Source of superoxide production in mammalian skeletal muscle at elevated temperature

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It is well established that at normal physiological temperature there is a marked increase in the production of superoxide (O_2^-) in skeletal muscle[1]. However, the major source of O_2^- production is not clear. For example, there are reports that suggest that O_2^- production is extracellular while other reports suggest that mitochondria or membrane bound NADH oxidase on the sarcoplasmic reticulum play the major role in O_2^- production[2].

In order to determine whether the main source of O_2^- production is intracellular or extracellular, different types of muscle fibre preparations in combination with specific mitochondrial inhibitors were used. Long-Evans hooded rats were killed under deep anaesthesia (2% v:v Fluothane), EDL muscles excised, single fibres or intact fibre bundles were prepared and volume measured. O_2^- was measured using the cytochrome C assay, based on changes in cytochrome C absorbance over the spectrum 540-560nm when cytochrome C becomes reduced by O_2^- [1].

Results show that removing the surface membrane results in more O_2^- being measured than when the surface membrane is intact (intact preps produced 40.92 ± 5.01% (n = 5) of membrane removed fibres). This strongly suggests that the source of O_2^- is mainly intracellular.

Addition of 50µM Rotenone, a mitochondrial inhibitor specific to complex I, caused the amount of O_2^- produced in skinned fibres to increase by a factor of 8.7 ± 0.7. This suggests that not only is O_2^- production intracellular but also that the primary source is from the mitochondria.

(1) van der Poel, C. & Stephenson, D.G. (2002) Journal of Physiology 544: 765-776.
(2) Zuo, L., Pasnciuc, S., Wright, V., Merola, A. & Clanton, T. (2003) Antioxidants and Redox Signalling 5: 667-675.