Different capacity for store-dependent Ca^{2+} influx and Ca^{2+} extrusion across the plasma membrane of wild-type and dystrophic mdx mouse muscle

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Store-operated Ca^{2+} entry (SOCE) is a ubiquitously expressed signalling system that is highly specialized in skeletal muscle to respond rapidly to depletions of Ca^{2+} within the internal store. "Dysregulated" SOCE had been proposed as a pathway for Ca^{2+} entry into dystrophic muscle that leads to fibre degradation. We recently showed that the integral SOCE proteins, STIM1 and Orai1, are upregulated approximately three-fold in dystrophic mdx mouse muscle. However the muscle still responds with normal activation and deactivation of SOCE during Ca^{2+} depletion from skinned fibres (Edwards *et al*., 2010). We now test our findings in intact fibres where conditions may be different to those in skinned fibres. Furthermore, a recent paper has described a previously unidentified STIM1 isoform that is specific to excitable cells, STIM1L, that could be conferring the fast kinetics of SOCE observed in muscle (Darbellay *et al*., 2011). We have also probed for STIM1L in mdx and wild-type (WT) muscle.

All experiments were approved by The Animal Ethics Committee at The University of Queensland. C57BL/6 and C57BL/10-mdx mice were killed by asphyxiation following inhalation of CO_2. Western blotting for STIM1L was conducted on homogenates of EDL muscle from WT and mdx. For Ca^{2+} imaging experiments, *interossei* muscle from these mice were removed and individual fibres were enzymatically isolated. Isolated fibres were loaded with 10 μM fluo-4AM and imaged on a confocal microscope. Fibres were depleted of Ca^{2+} in a K^+-based physiological solution with 20 μM cyclopiazonic acid (CPA) and no added Ca^{2+}, conditions favourable to Ca^{2+} extrusion by the plasma membrane Ca^{2+} pump (PMCA). After 20 min exposure to CPA, 10 mM caffeine was applied to the fibres to ensure thorough depletion of sarcoplasmic reticulum (SR). Store-dependent Ca^{2+} influx and subsequent deactivation were then observed upon reintroduction of external Ca^{2+} to the fibres in a 0 Ca^{2+}, Na^+-based physiological solution. The reintroduction of Ca^{2+} evoked fluo-4 transients in both mdx and WT fibres. 10 mM caffeine was subsequently applied to assay SR Ca^{2+} content. In other experiments cytoplasmic fura-2 was imaged by exciting at 340 and 380 nm during application of 50 μM CPA on a wide-field fluorescence microscope. We also imaged fluo-5N in the tubular system of skinned fibres during SR Ca^{2+} release in a low Mg^{2+} solution containing rhod-2 to estimate the store-dependent influx using confocal microscopy (Edwards *et al*., 2010). Statistical differences between data sets were determined by two-tailed *t*-test and *n* values are represented in parentheses.

We found that STIM1L was upregulated 1.8-fold in mdx muscle ($P<0.05$). Consistent with the higher levels of STIM in mdx fibres, the store-dependent Ca^{2+} influx recorded in skinned fibres was 2-fold greater in mdx compared with WT for the same amplitude of SR Ca^{2+} release ($P<0.05$). In contrast experiments in intact fibres showed a greater amplitude (1.16±0.07 (7) *vs* 1.60±0.13 (6); $P<0.01$) and d[fluo-4 fluorescence]/d*t* (1.02±0.5 (7) *vs* 5.1±1.4 (6) s^{-1}; $P<0.05$) during SOCE in WT compared to mdx fibres. However both fibre types displayed a similar time integral of the caffeine-induced fluo-4 transient (210±20 (7) *vs* 165±25 (6); $P>0.1$) following SOCE suggesting a similar level of SR Ca^{2+} reloading. Imaging of cytoplasmic fura-2 in the presence of 50 μM CPA and no added Ca^{2+} showed that more Ca^{2+} remained in the cytoplasm of mdx compared to WT fibres (fura-2 ratio after CPA application in WT and mdx: 0.50±0.05 (7) *vs* 0.56±0.02 (8); $P<0.05$) suggesting that Ca^{2+} extrusion by PMCA is restricted in mdx. This also explains the apparent lower store-dependent Ca^{2+} influx in mdx fibres, as the washout of caffeine and CPA resulted in more cytoplasmic Ca^{2+} re-entering SR to partially deactivate SOCE before externally applied Ca^{2+} could enter the fibre *via* SOCE. These results suggest that store-dependent Ca^{2+} influx is greater and PMCA is restricted in its capacity to extrude Ca^{2+} in mdx compared to WT fibres.

Edwards JN, Friedrich O, Cully TR, von Wegner F, Murphy RM, Launikonis BS (2010). *American Journal of Physiology - Cell Physiology* **299**: C42-C50.

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