Molecular mechanism of store-operated calcium entry (SOCE) in skeletal muscle and potential role in fatigue resistance

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In skeletal muscle, store-operated calcium entry (SOCE) is a trans-sarcolemmal calcium influx pathway activated when sarcoplasmic reticulum (SR) calcium stores are depleted. Recently, we demonstrated that SOCE activation in skeletal myotubes involves a functional coupling between STIM1 calcium sensor proteins in the sarcoplasmic reticulum (SR) and calcium-permeable Orai1 channels in the sarcolemma. However, the precise mechanism and physiological role of SOCE in adult skeletal muscle remains largely unknown. Here we investigated the mechanism and physiological role of SOCE in single *flexor digitorum brevis* (FDB) fibres from adult mice. Using a Mn^{2+} quench assay, we found that thapsigargin-induced SR calcium store depletion activates a calcium influx pathway in adult FDB fibres that is inhibited by: 1. multiple SOCE channel blockers (La^{3+}, BTP-2 and SKF96365); 2. prior expression of cherry-tagged dominant negative murine Orai1 (E108Q); or 3. STIM1 knockdown 12 days after *in vivo* electroporation of murine specific STIM1 siRNAs. To determine the potential role of SOCE in maintaining SR calcium stores during repetitive stimulation, we monitored myoplasmic calcium transients in mag-fluo-4 loaded mouse FDB fibres during repetitive high frequency tetanic stimulation (60 consecutive 500ms, 50Hz stimulation trains every 2.5s). Peak calcium transient amplitude was reduced dramatically with each tetanus by conditions that inhibit SOCE (*e.g.* addition of 0.5 mM Cd^{2+}/0.2 mM La^{3+}, 10 μM BTP-2, 50 μM SKF96365, and STIM1 knockdown).

To further assess the role of SOCE in skeletal muscle, we generated skeletal muscle-specific HA-tagged dominant negative murine Orai1 transgenic mice (HSAdnOrai) using a transgene containing the human skeletal muscle actin promoter (kindly provided by Dr. J. Molkentin). HSAdnOrai mice survive beyond weaning, grow and breed normally. Western blot analysis using an HA antibody confirmed dnOrai1 transgene expression in skeletal muscle, but not in heart, lung, brain, spleen kidney or liver. Primary skeletal myotubes derived from HSAdnOrai mice lack SOCE following SR calcium store depletion as assessed in Mn^{2+} quench, calcium influx, and whole cell patch clamp assays. In addition, compared to WT mice, the decline in peak calcium transient amplitude during repetitive tetanic stimulation was significantly increased in FDB fibres from HSAdnOrai mice. Together, these results demonstrate that STIM1-Orai1 coupling mediates SOCE in adult skeletal muscle and that this process limits SR calcium store depletion and the development fatigue during repetitive stimulation. In addition, muscle-specific HSAdnOrai transgenic mice are a powerful tool for future studies designed to assess the physiological role of SOCE in skeletal muscle.