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The mutation Gly99lys (E99K) in the cardiac actin (ACTC) gene was reported to cause Hypertrophic Cardiomyopathy in extensive clinical studies. Transgenic (TG) mice expressing 50% E99K mutant cardiac actin in their hearts were generated and studied. The mice show high mortality between 28 and 45 days old (70% females, 34% males).

Thin filaments reconstituted with purified mouse f-actin from the survivors and human heart tropomyosin and troponin were studied by *in vitro* motility assay. The E99K thin filaments were 2.5 ± 0.6 times more $Ca^{2+}$ sensitive than NTG thin filaments ($p = 0.05$). E99K actin thin filaments also exhibited a reduced response to troponin dephosphorylation ($EC_{50}$ E99K/E99KdP = 1.1 ± 0.1 compared with 3.0 ± 0.3 for NTG /NTGdP).

7 month-old E99K TG mice (n=9) and their NTG littermates (n=7) were studied using *in vivo* cine MRI. Abnormal cardiac morphology and significantly lower ejection fractions (56.5 vs. 65.2%) and reduced stroke volumes (26.0 vs. 42.3 μl) were observed in TG mice. Peak LV ejection rates were also reduced (188 ± 41 vs. 252 ± 49 μl/min). LV mass was similar between groups, but septal wall thickness was increased (1.5 vs. 1.0 mm).

Left ventricular function of 9 month-old female E99K NTG (n=4) and TG (n=5) mice were studied with an *in vivo* conductance catheter. In TG mice ejection fraction was 20.2% less, end-diastolic pressure was 39.6% higher and relaxation rate was 50.0% slower.

We conclude that the basic effect of E99K mutation is increased $Ca^{2+}$-sensitivity and blunted response to troponin dephosphorylation and this leads to the high rate of sudden death at early ages, alterations to cardiac function and hypertrophy as observed in patients with hypertrophic cardiomyopathy.

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**2576-Pos Board B546**

**Protein Kinase A Catalyzed Phosphorylation of Cardiac Myosin Binding Protein C Decreases Calcium Sensitivity of Force and Increases Cross-Bridge Cycling Kinetics in Murine Myocardium**

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At the myofilament level, increases in cardiac output in response to increased sympathetic tone is achieved via protein kinase A (PKA)-mediated phosphorylation of cardiac myosin binding protein C (cMyBP-C) and cardiac troponin I (cTnI). However, despite the physiological importance of β-adrenergic stimulation in maintaining cardiac performance, the respective roles of cMyBP-C and cTnI phosphorylations in the myofibrillar force response of working myocardium are not completely understood. Using transgenic mouse lines either (1) expressing mutant non-phosphorylatable cTnI ($cTnI_{ala5}$) or (2) expressing $cTnI_{ala5}$ on a cMyBP-C null background ($cMyBP\text{-}C^{-/-}/cTnI_{ala5}$), we assessed the calcium sensitivity of force ($pCa_{50}$) and the rate of force redevelopment ($k_{tr}$) in skinned myocardial preparations following treatment with PKA and/or reconstitution with purified recombinant cMyBP-C. Before mechanical measurements, all preparations were treated with 2,3-butanedione monoxime (BDM) to reduce regulatory light chain (RLC) phosphorylation to near zero. In $cTnI_{ala5}$ myocardium, PKA phosphorylation of cMyBP-C resulted in a decrease in $pCa_{50}$ and an increase in $k_{tr}$. However, no changes in either variable were observed in $cMyBP\text{-}C^{-/-}/cTnI_{ala5}$ myocardium in response to PKA treatment. Following reconstitution of $cMyBP\text{-}C^{-/-}/cTnI_{ala5}$ myocardium with cMyBP-C, $k_{tr}$ decreased to the values observed in $cTnI_{ala5}$ myocardium, demonstrating that incorporation of cMyBP-C slowed the rates of cross-bridge attachment and transitions to strongly bound, force generating states. Subsequent treatment of reconstituted $cMyBP\text{-}C^{-/-}/cTnI_{ala5}$ myocardium with PKA produced a rightward shift in $pCa_{50}$ and an increase in $k_{tr}$. Together, these results suggest that in the absence of cTnI phosphorylation (and RLC phosphorylation), PKA phosphorylation of cMyBP-C decreases calcium sensitivity of force and speeds cross-bridge cycling kinetics in murine myocardium.

**2577-Pos Board B547**

**PKA Phosphorylates Serine 307 of Murine Cardiac Myosin Binding Protein-C In Vitro**

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Cardiac myosin binding protein-C is a regulatory protein associated with sarcomere A-bands that modulates actomyosin interactions in a phosphorylation dependent manner. The MyBP-C motif, a highly conserved sequence in the N-terminus of cMyBP-C, contains three to five protein kinase A (PKA) phosphorylation sites, depending on species. In the human isoform, three PKA sites have been identified (S275, S284, and S304). Three homologous sites exist in the murine isoform (S273, S282, and S302) along with a potential fourth site, S307, which is not present in human cMyBP-C. In this study, we investigated the effects of PKA phosphorylation of murine cMyBP-C by treating a recombinant protein, C1C2 (which contains the C1, motif, and C2 domains), with PKA and assessing phosphorylation levels using IEF gels, ProQ Diamond staining, and mass spectrometry. The wild-type C1C2 has a pI of ~8 and PKA treatment (C1C2P) shifted the pI to ~5-6 as determined by 1-D IEF gels. A mutant C1C2 (3S/D), containing aspartic acid for serine substitutions at S273D, S282D, and S302D, was still phosphorylated upon treatment with PKA as indicated by increased ProQ Diamond staining. However, a mutant 4S/D C1C2 (containing the additional mutation S307D) showed a pI near that of C1C2P and was not further phosphorylated by PKA. Mass spectrometry and MASCOT analysis of C1C2P confirmed that S307 was phosphorylated by PKA. These results suggest that murine S307 can be phosphorylated *in vitro*. Further studies are needed to investigate the phosphorylation state of murine cMyBP-C *in vivo*.

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**Functional Effects of cMyBP-C Phospho-Mimics in Permeabilized Trabeculae**

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Myosin Binding Protein C (MyBP-C) is a sarcomeric protein that has both structural and regulatory roles in striated muscle contraction. Cardiac (c) isoforms of MyBP-C can be phosphorylated by protein kinase A (PKA) at three to five sites within a unique regulatory region referred to as the MyBP-C motif. We have previously shown, using permeabilized rat trabeculae, that the recombinant protein C1C2, which contains the motif, significantly increased Ca2+ sensitivity of force and increased rates of tension redevelopment (ktr) at submaximal [Ca2+]. To investigate whether these effects are modulated by phosphorylation of the motif, we used the catalytic subunit of PKA to phosphorylate C1C2. In addition, we used site directed mutagenesis to mutate three key serine residues (Ser273, 282, 302) to aspartic acids to mimic phosphorylation at these sites. Results demonstrated that either 10uM phosphorylated C1C2 (C1C2P) or 10uM phospho-mimic C1C2 (C1C23S/D) increased Ca2+ sensitivity of force and increased rates of tension redevelopment (ktr) at submaximal [Ca2+]. However, the phospho-mimic C1C23S/D was more effective than C1C2P in producing these effects. Together these results indicate that the 3 Ser to Asp phospho-mimic does not fully mimic effects of PKA phosphorylation of C1C2 and that the functional effects of C1C2 in permeabilized cardiac trabeculae are mediated at least in part through phosphorylation-independent mechanisms.

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**Altered Myofilament Targeting with Differential PKCδ Activation**

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Post-translational modification of troponin (Tn) and other myofilament proteins by protein kinase C (PKC) isozymes plays a prominent role in regulating myocardial contraction. Conventionally, phosphorylation of conserved serine and threonine residues in the c-terminus activates PKCdelta, a novel calcium independent isozyme. Several recent investigations including our work (Sumandea et al. J Biol Chem. 2008;283(33):22680-9) have led to the probability of alternative activation of PKCdelta by tyrosine residue phosphorylation through a redox-sensitive mechanism. Previous work determined PKCdelta phosphorylation of adult, cardiac rat myocytes in vitro reduces skinned myocyte tension generation at sub-maximum with no change at maximum calcium concentrations. Conversely, tyrosine phosphorylated PKCdelta reduces the maximal Ca-activated tension with no decrease in submaximal tension production. Biochemical data indicated a shift in TnI residue targeting with tyrosine phosphorylated PKCdelta from exclusive phosphorylation of S23/24, to include T144. To test the hypothesis that kinase targeting to TnI-T144 was sufficient to blunt effects of conventional PKCdelta targeting to TnI-S23/24, myofibrillar function was assessed following exchange of TnI with pseudo-phosphorylated residues. Pseudo-phosphorylation at residues S23/24 decreased Ca-sensitivity of force production and increased tension cost. Pseudo-phosphorylation at TnI-144 had minimal affect on mechanical parameters. However, the combination of pseudo-phosphorylations at residues S23/24 and T144 did not successfully blunt desensitization. Further biochemical assessments have determined