Assessment of RyR leak and t-system Ca^{2+} uptake in dystrophic skeletal muscle

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The tubular (t-) system of skeletal muscle is an internalization of the plasma membrane that forms a junction with the terminal cisternae of the sarcoplasmic reticulum (SR) at every sarcomere of skeletal muscle. In the resting muscle, $[Ca^{2+}]$ within the small volume bound by the junctional membranes will be determined by the leak of Ca^{2+} through the SR ryanodine receptors (RyRs), the Ca^{2+} handling ability of the t-system and diffusion of Ca^{2+} from the junctional space (js). The value of $[Ca^{2+}]_{js}$ could be expected to change under conditions of RyR leak, which is believed to occur in the DMD mouse model (mdx). Our aim was to use a method recently developed in the lab to determine the $[Ca^{2+}]_{js}$ in dystrophic mouse muscle fibres.

All experimental procedures were approved by The Animal Ethics Committee of The University of Queensland. 4 – 6 week old Mdx and C57Bl10 mice were euthanized by CO_2 asphyxiation and the extensor digitorum longus muscle rapidly excised. Muscles were pinned down in a Petri dish above a layer of Sylgard under a layer of Paraffin oil. Fibre bundles were isolated and a Ringer solution containing rhod-5N was applied to the fibres. Fibres were isolated and mechanically skinned and placed in a custom-built experimental chamber for imaging on an Olympus FV1000 confocal microscope employing GaAsp detectors.

Chronic depletion of $[Ca^{2+}]_{SR}$ with caffeine reduced $[Ca^{2+}]_{t\text{-}sys}$ to 0.03 mM and 0.08 mM respectively in wt and mdx via chronic activation of store-operated Ca^{2+} entry, providing a consistent starting point for tracking t-system Ca^{2+} uptake. We then exposed Ca^{2+}-depleted preparations to a solution containing either 50, 100, 200 or 800nM $[Ca^{2+}]$ in 50mM EGTA to allow observation of t-system Ca^{2+} uptake rates at known $[Ca^{2+}]_{bulk}$. The t-system was subsequently depleted of Ca^{2+} to return $[Ca^{2+}]_{t\text{-}sys}$ to depletion and the cycle was repeated. Experiments were repeated in the presence of 1mM tetracaine to block RyR Ca^{2+} leak and allow $[Ca^{2+}]_{js}$ to equilibrate with $[Ca^{2+}]_{bulk}$. Rhod-5N signals and $[Ca^{2+}]_{t\text{-}sys}$ were calibrated and t-system Ca^{2+} fluxes were derived. Mdx mice in this early pathological state were found to have lower t-system Ca^{2+} compared to wt, indicating a trend towards increased t-system Ca^{2+} leak. However this data is currently not statistically significant as the wt data set is underpowered. The mdx t-system Ca^{2+} could be further reduced with the application of 1mM tetracaine. Maximally activated SOCE due to caffeine application was found to be the same between wt and mdx mice when peak store dependent flux was analysed against t-system Ca^{2+}. 100nM $[Ca^{2+}]_{bulk}$ gave a $[Ca^{2+}]_{js}$ of between 150 and 200nM in mdx. These results may indicate that at this early stage of the disease phenotype the Ca^{2+} handling by the t-system and sarcoplasmic reticulum of the mdx mouse is able to function similarly to healthy muscle.