Adeno-associated virus directed expression of Orai1 to examine store operated calcium entry in wild-type and dystrophic skeletal muscle

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Calcium signalling in muscle is highly adaptable to the surrounding environment. Store-operated calcium entry (SOCE) is likely to be an important component of this in muscle. Edwards *et al.* (2010) previously reported an upregulation of SOCE in the mdx mouse, a model of Duchenne Muscular Dystrophy (DMD). The proteins responsible for conducting SOCE are STIM1 and Orai1, both of which had increased expression levels in mdx mouse muscle. The normal functioning SOCE mechanism raised the possibility that the mdx mouse may be adapting to its pathophysiological state by altering the SOCE proteins to continue normal Ca^{2+} signalling making this compensation an important avenue to explore. Our approach was to alter Orai1 protein expression in mouse whole muscle using adeno-associated virus (AAV), a potential vehicle for gene therapy in human disease, and to then examine the physiology of SOCE on isolated, mechanically skinned fibres. As mdx mice undergo a major phase of muscle degeneration-regeneration at approximately 4 weeks of age, we investigated whether upregulation of Orai1 pre- and post-degeneration-regeneration phase would affect Ca^{2+} signalling.

The Animal Ethics Committee at The University of Queensland approved all experimetal procedures used in this study. An Orai1 expression cassette was delivered to the *extensor digitorum longus* (EDL) muscle of C57BL10 (wild-type, WT) and mdx mice at 3 weeks and 2.5 months of age using recombinant adeno-associated viral vectors. All surgery was performed under anaesthesia with isoflurane (carprofen S.C). Four weeks after administration of the vectors, the mice were killed by CO_2-induced asphyxiation. EDL muscles were rapidly dissected, small bundles of intact fibres were isolated and exposed to a physiological solution containing fluo-5N and single fibres were skinned. Ca^{2+} was released using a 'low Mg^{2+}' solution. Cytoplasmic rhod-2 and t-system fluo-5N were continuously imaged on a confocal microscope. The decrease in t-system fluo-5N signal during the cytoplasmic Ca^{2+} transient reflected SOCE activity.

An age-dependent effect of Orai1 over-expression on SOCE rate in the muscles of WT and mdx mice was observed, with a mean increase in SOCE rate in 2.5 month-old mice of 0.02±0.001 SFU s^{-1} (mean±SEM) in mdx control EDL and 0.04±0.006 SFU s^{-1} in the treated EDL. In WT, we observed a rate of 0.02±0.001 SFU s^{-1} in the control EDL and 0.05±0.014 SFU s^{-1} in the treated EDL. However, no difference was observed in animals treated similarly at 3 weeks of age (mdx control 0.03±0.006, treated 0.03±0.006, wt control 0.026±0.005, treated 0.028±0.004). One possibile explanation for the increase seen in the older mice is an alteration in the amount of Orai1 molecules available to bind to the STIM1 that is in excess in adult mice. These results also suggest STIM1 may be limiting in the young mice.

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