

## Abnormal calcium transients and calcium handling protein expression in cardiomyocytes from *mdx* (dystrophic) mice

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Duchenne muscular dystrophy (DMD) is a fatal X-linked genetic disorder caused by deficiency of the cytoskeletal protein dystrophin. DMD patients have extensive skeletal muscle degeneration and a dilated cardiomyopathy (DCM). The *mdx* mouse also lacks dystrophin and in skeletal muscle it exhibits membrane damage and an abnormal influx of  $\text{Ca}^{2+}$ . The present study was aimed at characterising ventricular performance which may contribute to DCM in *mdx* mice. Ventricular myocytes were isolated from 8-week old wild-type and *mdx* mice and intracellular  $\text{Ca}^{2+}$  measured with the fluorescent indicator fluo-4 during electrical and caffeine (10 mM) induced stimulation. Protein expression of the ryanodine receptor (RyR), the sarco endoplasmic reticulum calcium ATPase (SERCA) and phospholamban were analysed using immunoblotting techniques. The peak of the electrically stimulated  $\text{Ca}^{2+}$  transient was significantly greater in *mdx* mice, but the time to peak was significantly shorter. These findings were not the result of increased sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  loading as the caffeine-induced  $\text{Ca}^{2+}$  transient peak was unchanged in *mdx* mice. The increase in peak calcium transient and the decreased time to peak could be due to the significantly increased levels of RyR protein expression (4-fold), allowing more rapid  $\text{Ca}^{2+}$  release from the SR during excitation. However, this is not usually found in established DCM (Kubo *et al.*, 2001). It was found that the rate of decline of the electrically stimulated  $\text{Ca}^{2+}$  transient was significantly slower in *mdx* mice, but the rate of decline of the caffeine-induced  $\text{Ca}^{2+}$  transient was unchanged, suggesting that the slower removal of  $\text{Ca}^{2+}$  from the intracellular milieu was a result of decreased SERCA activity, and not decreased sodium-calcium exchanger activity. SERCA protein levels were unchanged, but phospholamban levels were increased significantly (2-fold). The slower rate of decline of the  $\text{Ca}^{2+}$  transient in *mdx* mice is therefore possibly a result of increased inhibition of SERCA by phospholamban, a finding which is consistent with other studies of DCM (Meyer *et al.*, 1995). This is further supported by the finding that the SERCA/phospholamban ratio was significantly smaller in *mdx* mice. It is concluded that dystrophin deficiency causes impairment in the  $\text{Ca}^{2+}$  handling properties of *mdx* ventricular myocytes, which may play a role in the development of DCM. Future work will test whether the increase in peak  $\text{Ca}^{2+}$  transients in the *mdx* mouse is an early compensatory mechanism that reverses as the cardiomyopathy progresses, by investigating myocyte  $\text{Ca}^{2+}$  handling in older mice.

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