Integral role of Mg^{2+} in observing the inhibitory effect of dantrolene

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Malignant hyperthermia (MH) is a clinical syndrome of the skeletal muscle that presents as a hypermetabolic response to volatile gas anaesthetics. Currently dantrolene, a long acting muscle relaxant, is the only FDA approved drug to treat MH. Although dantrolene has robustly been used to treat MH its mechanism of action is poorly understood. Evidence suggest that dantrolene binds directly to the RyR-1 to inhibit Ca^{2+} release (Fruen *et al.*, 1997), and indeed recent studies have found the corresponding RyR-1 binding sites for dantrolene (Kobayashi *et al.*, 2005; Paul-Pletzer *et al.*, 2001), however the inhibitory effect of dantrolene is all but absent in isolated RyR-1 reconstituted in lipid bilayers (Wagner *et al.*, 2014; Diaz-Sylvester *et al.*, 2008; Szentesi *et al.*, 2001). Therefore, it is unclear if the action of dantrolene targets the RyR-1 or some other upstream processes involved in excitation contraction coupling. To examine the mechanism of dantrolene action on the RyR we used mechanically skinned fibres where the imposed ionic conditions mimic those occurring in the body. The skinned fibre allowed us to track the effect of dantrolene on Ca^{2+} transients in the cytoplasmic elicited by action potentials; and a recently developed technique allowed the detection of the activity of the RyR under defined ionic conditions, including basal levels of activity, with a high degree of sensitivity.

The use of animals in this study was approved by the Animal Ethics Committee at the University of Queensland. 2-4 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. 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.*, 2016). Fluorescence signals were imaged on an Olympus FV1000 confocal microscope.

Under physiologically relevant $[Mg^{2+}]_{cyto}$ (1mM), $[Ca^{2+}]_{t\text{-}sys}$ steady states over a range of $[Ca^{2+}]_{cyto}$ (200-1342 μM), were reduced in the presence of 50μM dantrolene. In sub-physiological $[Mg^{2+}]_{cyto}$ however dantrolene had no observable effect on RyR channel gating. This indicates that dantrolene is a RyR antagonist, and is dependent on the presence of physiologically relevant concentrations of Mg^{2+}.

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