Lack of Neuronal Nitric Oxide Synthase Slows Phosphocreatine Recovery and Reduces Microvascular Function Following Muscle Contractions

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder that affects one in 3,500 male births, and results in a deficiency in the protein dystrophin on the membrane of muscle fibers (1). DMD is characterized by progressive muscle weakness, deteriorating functional capabilities, loss of independence, and early death. Deficiency in dystrophin is accompanied by lack of sarcolemma-localized neuronal nitric oxide synthase (nNOS), which has been suggested to contribute to impaired local perfusion to skeletal muscle following exercise (2). In this study we tested the hypothesis that mice lacking nNOS (nNOS-/-) would have slower recovery of phosphocreatine (PCr) and reduced microvascular response following muscle contractions.

Experimental

Phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) data were acquired (TR 1 s, 15 NSA, 2048 data points, 8k sweep width) in male wild-type (n=22) and nNOS-/- (n=14) mice of 9-27 weeks of age using an 11.1 T 40 cm horizontal bore with a Varian/Agilent spectrometer. An oblong transmit/receive surface coil (6 mm X 12 mm) was centered on the posterior region of the lower hindlimb. Measures were obtained from the posterior lower hindlimb during three bouts of muscle contractions at 0.75 Hz for 10 min with 10 min recovery (low-intensity) and 5 Hz for 2 min with 15 min recovery (high-intensity). In a subset of mice, the peak blood oxygenation level dependent (BOLD) response was measured following 5 tetanic contractions separated by 3 minutes using a 4.7T Varian/Agilent spectrometer.

Results and Discussion

The time constant (τ) of PCr recovery was slower following the contractions ($p<0.05$) in nNOS-/- than wild-type after both low- (139±9vs.221±14s) and high-intensity (143±9vs.196±13s) (**Figure 1**). No differences were observed between groups in pH at rest and end olow-intensity (con 7.03±0.16; nNOS-/- 7.03±0.09) or high intenstiy (6.95±0.11; nNOS-/- 6.94±0.08) exercise. Furthermore, the peak postcontractile BOLD response was lower ($p<0.05$) in nNOS-/- (1.7±0.1%) than wild-type (3.0±0.4%).

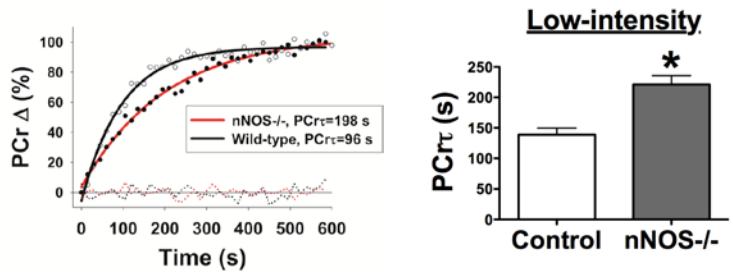

Figure 1. Representative recovery of PCr in an nNOS-/- and Wild-type mouse with PCr breakdown normalized to end-exercise levels. Data were fit with a mono-exponential model and residuals of the fits are plotted.

Conclusions

The slower PCr kinetics may be due to a reduced delivery of oxygen to skeletal muscle as a result of an inability to blunt sympathetic vasoconstriction (2, 3). Future studies aimed at testing the effects of compensating for lack of nNOS by administration of PDE5a inhibitors may be beneficial for enhancing muscle function in DMD.

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