SR pump function and fatigue characteristics of fast fibres from α-actinin-3-deficient speed gene knockout mice

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Background: Absence of 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. Fast-twitch muscles from KO mice display longer twitch half-relaxation times than fast-twitch muscles from wild-type (WT) mice (Chan *et al.*, 2011).

Aim: Our present study investigates the Ca^{2+} kinetics of fast-twitch fibres from KO and WT mice. Specifically, we examine the decay of the Ca^{2+} transient during tetanic stimulation as an indication of the SR pump function of fibres from KO and WT mice, and SR function during fatigue.

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 minimise 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. SR pump function curves were derived from recordings of 100 Hz, 500 ms tetani using the derivation procedure outlined in Allen & Westerblad (1995). Briefly, the trace was first smoothed by fitting a double exponential function to the long tail of the transient decay. The $[Ca^{2+}]$ and the rate of $[Ca^{2+}]$ decline $(-d[Ca^{2+}]/dt)$ were then measured at various points along the fitted double exponential curve, to give a plot of $-d[Ca^{2+}]/dt$ *versus* $[Ca^{2+}]$. A power function was fitted to these plotted points to give the SR pump function curve. For the fatigue runs, fibres were stimulated by 50 Hz tetani, 500 ms on, 500 ms off until the $[Ca^{2+}]$ transient had dropped to half its original level.

Results: In our study, the SR pump function curves were significantly steeper than those obtained by Allen & Westerblad (1995). In the study of Allen & Westerblad (1995), the rate of $[Ca^{2+}]$ decline changed from 100 nM/s to 0 nM/s as $[Ca^{2+}]$ changed from 120 nM to 25 nM. In our present study, however, the same drop in $[Ca^{2+}]$ decline rates occurred over a range of $[Ca^{2+}]$ that was only 100 nM to 75 nM. In our study, there were no significant differences between the SR pump function curves of fibres from WT and KO mice. During fatigue recordings from 2 KO fibres, the rate of $[Ca^{2+}]$ decay decreased by 75% (4.15 ± 0.25 nM/ms to 1.05 ± 0.25 nM/ms, when fitted with a single exponential decay function).

Conclusions: In our study, the SR pump function was significantly faster than the rates reported in Allen & Westerblad (1995). The decrease in rate of $[Ca^{2+}]$ decay during fatigue was most likely the result of depletion of ATP and failure of Ca^{2+} release from the SR.

Allen DG & Westerblad H. (1995) *Journal of Physiology* **487,** 331-342.
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