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