normal $[Ca^{2+}]_i$ transient that is stimulated by field shocks as described above. Since the benefit of MSC co-culture are found even when a solute permeable / cell impermeant membrane separates the MSC from the LPS treated cardiac myocytes, we conclude that a paracrine action of the MSC can account for the treatment attributed to the MSCs. We continue to investigate possible beneficial signaling pathways that may explain the paracrine effect of MSCs.



3734-Pos

Mechanical and Biochemical Characteristics of Human Stem Cell-Derived Cardiomyocytes

Scott D. Lundy[1], Marieke J. Bloemink[2], Samantha C. Lynn[2], Michael A. Geeves[2], Michael A. Laflamme[1], Michael Regnier[1].
[1]University of Washington, Seattle, WA, USA, [2]University of Kent, Canterbury, United Kingdom.

Cell-based cardiac repair following myocardial infarction has gained considerable interest recently, and the human pluripotent stem cell is an attractive cell source due its efficient differentiation into immature but functional cardiomyocytes. We examined the biophysical characteristics of cardiomyocytes generated from human embryonic stem cells (hESC-CMs) by measuring calcium transients, single cell contractions, and actomyosin interactions via flash photolysis. Furthermore, we compared these characteristics with those obtained from a second promising but still poorly characterized cell type, the human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM). We hypothesized that understanding fundamental biochemical and mechanical characteristics of these cells would provide insight into potential strategies to induce further cell maturation *in vitro*.

Our results suggest that hESC-CMs and hiPSC-CMs exhibit spontaneous contractions and calcium transients with similar kinetics, including time to peak $[Ca^{2+}]_i$ (116 ± 34ms vs. 155 ± 40ms) and time to 50% $[Ca^{2+}]_i$ decay (352 ± 87ms vs. 296 ± 49ms). Furthermore, quantitative videomicroscopy of resulting single cell contractions suggests that cardiomyocytes from both sources demonstrate similar resting cell size (17.1 ± 1.4um vs. 16.5 ± 4.6um), contraction amplitude ($4.2 \pm 1.6\%$ vs. $4.4 \pm 2.1\%$), time to peak contraction (0.346 ± 0.135sec vs. 0.339 ± 0.214sec), maximum contraction velocity (6.34 ± 3.50um/sec vs. 7.46 ± 4.81um/sec), and maximum relaxation velocity (3.21 ± 2.49um/sec vs. 3.40 ± 2.49um/sec).

We have also successfully isolated and purified 20 ug of myosin per million hESC-CMs. Using flash photolysis to liberate ATP in a solution of actomyosin, we have shown that the myosin binds actin and is dissociated from the complex by ATP with the expected 2nd order rate constant (~1 $uM^{-1}sec^{-1}$).

In summary, the contractile properties of hESC-CMs and hiPSC-CMs are similar to each other but differ from values published for adult human cardiomyocytes, suggesting that they are functionally immature and may benefit from *in vitro* maturation efforts.

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3735-Pos

Cell-Seeded Fibrin Scaffolds for Cardiac Tissue Engineering

Kassandra S. Thomson, Gabrielle Robinson, F. Steven Korte, Cecilia Giachelli, Buddy D. Ratner, Marta Scatena, Michael Regnier.
University of Washington, Seattle, WA, USA.

Cellular cardiomyoplasty to replace non-functional tissue following cardiac infarction appears clinically viable. Current strategies utilizing direct injection of cell suspensions are limited by low cell retention, poor cell localization, and high cell death. Synthetic biomaterials developed to enhance cell delivery can lead to problems with immune rejection, degradation, and mechanical mismatch, preventing functional integration of constructs with host myocardium. The goal of this project is to develop a functional cardiac tissue construct with enhanced host integration capabilities as a novel strategy to replace damaged myocardium. We have developed a novel templated fibrin scaffold seeded with cells to promote functional integration. Fibrin is an ideal scaffold material because it can be autologous, improves cell attachment and growth, and degrades into natural byproducts that can induce angiogenesis. The novel scaffold architecture includes 1) microchannels spanning the length of the scaffold, allowing alignment and organization of cells to mimic native cardiac tissue structure, and 2) micropores to enhance construct survival by improving nutrient delivery and waste removal. The dense fibrin scaffolds (stiffness = 16.0 ± 3.0 kPa) had mechanical properties closer to native myocardium than fibrin gels (0.5 to < 7 kPa). Centrifuge seeding with a tri-cell mixture of cardiomyocytes, endothelial cells, and fibroblasts increased scaffold stiffness (38.3 ± 8.9 kPa) to values near neonatal myocardial tissue (~40 kPa). Stiffness decreased over time in culture (25.2 ± 3.1 kPa, Day 6), which may indicate ECM formation and scaffold degradation. Patches of beating cells were observed inside channels within two days in culture. After three days in culture, histology showed cardiomyocyte and fibroblast alignment and immature lumen formation. These results indicate micro-templated fibrin scaffolds are a unique and viable platform for cardiac tissue engineering. This work supported by NIH HL064387 (MR, MS, BR) and NSF GRFP (KT).

3736-Pos

Cardiac Specific Overexpression of N-RAP in Transgenic Mice

Garland Crawford, Shajia Lu, Justin Dore, Robert Horowits.
National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA.

The muscle specific protein NRAP plays a role in myofibril assembly and is upregulated in mouse models of dilated cardiomyopathy. We sought to determine if increased N-RAP expression would directly lead to a cardiomyopathy phenotype. Novel transgenic lines were developed using the tet-off system with transgenic N-RAP expression requiring the tetracycline transactivator (tTA). tTA was introduced by mating the N-RAP transgenic animals with well-characterized animals carrying the tTA transgene controlled by the cardiac specific alpha-myosin heavy chain promoter. Multiple founder lines were examined and lines showing the most significant increase in NRAP expression were used for further investigation. N-RAP expression in theses animals was up to 2.5 times greater than control littermates as determined by western blot analysis. Histological examination of hearts from ~12 week old transgenic mice