The effect of intracellular [Mg^{2+}] on Ca^{2+} handling by the sarcoplasmic reticulum

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In skeletal muscle, the release of Ca^{2+} from the sarcoplasmic reticulum (SR) is mediated by the ryanodine receptors (RyR) which are activated by the voltage-sensors of the transverse tubule (t-sys). Cytoplasmic Mg^{2+} provides inhibition of the RyR at rest to reduce Ca^{2+} leakage by binding to a low affinity inhibitory site. Ca^{2+} leaked from the RyR is resequestered by the SR Ca^{2+}ATPase (SERCA) pump. In the present study we aimed to assess the effect of $[Mg^{2+}]_{cyto}$ on SR Ca^{2+} handling and specifically RyR Ca^{2+} leak. To do this, mechanically skinned fibres were loaded with Ca^{2+} dependent indicators inside the SR or t-sys. Measurements of Ca^{2+} dependent fluorescence from inside the t-sys allowed determination of t-sys Ca^{2+} handling activity that was directly dependent on the [Ca^{2+}] in the junctional space, set by RyR Ca^{2+} leak (Cully *et al.*, 2015).

The use of animals in this study was approved by the Animal Ethics Committee at the University of Queensland. 2 month old Wistar rats were culled by $C0_2$ asphyxiation and the *extensor digitorum longus* (EDL) muscles were removed. Bundles of fibres were isolated and exposed to a Ringer solution containing Rhod-5N and then mechanically skinned, trapping the dye in the t-sys. For SR dye loading, mechanically skinned fibres were mounted to an experimental chamber, and bathed in an internal solution with 10μM Fluo-5N AM, 10μM carbonilcyanide p-triflouromethoxyphenylhydrazone (FCCP) and 0.05% Pluronic detergent for 1 hour at 30°C. An additional 1 hour incubation at room temperature in the absence of Fluo-5N AM was given to allow for complete hydrolysis. The release of SR Ca^{2+} was induced by exposing the cell to a 0.01mM Mg^{2+} solution containing 30 mM caffeine. The SR and t-sys were then loaded in solutions with varying amounts of free [Ca^{2+}] and with the free [Mg^{2+}] set at 0.13, 1, or 10mM. Rhod-5N t-sys signals were calibrated with [Ca^{2+}] as described (Cully *et al.,* 2013). Fluorescence signals were imaged on an Olympus FV1000 confocal microscope.

Steady state $[Ca^{2+}]_{t\text{–}sys}$ over a range of $[Ca^{2+}]_{cyto}$, were reduced in the presence of 10mM $[Mg^{2+}]_{cyto}$, presumably due to reduced [Ca^{2+}] in the junctional space. This indicates blocked RyR Ca^{2+} leak by 10mM Mg^{2+}. An *in-situ* calibration of intra-SR Fluo-5N determined a K_d value of 418±36μM, allowing direct comparison of $[Ca^{2+}]_{SR}$ between fibres. At 1 and 10 mM $[Mg^{2+}]_{cyto}$ the free $[Ca^{2+}]_{SR}$ was similar. This suggests that the block of RyR Ca^{2+} leak do not significantly change $[Ca^{2+}]_{SR}$. Lowering the $[Mg^{2+}]_{cyto}$ to 0.13mM significantly reduced the steady state $[Ca^{2+}]_{t\text{-}sys}$, and this was partially restored in the presence of 1mM tetracaine (0.13mM Mg^{2+}), an RyR inhibitor. This suggests that the potentially increased uptake of Ca^{2+} in the presence of greater junctional space [Ca^{2+}] (due to increased RyR Ca^{2+} leak) was offset by Ca^{2+} influx from the t-sys *via* a store dependent entry pathway (Cully *et al.,* 2015). Lowering $[Mg^{2+}]_{cyto}$ from 1 to 0.13mM minimally altered the free $[Ca^{2+}]_{SR}$, demonstrating that SOCE is active despite the presence of high free $[Ca^{2+}]_{SR}$.

Cully TR, Edwards JN, Shannon TR, Launikonis BS. (2013) Ca^{2+} uptake by the tubular (t-) system membrane of rat fast-twitch muscle. *Proc Aust Physiol Soc* **44**, 61P.

Cully TR, Roberts L, Fassett R, Raastad T, Sax J, Coombes JS, Launikonis BS, (2015) The tubular (t-) system is a dynamic Ca^{2+}-buffer in human skeletal muscle fibres. *Proc Aust Physiol Soc* **46**, 104P.