The effects of membrane potential and cytoplasmic calcium concentration on calcium extrusion across the tubular system in mammalian skeletal muscle

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The regulation of cytoplasmic Ca^{2+} is essential in the maintenance of skeletal muscle function and survival. This is achieved by the combined activities of ion transporters such as SERCA, NCX, PMCA, found embedded within the sarcoplasmic reticulum (SR) and plasma membranes. Upon Ca^{2+} release from the SR, $[Ca^{2+}]_{cyto}$ can globally reach ~2µM and it is during such an event that highlights Ca^{2+} extrusion is an essential regulatory mechanism, as this is ~20 times the resting $[Ca^{2+}]_{cyto}$. We have shown that during cell wide Ca^{2+} release events, there is an initial increase in t-system (extracellular) Ca^{2+}, followed by a net decline due to the activation of a store-operated calcium entry (SOCE) current. Although the SOCE current has recently generated significant levels of interest, information on the rates of t-system Ca^{2+} uptake (cytoplasmic Ca^{2+} extrusion) remains limited. Therefore, we aimed to characterise the effects of membrane potential and $[Ca^{2+}]_{cyto}$ on the rate of cytoplasmic Ca^{2+} extrusion in mammalian skeletal muscle.

Wistar rats were killed by asphyxiation in accordance to the guidelines set by the Animal Ethics Committee of the University of Queensland. *Extensor digitorum longus* and *soleus* muscles were rapidly excised, pinned out and fully immersed in paraffin oil. Small bundles of intact fibres were isolated and exposed to a Na^{+}-based physiological solution containing the fluorescent dye, fluo-5N salt. Single fibres were then isolated and mechanically skinned (resulting in the trapping the dye in the t-system) and transferred to a chamber containing a K^{+} or Na^{+} -based internal solution to set the membrane potential. A 'release solution' with low Mg^{2+}, 5mM BAPTA and 5mM caffeine was used to chronically deplete sarcoplasmic reticulum Ca^{2+} stores and activate SOCE. T-system fluo-5N fluorescence was imaged on an Olympus FV1000 confocal microscope in either xy or xyt mode in polarized and depolarized fibres with known cytoplasmic Ca^{2+} concentrations, at rest or during SR Ca^{2+} release. The net change in t-system fluo-5N signal was used as an indicator of Ca^{2+} movements across the t-system.

Fluo 5N trapped in the sealed t-system was calibrated (*in situ*) and in the presence of different concentrations of bovine serum albumin. Following low $[Ca^{2+}]_{t\text{-}system}$ (achieved by chronic activation of SOCE with 'release solution') the t-system could be reloaded with internal solutions containing 1 mM EGTA (either 100, 200 or 800 nM free Ca^{2+}). This rate of uptake was markedly greater in depolarized cells (Na+ based solutions) compared to polarized cells (K^{+} based solutions), most likely due to an increased driving force for Ca^{2+} to exit the cell. Interestingly, vacuoles were seen in some fibres. Vacuoles retained fluo 5N for > 20mins in the presence of a 'release solution' whereas a fluorescence signal from transverse tubules was rapidly lost. We have also measured for the first time, a SOCE flux and t-system uptake in slow-twitch fibres from the *soleus* muscle which are currently being explored.