Key Findings: Pargyline increased dialysate 5-HT concentration from 1.8 ± 0.3 at baseline to 3.9 ± 0.5 nM but decreased dialysate 5-HIAA concentration from 20.7 ± 1.0 at baseline to 15.8 ± 1.4 nM at 60-80 min of administration. Fluoxetine increased dialysate 5-HT concentration from 1.9 ± 0.4 at baseline to 6.5 ± 0.9 nM at 60-80 min of administration, but did not change dialysate 5-HIAA concentration. Local administration of ADP (100 mM) increased dialysate 5-HT and 5-HIAA concentrations. Pargyline did not affect ADP-induced increase in dialysate 5-HT concentration but suppressed ADP-induced increase in dialysate 5-HIAA concentration during 60 min of ADP administration. Fluoxetine increased dialysate 5-HT concentration at 40-60 min of ADP administration, but did not affect ADP-induced increase in dialysate 5-HIAA concentration.
Significance: Simultaneous monitoring of myocardial interstitial 5-HT and 5-HIAA levels provides valuable information on 5-HT kinetics including reuptake and enzymatic degradation by MAO, which play a role in the regulation of myocardial interstitial 5-HT levels at baseline and when 5-HT levels are elevated.

3004-Pos Board B434
The Treatment Benefit of Ghrelin on a Mouse Model of Inherited Dilated Cardiomyopathy Caused by Troponin Mutation
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The therapeutic effect of ghrelin has been reported in humans as well as in animal models of chronic heart failure. However, little is known about the therapeutic efficacy of ghrelin for the treatment of inherited forms of dilated cardiomyopathy (DCM). We aim to examine whether ghrelin is beneficial for the treatment of inherited DCM with a deletion mutation ΔK210 in the cardiac troponin T (cTnT) gene using a knock-in mouse model. Ghrelin (150 μg/kg/day) was administered subcutaneously to the mouse model of inherited DCM. The therapeutic effects were examined on the basis of survival and myocardial remodeling. Ghrelin administration prolonged the life span of DCM mice compared to the saline-treated controls. Echocardiography data showed that ghrelin reduced left ventricular (LV) end-diastolic dimensions and increased LV ejection fraction. Moreover, histoanatomical data revealed that ghrelin decreased the heart-to-body weight ratio, prevented cardiac remodeling and fibrosis, and markedly decreased the expression of brain natriuretic peptide. Telemetry recording and heart rate variability analysis showed that ghrelin suppressed the excessive cardiac sympathetic nerve activity (CSNA) and recovered the cardiac parasympathetic nerve activity. Ghrelin has therapeutic benefits for the treatment of DCM with ΔK210 mutation in cTnT. Importantly, these cardiovascular benefits of ghrelin are likely linked to the suppression of CSNA and recovery of cardiac parasympathetic nerve activity.

3005-Pos Board B435
The Cardiac Troponin T Mutant Missing the N-Terminal Extension Causes Dose-Dependent Effects on Cardiac Function and Remodeling in Transgenic Mice
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The N-terminal extension (NTE; residues 42-73) of mouse cardiac troponin T (TnT) desensitizes cardiac myofilaments to Ca^{2+} by stabilizing thin filaments in the blocked-state. We arrived at this conclusion using detergent-skinned muscle from transgenic (TG) mouse hearts that expressed 54% of chimeric TnT (residues 1-73 of mouse cardiac TnT were replaced by residues 1-41 of mouse fast skeletal TnT). Here, we extended our investigation to include higher dose effects of the modified TnT on cardiac myofilament function/phenotype using detergent-skinned fiber studies and echocardiography measurements in two different TG mouse lines (TG-55 and TG-64 that expressed 55% and 64% of chimeric TnT, respectively). Both TG-55 and TG-64 mice showed a similar increase in myofilament Ca^{2+} sensitivity at sarcomere lengths (SL) of 1.9 and 2.3 μm. However, Ca^{2+}-activated maximal tension increased significantly only in TG-64 mice at either SL. There was a progressive decrease in the overall heart size and heart-to-body weight ratios in both TG-55 and TG-64 mice. Left ventricular diastolic functional parameters (isovolumic relaxation time and E-wave deceleration time) showed a graded increase in TG-55 and TG-64 mice; however, such effects were only significant in TG-64 mice, suggesting impaired relaxation. Systolic functional parameters (stroke volume, ejection fraction and fractional shortening) were unaffected in TG-55 mice, but significantly decreased in TG-64 mice. Thus, higher levels of chimeric TnT (64%) depressed both diastolic and systolic function significantly in TG-64 mice. We will discuss the link between the effects of the modified N-terminus of TnT on cardiac myofilament function and the resultant pathological remodeling of the heart. Our findings have pathological relevance because a growing number of disease-related mutations are found both in and near the NTE of cardiac TnT.

3006-Pos Board B436
Functional Effects of the H1-Helix of Rat Cardiac Troponin T on Cross-bridge Detachment Rate is Differently Modulated by α- and β-Myosin Heavy Chain Isoforms
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The primary structure of the H1-helix of troponin T (TnT) varies among different types of striated muscles. Moreover, these muscles also express different myosin heavy chain (MHC) isoforms. Recently, we demonstrated that pseudo-phosphorylation of residue 204 (near the H1-helix) of cardiac TnT affected the functional state of the thin filament differently in fibers that expressed either α- or β-MHC isoforms (Michael et al., Basic Res Cardiol, 109:442, 2014). In this follow-up study, we investigated how the isoform-specific function of the H1-helix of cardiac TnT was influenced by α- and β-MHC isoforms. We generated a mutant rat cardiac TnT (RfsH1) in which the cardiac H1-helix was replaced by the fast skeletal H1-helix. Recombinant RfsH1 was reconstituted into detergent-skinned cardiac muscle fibers from either normal rats (expressing α-MHC) or propylthiouracil treated rats (expressing β-MHC). Steady-state and dynamic measurements were carried out at sarcomere length 2.3 μm. Our results demonstrated that RfsH1 decreased Ca^{2+}-activated maximal ATPase activity differently in α-MHC (~33%) and β-MHC (~17%) fibers. Furthermore, RfsH1 decreased tension cost (~31%) and crossbridge (XB) distortion dynamics (~25%) in α-MHC but not in β-MHC fibers. Because the above mentioned parameters are indices of the rate of XB detachment, our results suggest that the interplay between the RfsH1- and α-MHC-mediated effects on the thin filament modulates XB detachment kinetics. Our findings suggest that the conformational changes in the H1-helix of TnT are sensitive to MHC isoform-mediated changes in the thin filament.

3007-Pos Board B437
Engineering Cardiac Troponin C: Potential Therapeutic for Heart Failure
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We have engineered cardiac TnCs with increased (L48Q) or decreased (D73N) Ca^{2+} sensitivity. To express these proteins in the *in vivo* heart we utilized an adeno-associated virus serotype 9 (AAV-9). The Ca^{2+} desensitized D73N TnC recapitulated a dilated cardiomyopathy phenotype and depressed function as observed by echocardiography and isolated cardiomyocytes. On the other hand, AAV-9 containing the Ca^{2+} sensitized L48Q TnC did not cause any disease phenotype or arrhythmias commonly associated with increased myofilament Ca^{2+} sensitivity. In healthy mice, L48Q TnC increased myocyte contraction and whole heart contractility with improved cardiovascular performance (increased V02max). Excitingly, L48Q TnC expressing mice were able to preserve higher contractility, ejection fraction, cardiac performance and decreased death rate even after undergoing trans-aortic constriction or myocardial infarction. Additionally, L48Q TnC was able to increase contractility, ejection fraction and cardiac performance in mice which expressed L48Q TnC after having a myocardial infarction. In summary, engineered TnCs show potential to be used as treatment strategies against different cardiomyopathies.

3008-Pos Board B438
Modeling the Response of Cardiac Troponin C to Calcium on the Thin Filament: Effects of Disease-Related and Post-Translational Modifications
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Calcium binding to and dissociation from cardiac troponin C (TnC) are essential steps leading to cardiac muscle contraction/relaxation. It is well documented that the calcium binding properties of TnC are not constant, but are sensitive to complex interactions between the additional thin and even thick filament proteins. There is a growing body of evidence that protein modifications/mutations within

different subunits of the troponin complex (troponin T, troponin I) exert their effects by altering the apparent TnC calcium sensitivity/exchange kinetics. There are a number of potential mechanisms that could alter the calcium binding properties of TnC, potentially the most significant being the ability of the regulatory domain of TnC to bind the switch peptide region of TnI. We have developed a rather simple mathematical model that can simulate the steady-state and kinetic calcium binding properties of a wide assortment of disease-related and post-translational protein modifications in the isolated troponin complex and reconstituted thin filament. We propose that roughly half of the studied modifications do not alter any of the intrinsic TnC calcium binding constants but rather alter the ability of TnC to "find" TnI in the presence of calcium. Considering TnI is essentially tethered to TnC and cannot diffuse away in the absence of calcium and that TnI also binds to actin, we suggest that the apparent calcium binding properties of TnC are highly dependent upon an "effective concentration" of TnI available to bind TnC.

3009-Pos Board B439

The Contribution of Myosin Binding Protein-C and Troponin I Phosphorylation to the β-Adrenergic Acceleration of Left Ventricular Contraction and Relaxation

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Activation of the β-adrenergic signaling pathway in the heart leads to an increased rate of pressure development in early systole and an acceleration of relaxation in diastole to increase cardiac output. These effects are mediated by protein kinase A (PKA), which primarily targets myosin binding protein-c (MyBP-C) and troponin I (TnI) in the sarcomere for phosphorylation. However, the relative contributions of MyBP-C and TnI phosphorylation to the β-adrenergic-mediated increases in ionotropy and lusitropy remains unclear. To investigate, we used mice expressing non-phosphorylatable TnI (TnI_{Ala2}), non-phosphorylatable MyBP-C ($MyBP\text{-}C_{3SA}$), and mice expressing non-phosphorylatable TnI and MyBP-C ($TnI_{Ala2}/MyBP\text{-}C_{3SA}$), as well as control WT mice. Pressure-volume loop analysis was performed to measure early systolic pressure development and ventricular relaxation at baseline and in response to dobutamine (a β-agonist) administration. At baseline there was no difference in systolic pressure development between the groups, but the acceleration of dp/dt_{max} after dobutamine administration was blunted in $MyBP\text{-}C_{3SA}$ and $TnI_{Ala2}/MyBP\text{-}C_{3SA}$ mice (mmHg/s; 14,414 ± 1,016 in WT, 12,921 ± 982 in TnI_{Ala2}, 7,830 ± 899 in $MyBP\text{-}C_{3SA}$, and 8,803 ± 1,001 in $TnI_{Ala2}/MyBP\text{-}C_{3SA}$ after dobutamine; $p<0.05$). We examined elastance (E) decay from 75% to 25% of maximal E ($T_{75\text{-}25}$; normalized to cardiac cycle duration) to measure ventricular relaxation. At baseline there were no differences between the rate of relaxation in any of the groups; however, after dobutamine administration $MyBP\text{-}C_{3SA}$ and $TnI_{Ala2}/MyBP\text{-}C_{3SA}$ showed a significantly longer relaxation time compared to WT and TnI_{Ala2} (6.8 ± 0.3% in WT, 6.9 ± 0.3% in TnI_{Ala2}, 8.3 ± 0.2% in $MyBP\text{-}C_{3SA}$, and 8.5 ± 0.4 in $TnI_{Ala2}/MyBP\text{-}C_{3SA}$ after dobutamine; $p<0.05$). These results show that MyBP-C phosphorylation is necessary to fully accelerate contraction and relaxation in response to β-adrenergic signaling.

3010-Pos Board B440

Molecule Specific Effects of PKA-Mediated Phosphorylation on Myofibrillar Function

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Increased cardiac myocyte contractility by the beta-adrenergic system is an important mechanism to elevate cardiac output to meet greater hemodynamic load and this process is often depressed in failing hearts. While increased contractility involves augmented myoplasmic calcium transients, the myofilaments also adapt to boost the transduction of the calcium signal. Accordingly, ventricular contractility is tightly correlated with PKA-mediated phosphorylation of two myofibrillar proteins, cardiac myosin binding protein-C (cMyBP-C) and cardiac troponin I (cTnI), implicating these two proteins as important transducers of hemodynamics to the cardiac sarcomere. Consistent with this, we have previously found that phosphorylation of myofilament proteins by PKA (a downstream signaling molecule of the beta-adrenergic system) increased force, slowed force development rates, sped loaded shortening, and increased power output in rat skinned cardiac myocyte preparations. Here, we sought to define molecule-specific mechanisms by which PKA-mediated phosphorylation modulates these contractile properties. Regarding cTnI, the incorporation of thin filaments with a majority of unphosphorylated cTnI (as observed in some models of late stage heart failure) decreased isometric force production at any given activator [Ca^{2+}] and these changes were reversed by PKA-mediated phosphorylation in skinned cardiac myocytes. In addition, incorporation of unphosphorylated cTnI sped rates of force development, which suggests less cooperative thin filament activation and recruitment of non-cycling cross-bridges into the pool of cycling cross-bridges, a process that would tend to depress myocyte force and power. Regarding cMyBP-C, PKA treatment of slow-twitch skeletal muscle fibers caused phosphorylation of MyBP-C (but not TnI) and yielded faster loaded shortening velocity and an ~30% increase in power output. These results add novel insight into the molecular specificity by which the beta-adrenergic system controls myofibrillar contractility and how attenuation of PKA-induced phosphorylation of cMyBP-C and cTnI may contribute to ventricular pump failure.

3011-Pos Board B441

Length-Dependent Contractile Dynamics are Blunted Upon Ablation of Cardiac Myosin Binding Protein-C

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Enhanced cardiac contractile function due to increased sarcomere length (SL) is, in part, mediated by a decrease in the radial distance between myosin heads and actin. The radial disposition of myosin heads relative to actin is modulated by cardiac myosin binding protein-C (cMyBP-C), suggesting that cMyBP-C contributes to the length-dependent activation (LDA) in the myocardium. However, the precise role of cMyBP-C in modulating cardiac LDA is unclear. To determine the impact of cMyBP-C on LDA, we measured isometric force, myofilament Ca^{2+}-sensitivity (pCa50) and length-dependent crossbridge (XB) contractile dynamics in skinned ventricular muscle fibers isolated from the hearts of wild-type (WT) and cMyBP-C knockout (KO) mice, at SL's 1.9μm or 2.1μm. Our results show that maximal force was not significantly different between KO and WT fibers. pCa50 was not significantly different between WT and KO fibers at long SL (5.82 ± 0.02 in WT vs. 5.87 ± 0.02 in KO), whereas pCa50 was significantly different between WT and KO fibers at short SL (5.71 ± 0.02 in WT vs. 5.80 ± 0.01 in KO; $p<0.05$). The rate of force redevelopment (ktr), measured at submaximal Ca^{2+}-activation, was significantly accelerated at short SL in WT fibers (8.74 ± 0.43s-1at 1.9μm vs. 5.71 ± 0.40s-1at 2.1μm, $p<0.05$). Furthermore, the rates of stretch-induced XB relaxation (krel) and XB recruitment (kdf) were accelerated by 32% and 70%, respectively at short SL in WT fibers. In contrast, ktr was not significantly different between both SL's in KO fibers (8.03 ± 0.54s-1at 1.9μm vs. 8.90 ± 0.37s-1at 2.1μm). Furthermore, KO fibers did not exhibit length-dependent differences in krel and kdf -suggesting that LDA is severely depressed in KO fibers. Collectively, our data indicate that cMyBP-C plays a central role in modulating cardiac LDA.

3012-Pos Board B442

Cardiac Troponin I Ser-23/24 and Tyr-26 Phosphorylation Crosstalk

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The troponin complex is a critical molecular switch involved in transducing the calcium activating signal into contraction. Troponin I (TnI), the inhibitory subunit of the complex, is phosphorylated as a key regulatory mechanism to alter the calcium regulation of contraction. Recent work identified a novel phosphorylation of TnI at Tyr-26 that is decreased from its normal basal level in heart failure with unknown functional effects. Similar to the desensitizing TnI Ser-23/24 phosphorylation, TnI Tyr-26 is located in the unique cardiac TnI N-terminal extension. Employing TnI containing actual phosphate at Tyr-26 and Tyr-26 phosphomimetics, we demonstrate that TnI Tyr-26 phosphorylation decreases thin filament calcium sensitivity and accelerates deactivation. To assess the functional integration of TnI Ser-23/24 and Tyr-26 phosphorylation, we generated recombinant TnI with phosphomimetic substitution at all three residues. Calcium sensitivity measurements demonstrate no additional effect on calcium binding to troponin C nor calcium sensitive force development of the triple phosphomimetic TnI. However, the integration of Tyr-26 with Ser-23/24 pseudo-phosphorylation further accelerated thin filament deactivation. The kinase responsible for TnI Tyr-26 phosphorylation remains to be elucidated. Considering that the Src kinase recognition sequence (EEXY) is similar to that surrounding TnI Tyr-26 when Ser-23/24 are phosphorylated (S(p)S(p)NY), we hypothesize that Ser-23/24 phosphorylation would exhibit signaling crosstalk to alter the rate of Tyr-26 phosphorylation. Current efforts are directed towards determining the ability of Ser-23/24 phosphorylation to modulate Src-family kinase phosphorylation of Tyr-26. Our findings suggest that TnI Tyr-26 phosphorylation in isolation functions similarly to Ser-23/24 N-terminal