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Saline tonicity affects cross-bridge structure during isometric tetani

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In skeletal muscle cells, a synchronised myosin S1 power stroke causes a change in intensity (I_{M3}) of the meridional X-ray reflection at 14.5 nm, which is thought to indicate both elastic and active changes in the myosin lever arm tilt. During sinusoidal length oscillations (0.1–1 kHz), I_{M3} signals are approximately sinusoidal and in phase opposition to fibre length, but are distorted during shortening by the passage of I_{M3} through an intensity maximum ($I_{M3\,max}$), forming a secondary I_{M3} minimum at the point of maximum shortening. The extent of shortening (Δy) required to reach $I_{M3\,max}$ has been shown to depend upon the force per cross-bridge (P_{S1}), when isometric tetanic tension is varied by temperature change (Griffiths *et al.* 2002). However, since temperature effects on relaxed I_{M3} (i.e. unrelated to the power stroke) have been reported (Lowy *et al.* 1991), we have now examined the effect of Ringer's tonicity on Δy, which is an alternative method of varying P_{S1}.

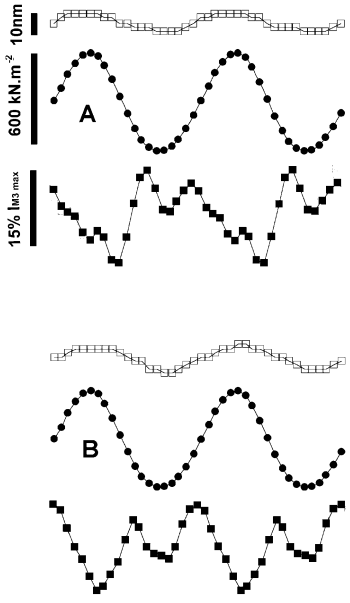

Figure 1. I_{M3} (■), sarcomere length (□) and force (●) signals during 1kHz sinusoidal length oscillations. Panel *A*, 1.4x standard tonicity; panel *B*, standard tonicity. Temperature 12°C.

Intact fibre bundles from tibialis anterior muscles of *Rana temporaria* (sacrificed by decapitation) were mounted horizontally between a moving coil motor and a capacitance force transducer. Synchrotron radiation (source Elettra, Trieste, Italy; wavelength 0.15 nm, dimensions 0.3 × 3.0 mm) was admitted to the experimental chamber by a fast shutter mechanism at the plateau of an isometric tetanic contraction, while sinusoidal length oscillations were imposed simultaneously at 1 kHz. I_{M3} was monitored by a 1D delay line detector at 2.6 m from the preparation, with a sampling time resolution of 50 μs (Fig. 1). Bundles were exposed to 0.8 or 1.4 fold standard Ringer's tonicity by variation of [NaCl]. Hypertonicity reduced P_{S1} by 17 % and increased Δy by 0.15 nm (12 °C); hypotonicity increased P_{S1} by 12 % and reduced Δy by 0.24 nm (2 °C). These findings are consistent with a P_{S1} dependence of S1 lever arm tilt, but indicate a less steep dependency under hypertonic conditions than that observed by temperature variation.

Griffiths PJ *et al.* (2002). *Proc Nat Acad Sci* **99**, 5384–5389.
Lowy J *et al.* (1991). *Biophys J* **60**, 812–824.

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All procedures accord with current national and local guidelines

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Activated signal transduction pathways in rat extensor digitorum longus after six weeks of electrical stimulation

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Chronic electrical stimulation leads to a fast-to-slow phenotype transformation and reduces muscle mass in rat extensor digitorum longus muscle (EDL). These changes are likely to result from the activity of signal transduction pathways that are activated by contraction and regulate the adaptive process. The aim was to characterise signal transduction pathway activation in rat EDL after 6 weeks of electrical stimulation when signalling maintains a slower, transformed phenotype and lower muscle mass.

We measured the phosphorylation of proteins involved in the regulation of motor proteins (extracellular signal regulated kinase, ERK1/2), mitochondrial biogenesis (AMP-activated kinase, AMPK; p38 kinase, p38), translational signalling (protein kinase B, PKB; glycogen synthase kinase, GSK3beta; 4E-binding protein, 4E-BP1; p70 S6 kinase, p70 S6k), myostatin growth regulation (myostatin; SMAD2/3) and ribosome biogenesis (upstream binding factor, UBF). Miniature stimulators were implanted into the peritoneal cavity under isoflurane/nitrous oxide anaesthesia with their integral electrodes placed close to the common peroneal nerve. Intramuscular buprenorphine (0.1-0.5 mg/kg) was used to provide post-operative analgesia. Rat EDL muscles were chronically stimulated at 10 Hz for 6 weeks. Rats were killed humanely and both EDLs were removed and quickly frozen in liquid nitrogen. Protein and phospho-protein levels were measured by densitometry of Western blots.

Electrical stimulation increased the phosphorylation of ERK1 5.80 ± 5.91-fold, p38 4.31 ± 1.82-fold and c-jun kinase (JNK) mitogen-activated protein kinase 1.99 ± 0.71-fold whereas the phosphorylation of ERK2 decreased to 0.60 ± 0.21 of control. It is unclear how ERK1 activation and ERK2 inhibition affect motor protein isoform expression. AMPK and p38 phosphorylation increased 3.78 ± 2.45 and 4.31 ± 1.82-fold, respectively, which is consistent with the proposed involvement of AMPK and p38 in mitochondrial biogenesis. AMPK activation can also explain the decrease in phosphorylation of the translational activator p70 S6k to 0.33 ± 0.08 of control (Bolster *et al.* 2002). Myostatin increased 2.68 ± 1.32-fold and increased Smad2 and 3 phosphorylation by 2.74 ± 1.63 and 2.24 ± 0.86, respectively, which suggests atrophy signalling. UBF content increased to 2.05 ± 0.09 of control which is consistent with

increased (r)RNA synthesis and ribosome biogenesis seen in chronically stimulated muscle (Cummins & Salmons, 1999). However, UBF phosphorylation was only 0.83 ± 0.10 of control.

Bolster *et al.* (2002). *J Biol Chem* **277**, 23977–23980.
Cummins B & Salmons S (1999). *Basic Appl Myol* **9**, 19–28.

All procedures accord with current UK legislation