Calcium transients in fast-twitch FDB skeletal muscle from old dystrophic mice

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Duchenne muscular dystrophy (DMD) is the second most common fatal genetic disease in humans with an occurrence of 1:3500 live male births. The disease is characterised by a cyclic degeneration and regeneration of the skeletal musculature, there is also a CNS involvement with the boys having an average IQ of 85 and associated cognitive defects. The *mdx* mouse is the most commonly used model of DMD, it has a point mutation in the dystrophin gene and lacks all long forms of the protein dystrophin. By 6 weeks of age the majority of the skeletal musculature in the *mdx* mouse has undergone at least 1 cycle of degeneration and regeneration. The regenerated muscle fibres have centrally located nuclei and the fibres exhibit a range of deformities from simple splits to more complex syncytiums of branching. There are several reports that the dystrophic process is more pronounced in old *mdx* mice, with old muscles being more susceptible to damage by lengthening contractions. Here I examine Ca^{2+} transients in single isolated fast-twitch fibres from FDB muscles from old *mdx* mice 20-24 months of age. The *mdx* mice (C57BL/10ScSn-DMD) have been produced by a backcross in order to provide age matched littermate controls (C57BL/10ScSn) on identical genetic backgrounds. This circumvents deficiencies in studies which use separate colonies as wild-type controls which have been bred separately from the *mdx* mouse for over 20 years and contain an unknown number of additional mutations. Immediately before experimentation, animals were killed with an overdose of halothane. The FDB muscle was dissected out and digested to yield individual fibres. The digestion solution was Krebs solution containing 3 mg/ml collagenase Type I (Sigma) and 1 mg/ml trypsin inhibitor (Sigma), continuously bubbled with 95% O_2–5% CO_2 and maintained at 37°C. After 30 min, the muscle was removed from this solution and rinsed in Krebs solution. Fibres were plated onto a chamber placed on a Nikon inverted microscope attached to a Cairn spectrophotometer to monitor calcium transients using a PMT, during the experiment fibres were videotaped using infrared illumination to monitor their contraction and viability. Fibres were continually superfused with bubbled Krebs. After fibres had attached to the glass coverslip the central area of the fibre was bracketed to minimise any movement artefacts. An intracellular microelectrode was used to ionophoresed the free acid form of fura-2 (molecular probes) to give a final concentration 5-50 μm. Fibres were rejected if they had a RMP more depolarised than -65 mV. Fibres were electrically stimulated using a bipolar concentric electrode positioned near the neuromuscular junction. In some cases after the first Ca^{2+}ratio/freq had been constructed the fibre was immobilised with BDM to stop contractions, the BDM Ca^{2+}ratio/freq curve was essentially the same. Fibres were fatigued by stimulating at 50 Hz, 1 second on 1 second off, for 3 minutes. The resting ratio (Ca^{2+}) was the same in old *mdx* and old littermate controls; 0.46 ± 0.02 (n = 5), 0.45 ± 0.03 (n = 4). Ca^{2+}ratio /freq curves were produced by stimulating fibres in the range 2-100 Hz.

Freq(Hz)	2	5	10	15	20	25	30	50	60	75	100
Mdx ratio n=5	0.59±.03	0.66±.02	0.75±.02	0.88±.04	1.00±.02	1.16±.04	1.20±.02	1.38±.02	1.42±.05	1.44±.04	SAG*
Cont. ratio n=4	0.65±.02	0.68±.01	0.77±.03	0.95±.05	1.02±.02	1.12±.08	1.19±.01	1.37±.01	1.39±.04	1.44±.04	1.49±.02

The table shows ratio/freq curves for rested *mdx* (*mdx* fibres were centrally nucleated but had no splits) and control fibres. There was no statistical difference at any frequency apart form 100 Hz where the plateau of the tetanus peaked (1.62 ± 0.02) and then sagged to a new stable level (1.46 ± 0.03) for the duration of the stimulus. The peak in the *mdx* at 100 Hz was significantly higher than in littermates. When fatigue fibres were stimulated at 100 Hz immediately after fatigue *mdx* tetanus sag was more pronounced, (peak 1.34 ± 0.05 plateau 0.75 ± 0.04 n = 5) *c.f.* littermate control which had no sag (peak 1.21 ± 0.03 n = 4). The resting ratio (Ca^{2+}) during fatigue was the same in *mdx* and control. When deformed split fibres were examined there was a dramatic difference in that they did not survive the fatiguing stimulus and the base line ratio (Ca^{2+}) rose to 0.7 ± 0.12 and the fibres stopped contracting, although ratio (Ca^{2+}) transients were still present on stimulation. It appears that there is a pathology of Ca^{2+} kinetics in old dystrophin deficient *mdx* fast-twitch fibres.