STRESS-DEPENDENT AND STRESS-INDEPENDENT GENE EXPRESSION IN RAT SKELETAL MUSCLE AFTER A SINGLE BOUT OF “EXERCISE”

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INTRODUCTION

The mechanical factors that affect skeletal muscle gene expression are largely unknown. Skeletal muscle tissue affords the unique opportunity to investigate the role of mechanical events on signaling and gene expression. Because muscle has well-defined contractile properties during isometric (IC) or eccentric (EC) modes (Close, 1972) and because muscle stress increases as a function of frequency (Rack & Westbury, 1969), it is possible to combine frequency and contraction mode in such a way as to vary the levels of stress and injury on the muscles. This approach permits investigation of the relationship between muscle mechanics during exercise, injury (if any), and gene expression.

METHODS

Experimental subjects were adult male Sprague-Dawley rats (n=40). All procedures were approved by the local Committees on the Use of Animal Subjects in Research. Five experimental groups were studied composed of rats exposed to ECs at either 40 Hz, 100 Hz or 150 Hz (EC40, EC100 and EC150) and those exposed to isometric contractions at either 40 Hz or 150 Hz (IC40 and IC150). Exercise consisted of 30 isometric or eccentric contractions of ankle dorsiflexors, with maximal isometric torque recorded before, immediately after and 24 hours after the exercise bout. For muscle stimulation, the peroneal nerve was stimulated for 650 ms once every two minutes. For the isometric group the foot was held stationary. For the eccentric group the foot was plantarflexed ~40° 200 ms after nerve stimulation commenced. During treatment (isometric or eccentric) dorsiflexion torque was measured on-line to infer tissue stress.

Twenty-four hours after the exercise bout, isometric torque was again measured, and the tibialis anterior muscle was harvested and immediately frozen. The quantitative polymerase chain reaction (QPCR) was used to quantify transcript levels of two isoforms of the muscle ankryn repeat protein (MARP) family. This gene family was our focus based on a recent gene profiling study that revealed tremendous upregulation of these genes after a single EC bout (Barash \textit{et al.} 2004).

Analysis of variance (ANOVA; Statview, SAS Institute, NC) was used to compare transcript levels and functional measurements between groups. Statistical significance level ($\alpha$) was set to 0.05, statistical power (1-$\beta$) exceeded 85%, and all values are presented as mean ± SEM.

RESULTS AND DISCUSSION

As expected based on well-known muscle mechanics, peak torque measured during exercise was highest for the EC150 group, intermediate for the EC40 and IC150 groups, and lowest for the IC40 group (Fig. 1A). The EC40 and ISO150 groups were “exercised” at the same torque levels. Similarly, muscle injury, as indicated by the
torque change 24 hours after the exercise bout (Fig. 1B) was highly correlated with peak torque, independent of stimulation frequency and contraction type (Fig. 1B; $r^2=0.72$, $p<0.001$).

In contrast to the consistent effects of peak stress on injury, the MARPs examined showed a distinct pattern of gene expression (Fig. 2). For example, MARP1 was highly correlated with torque, independent of contraction mode (Fig. 2A; $r^2=0.81$, $p<0.001$).

MARP2, on the other hand, was highly elevated in all EC groups and significantly lower in both IC groups, independent of stress (Fig. 2B).

These data demonstrate the rapid and gene-specific response of muscle to stress and/or contraction mode. Thus, skeletal muscle sensors can transduce not only tissue stress, but the nature of the contraction itself. Further studies are required to identify the structures and processes involved in this signal transduction.

**REFERENCES**


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