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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