Conformational coupling of store-operated Ca^{2+} entry in skeletal muscle

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that allows the entry of extracellular Ca^{2+} upon depletion of the internal stores in order to refill them. This mechanism has been described in excitable and non-excitable cells and the two main molecular players, Stim1 and Orai1, have been identified. In non-excitable cells the response to depletion of internal Ca^{2+} stores involves aggregation of Stim1 on the endoplasmic reticulum (ER) membrane and translocation of the ER towards the plasma membrane to allow Orai1 to conduct the entry of Ca^{2+} in a process taking tens of seconds (Lewis, 2007). In contrast, 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.

The Animal Ethics Committee at The University of Queensland approved the use of animals in this study. 7-12 week old C57 mice were killed by asphyxiation and the extensor digitorum longus (EDL) muscles were removed. Intact fibres were exposed to a Na^{+}-based physiological solution containing fluo-5N salt. Fibres were mechanically skinned, trapping the dye in the t-system, and transferred to a chamber containing a K^{+}-based internal solution with 1 mM EGTA (100 nM Ca^{2+}), 1 mM Mg^{2+} and 0.05 mM rhod-2. Release of SR Ca^{2+} was evoked by substitution of the bathing solution with a 'low Mg^{2+}' solution, containing 0.01 mM Mg^{2+} and being nominally free of Ca^{2+}. Cytoplasmic rhod-2 and t-system fluo-5N were continuously imaged on an Olympus FV1000 confocal microscope in xyt mode during Ca^{2+} release. The net change in t-system fluo-5N signal was used as an indicator of SOCE activity (Launikonis & Rios, 2007).

As described previously, low Mg^{2+} solution induced cell-wide SR Ca^{2+} release that was accompanied by an initial uptake of Ca^{2+} by the t-system, followed by a depletion (due to SOCE) and a reuptake of Ca^{2+} as the cytoplasmic Ca^{2+} transient declined and the SR refilled with Ca^{2+} (SOCE deactivation). In some experiments we observed subsequent Ca^{2+} waves. These waves were associated with a lower SR release flux than cell-wide release and allowed observation of the latency between Ca^{2+} release, which had a defined front and the defined onset of SOCE. The line acquisition rate of 2 ms along the y-axis of each image allowed for analysis of Ca^{2+} fluxes at this temporal resolution. Thus SOCE "Coupling delay" following the initiation of SR Ca^{2+} release was determined to be 27 ± 3.6 ms ($n = 6$). This rapid activation of SOCE is too fast to involve diffusible messengers and is consistent with conformational coupling of SOCE in skeletal muscle. A rapid deactivation mechanism in this cell (Friedrich *et al.*, 2008) also supports this conclusion.

Friedrich O, Edwards JN, Murphy RM, Launikonis BS. (2008) *Proceedings of the Australian Physiological Society,* **39:** 19P.

Launikonis BS, Rios E (2007) *Journal of Physiology,* **583:** 81-97.

Lewis RS (2007) *Nature,* **446:** 284-287.