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.