Specialization of the skeletal muscle junctional membranes allow rapid activation of store-operated calcium entry, tightly controlled by $[Ca^{2+}]_{SR}$

B.S. Launikonis, School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.

The mechanism store-operated Ca^{2+} entry (SOCE) appears a ubiquitously expressed mechanism, currently best described in non-excitable cells. Non-excitable cells typically have a endoplasmic reticulum, ER, which stores Ca^{2+}. The ER may not be highly abundant in non-excitable cells and may not form any permanent junction with the plasma membrane at rest. Under situations where the ER is depleted of Ca^{2+}, Ca^{2+} sensor proteins that span the ER membrane, known as stromal interacting molecule 1 (STIM1), aggregate at sites on the ER membrane. The ER membrane translocates to form a junction with the plasma membrane. These two events allow the cluster of STIM1 molecules to interact with Orai1 proteins on the plasma membrane to initiate the entry of Ca^{2+}. This is store-dependent Ca^{2+} entry.

This brief description of SOCE does not hold for skeletal muscle because skeletal muscle has highly specialized membranes, significantly changing the landscape for SOCE from the picture in non-excitable cells. In muscle, the sarcoplasmic reticulum, SR, is the specialized internal Ca^{2+} store, which holds large amounts by Ca^{2+} buffered by calsequestrin (CSQ). The SR makes a standing junction throughout the muscle fibre with the tubular (t-) system, which is the internalization of the plasma membrane. The purpose of these specializations in muscle is to allow an even and rapid conduction of action potentials throughout the t-system, to all sarcomeres so that the SR in each will release Ca^{2+} rapidly and evenly to initiate contraction.

SOCE in skeletal muscle does not require translocation of SR to plasma membrane, as a standing junction already exists. SOCE has been shown to be activated very rapidly in skeletal muscle fibres upon depletion of $[Ca^{2+}]_{SR}$ below an activation threshold due to a prepositioning of Orai1 and STIM1L uniformly across the junctional membranes (Edwards *et al.*, 2010; Darbellay *et al.*, 2011). It has been suggested by other groups that the rate of the store-dependent Ca^{2+} influx is regulated by ryanodine receptor (RyR) and CSQ, based on studies in the presence and absence of these proteins. However, the absence of either of these proteins will significantly affect Ca^{2+} release from SR. The aim of this work was to determine whether the rate of change of Ca^{2+} in the SR would be matched by the rate of the store-dependent Ca^{2+} influx, independent of stimulation type.

C57/Bl6 mice were killed by asphyxiation with CO_2 under procedures approved by The Animal Ethics Committee of The University of Queensland. The *extensor digitorum longus* were removed and placed in a Petri dish under a layer of Paraffin oil. Bundles of fibres were isolated and exposed to a physiological solution containing 1 mM fluo-5N salt for > 10 min, then individual fibres were isolated and mechanically skinned; or individual fibres were isolated without prior exposure to an exogenous solution and moved to a chamber with a coverslip base under a small volume of internal solution containing 0.01 mM fluo-5N-AM. To examine the regulation of SOCE rate we imaged cytoplasmic Ca^{2+} with rhod-2 simultaneously with fluo-5N inside the t-system or SR of skinned fibres to measure cytoplasmic Ca^{2+} release with SOCE rate ($-d[Ca^{2+}]_{t\text{-}sys}/dt$) or SR Ca^{2+}, respectively. Ca^{2+} release was induced by lowering cytoplasmic Mg^{2+} in the presence of 1 mM EGTA. This manoeuvre caused CSQ to depolymerize in the progressively lowered calcium environment, resulting in a reduction of SR Ca^{2+}-buffering power (Launikonis *et al.*, 2006).

Fluo-5N fluorescence from the SR or t-system of skinned fibres was imaged simultaneously with cytoplasmic rhod-2 in *xyt* mode on a confocal microscope. Cell-wide Ca^{2+} transients and propagating waves were tracked using this method of Ca^{2+} release and combination of imaging protocols. On the same fibre it was observed that Ca^{2+} released from SR under the same RyR stimulus had three distinct rates, $-d[Ca^{2+}]_{SR}/dt$, indicating three states of SR Ca^{2+}-buffering in the SR. This is probably due to stepwise decreasing proportions of CSQ polymers: dimers: monomers in the SR resulting in a stepwise reduction in SR Ca^{2+}-buffering power. SOCE was active in the second and third phases of CSQ depolymerization as total calcium declined. SOCE rate was proportional to $-d[Ca^{2+}]_{SR}/dt$ in the two latter phases (that were about 5-fold different) of declining SR Ca^{2+}-buffering capacity during Ca release. It is concluded that SOCE rate is regulated by, and can match, $-d[Ca^{2+}]_{SR}/dt$.

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