The effect of dithiothreitol (DTT) application on isolated mouse muscle fatigued at 37°C

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We have previously shown that muscle fatigue at 37°C *in vitro* is associated with a reduction in calcium sensitivity and that this process could be prevented by the antioxidant Tiron (Moopanar & Allen, 2005). Previous studies have also found that application of the reducing agent dithiothreitol (DTT) to the rat diaphragm improves recovery post-fatigue (Diaz *et al.*, 1998). The aim of the current study was to determine whether DTT could reverse the effect of temperature- and fatigue-induced myofibrillar desensitization.

Single muscle fibres were isolated from the foot of balb-C mice and were attached to a force transducer. The temperature in the muscle chamber was raised to 37°C prior to each experiment. Fibres were microinjected with indo-1 to measured intracellular calcium ($[Ca^{2+}]_i$), and were stimulated at a range of frequencies (20, 30, 50, 70 and 100 Hz and 100 Hz in the presence of 10 mM caffeine) to establish myofibrillar sensitivity to calcium. The preparation was then fatigued and sensitivity was immediately reassessed. Finally, the muscle preparation was treated with DTT (0.5 mM) for two minutes and myofibrillar sensitivity was again tested.

The Ca_{50}, which is the level of $[Ca^{2+}]_i$ that produces half maximum force and a measure of Ca^{2+}-sensitivity, was initially found to be 649 ± 40 nM (n=18). This value was increased post fatigue to 872 ± 40 nM (n=9). There was no change to the Ca_{50} in the absence of fatiguing stimuli. Application of DTT to the fatigued muscle caused the Ca^{2+}- sensitivity to return to prefatigue values (683 ± 40 nM (n=6)). In order to determine whether the decline in muscle function was due to a change in maximum calcium activated force (F_{max}), fibres were stimulated at 100 Hz in the presence of caffeine (10 mM). There was no significant change in F_{max}.

These results indicate that the process of myofibrillar desensitization at 37°C requires repeated stimulation to occur. In addition, we show that the desensitization can be reversed by DTT. This suggests that a protein involved in calcium sensitivity has critical S-H groups which can be oxidized to form disulphide bonds (S-S) with loss of Ca^{2+}-sensitivity. This reaction can be reversed by the reducing agent DTT.

Diaz, P.T., Costanza, M.J., Wright, V.P., Julian, M.W., Diaz, J.A. & Clanton, T.L. (1998) *Medicine and Science in Sports and Exercise* **30**, 421-426.

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