Effect of changing the [Mg^{2+}] on ryanodine receptor leak activity in rat skeletal muscle

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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-tubule). 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. The net leak of Ca^{2+} contributes minimally to the overall $[Ca^{2+}]_{cyto}$ at rest, but increases metabolic rate and ATP turnover. Importantly the RyR Ca^{2+} leak sets a standing Ca^{2+}gradient, with a high $[Ca^{+2}]$ in the junctional space between the SR and t-tubule membrane. In situations such as metabolic fatigue $[Mg^{2+}]_{cyto}$ can increase following the consumption of MgATP to Mg^{2+}, ADP and inorganic phosphate. In the present study we aimed to assess the effect of $[Mg^{2+}]_{cyto}$ on RyR Ca^{2+} leak. To do this mechanically skinned fibers with the t-tubule loaded with a Ca^{2+} indicator were used. Measurements of Ca^{2+} dependent fluorescence from inside the t-tubules allowed determination of t-tubule Ca^{2+}ATPase (PMCA) activity. This activity is directly dependent on the $[Ca^{2+}]$ in the junctional space, set by RyR Ca^{2+} leak. Thus the effect of changing $[Mg^{2+}]_{cyto}$ on RyR Ca^{2+} leak can be determined from these measurements.

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 CO_2 asphyxiation and the *extensor digitorum longus* (EDL) muscles were removed. The t-tubule were loaded with a Ringer solution containing Rhod-5N and then mechanically skinned, trapping the dye in the t-tubule. The preparation was then transferred to an experiment chamber containing a physiological internal solution. Rhod-5N fluorescence signals were imaged on an Olympus FV1000 confocal microscope. The release of SR Ca^{2+} was induced by treating the cell with a 30 mM caffeine solution. The SR and t-tubule were then loaded in solutions with 50-800nM $[Ca^{2+}]$ and 0.2-3mM $[Mg^{2+}]$. In some experiments the skinned fiber were exposed to the same levels of $[Ca^{2+}]$ and $[Mg^{2+}]$ but in the presence of 1mM Tetracaine, a RyR inhibitor. Rhod-5N t-tubule signals were calibrated with $[Ca^{2+}]$ as described previously (Cully *et al.*, 2013).

Exposing the preparation to a 30mM caffeine solution induced store-dependent entry and reduced $[Ca^{2+}]_{t–tubule}$ to 0.1mM. The t-system Ca^{2+} uptake rate and steady state $[Ca^{2+}]_{t-tubule}$ was not changed by increasing $[Mg^{2+}]_{cyto}$ from 1mM to 3mM. In the presence of 1mM Tetracaine, both the $[Ca^{2+}]_{t-tubule}$ and t-tubule peak uptake flux were reduced. Collectively, this suggests that RyR leak rate was not effected by raising $[Mg^{2+}]_{cyto}$ from 1 to 3mM. Interestingly, lowering the $[Mg^{2+}]_{cyto}$ to 0.2mM significantly reduced the steady state $[Ca^{2+}]_{t-tubule}$, suggesting that the potentially increased uptake of Ca^{2+} in the presence of greater junctional space $[Ca^{2+}]$ was offset by Ca^{2+} loss from the t-tubule *via* a store dependent entry pathway. Reducing RyR leak with 1mM Tetracaine in 0.2 mM Mg^{2+} partially restored the t-tubule $[Ca^{+2}]$.

Cully TR, Edwards JN, Shannon TR, Launikonis BS. (2013). *Proceedings of the Australian Physiological Society* **44**: 61P.