C20

Acute endotoxaemia does not impair voluntary or electrically stimulated human quadriceps femoris contractions.

R.G. Cooper, F.J. McNicol and G.L. Carlson

Injury, Repair and Rehabilitation Research Group, University of Manchester, Manchester, M13 9PL, UK

Respiratory failure is a serious feature of sepsis, and animal studies suggest this is due to skeletal muscle dysfunction (e.g Hussain *et al*, 1985). The effects of sepsis on human muscle contractility are unclear, and patient studies are likely to be confounded by malnutrition and therapeutic neuromuscular blockade. This Local Research Ethics Committee-approved study used an *in vivo* sepsis model to assess the effects of this, and the resulting systemic inflammatory response, on quadriceps contractile function. Eight healthy males, mean (SD) age 32.0 (4.4) yr were examined twice, in random order and 2 weeks apart. On one occasion they received 4 ng/kg *i.v* E.coli lipopolysaccharide (LPS), and on the other the same volume of *i.v* sterile normal saline. Pulse rate, rectal temperature and oxygen consumption (VO_2), and arterialised venous plasma concentrations of cortisol, growth hormone (GH), IL-6 and TNF-α were monitored/measured hourly for one hour before and 6 hours following *i.v* LPS/saline. Also assessed hourly were isometric quadriceps contractile properties: maximum voluntary contractile force (MVC), and 10:50 and 20:50 Hz force ratios and maximum relaxation rate (MRR) determined from computer-controlled electrically stimulated contractions (6s trains comprising 10, 20 and 50Hz, arranged contiguously). Statistical comparisons used repeated measures ANOVA and, where treatment/time interactions were apparent, post-hoc Student's t-test using Bonferroni corrections for multiple comparisons.LPS caused fever, tachycardia and increases in plasma cortisol, GH, IL-6 and TNF-α concentrations (saline/LPS comparisons made at peak LPS-induced change): pulse rate 64.4 (8.8) *vs* 90.6 (10.3) bpm, core temperature 36.4 (0.2) *vs* 37.8 (0.3) ^oC, VO_2 120.2 (8.3) *vs* 145.9 (18.7) ml/min/m^2, cortisol 159.8 (46.6) *vs* 721.6 (105.6) nmol/L, IL-6 11.0 (8.7) *vs* 1074.0 (754.9) pg/mL, GH 4.8 (6.7) *vs* 33.6 (19.7) mU/L and TNF-α 13.9 (7.7) *vs* 960.7 (589.6) pg/mL, $p<0.01$ for each comparison. LPS had no effect on quadriceps contractile performance (saline/LPS comparisons shown made at 3 hours): MVC 750 (80.1) *vs* 741.8 (82.5) N, 10:50 ratio 27.3 (7.3) *vs* 29.7 (4.2), 20:50 ratio 72.4 (7.8) *vs* 73.4 (8.9) and MRR 4.71 (0.56) *vs* 5.00 (0.73) % per 10ms, $p>0.05$ for each comparison. The LPS doses given were small, compared with those previously given to animals (e.g Hussain *et al*), but the systemic physiological effects were large and highly significant. These results suggest that sepsis-induced neuromuscular and/or contractile dysfunction may only occur if sepsis is intense or prolonged.

Hussain SNA *et al.* (1985) *J Appl Physiol* **58**: 2033-2040.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

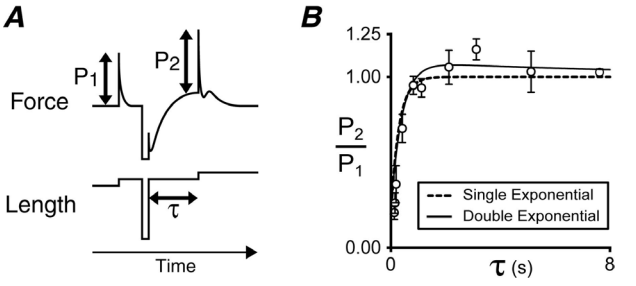
C21

Muscle stiffness transiently exceeds its steady-state value following a rapid shortening/re-stretch protocol

K.S. Campbell and R.L. Moss

Department of Physiology, University of Wisconsin-Madison, Madison, WI, USA

When contracting muscle fibres are allowed to shorten and then rapidly re-stretched, tension often transiently exceeds the steady-state isometric level during the recovery process. The effect is particularly prominent in permeabilized muscle fibres at sub-maximal levels of Ca^{2+} activation. We have used stiffness measurements to investigate whether this tension overshoot may reflect a temporary increase in the number of attached cross-bridges.Bundles of soleus fibers were isolated from a rat (anaesthetized by inhalation of 3-4% isofluorane and subsequently killed by a pneumothorax) and skinned using 1% Triton X-100. Individual fibres (n=7, initial sarcomere length 2.55 ± 0.04 μm) were connected between a motor (Aurora 312B, step-time ~0.5 ms) and a force transducer (AE801, resonant frequency 2.5 kHz). Fibres were activated in solutions with pCa (= $-\log_{10}[Ca^{2+}]$) values in the range 9.0 to 4.5. Once tension had stabilized in each solution, ten trials, each of the form shown schematically in Fig 1A were imposed at regular 18 s intervals. The fibre was subjected to two 1% length changes during each trial. A large shortening/re-stretch perturbation (20% muscle length, 20 ms duration) was applied 60 ms after the first length change. The time interval τ (Fig 1A) was adjusted between pre-set values in a pseudo-random manner in successive trials. The muscle was returned to its initial length at the end of each 8 s recording. Experiments were performed using SLControl software (Campbell & Moss, 2003).The P2/P1 ratio (Fig 1A) is a measure of the muscle's relative stiffness. Fig 1B shows this ratio plotted as a function of the time interval τ at a sub-maximal level of Ca^{2+} activation. A double exponential function (which transiently exceeds unity) fits the experimental data significantly better (F-test, $p<0.001$) than a single exponential function (which approaches unity asymptotically).This result suggests that the number of attached cross-bridges temporarily exceeds the steady-state value following release and re-stretch of the muscle. Possible mechanisms include transient cooperative recruitment of new force-generating cross-bridges or a combination of new cross-bridge attachments at the longer lengths and slower detachment of cross-bridges strained by the stretch.



All results are quoted as mean ± SD. Fig 1. A) Schematic diagram illustrating experimental protocol. B) Mean ± SD values of P2/P1 plotted as a function of the delay interval τ. pCa 5.9. Isometric tension was 0.57 ± 0.03 of maximum Ca^{2+} activated tension.

Campbell, K.S. & Moss, R.L. (2003) Am. J. Physiol. 285: H2857-2864.

Supported by NIH HL 47053.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C22

Effects of N-benzyl-p-toluene sulphonamide (BTS) on tension responses in intact mammalian skeletal muscle.

G. Pinniger and K. Ranatunga

Department of Physiology, University of Bristol, Bristol, BS8 1TD, UK

The present study examined stretch-induced tension responses in muscle when its active tension was significantly reduced using N-benzyl-*p*-toluene sulphonamide (BTS), a compound known to inhibit myosin-II ATPase without affecting calcium release (Cheung, *et al.*, 2002). Adult male rats were humanely killed with an intra-peritoneal injection (>200 mg kg^{-1}) of an overdose of sodium pentobarbitone (Euthatal). Bundles of ~5 intact (fast) fibres (fibre length, L_0, ~2 mm) were isolated from the flexor hallucis brevis muscle of the rat and mounted horizontally between a force transducer and a servomotor (initial sarcomere length = 2.5 μm; 20 °C). Fibre bundles were tetanized and a ramp stretch of 5% L_0 applied on the tension plateau. Stimulation was maintained for a further 500 ms where residual force enhancement (RFE; see Edman & Tsuchiya, 1996) was measured. Experiments (n=5) show that 10 μM BTS produced a reversible depression of tetanic tension. Mean ± S.E.M. (kN m^{-2}) for peak twitch tension (P), tetanic tension before stretch (P_0), peak tension during stretch (P_k), and steady tension at the maintained stretch length (i.e. RFE) are shown in the table.Student's paired t-tests revealed significant decreases in P, P_0, and P_k, whereas RFE was not significantly different, in the presence of BTS, suggesting the involvement of non-crossbridge mechanism(s) in the tension response to stretch. To further investigate these findings, additional experiments were conducted at different initial muscle lengths. RFE was clearly seen on the ascending limb, plateau and descending limb of the length-tension relationship in both the presence and absence of BTS. To our knowledge, these are the first data to indicate the reversible suppression of active tension in intact mammalian skeletal muscle by BTS. Our findings indicate that RFE does not vary in proportion to steady active tension. Similar observations have been made in frog fibres (Bagni, *et. al.*, 2002); however, the exact mechanism of non-crossbridge contributions to the stretch-induced tension response remains unclear.

	P	P_0	P_k	RFE
Control	32.3 ± 3.0	209 ± 23.4	164 ± 20.1	26.6 ± 4.9
10 μM BTS	2.9 ± 0.6	42.7 ± 6.5	111 ± 17.2	35.8 ± 7.3
(BTS/Control) %	8.9 ± 1.4 (P<0.001)	20.9 ± 3.3 (P<0.001)	70.2 ± 7.3 (P<0.02)	158 ± 34.6 (P>0.05)

Bagni MA *et. al.* (2002) *Biophys J.* **82**, 3118-3127.
Cheung A *et. al.* (2002) *Nature Cell Biol.* **4**, 83-88.
Edman KA & Tsuchiya T (1996) *J Physiol.* **490**, 191-205.

We thank The Wellcome Trust for financial support.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.