PC103

Tension responses to ramp shortening in tetanized rat muscle fibres: effects of caffeine

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We previously reported that the tension response to a 20% ramp shortening consisted of an initial fast tension decline followed, typically, by a slow tension decline; the tension at the transition between the two phases (P_2) could be used to construct the force – shortening velocity curve (Roots & Ranatunga, 2005). The cause of the continued slow tension decline after P_2 transition, however, was not clear. Thin filament deactivation has been shown to occur during and after shortening but typically with sub-maximal activation (Edman, 1975 and refs therein). In skinned fibres, the increase of calcium level abolished this type of deactivation (Eklund & Edman, 1982) and, in intact fibres, such deactivation was reduced by caffeine (Edman, 1980). Millimolar caffeine is known to increase Ca^{2+} release and myofilament Ca^{2+} sensitivity; therefore, we have now examined the effects of caffeine on the tension response to ramp shortening in tetanized rat muscle fibres.

Adult male rats (~250 g) were terminally anaethetised; small bundles (~5 fibres) were isolated from the flexor hallucis brevis (a fast muscle) of the foot and mounted horizontally between a force transducer and servo-motor at 20 ºC. Initial fibre length (L_0, ~2 mm) was set for maximal tetanic tension; the sarcomere length was ~2.5 μm. Experiments were done when the fibre bundle was immersed in normal Ringer solution (control) and in Ringer containing 2.5–5 mM caffeine. A fibre bundle was tetanized and, on the tension plateau, a ramp shortening (up to 20% L_0 in amplitude and at velocities of 0.01 to ~5 L_0/s) was applied and the tension changes monitored.

With 2.5–5.0 mM caffeine, the twitch tension was potentiated (>75%) indicating an increase in $[Ca^{2+}]$ release on activation; however, the tetanic tension was not significantly changed. In experiments on 5 fibre bundles, the mean (± s.e.m.) tetanic tension (in kN/m^2) was 236 (± 24.4) in control solution before exposure to caffeine and it was 233 (± 27.1) after recovery; the tension in the presence of caffeine was 234 (± 25.7) and the differences were not significant (P>0.7, paired t test). The continued slow tension decline during ramp shortening was seen in the presence of caffeine; with increase of shortening velocity, the rate of tension decline increased to a similar extent both in the presence of caffeine and in the control condition. When the force–shortening velocity curves were constructed using the tension level at the P_2 transition and analysed using Hill equation, the V_{max} (in L_0/s) was 3.28 (± 0.32) before caffeine and 3.23 (± 0.44) after recovery from caffeine: the V_{max} with caffeine (3.33 ± 0.59) was not significantly different (P>0.6). There was also no significant difference in the a/P_0 ratio (Hill's equation) indicating the curvature of the relation was similar between control and caffeine data. Thus, our data suggest that the tension response during ramp shortening is not significantly altered by caffeine; preliminary findings indicate that the tension decline is not seen at fibre lengths >15% longer than L_0.

Edman KAP (1975). J Physiol 351, 517-529.

Edman KAP (1980). Acta Physiol Scand 109, 15-26.

Ekelund MC & Edman KAP (1982). Acta Physiol Scand 116, 189-199.

Roots HR & Ranatunga KW (2005). J Physiol 567P, PC80.

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

PC104

Expression of *RND2* and *RND3* mRNA in human myometrial and placental tissues

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RHO GTPases are key regulators of the actin cytoskeleton and stress fibre formation in smooth muscle. In human myometrium, activated RHOA forms a complex with RHO-kinase (ROCK) which phosphorylates and inhibits myosin phosphatase (MYPT), causing a calcium-independent increase in myosin light chain phosphorylation and tension (Ca^{2+} sensitization) [1,2]. We have recently reported that new family small GTP binding proteins, RND proteins, can inhibit RHOA-ROCK interaction to reduce Ca^{2+} sensitization [3]. There is very little information regarding the mRNA expression of RND proteins in human myometrial or placental tissues.

We aim to quantify the relative expression of RND mRNA in non-pregnant, pregnant myometrial and placental tissues using quantitative RT-PCR. mRNA will be extracted from five independent myometrial and placental tissues and differential expression of RND mRNA will be determined using SYBR green detection and normalised to RNA polymerase II (house keeping gene) expression in NP tissue.

RND2 and *RND3* mRNA were expressed in both non-pregnant and pregnant myometrium, and although there was an increasing trend in mRNA expression in pregnant myometrium relative to non-pregnant myometrium, this was not significant by one-way ANOVA analysis (p> 0.05). This is in marked contrast to previous reports demonstrating significant increases in RND protein expression in pregnancy[3]. *RND2* and *RND3* mRNA were also expressed in placental tissues. Further work is needed to explore the regulation of the expression on RND GTPases in human myometrium.

Somlyo AP & Somlyo AV (1994). Nature 372, 231-236.

Moore F, Da Silva C, Wilde JI, Smarason A, Watson SP & Lopez Bernal A (2000). Biochem Biophys Res Commun 269, 322-326.

Lartey J, Gampel A, Pawade J, Mellor H & Lopez Bernal A (2006). Biol Reprod, DOI: biolreprod.105.049130.

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PC105

Characterisation of a novel endosomal RHO GTPase family member RHOB, and its effectors DIAH1 and DIAH2 in human myometrium

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RHO GTPases are small monomeric proteins that play a key role in the regulation of the actin cytoskeleton. In human myometrium, the RHOA–RHO kinase pathway in response to oxytocin and other stimulatory agonists can inhibit myosin light chain phosphatase to produce a calcium-independent increase in myosin light chain phosphorylation and tension [1,2]. Transfection of activated RHOA and RHOB GTPases into quiescent fibroblasts induces the formation of actin stress fibres and adhesion complexes [3,4]. Thus RHOB is a potential regulator of smooth muscle contraction.

We aim to quantify the presence and localisation of RHOA and RHOB GTPases in non-pregnant (NP), pregnant at term not in labour (NIL), pregnant at term in labour (SL) and spontaneous preterm labouring human myometrium using immunoblotting and immunohistochemistry. Four individual samples from each group will be analysed. Expression of their related effector proteins (DIAPH1, DIAPH2, ROCK1 and ROCK2) will be determined using immunoblotting and densitometry. mRNA will be extracted from myometrial tissue, differential expression of RHOA and RHOB mRNA will be determined using SYBR green detection and normalised to RPII RNA polymerase II (house keeping gene) expression in NP tissue.

RHOA and RHOB GTPase protein expression was similar in the NP, NIL, SL and SPT samples. DIAPH1, DIAPH2, ROCK1 and ROCK2 were also expressed in myometrial tissues with no change in expression between the four groups of samples examined. There was a two-fold increase in RHOA and RHOB mRNA expression in pregnant relative to non-pregnant myometrium; however, this was not significant by analysis by ANOVA (RHOA 95% CI -0.2029 to 2.906 ($p>0.05$); RHOB 95% CI -0.4570 to 2.652 ($p>0.05$). Immunohistochemical analysis from the four individual samples revealed that RHOB GTPase has a cytoplasmic distribution in myometrial cells, with staining localised to the perinuclear region in endometrial cells.

To our knowledge, this is the first time RHOB GTPase mRNA and protein expression has been described in human myometrium. This is also the first characterisation of known effectors of RHOB (mDia1 and mDia 2) in human myometrial tissue samples. RHOA and RHOB GTPase protein expression is similar in non-pregnant, pregnant preterm and term labouring myometrium. We aim to further explore RHOB GTPase function in myometrial contraction using agonist stimulation and pull down assays.

Moore F, Da Silva C, Wilde JI, Smarason A, Watson SP & Lopez Bernal A (2000). Biochem Biophys Res Commun 269, 322-326.

Friel AM, Curley M, Ravikumar N, Smith TJ & Morrison JJ (2005). J Soc Gyneco Investig 12, 20-27.

Hall A (1998). Science 279, 509-514.

Ridley AJ (1996). Curr Biol 6, 1256-1264.

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PC106

Effect of streptozotocin-induced diabetes on contraction and cation levels in isolated rat soleus and EDL skeletal muscles

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Two major long-term complications of Diabetes mellitus (DM) are neuropathy and skeletal atrophy which decrease the quality of life for the diabetic patients. This study investigated the effect of streptozotocin (STZ)-induced DM on EDL and soleus skeletal muscle contraction and on sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), zinc (Zn^{2+}), iron (Fe^{2+}) and copper (Cu^{2+}) levels in the two muscle types of male Wistar rats compared to age-matched controls. DM was induced by a single (i.p.) injection of STZ (60 mg kg^{-1}). Control rats received an equivalent volume of citrate buffer. DM was confirmed 4-5 days after STZ injection and 6-8 weeks later using a glucose meter. Contraction was measured using an isometric transducer and the muscles were stimulated electrically (EFS) at an amplitude of 50 V, 1 ms pulse duration and at 1-100 Hz. At the end of the experiment, each muscle was blotted, weighed and dissolved in concentrated nitric acid. Cation contents in the muscles were measured using an atomic absorbance spectrophotometer. Data are presented as mean ± SEM, n= 8-10 rats for each parameter. Prior to STZ injection, the rats weighed 161.13±1.41 g, (n=22). Diabetic rats gained significantly ($p<0.05$) less body weight (222.19±10.51 g) and muscle weights (EDL; 0.17±0.02 g; soleus 0.17±0.02 g) compared to age-matched control body (501.83±25.91 g) and EDL (0.21±0.05 g) and soleus (0.23±0.02 g) weights. Diabetic rats also have significantly ($P<0.05$) elevated blood glucose (29.65±0.33 mM) and significantly ($p<0.05$) reduced plasma insulin level (4.81±1.28 mg dl^{-1}) compared to control (glucose, 6.06±0.21 mM; insulin, 20.63±7.52 mg dl^{-1}). EFS evoked frequency-dependent (10-100 Hz) contraction in both soleus and EDL muscles of healthy control and diabetic rats with a maximum response at 40-50 Hz. Higher frequencies caused a small reduction in contraction. The force of contraction was significantly ($P<0.05$) elevated in EDL muscle (4.39+0.36 N cm^{-2}, n=8) compared to soleus muscles (1.53±0.26 N cm^{-2}, n=8) at 50 Hz. In both diabetic EDL (1.19±0.05 N cm^{-2}, n=8) and soleus muscles (0.31±0.01 N cm^{-2}, n=8), the force of contraction was significantly ($P<0.05$) reduced compared to the respective control at 50 Hz. The levels of total Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+} in both muscles were significantly ($P<0.05$) reduced in diabetic rats compared to control animals. Typically, the levels of Na^+, K^+, Mg^{2+} and Ca^{2+} in control EDL muscle were 0.432±0.014, 0.121±0.004, 0.037±0.002 and 0.025±0.0007 mM (100 mg tissue)$^{-1}$ compared to 0.210±0.004, 0.071±0.001, 0.022±0.0004, 0.005±0.0008 mM (100 mg of tissue)$^{-1}$ in diabetic EDL muscle, n=8-10. Similarly, levels of Na^+, K^+, Mg^{2+} and Ca^{2+} in control soleus muscle were 0.351±0.023,

0.131±0.008, 0.029±0.002 and 0.023±0.0005 mM (100 mg tissue)$^{-1}$ compared to 0.100±0.001, 0.03±0.001, 0.004±0.0007, 0.003±0.0004) mM (100 mg of tissue)$^{-1}$ in diabetic soleus, n=8-10. The results indicate that diabetes is associated with significant reductions in the force of contraction and cation contents in EDL and soleus muscles. The reduced cation contents in turn may be responsible for the development of muscle atrophy and reduced force of contraction since they are associated with electrical activities and cell signalling processes in skeletal muscles.

Kumar PJ & Clark M (2002). Textbook of Clinical Medicine, pp. 1099-1129. Saunders, London.

Zimmet P (2000). J Medicine 247, 301-310.

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PC107

Store-operated Ca^{2+} entry following halothane-induced Ca^{2+} waves in mechanically skinned rat muscle fibres

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Recent work on mechanically skinned toad skeletal muscle fibres has shown that depletion of SR Ca^{2+} by 30 mM caffeine can induce store-operated Ca^{2+} entry (SOCE, Launikonis et al. 2003). SOCE was detected as a decreased in the fluorescence of fluo-5N, which was trapped within the t-tubules during skinning and subsequent resealing.

We have shown previously that (i) in human fibres obtained from malignant hyperthermia (MH) susceptible patients, application of 1 mM halothane induces SR Ca^{2+} release, which takes the form of a propagated Ca^{2+} wave and (ii) similar Ca^{2+} waves can be induced in normal muscle by application of halothane in the presence of reduced cytosolic [Mg^{2+}] (Duke et al. 2004). In this study, we have investigated whether SOCE occurs as a consequence of halothane-induced Ca^{2+} waves.

Rats (200-250 g) were humanely killed. Single extensor digitorium longus (EDL) muscle fibres were mechanically skinned under oil and then perfused with solutions approximating to the intracellular milieu. Exposure of skinned fibres to 50 μM Fluo-5N (pentapotassium salt) resulted in a progressive increase in fluorescence and the emergence of a characteristic sarcomeric pattern (Fig. 1 left), detected using confocal microscopy. Subsequent treatment with saponin abolished the sarcomeric pattern, suggesting that the dye accumulates within the resealed t-system, rather than the SR (not shown). Similar results were obtained in 6 other preparations.

In the presence of 0.1 mM [Mg^{2+}], introduction of 0.2 mM halothane resulted in a Ca^{2+} wave, apparent as localised sarcomere shortening (not shown). The wave was followed by a sustained decrease in fluo-5N fluorescence, consistent with Ca^{2+} depletion of the resealed t-tubules (Fig. 1 right). Similar results were obtained in 6 other preparations. The t-tubules re-loaded with Ca^{2+} over 5-10 min and this process could be accelerated by raising the cytosolic [Ca^{2+}] (not shown).

In conclusion, the entry of fluo-5N into the resealed t-tubules may occur via anion transporters, which have been implicated in extrusion of fluorescent dyes from the cytosol in intact cells. The decrease in fluo-5N fluorescence following the halothane-induced Ca^{2+} wave suggests that SR Ca^{2+} depletion is sufficient to activate SOCE. If SOCE occurs during MH, then Ca^{2+} entering the cell via this mechanism might contribute to the sustained activation of the myofilaments.

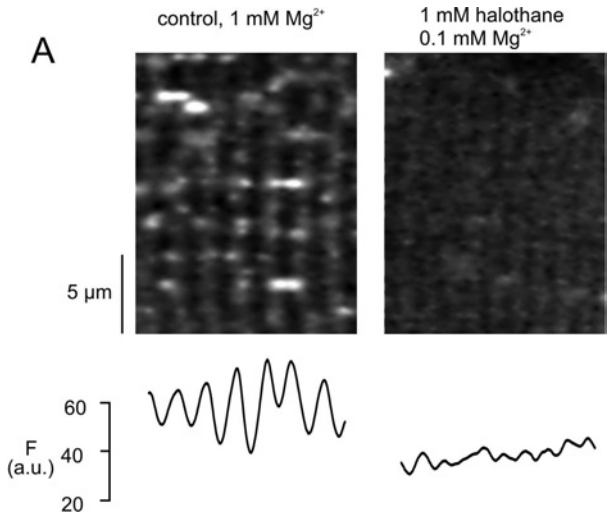

Figure 1

Confocal image of an EDL fibre after 15 min exposure to fluo-5N (left). The same fibre is shown 1 min after addition of 0.1 mM Mg^{2+}, 0.1 mM halothane (right).

Launikonis BS et al. (2003). Proc Natl Acad Sci U S A 100, 2941-2944.

Duke AM et al. (2004). Anesthesiology 101, 1339-1346.

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