Myoplasmic and sarcoplasmic reticulum calcium in intact mouse muscle during fatigue

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Studies on isolated single muscle fibres at room temperature have established that tetanic calcium declines during repeated tetani and that this decline is one contributor to the reduced force in fatigued muscles (Allen *et al.*, 2008). The aim of the present experiments was to determine whether similar changes occur in intact muscles perfused with blood and operating at body temperature. For this purpose we used genetically encoded Ca^{2+} sensors, cameleons (Miyawaki *et al.*, 1997), targeted to the myoplasm or the sarcoplasmic reticulum (SR). Plasmids for the sensors were transfected into the tibialis anterior 2 weeks before the experiments under anaesthesia (Zoletil + Rompun i.p.). On the day of the experiment, mice were anaesthetised, the tibialis anterior exposed and the distal tendon detached and connected to a force transducer. The muscles were stimulated directly by near-maximal pulses through platinum electrodes touching the upper surface of the muscle. Ca^{2+} signals were obtained from those fibres which expressed the sensors using FRET and a Leica multiphoton microscope (Rudolf *et al.*, 2006). This is an upright microscope and the objective was optically coupled to the muscle with artificial tears (a viscous salt-containing solution).

Many fibres expressed the indicators but movement of the muscle during tetani prevented optical measurements of the same fibre at rest and during tetani. Thus tetanic measurements were only possible irregularly. Tetanic force (1 s tetani at 100 Hz repeated every 4 s) generally fell monotonically to 49 ± 5 % of the control and was then usually stable for some minutes. Recovery, measured by tetani at 2 min intervals, was to 86 ± 4% after 4 min. Peak tetanic myoplasmic $[Ca^{2+}]$ declined during fatigue from 1.36 ± 0.08 (mean ± SE, $n = 5$) to 1.27 ± 0.09 ratio units ($p < 0.01$, paired t test) and in two experiments, in which it could be measured, the peak tetanic $[Ca^{2+}]$ recovered by 0.075 units. The free calcium in the SR ($[Ca^{2+}]_{SR}$) declined during fatigue from 1.42 ± 0.08 ($n = 9$) to 1.12 ± 0.11 ($p < 0.02$, paired t test) and showed a partial recovery to 1.26 ± 0.11 ($p < 0.02$).

These data show for the first time that calcium handling changes during fatigue in intact mammalian muscle at body temperature. The fact that $[Ca^{2+}]_{SR}$ declined during fatigue suggests that precipitation of calcium phosphate in the SR might make a contribution to this process.

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