Store-dependent Ca^{2+} influx in intact healthy and dystrophic skeletal muscle

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that involves an extracellular Ca^{2+} influx in response to store-depletion to allow refilling of internal stores to physiological levels. It was suggested that SOCE may be enhanced in dystrophic skeletal muscle, activating proteolytic enzymes and triggering necrosis. However, those studies were exclusively performed in myotubes that do not compare to the fully differentiated muscle cell, where the dystrophic phenotype is primed. A recent study by Boittin *et al.,* (2006) claimed that SOCE flux rates were increased by an order of magnitude in adult single skeletal muscle fibres from dystrophic mdx mice compared to wild-type (wt). This SOCE flux rate was based on fluorescent myoplasmic signals from the high affinity Ca^{2+} dye fura-2. The plateau was reached within ~10 s in mdx, probably saturating the dye, and ~2 min in wt fibres. Having observed a robust and normally regulated SOCE in skinned fibres from mdx (Friedrich *et al.,* 2008), we re-examined SOCE fluxes in intact single wt and mdx muscle fibres.

C57BL/10 mice (8-20 weeks old) were killed by asphyxiation, in accordance to guidelines set by the Animal Ethics Committee of the University of Queensland. Interossei muscles were dissected and placed in a physiological saline. Isolated fibres were obtained by mild enzymatic treatment and loaded with 10 μM fluo-4AM (15 min) to allow for imaging of cytoplasmic Ca^{2+}. Sarcoplasmic reticulum $[Ca^{2+}]$($[Ca^{2+}]_{SR}$) was depleted by bathing the cells in a 0 Ca^{2+}, K^+-based physiological solution with 20 μM cyclopiazonic acid (CPA), followed by caffeine (10 mM) application. Fibres were then transferred into a 0 Ca^{2+}, Na^+-based solution containing Rhod-5N (10 μM) and 2 mM Ca^{2+} was added during imaging to measure SOCE. Fluorescence xyt images were obtained on an Olympus FV1000 confocal microscope. All solutions contained 50 μM BTS to prevent contraction.

After CPA incubation, caffeine evoked a significant fluorescence increase indicating that SR Ca^{2+} leakage alone does not fully deplete $[Ca^{2+}]_{SR}$. Therefore, all cells were depleted with CPA plus caffeine in a Ca^{2+}-free solution. By adding Ca^{2+} to the Rhod-5N containing solution, "Ca^{2+} arrival" at the depleted cell could be tracked. A very fast or very slow fluorescence increase was observed in response to SOCE regardless of cell type. The fast increase saturated the dye in ~50 s. All these cells vesiculated and were permeant to propidium iodide (PI). Thus, the high Ca^{2+} influx rates are a result of breakdown in membrane integrity. Cells that did not vesiculate also excluded PI entry and showed much slower fluorescence increases which did not saturate the dye. In these cells, there was three times larger SOCE flux into mdx compared to wt fibres. This result correlates well with increased expression of Stim1 and Orai1 in mdx compared to wt fibres (Friedrich *et al.,* 2008).

We conclude that there is an increased SOCE flux in dystrophic intact muscle that is, however, significantly lower than previously suggested (Boittin *et al.,* 2006). Furthermore, this increased influx will not contribute to Ca^{2+} overload in mdx cells due to the robust SOCE deactivation mechanism that persists in these cells (Friedrich *et al.,* 2008).

Boittin F, Petermann O, Hirn C, Mittaud P, Dorchies OM, Roulet E, Ruegg UT. (2006) *Journal Cell Science*, **119:** 3733-3742.

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