trabeculae. To assess cTnC-cTnI interactions that may underlie this functional effect, we have used both solution and modeling approaches. In solution, cTnC affinity for cTnI was assessed by spectrofluorimetry via labeling cTnC (C35S) with IANBD at Cys84. In both the absence and presence of Ca2+, C35S, L48Q cTnC had increased affinity for cTnI while C35S, I61Q cTnC (and other variants) had reduced affinity. To examine this in more detail we studied the molecular interactions between the (+/-Ca2+)-cTnC1-89-cTnI147-163 complex using molecular dynamics (MD) simulations. Multiple MD simulations (~ 100ns) of wild-type (WT) and the cTnC variants were performed to predict specific structural effects of each residue substitution. Results showed the 48 position of WT and the variant positions of cTnC had the most intermolecular contact pairs with cTnI(147-163). L48Q greatly increased time of contact with cTnI hydrophobic residues Ile148, Met153 and Leu157 compared with WT complex. At ~45ns simulation the B-helix of L48Q cTnC1-89 ''lifted", suggesting a move favorable ''opening up'' of the N-lobe hydrophobic patch compared with WT cTnC1-89. This could lead to stronger binding with the regulatory region of cTnI. Thus, our computational results provide novel details about specific structural alterations throughout L48Q cTnC and other cTnC variants. NIH-HL65497 (MR), AHA-09PRE2090056 (DW)

773-Pos

Structure of the Regulatory Domain of Human Cardiac Troponin C in Complex with the Switch Region of Cardiac Troponin I and the Drug W7: The Basis of W7 as an Inhibitor of Cardiac Muscle Contraction

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The solution structure of Ca^{2+}-bound regulatory domain of cardiac troponin C (cNTnC) in complex with the switch region of troponin I ($cTnI_{147-163}$) and the calmodulin antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfinamide (W7), has been determined by NMR spectroscopy. The structure reveals that the W7 chloronaphthalene ring interacts with the terminal methyl groups of M47, M60, and M81 as well as aliphatic and aromatic side-chains of several other residues in the hydrophobic pocket of cNTnC, while the N-(6-aminohexyl) tail interacts with the C- and D-helices of cNTnC and with $cTnI_{147-163}$. Compared to the structure of the cNTnC•Ca^{2+}•W7 complex (Hoffman, R. M. B. and Sykes, B. D. (2009) *Biochemistry* 48, 5541-5552), the tail of W7 moves toward the surface of cNTnC, in close proximity to the N-terminus of $cTnI_{147-163}$. As a result, the N-terminus of the peptide clashes with the positively charged amino group of the W7 molecule and this repulsive interaction diminishes the helical content of $cTnI_{147-163}$ when compared to the structure of cNTnC•Ca^{2+}•$cTnI_{147-163}$ (Li, M. X., Spyracopoulos, L., and Sykes B. D. (1999) *Biochemistry* 38, 8289-8298). Thus the ternary structure cNTnC•Ca^{2+}•W7•$cTnI_{147-163}$ reported in this study provides a structural basis for the inhibitory effect of W7 in cardiac muscle contraction. The structure also offers an explanation for the ~10-fold affinity reduction of $cTnI_{147-163}$ for cNTnC•Ca^{2+} in the presence of W7. This result generates insights into the features that are useful for the design of cTnC-specific Ca^{2+}-desensitizing drugs.

774-Pos

A Second Look at the Two Phases of Ca^{2+} Binding to Fast Skinned Fibers

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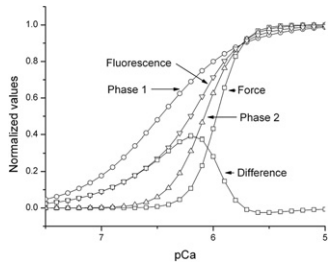
Experiments that replace native TnC with TnC_{danz} in skinned rabbit psoas muscle fibers generate pCa/Ca^{2+} binding curves that are left of and roughly parallel to the pCa/force curve (Guth & Potter, 1987). The fluorescence curve best fits two binding phases, #1 with a slope of about one and #2 with a slope of about 3 or more, a cooperative binding that is associated with major force development (Allen et al, 1992, Huang et al, 2001). Phase 1 is also accompanied with subtle increments in force. The force, fluorescence, phase 1 & 2 curves synthesized from mean fitted parameters for 22 experiments are shown. To demonstrate the difference between force and binding, we subtract the force from the fluorescence. The difference curve indicates that phase 1 Ca^{2+} binding dominates at high pCa then decreases as cooperative binding increases. We argue that the regulatory sites are shifting from normal to cooperative binding and this is why the phase 2 parameters can only approximate force. Because phase 2 binding begins on top of phase 1 the fluorescence curve is shifted left away from force.



775-Pos

Effect of D145E Mutation on Calcium Binding and Exchange with the C-Domain of Troponin C

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Recent discoveries of a number of hypertrophic cardiomyopathy related mutations in the C-terminal domain of cardiac troponin C suggest that sites III and IV might play a more important role than just anchoring troponin C into the troponin complex. We investigated the effects of hypertrophic cardiomyopathy related mutation D145E in human cardiac troponin C on calcium binding and exchange with the C-terminal domain sites III and IV. The calcium titration data indicated that the D145E substitution in the +z position of the calcium binding site IV dramatically decreased calcium binding affinity of that site (~1, 856-fold), and virtually eliminated magnesium binding to that site. Furthermore, the D145E substitution significantly decreased the calcium affinity of site III (~1.4-fold), correlating with ~1.6-fold faster rate of calcium dissociation from site III. Stopped-flow studies utilizing fluorescent calcium chelator Quin-2 demonstrated that the D145E mutation reduced the stoichiometry of moles of calcium per mole of the C-terminal domain by ~2-fold, both in the absence and presence of cardiac troponin I peptide (residues 34-71). Thus, binding of troponin I peptide to the C-terminal domain of D145E troponin C was not able to restore normal calcium binding to site IV. These results indicate the conservative D145E substitution has detrimental effects on calcium and magnesium binding to site IV of troponin C.

776-Pos

Effect Of Down-Regulation of a Stretch-Activated TnC Isoform on Flight of Drosophila

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Both *Drosophila* and *Lethocerus* have indirect flight muscle (IFM) that is activated by sinusoidal length changes at constant [Ca^{2+}]. IFM has two TnC isoforms. F1 binds a single Ca^{2+} in the C-lobe and is needed for the periodic stretch-activation of fibres to produce oscillatory work. F2 binds Ca^{2+} in both N- and C-lobes and is needed for producing Ca^{2+}-dependent isometric tension. We have obtained flies (from VDRC, Vienna) in which F1 is down-regulated by RNAi. Male flies of the F1 RNAi line were crossed with virgin female flies having the Dmef2 driver, which is expressed in all muscles, or a UH3 driver, which is expressed only in IFM. Crosses were maintained at 25°C and 29°C to get different levels of RNAi expression. The proportion of flies unable to fly was: wt 0%; Dmef2 87% at 25°C, 90% at 29°C; UH3 70% at 25°C, 100% at 29°C. There was no difference in time of development or viability of the different lines. Confocal microscopy of Dmef2 and UH3 flies showed myofibrils of both lines were narrower than wt; sarcomere length was normal, but Z-disc and M-line were not straight. Electron microscopy showed that sarcomere structure was disrupted more than expected. Troponin was regularly spaced at 38 nm along thin filaments, but thick and thin filaments were misaligned and Z-and M-lines shifted. Blots of IFM with anti-F1 and F2 showed F1was absent in Dmef2 flies, and greatly reduced in UH3 flies; F2 content of IFM was the same as wt. Therefore, F1 is essential for maintaining normal sarcomere structure of IFM, as well as for stretch-activation. Evidence for cross-linking between troponin components and thick filaments of *Lethocerus* IFM will be presented. Lack of F1 may affect these links.

777-Pos

The Effect of Glutathione on Skeletal Muscle Calcium Sensitivity and Myofilament Sulfhydryl Groups

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Glutathione, a critical reducing agent present in relatively high levels(~5mM) in skeletal muscle, can also attach to protein thiols via a disulfide bond. This process is referred to as glutathionylation, and is thought to be a protective mechanism to prevent irreversible protein oxidation. Prior studies have shown that when skinned fibers are exposed to reduced glutathione there is an increase in calcium sensitivity with no significant change in maximal force. These calcium sensitivity changes were largely reversible by the reducing agent DTT, indicating modification of protein thiols. We measured the force-pCa relationship of permeabilized rabbit psoas fibers treated with DTDP a thiol-specific oxidizing agent and glutathione (5mM). 2D gel electrophoresis using either IEF 4-6.5 or NEPHGE 3-10 in the first dimension was used to identify myofilament proteins whose sulfhydryl groups were modified with the oxidant-glutathione treatment. Additionally, phosphorylation of the regulatory myosin light chain was analyzed using 2d gels (IEF 4-6.5) and the phosphorylation specific stain Diamond Pro-Q. The pCa 50 of skinned psoas fibers was decreased upon exposure to DTT. Following DTT, the addition of DTDP and GSH sequentially,

increased the calcium sensitivity. 2D gel electrophoresis, indicated myosin as a critical target for glutahionylation under these conditions. The RMLC was phosphorylated in the psoas fibers and the level remained constant during oxidant - glutathione treatment. In summary, our data suggest that glutathionylation of myofilament proteins can modulate calcium sensitivity, and may play an important role in maintaining muscle function during oxidative stress.

778-Pos

The Small Molecule Skeletal Sarcomere Activator, CK-2017357, is a Calcium Sensitizer that Binds Selectively to the Fast Skeletal Troponin Complex

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Striated muscle contraction is governed by the release of Ca^{2+} from the sarcoplasmic reticulum via the sarcomeric calcium sensor, the troponin complex. A trimer consisting of troponins T, I, and C, the complex undergoes calcium-dependent conformational changes that regulate the accessibility of myosin binding sites along actin filaments. We used a high throughput screen to identify compounds that activate the ATPase activity of skinned fast skeletal myofibrils; optimization of the initial hit compounds has resulted in compounds with improved potency and medicinal chemical properties. The most advanced exemplar of this chemical series, CK-2017357, shifts the calcium sensitivity of detergent-skinned fast skeletal myofibrils by >10-fold in a concentration dependent manner. This compound specifically activates fast skeletal myofibrils, with no effect on either slow skeletal or cardiac myofibrils. A reconstituted sarcomere assay using combinations of fast skeletal, slow skeletal, and cardiac components demonstrates that the activity of CK-2017357 requires the presence of fast skeletal troponin. Isothermal titration calorimetry indicates the compound binds directly to fast skeletal troponin with a sub-micromolar dissociation constant, while experiments with the fluorescent calcium chelator Quin-2 demonstrate that CK-2017357 slows calcium dissociation from troponin. Consistent with this ability to stabilize the calcium-troponin complex, CK-2017357 increases sub-maximal force development *in vitro* and *in vivo*, suggesting this mechanism may increase power or strength in diseases where muscle function is compromised due to injury, disease or age.

779-Pos

Effect of Temperature on The Rates of Calcium Dissociation and Cross-Bridge Detachment in Cardiac Myofibrils Reported by Troponin C

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It has been proposed that the rate limiting step of cardiac muscle relaxation resides in the myofilaments. The primary mechanism is thought to be the rate of cross-bridge detachment (strongly governed by ADP) since it is generally assumed to be substantially slower than the rate at which the thin filament inactivates (governed by Ca^{2+} dissociation from TnC). This stems from the fact that the rate of Ca^{2+} dissociation from isolated TnC is orders of magnitude faster than the rate of relaxation. However, TnC does not function in isolation but as an integral component of the myofilament contractile system. Furthermore, the Ca^{2+} binding properties of TnC can be drastically affected by the other thin filament proteins and by myosin binding to actin. Thus, we wanted to determine the Ca^{2+} dissociation rate from TnC in cardiac myofibrils during different cross-bridge states. To achieve this goal, rabbit ventricular myofibrils were exchanged with human cardiac troponin containing a TnC (C35S, C84S, T53C) fluorescently labeled with IANBD. Unexpectedly, via the change in TnC fluorescence, not only could we observe the rate of Ca^{2+} dissociation from TnC in the myofibrils, but also what we think is the rate of cross-bridge detachment. At 15^{o}C and in the presence of ADP, the cross-bridge detachment rate was ~7/s, three times slower than the rate of Ca^{2+} dissociation from TnC (~21/s). However, at near physiological temperature (35^{o}C) the two rates were very similar (~60/s). Based on the temperature dependence of the rates, at temperatures below 25^{o}C, cross-bridge detachment may very well be rate limiting for relaxation, but at higher temperatures both rates may be able to modulate the rate of relaxation.

780-Pos

The Effect of Rigor Myosin Upon the pCa of Calcium Binding to Native Cardiac Thin Filaments

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We have used double mixing stopped-flow fluorescence to measure the effect of calcium on the kinetics of the dissociation of the hydrolysis product deoxymantADP (mdADP) from cardiac myosin-mdADP and cardiac myosin-mdADP-Pi by native cardiac thin filaments. Increasing the calcium concentration from pCa > 7 to pCa < 4 increased the rate of dissociation of mdADP from cardiac myosin-S1-mdADP-Pi ~100 fold from 0.5 s^{-1} to 50 s^{-1} while the rate of dissociation of mdADP from cardiac myosin-S1-ADP increased only ~10 fold from 15 s^{-1} to 150 s^{-1}. Rigor myosin-S1 bound to the thin filaments increased the apparent pCa of mdADP dissociation myosin-S1-mdADP-Pi from 0.12 to 0.79 uM. The change in pCa is similar to the increase in the rate of ADP dissociation but is much less than the acceleration in the rate of rate of product dissociation from myosin-ADP-Pi, ~ 100 fold. These results indicate that slow dissociation of phosphate limits the rate of ADP dissociation from acto(thinfilaments)myosin-ADP-Pi and that there are different mechanisms for the calcium regulation of dissociation of the two products of myosin ATP hydrolysis, ADP and phosphate. These results support a mechanism in which phosphate dissociation from actomyosin-ADP-Pi is the step of the hydrolysis cycle that is principally regulated by calcium and do not support a mechanism such as the three state mechanism in which the regulation is a result different distributions of thin filament states in presence and absence of bound calcium that occur prior to myosin binding. This work is supported by a NIH HL84604.

781-Pos

Model for Transient Activation of Isometric Force by Calcium

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The purpose of this study is to model force transients generated by vertebrate striated muscle in response to calcium pulses. We have developed an equilibrium model for calcium activation of isometric force based on three positions of tropomyosin, i.e., troponin-dependent (B), central (C), and myosin-dependent (M). From the equilibrium model, we derived a complete set of ordinary differential equations that can be solved simultaneously given arbitrary calcium. By setting the differential equations equal to zero, steady-state activation was found to reproduce the equilibrium results of the parent model. A time-dependent solution resulted by providing a pulse of calcium using a Gaussian function to control the duration and amplitude of the calcium transient. The results report the fraction of tropomyosin in Position M (activation transient) as function of calcium changes over time. For a given calcium pulse, several characteristics of the activation transient varied with the rate constants used, including the amplitude of peak activation, the time lag in the peak activation, and the duration of the activation transient. If a train of calcium pulses were sufficiently separated in time, identical activation transients returned to baseline before each pulse. However, as the time between the pulses was shortened, the activation transients became progressively fused and the amplitude increased. Using a train of submaximum calcium pulses, the activation transients were seen to rise in amplitude with each pulse and approach plateau amplitude similar in appearance to the staircase phenomenon observed for tetanic muscle stimulation. Thus, we describe a model consistent with the known positions of tropomyosin that reproduces the transient behavior of force development of vertebrate striated muscle. A derivation of differential equations and application to muscle activation may be found online (www.westga.edu/STEMresearch). This work was supported by NSF grant MCB-0508203 (HGZ).

782-Pos

Equilibrium Model for Cooperative Activation of Muscle by Calcium

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The purpose of this study is to model the cooperative activation of muscle. Current models for calcium activation are based on three positions of tropomyosin, i.e., troponin-dependent (B), central (C), and myosin-dependent (M). Regulation of molluscan muscle, which lacks troponin, may be the basis for cooperative activation of all filamentous myosin systems. However, fitting actual calcium-dependent isometric force (F-Ca) data has been difficult to achieve for all muscle and, among existing models, none has been shown to be compatible with muscle that lacks troponin. We describe a mass action mechanism for cooperative activation that involves only Positions C and M. We show that our model fits F-Ca of scallop striated adductor muscle (RM Simmons and AG Szent-Gyorgyi, 1985, J. Physiol. 358: 47-64). Furthermore, given troponin that regulates simply by binding actin in Position B, we show that this same model will fit F-Ca of vertebrate striated muscle regulated by both native and mutant forms of troponin (MA Regnier et al., 2002, J. Physiol. 15: 485-497). Our model also fits paired myosin binding and thin filament activation data (KM Trybus and EW Taylor, 1980, Proc. Natl. Acad. Sci 77: 7209-7213). The results suggest that myosin binding couples energetically to a conformational change in tropomyosin that propagates in position M. Expansion of segments of tropomyosin in position M promotes the association of uncoupled myosin, which stabilizes one coupled myosin for each segment.