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

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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

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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

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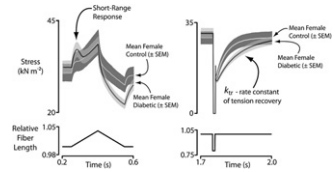
Diabetes mellitus is often associated with abnormalities in active relaxation and passive stiffness of the left ventricle but the molecular mechanisms responsible for the dysfunction are not yet clear. This study was designed to identify the molecular components that are responsible for the increased 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

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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

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