Increase in citrate synthase activity in electrically stimulated and contralateral non-stimulated soleus muscle of rat

A.D. Pimenta, L. Silveira, A.P. Filho and R. Curi

Physiology and Biophysics, University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

Increase in citrate synthase (CS) activity in skeletal muscle is a well-known adaptation to chronic aerobic exercise. However, skeletal muscle CS activity after a single bout of exercise is poorly understood. Some studies have shown no alteration (Cooper et al. 1986), increase (Tonkonogi et al. 1997; Leek et al. 2001) or even decrease (Ji et al. 1988) in CS activity in muscles extracted either from rodents or humans submitted to a single session of exercise. Here, we investigate the acute effects of muscle contractions on CS activity in rat skeletal muscle. We used male Albino rats (Wistar strain) weighing 250-275 g. The animals were anaesthetized by intraperitoneal injections of chloral hydrate (10% solution, 400 µl/100 g body wt). Subsequently, the hindlimbs muscles were exposed and subjected to a protocol of electrical stimulation (n = 8). The protocol consisted of supra-maximal contractions (32 V) of 200 ms delivered every 1 s for 60 min (Wojtaszewski et al. 1997). The impulse frequency and duration within the contractions were of 100 Hz and 0.1 ms, respectively. One hindlimb side was stimulated to contract and the contralateral non-stimulated hindlimb that had their soleus muscles extracted for the determination of CS activity. We also had another group of non-stimulated anaesthetized rats (n = 7) that served as control. After the experiment, all animals were humanely killed. CS activity (mean ± S.E.M.) significantly increased by 62.2% in the stimulated soleus muscles (357 ± 24 nmol min⁻¹ mg⁻¹) and a surprising increase of 53.6% was also found in the contralateral non-stimulated soleus muscle (338 ± 24 nmol min⁻¹ mg⁻¹) compared to non-stimulated rats (220 ± 22 nmol min⁻¹ mg⁻¹). In order to elucidate if the contralateral increase in CS activity was caused by a neural mechanism, a second experimental group (n = 5) with the sciatic nerve sectioned (ipsilateral) while the opposite (contralateral) was maintained under resting conditions and used as a control. Rats were kept anaesthetized throughout the experiment by supplementing 200 µl of chloral hydrate every 30 min. After 1 h, both the stimulated and contralateral hindlimbs had their soleus muscles extracted for the determination of CS activity. We also had another group of non-stimulated anaesthetized rats (n = 7) that served as control. After the experiment, all animals were humanely killed. CS activity (mean ± S.E.M.) significantly increased by 62.2% in the stimulated soleus muscles (357 ± 24 nmol min⁻¹ mg⁻¹) and a surprising increase of 53.6% was also found in the contralateral non-stimulated soleus muscle (338 ± 24 nmol min⁻¹ mg⁻¹) compared to non-stimulated rats (220 ± 22 nmol min⁻¹ mg⁻¹). In order to elucidate if the contralateral increase in CS activity was caused by a neural mechanism, a second experimental group (n = 5) with the sciatic nerve sectioned only in the contralateral hindlimb was investigated. Interestingly, it was found that denervation abolished the ipsilateral-contraction-induced increase in CS activity in the contralateral limb. Taken together these data provide evidence for a novel mechanism involving neural regulation of CS activity in skeletal muscle.


The authors are indebted to the technical assistance of J. Mendonca, G. de Souza and E. Portioli. This study was supported by CNPq.

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

Skeletal muscle ATP turnover is elevated at higher muscle temperatures during the development of maximal power output in humans

S.R. Gray, G. De Vito, M.A. Nimmo and R.A. Ferguson

Department of Applied Physiology, University of Strathclyde, Glasgow, UK

Passively elevating muscle temperature increases the maximal power output developed during cycle exercise (Sargeant et al. 1987). This is probably due to a greater rate of cross-bridge cycling that will require a greater rate of ATP turnover (Edwards et al. 1972). Such findings have not, however, been demonstrated in humans. The aim of this study was to investigate the effect of elevated muscle temperature on the rate of skeletal muscle ATP turnover during the development of maximal power output. Following local ethics committee approval six male subjects (age 25 ± 2 years, height 182 ± 3 cm, mass 77.1 ± 4.5 kg; means ± S.E.M.) volunteered to perform a 6 s maximal sprint on a mechanically braked cycle ergometer under conditions of normal (N) and elevated muscle temperature (ET). Muscle temperature (Tm) was elevated through passive heating of the legs. Prior to exercise, Tm of the vastus lateralis was measured at a depth of ~2-3 cm using a flexible thermistor. Biopsies were obtained from the vastus lateralis muscle before and immediately after each sprint and analysed for content of ATP, PCr and lactate from which anaerobic ATP turnover was calculated (see Spriet, 1995). Temperature co-efficient (Q10E) values were calculated for power output variables and ATP turnover. Statistical analyses were performed using two-way repeated measures ANOVA and paired t tests where appropriate.

Tm was 3.0 ± 0.3°C higher in ET compared with N (P<0.05).

Maximal power output, corrected for flywheel inertia (Lakomy, 1986) and pedal rate both increased by 258 ± 110 W (Q10E of 2.2) and 22.6 ± 6 rev min⁻¹ (Q10E of 1.6) respectively with elevated temperature (P<0.05). During exercise the rate of ATP turnover for both PCr hydrolysis and glycolysis were greater during ET (Q10E of 3.8 and 1.7, respectively; P<0.05). Consequently, the rate of anaerobic ATP turnover was greater during ET compared with N, with a Q10E of 2.9; Table 1; P<0.05).

We have demonstrated that the higher power output obtained with passive heating of the muscles was achieved at least partly through an increased rate of anaerobic ATP turnover.

Table 1: Rates of anaerobic ATP turnover during normal (N) and elevated (ET) temperature

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr ATP turnover</td>
<td>5.4 ± 0.3</td>
<td>7.9 ± 0.3*</td>
</tr>
<tr>
<td>Glycolytic ATP turnover</td>
<td>4.1 ± 0.4</td>
<td>4.8 ± 0.5*</td>
</tr>
<tr>
<td>Anaerobic ATP turnover</td>
<td>10.8 ± 0.8</td>
<td>14.6 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. (n=6). Significant differences between conditions denoted by * (P<0.05). Values are expressed as mmol.kg⁻¹ (dm)s⁻¹.
70 μM caffeine enhances isolated mouse extensor digitorum longus muscle power output

R. James¹, T. Kohlsdorf², V. Cox¹ and C. Navas³

¹Physiology and Sport Science, Coventry University, Coventry, UK and ²Departamento de Fisiologia, Universidade de Sao Paulo, Sao Paulo, Brazil

Caffeine ingestion by human athletes has been found to improve endurance performance primarily by acting via the central nervous system as an adenosine receptor antagonist (Graham, 2001). After caffeine ingestion the maximum normal concentration of caffeine in human blood plasma is 70 μM and a few studies have implied that such a concentration may directly affect skeletal muscle causing enhanced force production in both long term and short term activities (Graham, 2001). Our aim was to determine whether micromolar levels of caffeine enhance force production in isolated mammalian muscle. Eight- to ten-week-old female CD1 mice (Mus musculus) were humanely killed. An extensor digitorum longus muscle was isolated from each mouse and attached to a force transducer and a motor arm. The work loop technique was used to subject muscle preparations to cyclical sinusoidal length changes equivalent to 10% of resting muscle fibre length at a cycle frequency of 5 Hz (James et al. 2004). Electrical stimulation conditions were optimised to produce maximal work. Every 10 min for 130 min the muscle was subjected to three work loop cycles. For the first 30 min of this experiment the muscle was bathed in oxygenated (95% O₂-5% CO₂) Krebs-Henseleit solution at 35°C. The muscle was then subjected to a 60 min incubation in Krebs solution containing 70 μM caffeine, before a 40 min washout period in standard Krebs solution (Fig. 1). Each muscle was used as its own control by fitting a first order regression line through the power output data gained while in standard Krebs solution (excluding the first wash-out data point) and calculating power output during incubation as a percentage of the value indicated by the regression line. Single factor ANOVA was used to investigate the effect of caffeine on force and power using arcsine transformed data as the dependent variable, treatment as the independent variable and time as a fixed factor. Least significant difference pair-wise multiple comparisons were used as post-hoc tests.

70 μM caffeine caused a small but significant increase (2-3%; Fig. 1; P<0.05) in net power output produced between 30 and 60 min of caffeine incubation. This increase in power output was caused by an increase in force produced during the work loop. Our results indicate that micromolar levels of caffeine can directly enhance force and power output in mammalian skeletal muscle. These findings potentially confirm previous in vivo studies using humans that implied caffeine ingestion may cause acute improvements in muscle force and power (Graham, 2001).

Figure 1. Effect of 70 μM caffeine on mouse extensor digitorum longus muscle power output. Values represent mean ± SD. n = 8. *P<0.05 for post hoc test.


Where applicable, the experiments described here conform with Physiological Society ethical requirements.
Tension responses to ramp shortening in tetanised intact muscle fibres of the rat

H. Roots and K. Ranatunga

Physiology, University of Bristol, Bristol, UK

In our previous studies, we used after-loaded and isotonic release techniques to determine the force-shortening velocity relation in rat muscle fibres at different temperatures (Ranatunga, 1984). However, the shortening velocities were determined only for loads of up to 50-60% of the isometric force. In order to determine a fuller force-shortening velocity relation in rat muscle, as has been done in frog and fish fibres (Lannergren, 1978; Altringham & Johnston, 1982; see refs in Julian et al. 1986), we have now begun a study using ramp shortening.

Adult male rats (~250 g) were humanely killed. Small bundles (~5 fibres) were isolated from the flexor hallucis brevis (a fast muscle) of the foot and mounted horizontally in Ringer solution between a force transducer and servo-motor at 20°C. Initial fibre length (L₀) was ~2 mm and sarcomere length was ~2.5 μm. A fibre bundle was tetanized and on the tension plateau a ramp shortening up to 20% L₀ in amplitude was applied at different velocities (0.01 to ~5 L₀ per second), and the tension decline that continues until the end of the ramp.

Figure 1A and 1B show some experimental records from two separate preparations, in each of which the tension responses to three shortening velocities are shown. A ramp shortening caused a rapid initial decrease in tension followed by a steady level in A, as expected. Whereas in B the initial decrease was followed by a slow decrease that continued till the end of the ramp shortening and did not reach a steady level of shortening velocity. Preliminary analyses have shown that the tension level at the end of the initial rapid tension decline could be used to obtain the force-shortening velocity curve; the maximal shortening velocity was 3.87 ± 0.45 (mean ± s.e.m., n=6), comparable to previous estimates for rat fast muscle (see Ranatunga, 1984). The reason for the two types of behaviour, A (n = 2) and B (n = 7), remains unclear. The cause of the continued slow tension decrease during ramp shortening (as seen in B) seems to suggest the occurrence of shortening-deactivation in some tetanized rat muscle fibres; indeed, shortening deactivation has been shown to occur in other preparations but typically with submaximal activation (Edman, 1975; Julian et al. 1986).

Figure 1. Tension responses (upper traces) to three ramp shortening velocities (lower traces) from two experiments (A and B) at 20°C; a bundle was held isometric for ~0.3 to 0.4 s before a ramp shortening was applied. An isometric tetanus was superimposed in each case. Note that in A there is a rapid decrease in tension to a steady level, as expected, whereas in B the initial rapid decrease is followed by a slow tension decline that continues until the end of the ramp.

Supported by the Wellcome Trust.

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

Fibre length and stimulation scheme associated changes in muscle fibre fatigability

N. Radicheva1, K. Mileva1 and M. Vydevska-Chichova2

1Academy of Sport, Physical Activity and Well-being, FESBE, London South Bank University, London, UK and 2Department of Excitable Structures, Institute of Biophysics, Bulgarian Academy of Sciences, Sofia, Bulgaria

Muscle fatigue was studied during electrically stimulated long-lasting activity of single fibres isolated from m. gastrocnemius of humanely killed Rana ridibunda. The experimental protocol assessed individual and combined contribution of fibre stretch and stimulation frequency for fatigue-induced alterations in extracellular action potential (ECAP) parameters on different muscle fibre types.

Fibre activity was induced via 5 or 10 Hz frequency suprathreshold stimulation, applied for 180 s. The fibres were stretched by 25 or 35% from the initial length (L₀), determined as the length at which maximal twitch was evoked. ECAPs were recorded via pairs of electrodes with a fixed interelectrode distance, situated parallel to the fibre axis and close (15-20 μm) to the membrane, distant from the stimulating electrode and the fibre end. The rates of percentage change in propagation velocity of excitation (PV), ECAP time parameters and median frequency (MDF) of ECAP power spectrum were calculated for the period of uninterrupted activity (endurance time, ET). Non-parametric k-independent samples Kruskall-Wallis H test and 2-independent-samples Mann-Whitney U post-hoc test were applied to identify the statistical significance of main and interaction effects of fibre length and stimulation frequency. Paired Wilkoxon signed rank test was used to test the significance of fatigue induced parameter changes.

ET was followed by an interrupted activity period consisting of alternating action potential propagation failure and restoration. This pattern is dependent on fibre type and experimental protocol, thus fibres were classified as slow (SMF) or fast (FMF) fatigueable according to the rate of PV decrease at L₀ and 5 Hz-stimulation (Radicheva et al. 1998), and by histochemical assessment of their myosin ATPase activity. ET decreased (up to 92%) and the rates of parameter changes increased (>200%) significantly with fibre stretch and faster stimulation in both fibre types. At lower stimulation frequency and fibre stretch the rates of parameter changes of SMFs were less than those of FMFs, while simultaneous fibre stretch and higher stimulation frequency induced
lower rates of parameter changes in FMFs compared to SMFs (Fig. 1).
ECAPs reflect the changes in intracellular action potentials, which are dependent on the ionic conductance of the muscle fibre membrane. The differential effects of stimulation frequency and fibre stretch during fatigue on electrical activity of different muscle fibre types were most likely due to differences in the membrane properties of the fibres.

Figure 1. Rates of PV percentage change during ET at 3 fibre lengths and 2 stimulation frequencies; (mean ± S.E.M., n=24, *p<0.05 vs. L0, stimulation frequency effect †p<0.05 vs. 5 Hz).

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