$cTnI_{63-193}$ 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