amplitude oscillation (frequency: 1 −100Hz) and determined elastic and viscous moduli. Results showed that hysteresis was greatly increased in the KO over the WT (for example at a speed of 100 %/s and an amplitude of 0.3 μm/sarcomere, hysteresis was 1549 ± 379pJ/mm^2/sarcomere vs. 401 ± 94pJ/mm^2/sarcomere; $p<0.05$). It can be calculated that this differences in hysteresis is analogous to an energy difference in a 24 hour period of 600 BPM of ~16cal, or ~30% of the total energy consumed by the heart. We conclude that the N2B element greatly reduces energy loss during stretch/shortening cycles of the beating heart.

2849-Pos

Single Molecule Analysis of PKC Phosphorylation of Titin's PEVK Domain

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Titin's I-band region contains three spring-like domains that are primarily responsible for the development of passive tension in cardiac muscle. PKC phosphorylation targets one of these I-band domains_the PEVK region, which is rich in proline (P), glutamate (E), valine (V), and lysine (K). It has been shown that two serine residues within the PEVK are targeted by PKC phosphorylation, S26 and S170. We investigate the effects of PKC phosphorylation of these two residues on the single molecule level using force-extension curves generated by atomic force microscopy (AFM). We constructed four recombinant proteins: two PEVK single mutants (S26A and S170A), a PEVK double mutant (S26AS170A), and a wild-type PEVK segment. All constructs are flanked by immunoglobulin-like domains, Ig27 and Ig84, and the unfolding of these domains generates a single molecular ''fingerprint''. The force-extension curve leading up to the first unfolding peak describes the force-extension relationship of the PEVK. Preliminary data suggests that mutating either serine residue alters PEVK resistance to extension, which is quantified by the molecule's persistence length (PL). Wild-type PEVK underwent a large decrease in its PL after phosphorylation by PKC (by ~50%), and both single mutants have PLs similar to that of phosphorylated wild-type PEVK. Furthermore, phosphorylation of both single mutants resulted in a small PL decrease. Phosphorylation decreased PL for the S26A mutation by 16% (from 0.55 ± 0.02 to 0.46 ± 0.02 (mean ± SE)), and the serine-170 mutation PL by 11% (from 0.53 ± 0.04 to 0.47 ± 0.03). The double mutant was not affected by PKC (from 0.51 ± 0.04 to 0.51 ± 0.03). We conclude that both serines play a structural role in determining the relationship between longitudinal force and PEVK extension, and that this role is modulated through phosphorylation by PKC.

2850-Pos

Passive Stress in Myofibrils from Cardiomyopathic Hamsters

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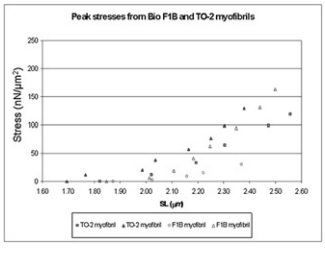
Dilated cardiomyopahty (DCM) is a frequent heart disease characterized by cardiac dilation and contractile dysfunction. The Bio TO-2 hamster is a genetic animal model of human DCM. The purpose of this research is to study the progression of DCM by comparing over time the passive mechanical properties of left ventricular wall myofibrils from TO-2 hamsters to those from F1B control hamsters. To date, we measured the passive stress-sarcomere length relations for two myofibrils each from experimental and control animals aged 36 weeks. Myofibrils were attached at one end to a glass needle controlled by a motor for stretching, and at the other end to a silicon-nitride nanolever of known stiffness for force measurements. Sarcomere lengths were measured from the myofibrillar striation patterns. Passive stresses in the experimental and control myofibrils were comparable. More passive mechanical experiments will be performed to confirm this result. In a single myofibril, titin is thought to be responsible for essentially all of the passive stress response to stretch (Linke et al., 1994; Bartoo et al., 1997). Titin depletion experiments and titin molecular weight determination will therefore also be performed to detect changes in titin isoform.



2851-Pos

Importance of Titin Based Viscosity in Cardiac Function: an Integrative Study on PEVK-Actin Interactions

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Viscosity has recently been hypothesized as an important regulator of diastolic relaxation during isovolumic relaxation and early rapid filling. A viscous interaction between the proline-glutamic acid-valine-lysine (PEVK) rich region of titin and the actin filaments has been shown at the protein level, but the physiologic relevance of such an interaction is unclear. A novel PEVK kockout (KO) mouse was utilized in order to investigate PEVK-actin based viscosity. KO and wild-type (WT) skinned cardiomyocytes were isolated subjected to ramp-hold protocols. Our data showed that the viscosity measured via stress relaxation was more than 2x greater in the WT vs KO cells and that WT cells showed a 2x faster relaxation to the steady state force at each of 4 stretch speeds, a hallmark of viscosity. Also using KO and WT mice, we examined the presence of viscosity in the intact ventricle. Using both ramp-hold and sinusoidal oscillations, we found that, in intact hearts, WT displayed greater viscosity than KO hearts. Ramp-hold analysis on isolated hearts again showed a 2x faster relaxation in WT (36ms) vs KO (53ms). Sinusoidal analysis provides KO viscosity nearly 30% lower than WT (Viscous modulus WT=0.97 vs KO=0.65 [mmHg/uL]). Because physiologic stretch speeds were probed in stretches on cells and isolated hearts, we analyzed in-vivo echocardiographic measurements utilizing kinematic models of stiffness and viscosity known as the Parameterized Diastolic Filling Formalism. As expected with a truncated titin, stiffness increased in the KO mouse (WT=10,700 vs KO=12,400 mass normalized stiffness [1/s2]). Importantly, a 30% reduction in viscous properties (WT=143 vs KO=99 mass normalized viscosity [1/s]). Titin based viscosity driven by PEVK-actin interactions are present in the ventricle and could play an important role in diastolic function and dysfunction.

2852-Pos

Titin Isoforms and Titin-Based Stiffness in Diastolic Heart Failure

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Diastolic heart failure (DHF) is a common heart disease characterized, e.g., by delayed relaxation, impaired left ventricular (LV) filling and increased LV stiffness. Titin is an established contributor to LV stiffness, but little is known about the protein's contribution to altered diastolic function in DHF. We investigated LV tissue samples of several animal models of DHF, as well as interventricular septum samples of aortic stenosis (AS) patients, for titin-isoform composition by loose-gel electrophoresis and titin-based stiffness by skinned-fiber mechanics. Induction of diastolic dysfunction in a small animal model, the ''two kidney one clip'' (2K1C) rat, which develops LV hypertrophy due to chronic afterload increase, caused no significant changes in the titin-isoform expression pattern, both 6 weeks and 8 months following surgery (~6% N2BA in both 2K1C and SHAM-operated LV, the remainder being N2B isoform; N2B contains a stiffer, N2BA a more compliant titin spring). Similarly, in a volume-overload mouse model created by aortocaval fistula surgery, cardiac titin isoforms remained unaltered compared to SHAM-operated animals (18.5% vs. 19.8% mean N2BA). However, in an old dog model (8-12 years) made hypertensive by bilateral renal wrapping, the cardiac N2BA proportion was significantly lower (41.6 ± 4.9%; mean ± SD) than in normal old dog LV (46.2 ± 4.2%; $p<0.020$). Mechanical measurements revealed passive-stiffness modulations consistent with the magnitude of titin-isoform switching. In contrast, in human AS samples, the titin isoform composition showed 42.0 ± 4.0% N2BA, significantly higher than in location-matched normal donor hearts (37.5 ± 5.0%; $p<0.025$). We conclude that diastolic dysfunction is associated with changes in cardiac titin isoform composition in a large animal model and in humans. The direction and the magnitude of the isoform shift appear to be determined by multiple factors not excluding, but clearly not restricted to, hemodynamic overload.

2853-Pos

Characterization of a Mutant Rat Model with Altered Titin Isoform Expression

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Titin isoform expression is related to human cardiac disease. A mutant rat model with dramatically altered titin isoform expression has been described (Greaser et al. J Mol. Cell. Cardiol. 44:483, 2009), and the ultrastructural and physiological properties of mutant and wild type rats were compared in this study. Electron micrographs of homozygous mutant ventricles showed normal structure in most areas, but occasional regions of Z line streaming, myofibrillar disarray, lipofuchin granules, and myofibril degeneration were observed as found previously in human heart failure. Dobutamine administration caused an increased heart rate in wild type (Wt), heterozygotes (Ht) and homozygote mutants (Hm), but

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