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Store-operated Ca^{2+} Entry In Skeletal Muscle Can Be Activated And Deactivated Within Milliseconds Of Ca^{2+} Release And Store Refilling

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that allows the entry of Ca^{2+} upon depletion of the internal stores. The skeletal muscle cell is built for the rapid delivery of Ca^{2+} to the contractile proteins. The cell microarchitecture allows this with the surface membrane invaginating into the cell forming the tubular (t-) system which apposes the sarcoplasmic reticulum (SR) for rapid signalling. In skeletal muscle SOCE has been shown to occur within 1 s of Ca^{2+} release (Launikonis & Rios, 2007) but this should be significantly faster if the molecular agonists are prepositioned for activation. To examine SOCE kinetics we used skinned fibres from C57 mice (7-20 weeks old) with t-system trapped fluo-5N, bathed in an internal solution with 50 μM rhod-2. These dyes were simultaneously imaged in xyt mode on a confocal microscope (2 ms/line) while Ca^{2+} release was induced by lowering [Mg^{2+}]. Global Ca^{2+} release induced SOCE activation and deactivation as the Ca^{2+} store refilled upon release inactivation (Launikonis & Rios, 2007). We also observed Ca^{2+} waves in the continued presence of low Mg^{2+}. These waves allowed an accurate observation of the latency between SR Ca^{2+} release and SOCE. Thus SOCE ''coupling delay'' following the initiation of SR Ca^{2+} release was determined to be 27 ± 4 ms (n = 6). SOCE deactivation already started to occur when myoplasmic Ca^{2+} levels dropped only by 10 % suggesting an intact switch-off signal for SOCE from the store. Also, SOCE deactivation rate depended upon SR Ca^{2+} refilling rate in a sigmoidal manner, indicating that binding of luminal Ca^{2+} to Stim1 effectively decoupled from Orai1 instantly during refilling. This suggests conformational coupling between Stim1 and Orai1 mediate SOCE.

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Down And Out. The Functional Effects Of Silencing Calsequestrin 1 Or Deleting Both Calsequestrin Genes In Mammalian Muscle

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Calsequestrins 1 and 2 are major calcium binding proteins of the SR in skeletal and cardiac muscle. We transiently suppressed synthesis of CSQ1 in fast twitch muscle of live adult mice by transfection with a plasmid coding for siRNA and a marker. Immunoblots showed reduction of CSQ1 by 40 to 100% in the whole treated (FDB) muscle. Ca^{2+} transients and Ca^{2+} release flux were measured in fibers selected for their high expression of the marker and patch clamped. Similar studies were done with FDB fibers from a double-null strain created by crossing CSQ1-null (Paolini et al. 2007) with CSQ2-null mice (Knollmann et al. 2006). Total Ca^{2+} releasable by maximal prolonged depolarization was decreased by up to 30% in silenced and 40% in KO muscles compared with the wild type. The reduction in CSQ had subtle kinetic consequences. The time course of release flux induced by long depolarization lost a ''shoulder'' (present in the WT; Royer et al. 2008). This shoulder reflects a component of the SR Ca^{2+} buffering power characterized by its dependence on $[Ca^{2+}]_{SR}$. Its loss here identifies the shoulder as a kinetic signature of the presence of CSQ. The KO presents additional anomalies, including asynchronous activation of different regions, and, occasionally, abnormally high initial release flux. Both features may be associated with structural changes like those found in the CSQ1 KO (Paolini, 2007). In conclusion, muscle either transiently or permanently devoid of CSQ is still capable of releasing large quantities of Ca^{2+}. Means of Ca^{2+} storage unrelated to CSQ appear to play a major role in skeletal muscle. Supported by NIAMS/NIH.

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Amyloid-β protein impairs Ca^{2+} release and contractility in skeletal muscle from Inclusion Body Myositis mice

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Inclusion Body Myositis (IBM), the most common muscle disorder in the elderly, is partly characterized by an abnormal, intracellular accumulation of β-amyloid precursor protein (βAPP) and β-amyloid epitopes. The present study examined the effects of β-amyloid accumulation on contractilioty and Ca^{2+} release in skeletal muscle from transgenic mice harboring human βAPP and assessed the consequence of $A\beta_{1\text{-}42}$ modulation of the ryanodine receptor Ca^{2+} release channels (RyR). Muscle from βAPP-transgenic animals produced less peak force, yet fatigued at slower rate than the non-Tg muscle. Analysis of sarcoplasmic reticulum (SR) Ca^{2+} release showed that transgenic myofibers consistently exhibited Ca^{2+} transients with smaller amplitude compared to the non-Tg cells. Although Ca^{2+} removal was slower in transgenic muscle, experiments with SR vesicles in the presence of synthetic β-amyloid peptide ($A\beta_{1\text{-}42}$) did not reveal an acute effect of this peptide on SR Ca^{2+} ATPase. To determine whether modification of RyRs by β-amyloid underlie the effects observed in muscle, in vitro Ca^{2+} release assays and RyR reconstituted in planar lipid bilayer experiments were conducted in the presence of synthetic $A\beta_{1\text{-}42}$. Application of $A\beta_{1\text{-}42}$ to resulted in modification of RyR properties in bilayers, while addition of $A\beta_{1\text{-}42}$ to the SR vesicles resulted in RyR-mediated Ca^{2+} release. These data may relate βAPP mismetabolism in IBM to altered RyR-mediated Ca^{2+} release and muscle contractility.

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Bin1, A Bar Domain Protein, Is Necessary For The Maintenance Of T-tubule Structure And Intracellular Ca^{2+} Homeostasis In Skeletal Muscle

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Efficient intracellular Ca^{2+} homeostasis in skeletal muscle requires establishment and maintenance of an intact triad junctional complex. Bin1, an amphiphysin family protein contains a conserved BAR domain that facilitates membrane curvature. Bin1 also contains a unique phosphatidylinositol-4,5-bisphosphate (PIP_2) binding domain that is required for transverse-tubule membrane biogenesis during C2C12 myogenic cell differentiation. The lethality associated with Bin1 knockout limits the opportunities to study the function of Bin1 in adult skeletal muscle. Using *in vivo* electroporation to deliver shRNA against Bin1, we knockdown Bin1 expression in the FDB muscle of adult mice. We find that transient loss of Bin1 alters the properties of Ca^{2+} sparks induced by osmotic stress. Specifically, we observe that the mean frequency of Ca^{2+} sparks per minute is reduced in fibers that exhibit a high level of Bin1 knockdown (33.1 ± 5.7), compared to control fibers (138.1 ± 21.7). Kinetic analysis of individual Ca^{2+} sparks shows that spark amplitude ($\Delta F/F_0$) is reduced in the Bin1 knockdown fibers (0.67 ± 0.02) when compared to control (0.85 ± 0.01). Bin1 knockdown also alters the full duration at half maximal (FDHM) of Ca^{2+} sparks. A two-exponential decay function fit of the FDHM histograms indicates that, in the control fiber, the corresponding time constants for Ca^{2+} sparks (t_{01}) are 38.0 ± 2.8 ms and 281.3 ± 40.0 ms for Ca^{2+} bursts (t_{02}). The time constant for Ca^{2+} bursts (t_{02}) are significantly reduced in the high Bin1 knockdown muscle fiber (140.9 ± 41.2 ms). Electron microscopy reveals Bin1 knockdown fibers exhibit vacuolation and swelling of t-tubule structures. Thus, alteration of triad junction structure can potentially affect the resting cytosolic Ca^{2+} levels and internal Ca^{2+} stores in Bin1 knockdown fibers.

Motions of the Cell Surface Molecules

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Effect of Energy Depletion and Antimicrobial Peptides on Single Protein Motility in Living *Escherichia coli*

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Using optical tweezers the motility of a single outer membrane protein in *E. coli* bacteria, the λ-receptor, was studied. The λ-receptor is a porin that transports nutrients (maltodextrins) across the bacterial membrane. By poisoning the cells with arsenate and azide the bacterial metabolism was stopped. The motility of the exact same λ-receptor was measured before and after energy depletion. After energy depletion there was a significant decrease in the spread of positions