Streptomycin reduces stretch-induced membrane permeability in isolated muscles from mdx (dystrophic) mice

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Duchenne muscular dystrophy (DMD) is a genetic disease, which causes severe muscle degeneration, leading to profound muscle weakness and early death. DMD is caused by the absence of a protein, dystrophin, which is attached to the surface membrane of muscle fibres.

The recent focus of our laboratory has been to investigate whether a component of the damage is caused by entry of Ca^{2+} through stretch-activated channels (SACs) in the surface membrane. We have recently shown in single muscle fibres from *mdx* (dystrophic) mice that following stretched (eccentric) contractions, Ca^{2+} influx can be prevented and isometric force improved by the addition of three known SAC blockers, one being the antibiotic streptomycin (Yeung *et al*., 2005). It is known that stretched contractions cause greater membrane permeability of *mdx* muscles compared to wild-type (Petrof *et al.,* 1993). In the present study we investigated whether this stretch-induced membrane permeability was due to Ca^{2+}-dependant membrane damage as a result of Ca^{2+} entry through SACs.

Extensor digitorum longus (EDL) muscles were dissected from 8-10 week old wild-type and *mdx* mice. Muscles with clips attached to the tendons were mounted in a chamber between a force transducer and the lever of a motor. In some experiments, streptomycin (200µM) was added to the perfusate 60 min before the stretched contractions. Muscles were set to the length that produced maximal isometric force (optimum length, L_o). Procion orange, a membrane impermeable fluorescent dye, was added to the perfusate in order to detect fibres with increased membrane permeability. Muscle damage was induced by 10 stretched contractions, where the muscle was stretched by 30% of its length from L_0, during a 400 ms tetanus. Following the stretched contractions, isometric force was measured at 30 and 60 min and the muscle was frozen in isopentane cooled in liquid nitrogen at either 0, 30 or 60 min. Muscle cross-sections (10µm) were viewed with a fluorescent microscope and the area of procion orange positive muscle fibres was calculated as a percentage of the entire muscle cross-sectional area.

Following the stretched contractions, isometric force measured from control *mdx* muscles (n=5) fell to 33.4% ± 3.3. In experiments on *mdx* muscles where streptomycin was added to the perfusate before the stretched contractions (n=8), the reduction in force was significantly less, reaching 44.6% ± 1.6 ($p<0.05$, t-test). Wild-type muscles had a smaller decrease in force than *mdx* muscles following the stretched contractions (60.3% ± 1.6, n=3) and there was no effect of streptomycin (60.3% ± 2.2, n=3). Procion orange uptake for control *mdx* muscles was 5.0% ± 0.9 (n=3) immediately after the stretched contractions and then increased to 10.3% ± 0.8 (n=4) at 30 min and 15.1% ± 2.5 (n=4) at 60 min. At all times, streptomycin significantly reduced procion orange uptake ($p<0.05$, t-test), with values of 1.6% ± 0.7 (n=3), 5.3% ± 1.4 (n=3), and 4.9% ± 1.4 (n=5) at 0, 30, and 60 min, respectively. Wild-type muscles had very little procion orange uptake, with mean values of 0.7% (without streptomycin, n=2) and 1.1% (with streptomycin, n=2).

This study showed that following stretched contractions, membrane permeability of *mdx* muscles increased progressively over 60 min, and importantly, most of this permeability could be prevented by the SAC blocker, streptomycin. Taken together, these results suggest that the increased membrane permeability is mainly due to Ca^{2+} entry through SACs and not the result of transient mechanical tears of the membrane during the stretched contractions (Petrof *et al.*, 1993). The mechanism by which increased intracellular Ca^{2+} causes muscle damage to dystrophic muscle is unclear but might be attributable to an increased production of reactive oxygen species and/or the activation of calcium-dependent proteases or phospholipase A_2. These damage pathways are now being explored in our current series of experiments.

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