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.

Exponential growth of segments by this feed-forward mechanism is limited by the finite supply of tropomyosin in Position C, which can be controlled by troponin in Position B. A derivation and application to thin and thick filament regulation may be found on-line (www.westga.edu/STEMresearch). This work was supported by NSF grant MCB-0508203 (HGZ).

783-Pos

Determinants of Loaded Shortening in Cardiac Myocytes

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Ventricular performance is dictated by stroke volume, which ultimately depends on the extent of myocyte shortening during loaded contractions. We propose that the extent of loaded shortening is determined by the balance between two processes: (i) Ca^{2+}-cross-bridge-induced cooperative activation of the thin filament and (ii) shortening-induced cooperative deactivation of the thin filament. Accordingly, any modulator that augments contractility (i.e., stroke volume) should favor process (i) and diminish process (ii). Since β-adrenergic stimulation is known to increase contractility, we tested whether PKA (the myofibrillar ligand of β-adrenergic signaling) would increase cooperative activation and diminish shortening-induced deactivation in rat permeabilized cardiac myocytes during submaximal Ca^{2+} activations. Regarding cooperative activation, PKA increased the slope of tension-pCa relationships ($n_H = 3.85 \pm 0.09$ before versus $n_H = 5.03 \pm 0.71$ after PKA). PKA also slowed rates of force redevelopment, increased the transient force overshoot after a slack-restretch maneuver, and increased the rate and amplitude of spontaneous oscillatory contractions (SPOCs); all of which are consistent with greater cooperative activation of the thin filament. Regarding cooperative deactivation, PKA increased the curvature of myocyte length traces during lightly loaded shortening ($k_{shortening} = 6.41 \pm 0.28$ before versus $k_{shortening} = 9.45 \pm 0.53$ after PKA) and steepened sarcomere length-tension relationships; both of which implicate enhanced (rather than diminished) shortening-induced cooperative deactivation. Taken together, PKA-induced myofibrillar phosphorylation appears to augment both Ca^{2+}-cross-bridge-induced cooperative activation of the thin filament and (ii) shortening-induced cooperative deactivation. Greater cooperative activation should lead to more cycling cross-bridges, which would speed loaded shortening against a given afterload. On the other hand, greater shortening-induced cooperative deactivation may be necessary to help accelerate relaxation and assist diastolic filling in the face of shorter systolic and diastolic times in the presence of higher heart rates induced by β-adrenergic stimulation.

784-Pos

The Role of Store-Operated Calcium Entry in Store Repletion During Repetitive High Frequency Tetanic Stimulation of Single Skeletal Muscle Fibers

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Store-operated Ca^{2+} Entry (SOCE) involves a trans-sarcolemmal Ca^{2+} influx mechanism triggered by Ca^{2+} store depletion. Recently, we demonstrated that SOCE activation in skeletal myotubes involves a functional coupling between STIM1 Ca^{2+} sensor proteins in the sarcoplasmic reticulum (SR) and Ca^{2+}-permeable Orai1 channels in the sarcolemma. However, the physiological role of SOCE in muscle remains unknown. Here, we monitored myoplasmic Ca^{2+} transients in mag-fluo-4 loaded mouse flexor digitorum brevis fibres during repetitive high frequency tetanic stimulation (60 consecutive 500ms, 50Hz stimulation trains every 2.5s). In normal Ringer's solution, tetanic Ca^{2+} transient amplitude decays in three phases: an initial rapid phase (trains 1-10), a second phase of maintained amplitude (trains 10-40), and a final phase of decay (trains 40-60). The maintained phase corresponds to a slightly elevated tail transient integral during each interpulse interval, consistent with activation of Ca^{2+} influx between tetani. Addition of 0.5mM $CdCl_2$ plus 0.2mM $LaCl_3$ did not alter the initial or final phases of Ca^{2+} transient decay, but significantly ($p<0.01$) compromised both the maintained Ca^{2+} transient ($4 \pm 3\%$ reduction from trains 10 to 40 in normal Ringer versus $30 \pm 3\%$ reduction with Cd/La) and the increase in tail transient integral (which decreased $21 \pm 7\%$ with Cd/La) observed during the second phase. Similar results were obtained following addition of either BTP-2 or SKF96365, two known SOCE inhibitors, consistent with SOCE mediating store repletion during the secondary phase of maintained release. Together, these results suggest that repetitive high frequency tetanic stimulation activate a SOCE flux used to replenish SR Ca^{2+} stores required to maintain subsequent Ca^{2+} release. Current experiments are testing the validity of this assertion using molecular interventions (transient STIM1 knockdown and dnOrai1 expression) to more selectively inhibit SOCE.

785-Pos

Disruption of Circadian Gene Expression in Skeletal Muscle but not Liver in Pre-Hypertensive SHR Vs. WKY Rats

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Recently, alterations of the molecular clock and circadian rhythms have been implicated as contributing factors to cardiovascular and skeletal muscle disease. Woon et al. (2007) determined that a polymorphism found in the congenic interval of the SHR rat is associated with hypertension and type II diabetes. Here, we examined the expression of circadian genes in striated muscle (cardiac and skeletal muscle) in young pre-hypertensive SHR (6 weeks old) and age-matched Wistar-Kyoto (WKY) male rats. The rats were entrained to a 12 hour light: 12 hour dark cycle for 2 weeks and then placed in constant darkness for 30 hours. Cardiac muscle (left ventricle), skeletal muscle (soleus) and non-muscle tissue (liver) were collected every 4 hours for 40 hrs, totally 10 time points. Expression of core clock genes (*Bmal1, Clock, Per2, Rorα, Rev-erb*) and the clock-controlled gene, *Dbp*, were analyzed using real-time quantitative PCR. Expression of *Bmal1* has a clear circadian pattern in muscle and liver tissue of rats. The pattern and amplitude of circadian expression of *Bmal1* were not altered between WKY and SHR strains in every tissue studied. In contrast, expression of the other clock genes, *Rorα, Dbp, Rev-erb, Clock and Per2*, were significantly dys-regulated in the soleus muscle from the SHR rat. In the left ventricle, circadian expression of *Per2* was dampened in the SHR but the other clock genes were unchanged. In liver, there were no differences in expression of any of the clock genes between the SHR and WKY rats. These data suggest that components of the molecular clock are disrupted in striated muscle prior to overt signs of hypertension. The contribution of this disruption in the clock to hypertension and type II diabetes are to be determined.

786-Pos

Effect of Cannabinoids on Choline Induced Contractures in Slow Skeletal Muscle Fibers of the Frog

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Cannabinoids interact with membrane receptors causing, among others, psychoactive and motor effects. Recently it has been shown the presence of CB_1 receptors in amphibian skeletal muscle. The aim of our study was to approach the role of acetylcholine receptors (AchR) on the mechanism of cannabinoid action in skeletal muscle contraction. We isolated bundles of the slow portion of *cruralis* muscle of the frog and induced contractures by incubating with Ringer solution with choline 115 mM. Choline induces maintained contractures with a slow relaxation phase. As reported recently for other frog slow skeletal muscle, the choline-contracture in *cruralis* bundles depends on the interaction with AchR as the contracture is reduced almost completely (~95%, n=3) by blocking these receptors with tubocurarine 100 μM. To test the effect of cannabinoids we incubated with the CB_1 agonist ACPA (1 μM) and the choline-contracture was diminished by around 40% ($p<0.05$; n=4). This effect was blocked partially (~20%) by preincubating the bundles with the CB_1 antagonist AM281 (1 μM; n=3). Also, pretreating the bundles with *pertussis* toxin (2 μg/ml) causes a partial blockade of the ACPA effect (~20%). Both results strongly suggest that part of the effect is caused through a mechanism involving the activation of CB_1 receptors, being the rest a receptor-independent effect. On the other hand, blocking the Ryanodine receptor-α (RyR-α) with Dantrolene (150 μM) causes a reduction of the choline-contracture by approximately 45%. Once the RyR-α are blocked, ACPA did not cause further decrease, suggesting the involvement of the RyR-α in the effect caused by ACPA on the choline-contractures. Our results show that ACPA modulates choline-contractures and suggests that this effect involves the participation of CB_1, AchR and RyR-α.

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787-Pos

The Alterations of Store-Operated Calcium Entry in TRPC1-Overexpressing C2C12 Myotubes

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When the endoplasmic reticulum (ER) calcium store is depleted, a Ca^{2+} influx is activated from the extracellular milieu to refill the intracellular stores. This well-regulated Ca^{2+} uptake mechanism, called store-operated Ca^{2+} entry