The response of healthy and dystrophic mdx mouse muscle to prolonged elevations of cytoplasmic Ca^{2+}

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Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy. It is caused by a mutation in the dystrophin gene resulting in the lack of the structural protein dystrophin. This results in numerous abnormalities in the dystrophic muscle, including the response of the membrane to stretch, calcium handling and the turnover of fibres within the skeletal muscle. A number of membrane proteins are responsible for shaping the Ca^{2+} transient in the cytoplasm. Therefore, with Ca^{2+} handling affected in dystrophic fibres, this signal can be different from that in healthy muscle fibres. This could lead to different downstream outcomes. We aimed to trigger a prolonged elevation of cytoplasmic Ca^{2+} in healthy and dystrophic muscle fibres, under different conditions, to observe the effect on the activation of signalling pathways by measuring cytoplasmic Ca^{2+} and observing membrane integrity and fibre status.

All experiments were approved by The Animal Ethics Committee at The University of Queensland. C57BL/10 (WT) and C57BL/10-mdx (mdx) mice were killed by asphyxiation following inhalation of CO_2. *Interossei* muscle from these mice were dissected out and individual fibres were enzymatically isolated. Selected isolated fibres were loaded with 10 μM Fura-2AM in a physiological solution and placed above a 20x objective of a Nikon inverted microscope in a customised experimental chamber with a glass coverslip as a base. The physiological solution used in experiments was either Na^+ or K^+-based, to polarise and depolarise the fibre, respectively, and had either 2 mM Ca^{2+} or was nominally Ca^{2+}-free. Ca^{2+}-free solution contained 1 mM EGTA and had raised Mg^{2+} from 1 to 3 mM. Cytoplasmic Fura-2 signals were collected from isolated fibres following excitation of the trapped dye with light at wavelengths of 340, 360 and 380 nm. Excitation wavelengths were generated in a Sutter DG4 monochromator that rapidly changed the pathway of light through the excitation filters. Light was passed through each excitation filter for <10 ms. Emitted light was collected by QuanTEM CCD digital camera and displayed on a computer monitor using MetaMorph software. Fura-2 was excited and fluorescence subsequently collected every 2 or 30 s during experiments. The ratio of the 340/380nm wavelengths was used to monitor cytoplasmic [Ca^{2+}] and the 360nm wavelength (isosbestic point) was used to monitor [Fura-2] in the fibre. Calcium was leaked from the sarcoplasmic reticulum using the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) blocker cyclopiazonic acid (CPA).

In 0 Ca^{2+}, K^+-based solution, a 100 μM CPA treatment induced cell death observed as a hypercontracture or loss of cytoplasmic Fura-2 through a lysed surface membrane in 18 of 33 WT and 1 of 18 mdx fibres. In addition, all 5 mdx fibres survived exposure to 250 μM CPA. The cytoplasmic Ca^{2+} transient induced by the introduction of CPA did not appear to be different between the fibre types. In a Na^+-based, 2 mM Ca^{2+} solution, the introduction of 50 μM CPA was sufficient to deplete the SR passed the activation threshold for store-operated Ca^{2+} entry (SOCE) in all 3 WT but not in all 3 mdx fibres. Store-operated Ca^{2+} entry was observed in all 4 mdx fibres when [CPA] was raised to 250 μM.

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