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).

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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.

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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.

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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.

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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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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