Cardiac Muscle I

1841-Pos

Abnormal Thin Filament Calcium Binding Associated with Cardiac Muscle Diseases Can be Corrected Through TnC Mutagenesis

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The Ca^{2+} sensitivity of cardiac muscle force development can be adversely altered during disease. Since troponin C (TnC) is the Ca^{2+} sensor for muscle contraction, TnC's Ca^{2+} binding properties may be affected by the disease related protein modifications. To test this hypothesis, a fluorescent TnC was utilized to measure the Ca^{2+} binding sensitivity of TnC in the physiologically relevant biochemical model system of reconstituted thin filaments. Consistent with the pathophysiology, the inherited restrictive cardiomyopathy (RCM) mutation TnI R192H and ischemia induced truncation of TnI (residues 1-192) increased TnC's Ca^{2+} binding sensitivity ~3 fold and ~7 fold, respectively; while the dilated cardiomyopathy (DCM) mutation TnT deltaK210 decreased TnC's Ca^{2+} binding sensitivity ~ 3 fold. Since the symptoms of the diseases may be caused by the abnormal Ca^{2+} binding, correcting the Ca^{2+} binding might improve cardiac function. To achieve this goal, we have engineered TnC constructs with a wide, yet adjustable, range of Ca^{2+} binding sensitivities by modulating the negatively charged residues in the Ca^{2+} chelating loop and/or by replacing key hydrophobic amino acids in the regulatory domain of TnC with polar Gln. We were able to correct both the increased and decreased thin filament Ca^{2+} sensitivities caused by the disease associated proteins via replacing the wild type TnC with specifically engineered TnC constructs. Additionally, engineered TnC constructs can correct the disease related abnormal Ca^{2+} sensitivity of the acto-myosin ATPase assay and the force-pCa relationship in skinned trabeculae. This study can potentially lead to a novel therapeutic strategy for treating cardiac muscle diseases.

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Sarcomere Length Dependent Contractile Activation is Reduced in Rat Trabeculae Exchanged with cTn Containing the L48Q cTnC Variant Independently of Strong Binding Cross-Bridges

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Calcium sensitivity of the force-pCa relationship depends strongly on sarcomere length (SL) in cardiac muscle. It can also be influenced by maneuvers that alter the distribution of cross-bridges within the cross-bridge cycle. We have demonstrated that cardiac trabeculae have left-shifted and virtually eliminated SL dependence of force-pCa relationship following passive exchange with cTn containing a mutant (L48Q) cTnC (with enhanced TnC-TnI interaction). Here we designed experiments to investigate the importance of strongly bound crossbridges and lattice spacing in modulating the force-pCa relationship of WT and L48Q cTnC-cTn exchanged trabeculae. Using 3% dextran at SL = 2.0 μm to osmotically compress preparations to widths ~SL = 2.3 μm, we observed increased maximal force but not increased pCa_{50} in L48Q cTnC-cTn exchanged trabeculae. Conversely, crossbridge inhibition with of 2,3-butanedione monoxime (BDM, 7 mM) at SL 2.3 μm decreased maximal force and Ca^{2+} sensitivity in native and WT-cTn exchanged trabeculae to levels measured at ~SL = 2.0 μm. L48QcTnC-cTn exchanged preparations treated with BDM also decreased maximal force to that seen at SL = 2.0 μm, but demonstrated no shift in Ca^{2+} sensitivity. This result is similar to decrease in maximal force but no shift in Ca^{2+} sensitivity for L48Q cTnC-cTn exchanged preparations at SL 2.0 vs. 2.3 μm. The combined results further support the idea that L48Q cTnC confers crossbridge independence on thin filament activation. It may also imply that native thin filaments are dependent on strong crossbridge binding for full activation because of relatively weak cTnC-cTnI interaction. Finally, the relative strength of cTnC-cTnI interaction may be an important determinant in length dependent activation of cardiac muscle. Support provided by NIH HL65497 (MR) and T32 HL07828 (FSK).

1843-Pos

Engineering Troponin C to Improve Cardiomyocyte Contraction and Relaxation Following Myocardial Infarction

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Gene based therapies targeting cardiac myofilaments offer a novel way to halt or even reverse cardiac dysfunction following infarction by enhancing contractility of the heart. Experiments here were designed to test the ability of an engineered troponin C (L48Q), with increased Ca^{2+} binding affinity to abrogate contractile deficits of isolated adult rat cardiomyocytes from rat hearts infarcted by permanent ligation of the left descending coronary artery. After 4 weeks cardiomyocytes from these and sham-operated, non-infarcted hearts were cultured and incubated with adenoviral constructs containing cDNA for either GFP + WT cTnC or GFP + L48Q cTnC. After 48-72 hours, stimulated cardiomyocyte contraction and relaxation were measured using video microscopy (IonOptix). In a sub-set of cardiomyocytes intracellular Ca^{2+} transients were measured following incubation with Fura-2. Myocardial infarction resulted in decreased extent (8.6 +/- 1.0 %) and velocity of shortening (93.4 +/- 13.0 μm/s) and relaxation velocity (51.3 +/- 6.4 μm/s), compared to control myocytes (12.1 +/- 2.4 % and 147.3 +/- 16.7 μm/s and 118.8 +/- 16.3 μm/s, respectively). Expression of L48Q cTnC in cardiomyocytes from infarcted hearts increased fractional shortening, shortening velocity, and relaxation velocity to near control values of 10.9 +/- 1.3 % and 165.0 +/- 23.6 μm/s and 141.7 +/- 32.2 μm/s, respectively. Interestingly, the peak Ca^{2+} transient amplitude was increased in cardiomyocytes from infarcted hearts, from 9.9 +/- 0.5 % in control cells to 23.0 +/- 2.69 % in cells from infarcted hearts, which was also restored to near control values with L48Q cTnC transfection to 11.9 +/- 0.8 %. These results suggest that targeted expression of L48Q cTnC may improve myocardial function in infarcted hearts by reversing contractile dysfunction and improving sensitivity to intracellular Ca^{2+}. Funding provided by NIH_HL091368 (MR, CEM), AHA_T32 HL07828 (FSK).

1844-Pos

Calcium Sensitivity, Force Frequency Relation and Cardiac Troponin I: Critical Role of PKA and PKC Phosphorylation Sites

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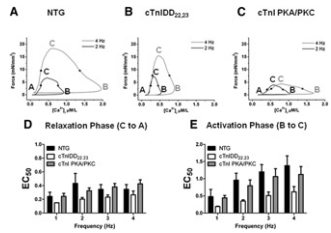
Transgenic models with mutants of cardiac troponin I, cTnI-PKA S22,23D ($cTnIDD_{22,23}$) or cTnI-PKC S22A,S23D,S42,S44D (cTnI PKA/PKC) displayed differential force-frequency relationships (FFR) *in vivo*. We hypothesized that these cTnI phospho-mimics would impact cardiac muscle force development and Ca^{2+} sensitivity in opposite directions in a rate dependent fashion. Our study shows that $cTnIDD_{22,23}$ increases (while cTnI PKA/PKC decreases) its ability to generate normal force per unit of $[Ca^{2+}]i$ when stimulation frequency increases. Force-$[Ca^{2+}]_I$ hysteresis-loops revealed that $cTnIDD_{22,23}$ shows an increased calcium sensitivity in the activation phase of force-$[Ca^{2+}]_i$ loops at 1 to 4 Hz when compared to NTG (Figure1E). An integrated computational model that encompasses electrophysiology, Ca^{2+} dynamics, contractile and mitochondrial activity (ECME model) indicates that these cTnI mutants might change the association-dissociation constants for Ca^{2+} binding, both the low- and/or high-affinity binding sites, of troponin complex. Our data indicate that cTnI phosphorylation at PKA sites is a crucial mediator of the FFR by increasing the frequency-dependent myofilament sensitivity; which might be achieved by adaptive changes on association-dissociation constants for Ca^{2+} binding of the troponin complex.



1845-Pos

Impact of Ischemia/Reperfusion Associated TnI Degradation on Cross-Bridge Dynamics in Skinned Rat Cardiac Trabeculae

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Proteolytic degradation of Troponin I (cTnI) may be the cause for the depressed contractility that is seen in myocardial stunning. Here, we studied the impact of a proteolytic fragment $cTnI_{63-193}$ (identified by McDonough et al, 1999) on cross-bridge cycling dynamics in isolated myocardium. Murine $cTnI_{63-193}$ mutant, as well as wild type cTnC and cTnT, were expressed in *E.coli*. Next, FPLC purified Troponin (cTn) complex containing either wild type cTnI or $cTnI_{63-193}$ was exchanged for endogenous cTn in skinned rat cardiac trabeculae; Western blot analysis confirmed that >75% cTn was exchanged. Myofilament chemo-mechanical cross-bridge dynamics were determined as function of $[Ca^{2+}]$ at SL=2.2 μm using an enzyme-coupled UV absorbance technique (de Tombe & Stienen, 1995).Compared to wild-type exchange, $cTnI_{63-193}$ exchanged fibers displayed ~30% decrease in myofilament Ca^{2+} sensitivity (EC_{50}). In contrast, neither maximal tension development, nor maximal ATPase activity (and consequently tension-cost), were significant different between the groups, nor was cooperative thin filament activation (Hill coefficient). We conclude that

$cTnI_{63-193}$ found following severe ischemic/reperfusion affects cardiac function predominantly via decreased myofilament Ca^{2+}-sensitivity. Our results may benefit rational drug development aimed to prevent ischemic/reperfusion injury in patients.

1846-Pos

TnI Switch Peptide Position within Cardiac Troponin as Studied by cw-Epr and DEER

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Muscle contraction is regulated by the troponin complex which is a heterotrimer protein consisting of a Ca^{2+} binding subunit (TnC), an inhibitory subunit (TnI) and a Tropomyosin binding subunit (TnT). Calcium binding to TnC ('ON' state) initiates a series of structural changes in the thin filament proteins leading to muscle contraction. In the low Ca^{2+} 'OFF' state, the TnI subunit induces muscle inhibition through its two actin binding domains (residues 133-148 and 166-210). Another region of TnI termed the 'switch peptide' (residues 150-161) is essential for a complete relieve of muscle inhibition in the ON state. The position of the switch peptide is vital since it affects the whereabouts of both TnI actin binding domains. In the $+Ca^{2+}$ cardiac Tn core crystal structure, the switch peptide is depicted to be close to the N-lobe of TnC (Takeda et al., Nature, 2003) but no or limited knowledge is known about its position in the absence of Ca^{2+}. We studied the proximity of the switch peptide to two domains within Tn in both the 'ON' and 'OFF' states. Two intermolecular distances from each end of the switch peptide back to the N-lobe of TnC (TnI152/TnC35, TnI152/TnC84, TnI160/TnC55) and one intramolecular distance (TnI129/TnI160) within TnI were measured with Conventional Electron Paramagnetic Resonance (cw) and Double Electron Electron Resonance (DEER) methods. In the 'ON' state, both the intramolecular and intermolecular distances were less than 2.5nm with narrow distance distributions indicative of restricted movement. Upon removal of Ca^{2+}, distances increased considerably (TnI129/160 to 5nm and TnI151/TnC35 to 3.3nm) with an accompanying increase in the distance distributions suggesting a more flexible, non-bound, switch peptide.

1847-Pos

Molecular Function of the C-terminal Domain of Cardiac Troponin I

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Ca^{2+} regulation of cardiac muscle contraction is dependent upon regulation by tropomyosin (Tm) and troponin (Tn); the extreme C-terminus of the inhibitory subunit of Troponin (cTnI) binds to actin at low [Ca^{2+}] and is presumed to hold Tm in a closed position preventing actomyosin interaction. cTnI's C terminus (''mobile domain'' (MD)) is the site of several human mutations that lead to familial hypertrophic cardiomyopathy (FHC), therefore it is of interest to clarify the specific function and importance of this domain in cardiac muscle contraction. We have demonstrated that even in the absence of Tm, Tn is able to enhance thin filament sliding speed and heavy meromyosin ATPase activity. To explore the possibility that the MD plays a role in enhancement of myosin activity in cardiac muscle, we have utilized an all-cardiac protein (porcine cardiac actin and myosin, recombinant human cardiac Tm-Tn) *In Vitro* Motility assay to detect alterations in Ca^{2+} regulation of cardiac actomyosin interaction in the presence of two specific human recombinant cTn MD structural mutants. ''K164Δ'' is truncated after cTnI K164 and ''LINK 2c2'' has an inserted 8-amino acid linker before cTnI K164. At pCa5, K164Δ showed no significant difference from WT in filament sliding speed at most Tn-Tm concentrations tested, while sliding speed with LINK 2c2 was significantly slower than WT. Conversely, at pCa9, K164Δ was unable to stop actomyosin interaction, with sliding speeds significantly faster than WT; LINK 2c2 regulated the same as WT at pCa9 for most concentrations tested. Our *in vitro* cardiac muscle experimental data suggest that (1) the MD of TnI is a key player in Ca^{2+} regulation of cardiac muscle contraction, and (2) the C-terminal Mobile Domain of cTnI is not responsible for observed functional enhancement of myosin at saturating [Ca^{2+}].

1848-Pos

Effect of Hypertrophic Cardiomyopathy Associated Troponin I Mutations on thin Filament Dynamics

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Troponin I plays an essential role in the regulation of cardiac muscle contraction. Together with troponin C and T, troponin I induces Ca^{2+} dependent cooperative transitions of thin filaments between a blocked, a closed and an open state. 29 mutations were found in cardiac troponin I in families with hypertrophic cardiomyopathy. Although unknown, the mechanism of molecular dysfunction is likely to involve an aberrant thin filament responsiveness to changes in intracellular level of Ca^{2+}. Our hypothesis is that these mutations modify important parameters in the cooperative-allosteric transitions of thin filaments. Here we aimed at using transient kinetics to assess the effect of hypertrophic cardiomyopathy TnI mutations (Q130R, R145G, and A157V) on the rate and equilibria of thin filament switching between the blocked, closed and open states. We found that TnIQ130R and TnIA157V did not affect the equilibrium constant between the blocked and the closed states (K_B). In contrast TnIR145G substantially increased K_B in the absence of Ca^{2+}. An increase in K_B is likely to lead to incomplete relaxation. We also investigated the effect of these mutations on the cooperative behaviour of thin filaments. TnIQ130R and A157V did not affect the size of the cooperative unit n while TnIR145G decrease n value to less than 7. Calcium binding to thin filaments was monitored by change in the fluorescence of IAANS-TnC^{C84S}. Thin filaments reconstituted with TnI mutations showed a change in calcium affinity and the rate of Ca^{2+} dissociation. These findings suggest that mutations in different regions of troponin I are likely to have different biochemical effect highlighting the unique molecular mechanism for each of these mutations.

1849-Pos

N-Terminal Truncated Cardiac TnI Extends Frank-Starling Response of the Heart

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Cardiac TnI (cTnI) has a unique N-terminal extension containing the PKA phosphorylation sites, and its removal by restricted proteolysis in cardiac adaptations to hemodynamic stress and β-adrenergic deficiency provides a functional compensation via improving myocardial relaxation (Barbato et al., JBC 2005; Feng et al., JBC 2008). By transgenic expression of N-terminal truncated cTnI (cTnI-ND) in the cardiac muscle of cTnI knockout mice, we examined the function of hearts containing purely cTnI-ND. Working hearts from the double transgenic mice showed no hypertrophy and normal baseline function as compared with the wild type controls, confirming the non-destructive nature of cTnI-ND. When preload was raised to examine the Frank-Starling response, left ventricular relaxation velocity was better maintained in cTnI-ND hearts than that in wild type controls. The effect of cTnI-ND on enhancing relaxation resulted in lower left ventricular end diastolic pressure and maintained left ventricular contractile velocity and end systolic pressure, especially at high preloads. The overall outcome was larger stroke volumes from cTnI-ND hearts at increased preloads than the responses of wild type hearts. The enhanced range and extent of positive response of cTnI-ND hearts to preload demonstrates that the removal of cTnI N-terminal extension by restricted proteolysis provides a novel mechanism to maximize the Frank-Starling effect in cardiac adaptation against hemodynamic and inotropic stresses.

1850-Pos

Functional Effects of Two Troponin I Mutations Linked to Restrictive Cardiomyopathy

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Mutations in cardiac troponin, a protein complex that regulates muscle contraction, have been shown to be linked to cardiomyopathies, which commonly lead to chest pains, myocardial infarction, or sudden cardiac death. The troponin complex consists of three proteins: Troponin T, Troponin I (TnI), and Troponin C (TnC). In recent clinical studies, two novel mutations in cardiac TnI were discovered co-segregated with cardiomyopathy, but their specific functional effects remain unknown. These mutations are the first frameshift mutations in cTnI known to be linked to restrictive cardiomyopathy (RCM). The deletion of two adenines at codon 177 (Delbp529AA) in cardiac TnI, was discovered in a six year old female RCM patient. The second cTnI mutation included in this investigation was the result of a deleted guanine in codon 168 which caused a frame shift and premature stop codon at 176 (DelG502). cTnI DelG502 was associated with sudden cardiac death. It was found during column purification that the 34 residue truncation of cTnI removed or greatly decreased its binding affinity for TnC. However, the Delbp529AA mutant protein, containing 32 alternate C-terminal residues, was successfully purified and formed a functional troponin complex. Actomyosin ATPase assays demonstrated similar maximal ATPase activity for complexes containing TNNI3 Delbp529AA compared to wild type complexes. cTnI Delbp529AA showed increased calcium sensitivity of ATPase and less inhibitory function compared to wild-type cTnI. Calpain