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 hypothesize 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 myobifilar 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 (nH = 3.85 ± 0.09 before versus nH = 5.03 ± 0.71 after PKA). PKA also slowed rate-force redevelopment. Incremental or decremental increases in force occurred 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 (kshortening = 6.41 ± 0.28 before versus kshortening = 9.45 ± 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 myobifilar 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.

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 ± 3% reduction from trains 10 to 40 in normal Ringer versus 30 ± 3% reduction with Cd/La) and the increase in tail transient integral (which decreased 21 ± 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 activates 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.