Contraction-induced changes to intracellular signalling in skeletal muscle

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The underlying mechanisms that trigger skeletal muscle adaptations to acute exercise are not well understood. The large mass and intrinsic metabolic capacity of skeletal muscle make it an important tissue in the maintenance of whole-body metabolic homeostasis. Skeletal muscle contraction is associated with increases in $[Ca^{2+}]_i$, energy use and force production. These stimuli are thought to activate intracellular signalling pathways that have downstream effects on gene and protein expression, thus promoting changes to muscle phenotype (Sandström *et al.*, 2007). The aim of this study was to utilize a potent inhibitor of cross-bridge cycling (in fast-twitch muscle), *N*-benzyl-*p*-toluenesulphonamide (BTS), to separate the influences of Ca^{2+}, energy usage, and mechanical force production during muscle contraction, on contraction-sensitive signalling proteins that have downstream effects on gene and protein expression.

Sprague Dawley rats (5-6 weeks of age) were anaesthetised deeply with pentobarbital sodium (60 mg/kg i.p.). *Extensor digitorum longus* (EDL) muscles were carefully excised and either incubated for 1 h in 500 μM BTS or vehicle control (DMSO). Rats were killed by cardiac excision. Following incubation in either BTS or vehicle, muscles underwent a fatigue protocol of 1 tetanic contraction every 5 s for 5 min at a frequency of 80 Hz. Fatigued muscles were either frozen immediately in liquid nitrogen or incubated in Ringer solution for 3 h post-fatigue for later determination of phosphorylated and total levels of calcium/calmodulin-dependent protein kinase isoform II (CaMKII), adenosine monophosphate-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK). These kinases are known to play roles in promoting skeletal adaptations to activity.

Phospho-AMPK levels were elevated from basal immediately post-contraction in control groups but not in BTS-treated muscles, indicating that cross-bridge cycling does influence phosphorylation of AMPK. Phospho-CaMKII levels were elevated from basal immediately post-contraction and three hours post-contraction in both groups ($p < 0.05$). The sustained elevations in phospho-CaMKII support previous studies that implicate the Ca^{2+} signal as an initiator of CaMKII activity. Phospho-p38 levels were unchanged from basal immediately post-contraction in both groups. Phospho-p38 was not different from basal in the BTS group at 3 h post-contraction but was increased significantly in the DMSO group.

These findings contribute to our understanding of the underlying mechanisms of exercise-associated metabolic improvements in skeletal muscle, revealing roles for calcium, energy usage, and force production. Specifically, energy and cross-bridge cycling mediate changes in AMPK and p38, and calcium signals mediate changes in CaMKII. Ultimately, the elucidation of pathways involved in improving skeletal muscle metabolism may allow us to identify novel drug targets or design specific exercise programs to assist in maintaining control of whole-body metabolic homeostasis.

Sandström ME, Zhang SJ, Westerblad H & Katz A. (2007) *Journal of Physiology* **579:** 527-34.

This work was supported by grants from the Australian Research Council and the National Health & Medical Research Council.