Store-operated Ca^{2+} entry in dystrophic skeletal muscle is not a source for Ca^{2+} overload due to robust deactivation

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Store-operated Ca^{2+} entry (SOCE) has been shown to be fully operational in intact and skinned skeletal muscle fibres. Previous work, mainly on myotubes, suggested that SOCE may become deregulated in dystrophic skeletal muscle and result in Ca^{2+} overload of the cell. The final result of such a process would be activation of proteolytic enzymes, cell necrosis and/or apoptosis. The assumption of studies exploring this is that an increase in SOCE flux or proteins, such as the Ca^{2+} sensing protein Stim1 or the Ca^{2+} channel, Orai1, will lead to Ca^{2+} overload. SOCE is a low conductance Ca^{2+} current feeding into the triad junction. On the other hand, the muscle cell can readily handle SR Ca^{2+} fluxes into the same microdomain that are much larger during excitation-contraction coupling. More importantly and generally less well characterized, a SOCE that deactivates properly following store refilling would prevent Ca^{2+} overload via this pathway regardless of flux rate. Therefore, under the same conditions, we compared the SOCE deactivation properties as well as the Stim1 and Orai1 protein levels in normal and dystrophic muscle.

7-12 weeks old C57 and mdx mice were killed by asphyxiation, according to the guidelines laid down by the Local Animal Care Committee, and the extensor digitorum longus (EDL) muscles were removed. Intact fibres were exposed to a Na^{+}-based physiological solution containing fluo-5N salt and were then mechanically skinned, trapping the dye in the t-system. Cytoplasmic SR Ca^{2+} release waves and tubular Ca^{2+} SOCE fluxes in response to exposure to 'low Mg^{2+}' solutions were monitored with a confocal microscope (Launikonis *et al.*, 2008). From the line averages in successive images, the time course of SOCE activation and deactivation with myoplasmic Ca^{2+} oscillations could be resolved at a much faster temporal resolution of 500 Hz. Western blotting was used to determine Stim1 protein levels in the same fibres used for individual physiological recordings as well as fibres collected specifically for protein detection from wt and mdx mice. Orai1 protein was measured in whole muscle homogenates from wt and mdx mice.

Stim1 and Orai1 protein amounts were 2-3 times higher in dystrophic compared to healthy muscle fibres and homogenates, respectively. Interestingly, during both cell-wide release and Ca^{2+} waves, SOCE activation rates were similar in wt and mdx muscle (see Launikonis *et al.,* previous abstract). Furthermore, upon inactivation of SR Ca^{2+} release, there was always a reuptake of Ca^{2+} by the t-system indicating that SOCE deactivated (Launikonis & Rios, 2007) in both healthy and dystrophic muscle. SOCE deactivation already started to occur when myoplasmic Ca^{2+} levels dropped only by 10 % suggesting an intact switch-off signal for SOCE from the store in both wt and mdx fibres. Also, SOCE deactivation rate depended upon SR Ca^{2+} refilling rate in a sigmoidal manner, indicating that binding of luminal Ca^{2+} to Stim1 effectively decoupled from Orai1 during refilling in both wt and mdx fibres. These results show that overexpression of Stim1 does not alter the SOCE mechanism in dystrophic muscle. Indeed a robust deactivation mechanism continues to exist in dystrophic muscle preventing this pathway to contribute to any Ca^{2+} overload in these cells.

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