Cardiac Muscle I

1841-Pos

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

Bin Liu[1], Ryan S. Lee[1], Jack A. Rall[1], Svetlana B. Tikunova[2], Jonathan P. Davis[1].
[1]The Ohio State University, Columbus, OH, USA, [2]University of Houston, Houston, TX, USA.

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

F. Steven Korte, **Erik R. Feest**, Maria V. Razumova, Michael Regnier.
University of Washington, Seattle, WA, USA.

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).

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

Kate O. Buckley.
University of Washington, Seattle, WA, USA.

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).

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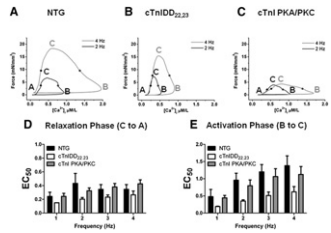
Calcium Sensitivity, Force Frequency Relation and Cardiac Troponin I: Critical Role of PKA and PKC Phosphorylation Sites

Genaro A. Ramirez-Correa, Sonia Cortassa, Brian Stanley, Wei Dong Gao, Anne M. Murphy.
Johns Hopkins School of Medicine, Baltimore, MD, USA.

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

Kittipong Tachampa[1,2], Helen Wang[1], Pieter P. de Tombe[1].
[1]Loyola University Chicago, Maywood, IL, USA, [2]Chulalongkorn University, Bangkok, Thailand.

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