Alterations in Ca^{2+} transients and SR function in isolated fast fibres from α-actinin-3-deficient speed gene knockout mice

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Background: Absence of the fast-twitch skeletal muscle Z-line protein, α-actinin-3, encoded by the ACTN3 speed gene, is associated with poorer sprinting performance in athletes and a slowing of relaxation in fast-twitch muscles of Actn3 knockout (KO) mice. Around 20% of the world's population are ACTN3 deficient. Fast-twitch muscles from KO mice display longer twitch half-relaxation times than muscles from wild-type (WT) mice. In mechanically skinned fast fibres the sarcoplasmic reticulum (SR) in fibres from KO mice load Ca^{2+} more slowly than the SR in fibres from WT mice (Chan *et al.*, 2011).

Aim: Our present study investigates the Ca^{2+} kinetics of fast-twitch fibres from Actn3-KO and WT mice and SERCA pump and parvalbumin protein expression, to see whether any changes in Ca^{2+} kinetics and associated SR and Ca^{2+} buffering proteins can account for the previously observed effects of α-actinin-3 deficiency on whole muscle relaxation and SR Ca^{2+} loading.

Methods: Mice were killed with an overdose of halothane, UNSW animal ethics approval 11/140B. *Flexor digitorum brevis* (FDB) muscles were dissected out and digested in collagense 1a to yield individual fibres. Fibres were plated onto a chamber placed on a Nikon inverted microscope attached to a Cairn spectrophotometer to monitor calcium transients using a photomultiplier tube (PMT). After fibres had attached to the glass coverslip the central area of the fibre was bracketed to minimize any movement artefacts. An intracellular microelectrode was used to ionophorese the free acid form of fura-2 or low affinity fura-ff to give a final concentration 5-50 μm. Fibres were electrically stimulated using a bipolar concentric electrode positioned near the neuromuscular junction. In some cases the fibre was immobilized with BTS (4-methyl-N-(phenylmethyl) benzenesulfonamide)to stop contractions. Due to the slow binding kinetics of fura-2 for Ca^{2+} *in vivo*, the peaks of the Ca^{2+} transients and their rate of rise will be underestimated if a correction process is not applied (Bakker *et al.*, 1997). We therefore used our deconvolution formula described in Bakker *et al.* (1997) to obtain corrected peak and rise time values for the Ca^{2+} transients in this study. Ca^{2+}-frequency curves were obtained using stimulation frequencies of 2 to 100 Hz. SR Ca^{2+} content was assessed using the method described in Loy *et al.* (2011). The SR was completely emptied of Ca^{2+} using a mixture comprised of 10 μM ionomycin, 30 μM cyclopiazonic acid and 100 μM EGTA (ICE mixture). SERCA and parvalbumin proteins were measured in whole muscle preparations by Western blotting.

Results: In FDB fibres of α-actinin-3-deficient KO mice, the Ca^{2+} transient in response to a single action potential displayed a lower peak, a slower rate of rise and a faster rate of relaxation than the Ca^{2+} transients from fibres of WT mice (n=15 for KO, n=19 for WT). Peak Ca^{2+} was 959 ± 26 nM in KO and 1737 ± 31 nM in WT (P<0.0001). Rise times (20% to 80% of peak) were 0.80 ± 0.03 ms in KO and 0.67 ± 0.02 ms in WT (P<0.0001). A double-exponential decay equation was fitted to the decay of the Ca^{2+} transient. For the fast decay phase, half-life and tau were 1.09 ± 0.05 ms and 1.57 ± 0.08 ms, respectively, in KO, and 1.97 ± 0.05 ms and 2.84 ± 0.08 ms, respectively, in WT (P<0.0001). For the slow decay phase, half-life and tau were 11.3 ± 0.1 ms and 16.3 ± 0.2 ms, respectively, in KO, and 20.3 ± 0.3 ms and 29.3 ± 0.4 ms, respectively, in WT (P<0.0001). Interestingly it was found that KO animals have 50% higher density of SERCA protein while the amount of parvalbumin protein is unchanged. The rate of ICE (calcium release cocktail)-induced SR Ca^{2+} release was higher in KO (1.82 ± 0.19 ratio units/s in KO, n=6, *versus* 1.01 ± 0.19 ratio units/s in WT, n=6). However there does not appear to be a difference in releasable Ca^{2+} (445 ± 101 ratio units·s in KO, n=6, *versus* 357 ± 110 ratio units·s in WT, n=6). Ca^{2+}-frequency curves showed no significant differences between fibres from KO and WT mice.

Conclusions: The faster decay of the Ca^{2+} transients in fibres of α-actinin-3-deficient KO mice is consistent with data showing an increased density of SERCA pumps in the SR of fibres from KO mice. However, the observations from previous studies that whole fast-twitch muscles from KO mice relax more slowly, and that SR loading is slowed in KO mice, suggest that there may be an increased Ca^{2+} leakage from the SR of KO mice *via* the SERCA pump Ca^{2+} leak pathway.

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