The effect of Phospholipase A_2 inhibition on contractile function in normal and dystrophic skeletal muscle

J. Lam, G.J. Pinniger and A.J. Bakker, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009, Australia.

Duchenne muscular dystrophy (DMD) is a devastating inherited muscle disorder that results in profound skeletal muscle weakness through increased atrophy and intrinsic contractile dysfunction. DMD results from a lack of a functional form of the protein dystrophin, which normally localizes to the inner sarcolemma of the muscle fibre. The loss of dystrophin has been shown to alter intracellular Ca^{2+} handling, which ultimately results in fibre damage through increased calpain activation and increased reactive oxygen species production. Phospholipase A_2 (PLA_2) is a critical enzyme in all cells. However, it can be cytotoxic at high levels of activation. PLA_2 activity is markedly elevated in skeletal muscle of DMD patients (Taggesson & Henriksson, 1984; Lindahl *et al.*, 1995), and PLA_2 activity has also been shown to alter Ca^{2+} handling in skeletal muscle fibres (Han *et al.*, 2003). Therefore, over-activation of PLA_2 in dystrophic muscle may play a significant role in the pathophysiology that is characteristic of DMD patients and dystrophic, mdx mice. The aim of this study was to determine the effect of PLA_2 inhibition on the contractile properties in skeletal muscle from dystrophic, mdx mice, using the non-specific PLA_2 inhibitor indomethacin.

Experiments were performed on 6 week old male mdx (dystrophic) and C57 (non-dystrophic) mice. Mice were anaesthetized (sodium pentobarbitone 40 mg/kg; IP) and *extensor digitorum longus* (EDL) muscles were surgically excised and attached to a force transducer system. The EDL muscles were bathed in Krebs mammalian Ringer solution (NaCl (137mM), $NaHCO_3$ (24mM), glucose (11mM), KCl (5mM), $CaCl_2$ (2mM), NaH_2PO_4 (1mM), $MgSO_4$ (1mM)) with d-tubocurarine chloride (0.025mM), which was bubbled with carbogen (95% O_2 and 5% CO_2) and maintained at 25°C. Muscles were exposed to Krebs solution containing either 300μM indomethacin (dissolved in DMSO), or a DMSO control solution for 60 minutes. The final concentration of DMSO in all solutions was 0.05%. The effects of indomethacin on maximum specific force ($N.cm^{-2}$) and the force frequency relationship (force expressed as a percentage of maximum force as a function of stimulation frequency) were determined.

A 60 min exposure to indomethacin significantly decreased maximum specific force in both C57 and mdx muscles by a similar amount (C57: 16.3 ± 1.4% decrease, n=5; mdx: 20.2 ± 3.0% decrease, n=6). In EDL muscles from C57 mice, exposure to indomethacin had no significant effect on the force-frequency relationship compared to DMSO alone. In EDL muscles from mdx mice, the force frequency curve under control conditions (DMSO alone) was situated to the left of the C57 control force frequency curve, with significantly greater relative forces at stimulation frequencies of 20, 40, 60 and 80 Hz ($P<0.05$). However, following exposure of dystrophic muscle to indomethacin, the force frequency curve was shifted to the right, such that there was no significant difference between the dystrophic (mdx) and non-dystrophic (C57) force frequency curves ($P>0.05$).

These findings indicate that indomethacin has detrimental effects on force production in both dystrophic and non-dystrophic muscles, possibly through inhibition of store operated Ca^{2+} entry (Boittin *et al.*, 2006). Indomethacin had no effect on the force frequency relationship in control muscles, but returned the force-frequency relationship to normal in mdx muscle. This suggests that PLA_2 activation may contribute to the altered contractile properties of dystrophic (mdx) skeletal muscle.

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