Determination of the junctional space [Ca^{2+}] set by ryanodine receptor Ca^{2+} leak in fast- and slow-twitch 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, at every sarcomere for the main purpose of conducting excitation-contraction coupling. 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 $[Ca^{2+}]_{js}$ is expected to be higher than the bulk cytoplasmic [Ca^{2+}] ($[Ca^{2+}]_{js}$) with a standing gradient set between the RyRs and SR Ca^{2+}-pumps in the resting muscle. The value of $[Ca^{2+}]_{js}$ is unknown but has significant implications for signalling cascades initiating in this nanodomain. The value of $[Ca^{2+}]_{js}$ could also be expected to change under conditions of RyR mutation, which underlie a number of myopathies. Our aim was to develop a method to determine the $[Ca^{2+}]_{js}$ in fast- and slow-twitch muscle fibres. To do this we exploited the fact that t-system Ca^{2+} uptake activity will be set by $[Ca^{2+}]_{js}$ (and the t-system Ca^{2+} gradient) to determine $[Ca^{2+}]_{js}$.

All experimental procedures were approved by The Animal Ethics Committee of The University of Queensland. Rats were euthanized by CO_2 asphyxiation and the *extensor digitorum longus* and *soleus* muscle were 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.

Chronic depletion of $[Ca^{2+}]_{SR}$ with caffeine reduced $[Ca^{2+}]_{t\text{-}sys}$ to 0.1 mM *via* chronic activation of store-operated Ca^{2+} entry, providing a 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 0.1 mM 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. $[Ca^{2+}]_{bulk}$ and peak t-system Ca^{2+} fluxes were fitted by Hill curves. V_{max} was significantly depressed in slow- compared to fast-twitch fibres. The k_D of Hill curves fitted to data for both fibre types was right-shifted by tetracaine compared to the absence of tetracaine. It followed that at 100nM $[Ca^{2+}]_{bulk}$, $[Ca^{2+}]_{js}$ was 165 and 220nM in slow and fast-twitch fibres, respectively. These results show that t-system Ca^{2+} fluxes can be used as a nanodomain sensor of RyR leak.