The role of ryanodine receptor Ca^{2+} leak in t-system Ca^{2+} handling in skeletal muscle fibres

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The tubular (t-) system of skeletal muscle forms a junction with the sarcoplasmic reticulum (SR), with some 12nm between the membranes. In the resting muscle, $[Ca^{2+}]$ within the small volume bound by the junctional membranes should 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 $[Ca^{2+}]_{js}$ is expected to be higher than $[Ca^{2+}]_{bulk}$ with a standing gradient set between the RyRs and SR Ca^{2+}-pumps. We aimed to detect the effects of RyR Ca^{2+} leak into the junctional space by harnessing the ability of the t-system to detect changes in this domain by monitoring a Ca-sensitive dye inside the t-system.

All experimental procedures were approved by The Animal Ethics Committee of The University of Queensland. Wistar rats were euthanized by CO_2 asphyxiation and the *extensor digitorum longus* and *soleus* muscle rapidly excised. Muscles were pinned down in a Petri dish above a layer of Sylgard under a layer of Paraffin oil. Bundles of fibres were isolated from the muscle and exposed to a physiological solution containing 2.5mM rhod-5N. After 10 mins a fibre was isolated and mechanically skinned, trapping the rhod-5N in the t-system as it sealed.

To detect the RyR Ca^{2+} leak and its effects on t-system handling we exploited the fact that t-system Ca^{2+} uptake activity will be set by $[Ca^{2+}]_{js}$. T-system Ca^{2+}-uptake activity was tracked with rhod-5N trapped in the t-system of mechanically skinned fibres of rat slow- and fast-twitch muscles on a confocal microscope. Chronic depletion of $[Ca^{2+}]_{SR}$ with caffeine reduced $[Ca^{2+}]_{t-sys}$ to 0.1 mM *via* chronic activation of store-operated Ca^{2+} entry. We then exposed Ca^{2+}-depleted preparations to 28nM-1.3μM $[Ca^{2+}]_{cyto}$ in 50mM EGTA to allow observation of t-system Ca^{2+} uptake rates at known $[Ca^{2+}]_{bulk}$. Experiments were repeated in the presence of 1mM tetracaine or 10mM Mg^{2+} to block RyR Ca^{2+} leak and allow $[Ca^{2+}]_{js}$ to equilibrate with $[Ca^{2+}]_{bulk}$. Rhod-5N signals and $[Ca^{2+}]_{t-sys}$ were calibrated and t-system Ca^{2+} fluxes were derived. $[Ca^{2+}]_{bulk}$ and peak t-system Ca^{2+} fluxes were fitted by Hill curves. Vmax was significantly depressed in slow- compared to fast-twitch fibres. The k_D for both fibre types was right-shifted by tetracaine. It followed that at 67nM $[Ca^{2+}]_{bulk}$, $[Ca^{2+}]_{js}$ was 165 and 220nM in slow and fast-twitch fibres, respectively. By lowering $[Mg^{2+}]_{cyto}$ to 0.13mM to increase RyR leak further than physiological levels in the presence of a broad range of $[Ca^{2+}]_{cyto}$, t-system Ca^{2+} uptake rate and steady state $[Ca^{2+}]_{t-sys}$ was reduced to below resting conditions but above that where SOCE was activated in the absence of Ca^{2+}. The addition of 25mM BAPTA with varied $[Ca^{2+}]_{cyto}$ also produced a decreased steady state $[Ca^{2+}]_{t-sys}$ and a reduced Ca^{2+} uptake rate.

We conclude that increasing RyR Ca^{2+} leak activates SOCE, allowing SOCE to simultaneously act as a counter-current against Ca^{2+} being extruded from the fibre. Thus SOCE holds Ca^{2+} within the fibre rather than sequentially recovering Ca^{2+} previously lost from the fibre, as commonly presumed. These results also highlight that t-system Ca^{2+} fluxes can be used as a nanodomain sensor of RyR Ca^{2+} leak, an important factor in many muscle diseases.