the dobutamine effects on preload recruitable stroke work and maximal systolic elastance were significantly blunted in the mutant groups. Maximal exercise capacity of Wt rats was significantly longer than that of Hm. Electrophoretic myosin heavy chain analysis of left ventricle (LV) samples showed no differences between Wt, Ht, or Hm in the beta myosin heavy chain proportions. Gene expression patterns in LV were conducted with Affymetrix GeneChip Rat Genome 230 2.0 microarrays using WT and Hm LV at three developmental stages (day 1, day 20 and day 49). A Student t-test with a p value cut-off of 0.05 and a minimum 1.5-fold change reveals changes in 372 mutation-specific transcripts (188 known and 96 un-annotated genes). A number of titin associated genes were up-regulated (Myot, T-cap, DARP, FHL1), and this up-regulation was verified by QPCR. Hierarchical clustering revealed gene expression patterns of Wt and Hm LV were related to their titin protein gel pattern. Predefined pathways and functional categories annotated by KEGG, Biocarta, and GO using the DAVID bioinformatic resource indicated involvement of TGF Beta 2, CTGF-regulated fibrosis, Trdn-Casq interaction-regulated RyR channel, and cAMP-dependent pathways. Supported by NIH HL77196.

2854-Pos

Binding of the N-Terminal Fragment C0-C2 of Cardiac MyBP-C to Cardiac F-Actin

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We have previously reported (Shaffer et al. 2009. J. Biol. Chem. 284:12318-27) that the N-terminal fragment C0-C2 of myosin binding protein-C (MyBP-C) can bundle actin, providing evidence for interaction of MyBP-C and actin. Neutron scattering studies (Whitten et al. 2008. PNAS 105:18360-5) also demonstrated the formation of ordered complexes of C0-C2 with actin, but these experiments were conducted under conditions that stabilized G-actin at reduced ionic strength and pH 8.0. To test whether C0-C2 also decorates F-actin at physiological ionic strength and pH, we incubated C0-C2 (5 - 30 uM, in a buffer containing in mM: 180 KCl, 1 $MgCl_2$, 1 EDTA, 1 DTT, 20 imidazole, at pH 7.4) with F-actin (5 uM) for 30 min and examined negatively-stained samples of the solution by electron microscopy (EM). Analysis of EM images revealed that C0-C2 bound to F-actin to form long helically-ordered complexes with a mean diameter of 16 nm. Fourier transforms indicated that C0-C2 binds with the helical periodicity of actin with strong 1st and 6th layer lines. The results provide evidence that the N-terminus of MyBP-C binds regularly to F-actin. Supported by NIH 5SC1HL096017 (RWK) and NIH HL080367 (SPH).

2855-Pos

Incorporation of the A31P Cardiac Myosin Binding Protein C Missense Mutation Into Feline Cardiac Sarcomeres

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Mutations in cardiac myosin binding protein C (cMyBP-C) are a frequent cause of hypertrophic cardiomyopathy (HCM), a major cause of sudden cardiac death and heart failure. Mutations include single amino acid substitutions and premature stop codons, but it is unclear whether dominant negative effects of mutant proteins, depletion of wild-type protein due to an affected allele (haploinsufficiency), or aberrant protein processing/degradation leads to disease. To distinguish among these possibilities, we investigated the sarcomeric localization and functional effects of a spontaneous cMyBP-C missense mutation in Maine Coon cats, a naturally occurring feline model of HCM. Immunofluorescent localization using an antibody specific for the A31P mutation showed that A31P cMyBP-C was incorporated into the sarcomeres of cats heterozygous and homozygous for the A31P mutation with similar distribution patterns as wild-type cMyBP-C. However, dominant negative effects due to incorporation of the mutant protein were not evident because myofilament Ca2+ sensitivity of tension and rate of tension development were not different in permeabilized myocytes from wild-type versus A31P cats. Actin binding and in vitro motility experiments also showed no difference between wild-type and A31P recombinant feline C0C2 proteins. By contrast, cytosolic proteasomes from a homozygous cat showed elevated β-5 (chymotrypsin-like) proteolitic activity compared to wild-type or heterozygous cats. Additional experiments are necessary to determine whether aberrant protein degradation of A31P cMyBP-C contributes to disease. Supported by NIH HL080367.

2856-Pos

Force, Ca-Sensitivity and Contractile Efficiency in Human Myocardium Expressing a Truncated Cardiac Myosin Binding Protein-C

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We have investigated contractile parameters of ventricular myocardium in samples from a patient diagnosed with hypertrophic cardiomyopathy (HCM) caused by a truncation mutation in *MYBPC3,* the gene encoding cardiac myosin binding protein C (cMyBP-C). The mutation truncates the protein in the C7 domain resulting in the loss of 408 residues. Our earlier work has shown that the truncated protein is not stably expressed and the disease is likely to be mediated by cMyBP-C haploinsufficiency.

We measured Ca^{2+}-sensitivity, isometric force generation, and myosin ATPase activity in tissue flash frozen in liquid nitrogen and subsequently stored in dry ice at −80°C before chemical demembranation. ATPase activity within the myocardium was measured simultaneously with force, using a fluorimetric technique and a linked-enzyme assay. Healthy human cardiac ventricular tissue served as control.

The mutant tissue exhibited an increased Ca^{2+}-sensitivity (pCa_{50} in control: 5.98 ± 0.02 (n = 12); mutant: 6.52 ± 0.07 (n=6), $p<0.001$) whereas the maximum isometric tension was reduced in mutant compared to control (control: 18.5 ± 3.0 kN.m^{-2} (n = 26); mutant 8.6 ± 0.8 kN.m^{-2} (n=7), $p<0.05$). There was no difference in the ATPase activity in maximally Ca^{2+}-activated tissue between the two groups (control, 131 ± 20μM.s^{-1} (n=26); mutant, 127 ± 9μM.s^{-1} (n=7), p=0.87). The dependence of ATPase activity on force was linear, with a slope (tension cost) of 7.32 ± 0.97μM.m^2.kN^{-1}.s^{-1} (mutant, n=6) and 3.46 ± 0.87 μM. m^2.kN^{-1}.s^{-1} (control, n=10), p = 0.01. The increased tension cost of the mutant sarcomeres may cause energetic compromise, which has been suggested to play an important role in the development of the HCM phenotype. Increased Ca^{2+}sensitivity has been reported in other investigations on HCM myocardium, and may be a direct effect of cMyBP-C haploinsufficiency or reflect compensatory changes.

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2857-Pos

Regulation of Contraction by PKA Phosphorylation of Myosin Binding Protein C and Troponin I in Murine Skinned Myocardium

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In skinned myocardium, cAMP-dependent protein kinase (PKA)-catalyzed phosphorylation of cardiac myosin binding protein-C (cMyBP-C) and troponin I (cTnI) leads to a decrease in myofilament Ca^{2+}-sensitivity and an acceleration in the kinetics of cross-bridge cycling. To examine the relative roles of cTnI and cMyBP-C phosphorylation in altering contractile function, we determined the Ca^{2+}-sensitivity of force (pCa_{50}) and the rate of force redevelopment (k_{tr}) in untreated and PKA-treated murine myocardium expressing: (1) phosphorylatable cTnI and cMyBP-C (WT), (2) non-phosphorylatable cTnI with serines$^{23/24/43/45}$ and threonine^{144} residues mutated to alanines ($cTnI_{ala5}$), (3) phosphorylatable cTnI on a cMyBP-C null background (cMyBP-C$^{-/-}$), and (4) non-phosphorylatable cTnI on a cMyBP-C null background ($cTnI_{ala5}$/cMyBP-C$^{-/-}$). A novel aspect of this study was the use of 2,3-Butandione Monoxime (BDM) treatments to reduce the basal levels of myosin regulatory light chain (RLC) phosphorylation to near zero in order to more accurately define the functional consequences of removing cMyBP-C and/or cTnI phoshorylation in transgenic myocardium. Our results showed that in the absence of RLC phosphorylation, PKA-treatment decreased pCa_{50} in WT, $cTnI_{ala5}$, and cMyBP-C$^{-/-}$ myocardium by 0.13, 0.08 and 0.09 pCa units, respectively, but had no effect in $cTnI_{ala5}$/cMyBP-C$^{-/-}$ myocardium. In WT and $cTnI_{ala5}$ myocardium, PKA treatment increased k_{tr} at submaximal levels of activation; however, treatment did not have an effect on k_{tr} in cMyBP-C$^{-/-}$ and $cTnI_{ala5}$/cMyBP-C$^{-/-}$ myocardium. Together, these results indicate that the attenuation of the myofilament force response following PKA treatment is due to phosphorylation of both cTnI and cMyBP-C and that the reduced Ca^{2+}-sensitivity of force mediated by phosphorylation of cMyBP-C is most likely due to an increased rate constant of cross-bridge detachment that also contributes to an acceleration of cross-bridge cycling kinetics.

2858-Pos

Endothelin as a Regulator of Phosphorylation of cMyBP-C

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The effect of endothelin, a powerful vasoconstrictor and enhancer of cardiac contractility, and hypoxia on the degree of phosphorylation of cardiac myosin binding protein C (cMyBP-C) has been studied in cardiac trabeculae isolated from rat hearts. Endothelin in concentrations that increase contractility increases phosphorylation in a dose-dependent fashion. Increase in sarcomere length itself increases phosphorylation and enhances the effect of endothelin on phosphorylation. Hypoxia decreases phosphorylation in a duration-dependent

manner and inhibits the ability of endothelin to produce phosphorylation of MyBP-C. These results suggest that phosphorylation of cMyBP-C may be a molecular component of the vascular endothelial cell - cardiac myocyte cross-talk. Coupled with already published work, the results also suggest that cMyBP-C phosphorylation may contribute to the regulation of the turnover of myofibrillar proteins.

2859-Pos

Comparative Effects of the Proline-Alanine Rich Regions of Human and Murine Cardiac Myosin Binding Protein-C

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The N-terminus of cMyBP-C can activate actomyosin interactions in the absence of Ca^{2+}, but it is unclear which sequences mediate the activating effects. Herron et al. (Circ Res, 98:1290-8, 2006) found that the Pro-Ala rich region (P-A) of human cMyBP-C could activate tension in the absence of Ca^{2+}, whereas Razumova et al. (J Gen Physiol, 132:575-85, 2008) found that murine C1 and M domains activated tension. The different results might be explained by isoform differences, especially in P-A which is only 46% identical between mouse and human cMyBP-C. The goal of this study was to determine if species-specific differences in P-A account for the different activating effects of murine and human cMyBP-C. Recombinant chimeric proteins containing the C0, P-A, and C1 domains (C0C1) from either human or murine cMyBP-C were engineered and their activating effects assessed using *in vitro* motility and ATPase assays. Consistent with previous observations, human C0C1 activated actomyosin interactions in the absence of Ca^{2+}, whereas murine C0C1 did not. However, substituting human P-A for murine P-A conferred activating properties to murine C0C1, whereas substituting murine P-A for human P-A depressed the activating effects of human C0C1. Activating effects of the chimera proteins were intermediate between those of murine and human C0C1, suggesting that C0 or C1 also contribute to activation properties. Further chimeric substitutions of C0 and C1 demonstrated that the human C1 domain also contributed to activation, whereas the C0 domain did not. These results suggest that the human P-A and C1 domains are sufficient to activate actomyosin interactions in the absence of Ca^{2+}, and that species-specific differences are likely to contribute to functional differences of cMyBP-C. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

2860-Pos

Single Sarcomere Imaging by Quantum Dots (Qdots) in the Heart

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Numerous studies have been conducted in tissues and cells to elucidate the molecular mechanisms of myocardial contraction. However, because of a number of differences between in vitro and in vivo conditions, the dynamics of myocardial sarcomere contractions in living animals is not yet understood. In the present study, we developed a novel system allowing for real-time single sarcomere imaging in the living heart. Male Wistar rats were anesthetized with pentobarbital sodium, and median sternotomy was performed under artificial ventilation. Qdots were conjugated with anti-α-actinin antibody and then transfected from the surface of the epicardium of the beating heart, for visualization of the Z-discs. An electron microscopic study confirmed the presence of Qdots in and around the T-tubles and Z-discs in the myocardial cells of the left ventricular wall. Consistent with this, we observed a striated pattern of Qdots (~2 μm spacing) in the heart under fluorescence microscopy. We are now performing real-time single sarcomere imaging in the beating heart of the rat.

2861-Pos

Single Sarcomere Imaging in Cardiomyocytes with Quantum Dots (Qdots): Physiological Significance of SPOC in Cardiac Beat

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Cardiac sarcomeres exhibit spontaneous oscillations (SPOC) over a broad range of intermediate activating conditions, namely, at pCa ~6.0 (Ca-SPOC), or at the coexistence of MgADP and Pi under the relaxing condition (ADP-SPOC). We have reported that the period of sarcomeric oscillations in skinned myocardium correlates with that of resting heart rate in various animal species [BBRC, 343, 1146-1152 (2006)]. In the present study, we analyzed sarcomeric oscillations in isolated single cardiomyocytes of the rat, by using Qdots conjugated with anti-α-actinin antibody for clear visualization of the Z-lines. First, we measured the period and amplitude of ADP- and Ca-SPOC at various sarcomere lengths (SLs) in skinned cardiomyocytes, and found that the amplitude of oscillations was inversely related to SL. We also conducted a SL measurement in intact cardiomyocytes at various stimulation frequencies, after transfection of Qdots into the cells. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during a contraction cycle simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (~5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to what was observed during SPOC in skinned myocytes. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to the regulation of cardiac beat *in vivo*.

2862-Pos

Modeling of Viscoelastic Properties of Isolated Myocardial Tissue Samples at Different Levels: Cardiomyocytes and Trabeculae

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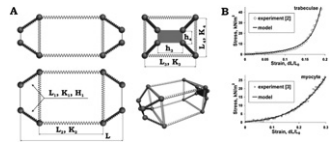
Viscoelastic properties of myocardium play an important role in a heart function. They determine the extent of filling of the heart, its subsequent stroke volume and contraction velocity. We present here the 3D model consisting of elastic springs and linear damping elements on basis of our earlier model [1] (Fig. 1A). Due to changes of geometry the model manifests nonlinear viscoelastic behavior in response to longitudinal stretch. Depending on set of input parameters, the model allows to describe quantitatively nonlinear viscoelastic behavior of both single cardiomyocytes and multicellular samples like trabeculae (Fig. 1B). Model volume stability is an essential condition for model parameter selection because in vivo a volume of cardiomyocytes is virtually unchanged. It is significant that viscoelastic parameters of structural elements of the model remain constant all over the range of investigated strains.

Thus, we can describe main viscoelastic properties of myocardial tissue at different organization levels within the basis of the simple mechanical model.



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2863-Pos

Radial Force and Lattice Spacing with Multi-Spring Crossbridge Models

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Previous spatially explicit models have used crossbridges consisting of single springs aligned to the axis of the thick and thin filaments. Such one-spring models cannot account for effects of lattice spacing or radial forces generated during axial shortening. We develop crossbridge models with multiple springs to examine how different mechanisms of crossbridge deformations affect radial forces, longitudinal forces, and the effects of changes in lattice spacing. A four-spring crossbridge (4sXB) treats the S2 region and light chain domain (LCD) as linear springs, linked to the thick filament and each other by torsional springs. Changing the rest angle of the S2/LCD linking spring models force generation via a power stroke. A two-spring crossbridge (2sXB) replicates the desired abilities of the 4sXB and is less computationally expensive. Unlike the 4sXB, the length and angle of the 2sXB's springs can be determined for any head position without iterative techniques. Both the 4sXB and the 2sXB use three state kinetics that, at resting lattice spacing, are similar to previous work, easing comparison to previous studies. In contrast to single spring crossbridges, the kinetics of the 4sXB and 2sXB change with lattice spacing. Notably, the axial offset (distance between the thick filament attachment site and myosin-binding site) at which the powerstroke becomes likely to occur varies by more than 5nm over physiological lattice spacings. Both the 4sXB and the 2sXB measure the axial and radial forces generated by during production of axial force. In a typical post-powerstroke position at resting lattice spacing, the axial forces exerted by the 4sXB and the 2sXB differ by approximately 10% while the radial forces are more divergent (differing by as much as 20%), making the choice of crossbridge a critical concern in measurements of radial force. HL65497 (MR), EB001650 (CDW).

2864-Pos

Sarcomere Velocity Regulates the Cross-Bridge Cycling Rate in Cardiac Muscle: a Novel Theory for the Muscle Molecular Motor

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