Nitric oxide and skeletal muscle regeneration in mice after injury – the role of muscular nNOS

J.E. Church, S.M. Gehrig, G.K. McConell and G.S. Lynch, Basic & Clinical Myology Laboratory, Department of Physiology, University of Melbourne, VIC 3010, Australia.

Nitric oxide (NO) plays a complex role in skeletal muscle biology and its role, particularly in muscular regeneration, is still not fully understood. Increased NO in skeletal muscle has been reported to be beneficial in models of muscle injury and degeneration/regeneration such as muscular dystrophy (Wehling *et al.*, 2001) or crush injury (Anderson, 2000), but NO also plays a prominent role in the muscle wasting (or cachexia) associated with inflammatory disorders (Marinez-Moreno *et al.*, 2007). These seemingly contradictory effects of NO in skeletal muscle may be dictated by a combination of subcellular localisation and/or concentration of NO, both of which are intimately related to the isoform of nitric oxide synthase (NOS) from which the NO is produced. Although skeletal muscle can express all three subtypes of NOS (nNOS, eNOS and iNOS), nNOS is the most highly expressed isoform of NOS in skeletal muscle.

The aim of this study was to clarify the role of nNOS-derived NO in skeletal muscle by examining muscle fibre regeneration after myotoxic injury in mice genetically lacking nNOS. Twelve week old C57BL/6J-Nos1^{tm1Plh} mice homozygously null for the nNOS gene (nNOS$^{-/-}$) and their littermate controls (nNOS$^{+/+}$) were used in this study. Muscle function of the *tibialis anterior* (TA) muscle of the right hindlimb was assessed *in situ* using methods described in detail previously (Schertzer *et al.*, 2007). Maximal isometric force (P_o) was determined from the frequency-force relationship and muscle fatiguability determined from a protocol of repeated, intermittent isometric contractions.

TA muscles from nNOS$^{-/-}$ mice were less susceptible ($P < 0.05$) to fatigue than nNOS$^{+/+}$ mice but specific force (P_o normalised to muscle cross-sectional area, sP_o) and the frequency-force relationships were not different between groups. To examine the contribution of nNOS to muscle regeneration after injury, mice were anaesthetised (ketamine 100mg/kg and xylazine 10mg/kg; *i.p.*) and the TA muscle of the right hindlimb injected with the myotoxin Notexin (1μg/ml, *i.m.*) to cause degeneration of all muscle fibres. Mice were allowed to recover for 10 days, after which function of the TA muscle was assessed *in situ*. At 10 days post-injury, the sP_o of the regenerating TA muscles, while lower ($P < 0.05$) than that of uninjured muscles, was unchanged between the two groups. These findings suggest that expression of nNOS does not appear to be a critical factor in successful regeneration of skeletal muscle with this mode of injury.

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