

## **Elevated temperature effects on sarcoplasmic reticulum function in mammalian skeletal muscle fibres**

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Superoxide (O<sub>2</sub><sup>-</sup>) has been shown to be produced by the muscle at elevated temperatures (40-46°C) and to cause marked reversible changes in contractile activation characteristics of mammalian skinned fibres (van der Poel & Stephenson, 2002). Here we examine the effect of similar temperature treatments on sarcoplasmic reticulum (SR) function in mammalian skeletal muscle. Long-Evans hooded rats were killed by an overdose of halothane in accordance with the procedure approved by La Trobe University Animal Ethics Committee. Extensor digitorum longus (EDL) muscles were dissected out and underwent temperature treatment at 40°C for either 5 or 30 min, 43°C for 30 min and 46°C for 5 min. Single muscle fibres were dissected from EDL muscles after exposure to elevated temperatures, mechanically skinned under paraffin oil and mounted on a force transducer. The endogenous SR Ca<sup>2+</sup> content was then estimated by releasing all SR Ca<sup>2+</sup> with 30mM caffeine and low Mg<sup>2+</sup> (Release Solution), and measuring the area under the force response as an indicator of the amount of SR Ca<sup>2+</sup> released. The fibre was then re-loaded with Ca<sup>2+</sup> under standard conditions ([Ca<sup>2+</sup>] 200nM and pH 7.10) for either 30, 60 or 90sec and the SR Ca<sup>2+</sup> was subsequently released in the Release solution. The relative area under the force responses was again used as the indicator of the relative amount of Ca<sup>2+</sup> in the SR prior to the exposure to the Release solution. In order to determine the extent of Ca<sup>2+</sup> leak out of the SR, the preparation was loaded with Ca<sup>2+</sup> for 90 sec and then washed for either 30 or 90 sec in a leak solution (pCa = 8, 0.5mM EGTA). The remaining Ca<sup>2+</sup> in the SR was then released in the Release solution and the ratio between the areas under the caffeine-induced responses after 90 and 30 sec exposure to leak solution was used to estimate the fraction of SR Ca<sup>2+</sup> remaining after 60 sec in the leak solution (Macdonald & Stephenson, 2001). Results show that after exposure of the EDL muscle to 40°C for 5 or 30 min, 43°C for 30 min and 46°C for 5 min the endogenous amount of Ca<sup>2+</sup> in the SR was greatly reduced. This was accompanied by a significant decrease in the rate and ability of the SR to load Ca<sup>2+</sup> and by a large increase in the rate of SR Ca<sup>2+</sup> leak, which could explain the decrease in both endogenous SR Ca<sup>2+</sup> and the rate of SR Ca<sup>2+</sup> loading. No significant recovery was observed in the parameters (0-3hrs after temperature treatment). Experiments using 20 µM TBQ (2,5-di(tert-butyl)-1,4-hydroquinone) to block the SR Ca<sup>2+</sup> pump and Ruthenium Red (5 µM) to block the RyR/SR Ca<sup>2+</sup> release channels indicated that the major route of the Ca<sup>2+</sup> leak was through the SR Ca<sup>2+</sup> pump. Pre-treatment of the muscles with the superoxide scavenger Tiron (20mM) markedly reduced the temperature-induced changes on the SR function suggesting that the observed temperature effects are influenced by O<sub>2</sub><sup>-</sup> production. The results can explain the earlier observations on isolated muscle preparations exposed to temperatures greater than 35°C, when force production becomes markedly and irreversibly depressed (Lännergren & Westerblad, 1986). Lännergren, J. & Westerblad, H. (1986) *Journal of Physiology*, 390: 285-293. Macdonald, W. A. & Stephenson, D. G. (2001) *Journal of Physiology*, 532: 499-508. van der Poel, C. & Stephenson, D. G. (2002) *Journal of Physiology*, 544: 765-776.