Effects of raising the temperature from 25°C to 37°C on twitch responses in fast-twitch mechanically skinned muscle fibres of the rat

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It has been known for many years that intact mammalian muscle fibre preparations brought to the normal body core temperature (37°C) rapidly and irreversibly deteriorate in their ability to produce force (Lännergren & Westerblad, 1987). Consequently, most experiments on isolated mammalian skeletal muscle are conducted at sub-physiological temperatures. In a previous study (van der Poel & Stephenson, 2004) we showed that as the temperature is brought to 37°C, the rate of mitochondrial production of superoxide ($O_2\cdot^-$), the parent molecule in the reactive oxygen species (ROS) cascade, rises and that a relatively large fraction of the superoxide produced in the mitochondria can be measured extracellularly. In this study we investigated the effect of raising the temperature to 37°C on the twitch responses induced by triggering action potentials in the sealed transverse tubular (t-) system of single fast-twitch mechanically skinned fibres of the rat by electric stimulation (Posterino *et al.*, 2000).

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 at room temperature, attached to a force transducer and placed in physiological solution at room temperature containing (mM): HEPES, 90; Mg^{2+}, 1; HDTA, 49.95; ATP, 10; CP, 8; Na^+, 36; K^+, 126; Ca^{2+}, $<10^{-6}$; pH 7.1. Fibres were initially equilibrated at 25°C and then transferred to equivalent solutions at 37°C. Twitch force responses at 37°C were obtained by electrically stimulating the fibres every 2 mins with supramaximal square pulses until the fibre failed to produce any force. After 7 min at 37°C, the amplitude of single twitches dropped to only 17.33 ± 10.22% (n = 5) of initial response. To test if this decrease was associated with $O_2\cdot^-$ production, an uncharged, membrane permeable SOD mimetic Tempol (1 mM), which effectively removes $O_2\cdot^-$ without being used as a substrate, was applied. In its presence, Tempol prevented to a large extent this decrease to only 61.31 ± 8.72% (n = 3).

The ability of the contractile apparatus to produce maximum Ca^{2+} activated force was not different between treatments as shown by the similar force responses per cross sectional area obtained at the end of each experiment in maximally Ca^{2+}-activating solutions at 22ºC (ANOVA, P = 0.82). Also the sarcoplasmic reticulum (SR) Ca^{2+} content was not different between treatments as indicated by the similarity of force responses elicited following direct activation of the SR Ca^{2+}-release channels in the presence of low [Mg^{2+}] (0.015 mM) ((ANOVA, P = 0.61) (low [Mg^{2+}] responses at 22°C as % of maximum Ca^{2+}-activated force at 22°C: 91 ± 14% max force for control fibres that were kept only at 22°C (n = 6) *vs* 96 ± 15% max force for fibres that became unresponsive to electrical stimulation at 37°C (n = 6)). Separate experiments indicated that neither the SR Ca^{2+} handling properties nor the sensitivity to Ca^{2+} of the contractile apparatus were affected by exposure to 40°C for up to 10 min. Thus, the results imply that $O_2\cdot^-$ production inside mitochondria at 37ºC is associated with the depression in Excitation-Contraction coupling at a step preceding the SR involvement.

The most likely interpretation of our results is that the intracellular $O_2\cdot^-$ production in the mitochondria decreases the excitability of the t-system in muscle fibres, thus explaining the deterioration in ability of the intact muscle fibre preparations to produce force at 37°C. The results also show that the use of Tempol, a membrane permeant $O_2\cdot^-$ dismutase (SOD) mimetic can markedly prevent muscle function deterioration at 37°C.

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