Disruption of EC-coupling by physiological [Ca^{2+}] is mediated by Ca^{2+}-activated proteases

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Short periods of supranormal cytosolic free [Ca^{2+}] have been shown to disrupt EC-coupling in mechanically skinned fibres[1], and in intact single fibres[2], and is proposed as a mechanism of long-lasting muscular fatigue. We investigated whether the effect of elevated [Ca^{2+}] is mediated by Ca^{2+}-activated proteases, by characterizing its effect and the effect of various inhibitors on the depolarisation-induced force response and the passive force in skinned fibres of toad iliofibularis muscles.

Cane toads were killed by double pithing, as approved by the Animal Ethics Committee at La Trobe University, and the iliofibularis muscles removed. Fibres were mechanically skinned, retaining normal EC-coupling. They were exposed to intracellular [Ca^{2+}] levels of 1-40μM for 1-3 min, in the absence of ATP so that the [Ca^{2+}] would remain unchanged and constant throughout the fibre. The effect of elevated [Ca^{2+}] on titin proteolysis was estimated from the rate of passive force decline in fibres stretched to double slack length, as titin is known to be responsible for this passive force and is a specific target of Ca^{2+}-activated proteases.

Levels of [Ca^{2+}] as low as 1.2μM disrupted titin, with higher [Ca^{2+}] being more effective. The effect of elevated [Ca^{2+}] could be switched on and off very rapidly and could be inhibited by 1mM leupeptin, but not by 10μM calpastatin. Oxidizing free cysteines in the fibre with dithiodipyridine also protected titin from the effect of elevated [Ca^{2+}]. Elevated [Ca^{2+}] also reduced the depolarisation-induced force response, apparently by interrupting the coupling between the voltage sensors in the t-tubuli and the Ca^{2+} release channels in the SR. The uncoupling effect of elevated [Ca^{2+}] had a similar dose-dependency as the effect on titin, with the fibres being half-uncoupled after exposure for three minutes at 1.7 μM [Ca^{2+}]. Importantly, the uncoupling at 2μM [Ca^{2+}] was also inhibited by leupeptin. These results strongly suggest that the disruption of EC-coupling and titin by elevated levels of [Ca^{2+}] is caused by Ca^{2+} activated proteases, most likely calpain 3.

(1) Chin ER et al. J.Physiol 491: 813-824, 1996
(2) Lamb GD et al. J.Physiol 489: 349-362, 1995