Skeletal muscle Na^+,K^+-pump activity, reactive oxygen species and fatigue in exercising humans

M.J. McKenna, School of Human Movement Recreation and Performance, Centre for Ageing, Rehabilitation, Exercise & Sport, Victoria University PO Box 14428, Melbourne, VIC 8001, Australia.

The Na^+,K^+-pump (Na^+,K^+-ATPase, NKA) plays a pivotal role in skeletal muscle function, regulating transmembrane Na^+ and K^+ concentration gradients, and membrane excitability. Increased NKA activity during contractions plays a vital modulatory role in these events, and thus also in minimising muscle fatigue. Despite this, substantial Na^+ and K^+ leaks occur during exhaustive exercise, with up to a 75% decline in the intracellular/extracellular [K^+] gradient and near-doubling of the extracellular/intracellular [Na^+] gradient. Furthermore, our laboratory has found that acute exercise also decreases the maximal NKA activity (Fraser *et al.*, 2002; McKenna *et al.*, 2006), which would both exacerbate Na^+/K^+ disturbances, and lessen the NKA electrogenic effects. Thus, we propose muscle Na^+ and K^+ ion disturbances together with NKA inactivation as important contributors to muscle fatigue.

Studies in recent years have highlighted a clear role of reactive oxygen species (ROS) in muscle force modulation and in fatigue, with enhanced ROS scavenging reducing muscle fatiguability (Reid, 2001; Smith & Reid, 2006). Support for a ROS-scavenging role in attenuating human muscle fatigue was gained from a landmark finding that intravenous infusion of the non-specific antioxidant N-acetylcysteine (NAC) attenuated fatigue during stimulation of tibialis anterior muscle (Reid *et al.*, 1994). Numerous target proteins for ROS effects have been identified, but whether skeletal muscle NKA is redox-sensitive, and whether ROS-sensitive mechanisms might be involved in exercise-induced NKA inactivation and fatigue were unknown. In two series of voluntary human exercise studies, we firstly investigated acute NKA inactivation and secondly, the role of ROS in fatigue. We developed a modified NAC infusion protocol to investigate whether enhanced ROS scavenging would attenuate muscle fatigue during intense intermittent exercise, and prolonged endurance exercise (Medved *et al.*, 2003; Medved *et al.*, 2004a). We have then fused these investigative lines together in a combined study into muscle redox status, ROS, NKA, K^+ and fatigue (McKenna *et al.*, 2006).

Intense intermittent exercise performance was unchanged by NAC, despite evidence of improved blood redox status by elevated glutathione (GSH) and lower oxidised GSH (GSSG) (Medved *et al.*, 2003). NAC effects on prolonged exercise performance were related to aerobic power (Medved *et al.*, 2004a). Studies in endurance athletes confirmed an ergogenic effect of NAC during prolonged exercise, together with elevated muscle GSH, cysteine and NAC, indicating greater redox buffering (Medved *et al.*, 2004b). This confirms a role for ROS in muscle fatigue and suggests antioxidant capacity may be an important factor affecting muscle performance. The exercise-induced decline in muscle NKA activity was markedly attenuated by NAC, and the rise in plasma [K^+] with exercise lowered, suggesting a strong link between ROS and NKA inactivation and further implicating ROS, NKA and K^+ in fatigue (McKenna *et al.*, 2006).

Finally, we recently investigated whether increased NKA gene expression with exercise (Murphy *et al.*, 2004) involves ROS-linked mechanisms. The acute upregulation of the NKA α2 isoform mRNA by exercise was abolished by NAC, whereas changes in mRNA of other isoforms were not. As the α2 isoform is the most abundant NKA isoforms in muscle, this further indicates a link between ROS and gene expression and thus likely also of adaptability, of this key regulatory protein.

In summary, muscle NKA inactivation may be important in exacerbating fatigue in exercising humans. NAC infusion studies indicate ROS scavenging can enhance exercise performance and establishes a link between ROS, NKA inactivation and muscle fatigue.

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