Rat skeletal muscle 3-O-MFPase activity is not decreased by fatiguing *in vitro* electrical stimulation

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Maximal 3-O-MFPase activity is a surrogate measure of the Na^+,K^+-ATPase activity and is commonly utilized in human exercise studies involving skeletal muscle biopsy samples. Studies to date (e.g. Fowles *et al.*, 2002; Petersen *et al.*, 2005), using a range of exercise intensities and durations, have shown that an acute bout of fatiguing exercise results in a decrease in maximal 3-O-MFPase activity at the point fatigue of between ~11 to 38%. These observations have been interpreted as evidence that the maximal activity of the Na^+,K^+-ATPase has declined thus contributing to skeletal muscle fatigue. It is speculated that endogenous factors such as reactive oxygen species (ROS) and calcium (Ca^{2+}) may be responsible for this observed reduction in 3-O-MFPase at fatigue. The aim of this study was to investigate whether rat skeletal muscle 3-O-MFPase activity could be reduced further than the above range from human studies by using large isolated skeletal muscles subjected to intense fatiguing *in vitro* electrical stimulation.

Sprague Dawley (260 ± 9g; Mean ± SE) fast twitch *extensor digitorum longus* (EDL) muscles (132 ± 4 mg) were dissected out under anaesthesia (Nembutal; ~85mg/kg) in accordance with Victoria University AEEC procedures and subjected to one of two different stimulation protocols: 1) two bouts of 10s continuous stimulation at a frequency of 100Hz (0.2ms pulse duration) separated by a 1hr recovery period; 2) two bouts of three min intermittent stimulation (1s stimulation at 100Hz followed by 4s recovery) separated by a 1hr recovery period. Tetanic force (500ms, 100Hz, 0.2ms pulse duration) was monitored during recovery. Fatigued muscles and their non-fatigued contra-lateral controls were blotted, weighed, frozen in liquid N_2 and maximal 3-O-MFPase activity analysed (Fraser & McKenna, 1998).

At the end of the first bout of 10s continuous stimulation tetanic force had declined by 51.8 ± 1.8% (n = 8) of initial force. Characteristic of high frequency fatigue, force had recovered to 81.2 ± 2.1% of initial after one min and remained relatively constant over the next hour (87.4 ± 2.6% of initial force at one hour). The second stimulation bout reduced force by 50.3 ± 1.3% of initial force, while 3-O-MFPase activity showed no decline (100.5 ± 3.4%; p = 0.9) compared to the non-fatigued, contra-lateral controls. Three minutes of high frequency intermittent stimulation resulted in tetanic force declining by 87.0 ± 1.0% (n = 8) of initial force. After one hour of recovery, tetanic force had gradually recovered to 62.7 ± 2.1% of initial force. At this time, force-frequency analysis showed the presence of low frequency fatigue with relative force being significantly lower at 10 (39.0 ± 2.1% *vs* 47.0 ± 1.2%; p = 0.005), 30 (44.4 ± 1.4% *vs* 60.7 ± 1.3%; $p < 0.0001$) and 50Hz (76.2 ± 1.4% *vs* 86.7 ± 0.7%; $p < 0.0001$) compared to pre-fatigue force. The second intermittent stimulation bout reduced force by 83.3 ± 1.3% of initial force while 3-O-MFPase activity was not significantly altered (94.4 ± 3.7%; p = 0.2) when compared to the non-fatigued contra-lateral controls.

In conclusion, under these conditions, rat EDL 3-O-MFPase activity was not reduced by either of the two fatiguing *in vitro* electrical stimulation protocols. Thus the decline in muscle force was not related to a depression in maximal 3-O-MFPase activity. Whether this reflects a species difference with resistance to Na^+,K^+-ATPase inactivation in the rat is unclear.

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