Long lasting muscle fatigue: partial disruption of EC-coupling by the elevated cytosolic calcium during contractions

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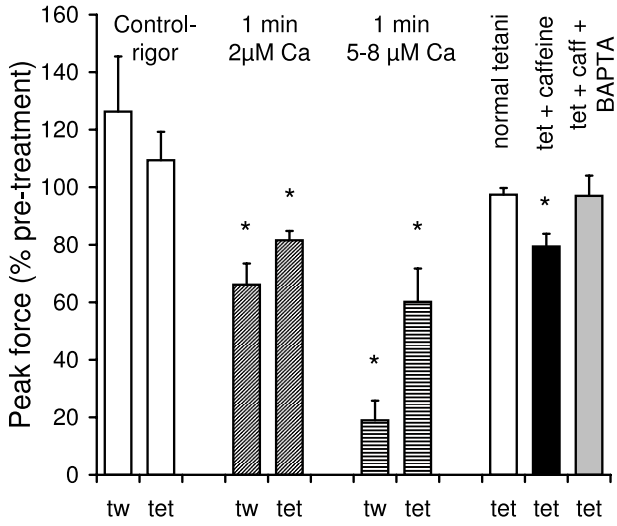
We have previously shown that a 10s period of very high cytosolic [Ca^{2+}] (20μM) can disrupt excitation-contraction (EC)-coupling at the signal transduction between the t-tubuli and the Sarcoplasmic Reticulum (SR) Ca^{2+}-release channels in the triad junction (Lamb *et al.*, 1995). It has also been shown that the repeated periods of elevated cytosolic [Ca^{2+}] during repeated tetani are associated with reduced Ca^{2+}-release and long-lasting fatigue (Chin & Allen, 1996). It is however unclear how low levels of [Ca^{2+}] can disrupt EC-coupling in mammalian muscle, and what aspects of the increased levels of cytosolic [Ca^{2+}] during contractions are causing the disruption of EC-coupling.

In this study we have used a mechanically-skinned fibre preparation, in which the normal EC-coupling system remains intact. Extensor Digitorum Longus muscles were excised from 4-11 month old Long-Evans Hooded rats that had been killed by an overdose of halothane. Single fibres were dissected from the muscle and skinned. The fibre was then transferred to a solution mimicking the cytosol. Twitch and tetanic force responses were triggered by depolarising the T-system with electrical field stimulation. Periods of elevated cytosolic [Ca^{2+}] were induced by transferring the fibre to a 'Ca^{2+}-rigor'-solution containing a set [Ca^{2+}]. In this solution no ATP or CrP was present, preventing Ca^{2+}-uptake by the SR, and thus applying a homogenous [Ca^{2+}] throughout the fibre. Alternatively, elevated [Ca^{2+}] was achieved by eliciting four or five 50Hz-tetani in the presence of 5 mM Caffeine.



The figure shows that even a concentration as low as 2μM free Ca^{2+} throughout the fibre can disrupt EC-coupling, with a bigger effect at concentrations that elicit > 90% of maximal force ($n \geq 4$ in each case). The time of elevated [Ca^{2+}] required for the effect was however quite long, 1 min. Fifteen normal 0.2 s long tetani, or 4-5 tetani with caffeine and 0.2 mM BAPTA present, did not result in a significant decrease in peak tetanic force. However, the total time at high [Ca^{2+}] eliciting > 90% force would have been ≤ 2s in the normal tetani. Only in the presence of caffeine, when tetani were at least twice as long as the normal ones and peak [Ca^{2+}] in the triad junction probably a lot higher, was EC-coupling partially disrupted. This shows that the [Ca^{2+}] has to be raised to a very high level and/or be applied for a relatively long period in order to have a deleterious effect. It also suggests that the relatively high [Ca^{2+}] attained locally in the triad junction is more important than the concentration attained in the bulk of the cytoplasm. During normal contractile activity, it can be expected that calcium-induced disruption of EC-coupling would have a significant impact after a very large number of contractions, and hence it may be one of the mechanisms causing the long-lasting muscle fatigue observed after prolonged hard exercise.

Chin E.R. & Allen D.G. (1996) *Journal of Physiology* **491**, 813-824.
Lamb G.D., Junankar P.R. & Stephenson D.G. (1995) *Journal of Physiology* **489**, 349-362.