in myofilament Ca^{2+} sensitivity was abolished by the expression of β-Tm. Furthermore, the McTnT 45-74 deletion-induced reduction in cooperativity of force production was more pronounced under a β-Tm background. Thus, our data shows that changes in the isoform expression of Tm modify T1-dependent cardiac function, indicating that T1-Tm interactions exert a modulatory role in regulating cardiac thin filament activation.

1814-Pos Board B584

Cardiac Troponin I Phosphorylation at ser149 by Protein Kinase A: A Potential Modulator of Myocardial Contractility

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In the heart, stimulation of β-adrenergic pathway and subsequent activation of protein kinase A (PKA) is known to increase myocardial contractility. The increase in contractility is, in part, due to target phosphorylation of troponin I (TnI). In this study, we sought to identify novel target sites for PKA that could potentially contribute to this increase in contractility. To induce phosphorylation of TnI, cardiac and fast skeletal muscle from 3-4 month old Sprague Dawley rats was mechanically disrupted and demembranated followed by incubation with the catalytic subunit of PKA (50U PKA/ 3mg tissue , 0-30 min). To identify target specific phosphorylation on fast skeletal (fsTnI) or cardiac (cTnI) TnI, western blot analysis with phospho-specific antibodies was performed. PKA treatment increased phosphorylation of cTnI at ser22/23, as expected, but also at ser149. Similarly, PKA treatment increased phosphorylation of fsTnI at ser117, which is the equivalent to ser149 in cTnI. Accordingly, fsTnI demonstrated no observable phosphorylation at ser22/23. Adenosine-monophosphate activated kinase (AMPK) has been shown to target ser149 of cTnI. Therefore, to validate PKA-dependent phosphorylation of cTnI at ser149, hearts were excised and perfused with AICAR, a known activator of AMPK. AICAR-perfused hearts demonstrated a time-dependent increase in phosphorylation of cTnI at ser149. These results demonstrate that PKA-dependent phosphorylation can target ser149 in cTnI and, equivalently, ser117 in fsTnI. The functional consequence of this target site phosphorylation and how it impacts contractility is currently under investigation.

1815-Pos Board B585

D230N Mutation in Tropomyosin and R92L Mutation in Cardiac Troponin T have Strikingly Different Impact on Calcium-Regulated Activation of Cardiac Myofilaments

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Dilated Cardiomyopathy (DCM) and Familial Hypertrophic Cardiomyopathy (FHC) are pathological heart conditions mainly associated with sarcomeric mutations that lead to contractile dysfunction. Despite the identification of several mutations associated with FHC and DCM, the role of these mutations in pathological cardiac-remodeling is still elusive. Therefore, we studied length-dependent contractile parameters of murine hearts expressing DCM-associated mutation (D230N in alpha-tropomyosin) and FHC-associated mutation (R92L in cTnT). Mechanical studies were carried out on detergent-skinned cardiac muscle fibers at sarcomere length (SL) 1.9 and 2.3 μm. Our preliminary results show that myofilament Ca^{2+} sensitivity and cooperativity are affected differently in both mutants, irrespective of SL. Ca^{2+} sensitivity was decreased in the D230N fibers, but increased in R92L fibers. Ca^{2+}-activated maximal tension was unaltered in both examples. Cooperativity of myofilament activation was significantly decreased in R92L fibers and significantly increased in D230N fibers. Our results suggest that single amino acid substitution mutations in Tm (D230N) and TnT (R92L) cause diverse functional effects, which may correlate with varied pathological remodeling. Further mechano-dynamic studies are planned to determine if other aspects of myofilament activation may be involved in the evolution of complications associated with DCM and FHC.

1816-Pos Board B586

Familial Hypertrophic Cardiomyopathy (FHC)-Related Cardiac Troponin C (cTnC) L29Q Mutation Alters the Contractility and the Functional Effects of the Phosphomimetric cTnI

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The FHC-related cTnC mutation, L29Q, is located at the dysfunctional binding site I of cTnC and interacts with the N-terminus of cardiac troponin I (cTnI) during muscle contraction. Recent studies suggest that the L29Q cTnC mutation affects Ca2+ activation and the transduction of PKA-dependent phosphorylation (S23/24) cTnI effect on cTnC, but the results are contradictory. Therefore, to investigate whether the L29Q cTnC mutation abrogates the effects of PKA-dependent phosphorylation of cTnI of the Ca2+ sensitivity of cTnC, we examined the Ca2+ binding properties in multiple biochemical systems of increasing complexity. Mouse cTn was reconstituted with phosphomimetic cTnI (S23/24D), cTnT, and IAANS-labeled control TnC (T53C, C84S, C35S) ± the L29Q mutation. Steady-state Ca2+ binding and Ca2+ dissociation rates were measured in the isolated cTn as well as the reconstituted thin filaments. There was no significant difference between steady-state Ca binding for the control cTn ($0.98 \pm 0.07\mu M$) and L29Q cTn ($0.99 \pm 0.08\mu M$), nor a difference in the Ca2+ dissociation kinetics of reconstituted thin filaments, control ($300 \pm 10/s$) and L29Q ($280 \pm 10/s$). However, L29Q cTnC in skinned cardiac myocytes increased Ca sensitivity by 1.2 fold and reduced the decrease in Ca2+ sensitivity caused by phosphomimetic cTnI by ~ 45%, as well as the enhanced length dependent Ca2+ sensitivity. These results suggest that the Ca2+ association rate, rather than the dissociation rate was affected by L29Q in the reconstituted thin filament. In conclusion, the cTnC L29Q mutation increased Ca2+ sensitivity of force generation and exacerbated the functional effects of TnI phosphorylation at S23/24 in skinned cardiac myocytes, but did not alter changes in the Ca2+ dissociation rate in the reconstituted thin filament caused by phosphomimetic cTnI.

1817-Pos Board B587

Mechanism of Doxorubicin-Induced Suppression of Cardiac Ankyrin Repeat Protein: Implications for Anthracycline Cardiomyopathy

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Doxorubicin (adriamycin) is an effective anti-cancer drug, but its clinical usage is limited by a dose-dependent cardiotoxicity characterized by widespread sarcomere disarray and loss of myofilaments. Cardiac ankyrin repeat protein (CARP, ANKRD1) is a transcriptional regulatory protein that is extremely susceptible to doxorubicin, however, the mechanism(s) of doxorubicin-induced CARP suppression and its specific role in cardiomyocyte biology remains to be elucidated. In this study, we report that treatment of cardiomyocytes with doxorubicin resulted in complete suppression of CARP promoter activity, decreased CARP protein levels, and marked sarcomere disarray. Transfection of CARP siRNA in cardiomyocytes resulted in a complete depletion of CARP and significant disruption of sarcomere ultrastructure. Adenoviral overexpression of CARP, however, was unable to rescue the doxorubicin-induced sarcomere disarray phenotype. GATA4 has previously been shown to regulate CARP, thus we examined the role of GATA4 in doxorubicin-induced CARP depletion. Cardiomyocytes treated with doxorubicin show a concomitant depletion of CARP and GATA4 protein levels. GATA4 siRNA inhibits while GATA4 overexpression enhances CARP promoter activity in cardiomyocytes. Both GATA4 and CARP siRNA significantly repressed titin and actin promoter activity. These data show that in cardiomyocytes transcription factor GATA4 is upstream of CARP and that doxorubicin induces a rapid down-regulation of GATA4 resulting in inhibition of CARP transcription. Our data further support a role for a GATA4/CARP signaling axis in sarcomere maintenance and that suppression of this pathway contributes, in part, to the overall pathophysiology of doxorubicin cardiomyopathy.

1818-Pos Board B588

MLCK moves on Actin Filament and Stress Fiber of Smooth Muscle Cells

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We are interested in the mechanism of phosphorylation of smooth muscle myosin (SMM) by the myosin light chain kinase -calmodulin-Ca^{2+} complex (MLCK-CaM-Ca^{2+}). This reaction is required for activation of SMM catalytic activity and smooth muscle contraction. In previous studies we characterized tightly-bound SMM-MLCK-CaM complexes in an in vitro model system and demonstrated that SMM-MLCK-CaM complexes co-purified from smooth muscle were functional, i.e. MLCK was able to phosphorylate SMM and the phosphorylated SMM resulted in actin filament motility in an in vitro assay. Moreover, using total internal reflectance fluorescence microscopy (TIRF), we observed dynamic interactions between single MLCK and SMM molecules by visualizing quantum dot-labeled MLCK (QD-MLCK) interacting with SMM aligned on actin filaments.We have also observed that QDs-MLCK moved along the pure actin filaments. We are currently using cultured human