The effect of suramin (a calmodulin antagonist) on caffeine-induced Ca^{2+}-release in mechanically skinned fast-twitch skeletal muscle fibres of the rat

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Calmodulin (CaM) is a ubiquitous, multifunctional calcium binding protein, which binds to the ryanodine receptor (RyR1) in skeletal muscle (Tripathy *et al.*, 1995). Ca^{2+}-free CaM binds to RYR1 and increases RYR1s affinity for calcium, while Ca^{2+}-bound CaM inhibits RYR1s affinity for calcium (Rodney *et al.*, 2000). The precise role of CaM in the regulation of calcium release in physiological preparations remains unknown. Suramin is a broad acting CaM antagonist known to displace bound CaM from RyRs (Sigalas *et al.*, 2009). In this study we investigated the effects of suramin on Ca^{2+} release from the sarcoplasmic reticulum (SR) in freshly mechanically skinned muscle fibres where the SR is intact under conditions where the suramin treatment did not affect the ability of the contractile apparatus to develop force.

All experiments conducted were approved by the La Trobe University Animal Ethics committee. Rats were killed by an overdose of isoflurane (4% volume: volume) and whole EDL muscles were removed and pinned out on a bed of Sylgard at resting length under paraffin oil. Single fibres were then isolated under a dissecting microscope, mechanically skinned, and mounted between a pair of forceps and a sensitive force transducer. After mounting to the force recording apparatus, fibres were bathed in a K-based solution that mimics the normal intracellular environment with respect to pH (7.10); monovalent ions (137 mM); Mg^{2+} (1 mM); divalent cations (60 mM); total ATP (8 mM); osmolality (295 mOsm/L); [Ca^{2+}] ~100 nM. The relative SR Ca-content and ease of Ca^{2+} moving through the RyR1s can be estimated from the force response following the transfer of the fibre to a SR-Ca^{2+}-depleting solution containing caffeine (30 mM) and low [Mg^{2+}] (50 μM) (Lamb & Stephenson, 1991). A similar area under the caffeine-induced force response is indicative of similar SR Ca-content and a faster rate of rise of the caffeine-induced force and/or a greater force peak (for same SR Ca-content) indicate a greater SR Ca^{2+}-efflux through the RyR1. Fibres were repeatedly bathed in a SR Ca-loading solution ([Ca^{2+}] ~300 nM) for increasing amounts of time (10-120 s) to reload the SR Ca to various levels and then the SR Ca^{2+} was fully released in the SR-Ca-depleting solution . Fibres were randomly divided into two groups (control and test) then exposed for 2 min to 100 μM suramin (test) or exposed to a like solution *sans* suramin (control). After the suramin/*sans* suramin treatment, the fibres were washed for 10 min and the SR Ca-loading/caffeine release protocol was repeated for both groups. Suramin treatment resulted in a marked reduction in the caffeine induced rate of rise (time between 20 and 80% of peak caffeine response decreased by 25.9 ± 7.1% (S.E.M.) of control ($p < 0.01$) at 30 s load without changing the area under the caffeine-induced force responses ($p > 0.1$). Suramin also increased the peak force of submaximal caffeine responses by 20.70 ± 5.99 % of maximum force for a 30 s load (n=4). Thus, the results show that treatment with 100 μM suramin for 30 s increases the SR Ca^{2+}-efflux through the RyR1, which is consistent with suramin removing the CaM from RyRs on the intact SR.

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