Influence of skeletal muscle ROS production on excitation-contraction coupling at physiological temperatures

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There is a long-standing issue in skeletal muscle research, which effectively limits experimentation on isolated mammalian skeletal muscle to sub-physiological temperatures. This issue arises from the rapid and irreversible functional deterioration of isolated skeletal muscle preparations from mammals when incubated at temperatures in the "normal" physiological range. The effects of physiological temperatures on individual excitation-contraction coupling events in fast-twitch skeletal muscle were investigated using isolated intact muscle preparations and freshly mechanically skinned muscle fibres from the rat.

Extracellularly released reactive oxygen species (ROS) were measured in intact isolated rat *extensor digitorum longus* (EDL) muscles at 22ºC, 32ºC, and 37ºC, and tetanic force was measured at 22ºC and 37ºC under the same conditions. The rate of ROS production showed a marginal increase between 22º to 32ºC, but increased fivefold when the temperature was increased from 22ºC to 37ºC. The increase in ROS was accompanied by a marked decrease in tetanic force after 30 min incubation at 37ºC (Edwards *et al*. 2007). Using mechanically skinned fibres we demonstrated that endogenously produced ROS acts on the contractile apparatus, reducing Ca^{2+}-activated force (van der Poel & Stephenson, 2002), and on the sarcoplasmic reticulum (SR), increasing the Ca^{2+} leak rate from the SR that is not *via* the ryanodine receptor (van der Poel & Stephenson, 2007). The resting membrane and intracellular action potentials of isolated muscle preparations were also significantly influenced by temperature, which was associated with an increase in ROS (van der Poel *et al*., 2007).

These results implicate the relatively high level of ROS production as a potential cause of the down-regulation of skeletal muscle function *in vitro* at physiological temperatures. The efficient perfusion of the muscle with blood *in vivo* would efficiently remove ROS from the muscle and prevent its accumulation to levels that cause depression in the force response at normal physiological temperature.

van der Poel C, & Stephenson DG. (2002) *Journal of Physiology*, **544:** 765-76.

van der Poel C, Edwards J, Macdonald WA, & Stephenson DG. (2007) *American Journal of Physiology*, **292:** C1353-C1360.

van der Poel C, & Stephenson DG. (2007) *American Journal of Physiology* **293:** C133-C141.

Edwards J, Macdonald WA, van der Poel C, & Stephenson DG. (2007) *American Journal of Physiology* **293:** C650-C660.