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TRANSCRIPTIONAL CHANGES IN RAT α-MOTONEURONS RESULTING FROM INCREASED PHYSICAL ACTIVITY

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Abstract—Electrophysiological properties of lumbar α-motoneurons change after chronic increases and decreases in hindlimb neuromuscular activity. Although modeling of these changes suggests that motoneurons probably alter gene expression in these situations, there is no evidence that this is the case. In this study, we measured the content of several mRNAs in lumbar motoneurons, harvested using laser capture microdissection, from rats previously subjected to normal cage activity, voluntary wheel exercise for 16 weeks, and forced treadmill training for 7 days and 16 weeks. As a result of the prolonged daily treadmill training, but not the voluntary wheel training, significant increases occurred in muscle peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α) mRNA, and in muscle PGC-1α and cytochrome oxidase proteins, in soleus and plantaris muscles. Significant changes in mRNA contents (decreases) were evident for the receptors 5-hydroxytryptamine (serotonin) receptor 1A (5HT1a), GABA A receptor, subunit alpha 2 (GABAAα2), and for the potassium conductance calcium-activated channel protein (SK2) in the motoneurons from 16-week-trained rats, and for glutamate receptor, metabotropic 1 (mGluR1) in the voluntary wheel-trained rats. Motoneurons from 16-week treadmill-trained rats also did not demonstrate the decreases in several mRNAs that were evident after 7 days of treadmill exercise, suggesting an adaptation of motoneurons to acute stress. The mRNA changes following prolonged daily treadmill training are consistent with a reduction in inhibitory influences onto motoneurons, and a transition toward motoneurons that innervate slower contracting muscle fibers. These results demonstrate that the previously reported physiological changes in motoneurons with altered activity are accompanied by changes in gene expression.

Key words: α-motoneurons, gene expression, exercise training, laser capture microdissection.

INTRODUCTION

Our previous research has revealed that α-motoneurones respond to increases and decreases in the activity of the organism by alterations in biophysical properties of the motoneurones innervating the hindlimbs, as measured in anesthetized rat preparations (Gardiner et al., 2006). The changes suggest fundamental adaptations in the membrane proteins that subtend these properties. Properties that we have shown to change (either increase or decrease depending on the model of increased or decreased chronic neuromuscular activity) include resting membrane potential (RMP), voltage threshold (Vth), afterhyperpolarization (AHP) amplitude, input resistance, rates of antidromic spike development, rheobase, minimum and maximum firing frequencies, frequency/current slopes, the amplitude of persistent inward currents, and the degree of late adaptation. Those motoneurones that are impacted the most are those whose normal activity shows the greatest change with the intervention. For example, low-threshold hindlimb motoneurones that are normally recruited extensively during normal movements demonstrate the highest levels of change when the animal is subjected to low-intensity voluntary activity, and to removal of weight-bearing. We have modeled these changes using a 5-compartment mathematical simulation of a motoneurone, and found that the results are consistent with changes in specific sodium and potassium conductances, and therefore most likely changes in the number, density, location, tonic activation, and/or voltage responsiveness of the ion channels involved (Gardiner et al., 2006).

In rat thoracic and lumbar spinal cord, 21 days of voluntary exercise results in increased expression of several genes, identified using microarrays. These genes involved "pathways promoting neuronal health, signaling, remodeling, cellular transport, and development of oligodendrocytes" (Perreau et al., 2005). Similar results were obtained using laser capture-harvested lumbar motoneurons from mice subjected to 21 days of voluntary exercise (Ferraiuolo et al., 2009). In rats

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Abbreviations: ATE, acute exercise training; AHP, afterhyperpolarization; BDNF, brain-derived neurotrophic factor; GEA, Gene Expression Assay; LCM, laser capture microdissection; mGluR, glutamate receptor, metabotropic; NT-3, neurotrophin-3; PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; qPCRs, quantitative polymerase chain reactions; RMP, resting membrane potential; TBST, Tris-Buffered Saline with Tween 20; Vth, voltage threshold.

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with a low thoracic spinal transection, passive cycling exercise of the hindlimbs resulted in increased expression of brain-derived neurotrophic factor (BDNF), GDNF, and NT-4, and an attenuation of the transection-induced increase in caspase-3 in laser-captured hindlimb motoneurons, further supporting the potential for activity-associated changes in motoneuronal gene expression (Keeler et al., 2012). Additionally, increased expression of BDNF, neurotrophin-3 (NT-3), TrkB, TrkC, synapsin I, GAP-43 and CREB mRNA, and BDNF, NT-3 and synapsin I protein has been observed in whole lumbar spinal cord homogenates after 7 days of endurance training (Gomez-Pinilla et al., 2001, 2002; Ying et al., 2003). While these relatively short-term changes demonstrate that spinal cord cells are responsive to a change in normal activity levels of the animal, no evidence exists yet that demonstrates changes in gene expression specifically in α-motoneurons, at a time when physiological adaptations of muscle and cardiovascular systems have reached a steady-state level following 12 to 16 weeks of increased daily physical activity, and when biophysical properties of motoneurons have adapted (Beaumont and Gardiner, 2003). The purpose of this study was to examine transcriptional changes in α-motoneurons, harvested using laser capture microdissection (LCM), from the lumbar spinal cords of rats subjected to 16–20 weeks of daily endurance-type treadmill exercise, or 16–20 weeks of voluntary wheel exercise. We targeted genes associated with specific ion channels, receptors, and neurotrophin systems, that we felt could be involved in the expression of meaningful physiological adaptations.

EXPERIMENTAL PROCEDURES

Ethical approval

All procedures were approved by the University of Manitoba (Bannatyne Campus) Animal Care Committee (approval documents available upon request) and were in accordance with the guidelines of the Canadian Council on Animal Care and with the National institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental design

Thirty-six female Sprague–Dawley rats (2 mo old) were obtained from the University of Manitoba (Winnipeg, Canada) and housed in pairs in standard plastic cages located in a temperature controlled room (maintained at 23 °C) with a 12:12-h light:dark cycle. Animals were provided food and water *ad libitum* and were allowed 7 days to adjust to their environment prior to the start of the exercise training protocol. Following the adjustment period, animals were assigned to exercise ($n = 18$) or sedentary control ($n = 18$) groups, and exercise animals were acclimated to treadmill running for 3 days (1 brief, low-intensity training session/day) prior to start of the exercise training protocols. Exercise consisted of three different types, involving three different groups of animals and their respective cage-confined controls. The acute exercise training (ATE) protocol consisted of 1 treadmill running session daily on a motor-driven rodent treadmill for 7 days, with duration and intensity being progressively increased (to a peak of approximately 23 m/min at a 10° incline for 30 min during the final training session). In this and other exercise groups, control animals were handled similar to exercised animals, but not subjected to the specific exercise. All exercised animals were euthanized approximately 4 h after the final training session.

A second group of rats ($n = 6$) was used for the long-term (16–20 weeks) endurance training program. The trained rats began their program as described for the ATE rats, after which time speed and time of treadmill exercise were increased gradually such that, by week 8, rats were running at 27 m/min at a 10° incline for 60 min, twice per day, 5 days per week. After 16 to 20 weeks, rats were euthanized and samples treated as described above.

A third group ($n = 6$) performed daily exercise in voluntary wheels (activity wheel model # 80859, Lafayette Instruments), for a period of 16–20 weeks. Rats were allowed access to the voluntary wheels for 24 h per day. At the end of the experiment, rats were euthanized and samples treated as described above.

Tissue extraction and LCM

Upon completion of the exercise training protocol, animals were euthanized with a combination of isofluorane anesthesia and decapitation. Because of sampling logistics and to attempt to standardize the stress level of the animals, treadmill-exercised animals were euthanized approximately 4 h after the final exercise session. The lumbar enlargement of the spinal cord was immediately removed, placed in a cryomold, covered in Tissue-Tec O.C.T. embedding compound (Gene Research Lab), fresh-frozen in melting isopentane and stored at −80 °C until further processing for LCM. Soleus and plantaris muscles were dissected bilaterally on ice-cold saline, blotted dry, placed in cryogenic vials and stored at −80 °C for future use.

Cross-sections (10 μm) of the lumbar enlargement were cut on a cryostat and mounted on polytetrafluoroethylene-coated (PTFE) glass slides. Prepared slides were either used immediately for LCM or stored at −80 °C for up to 7 days. Slides were immersed in pre-chilled acetone (−20 °C) for 2 min, treated according to the LCM Staining Protocol for Cresyl Violet (Ambion) and dried for 15 min in a vacuum desiccator prior to LCM. Individual motoneuron soma cross-sections were dissected bilaterally with the Arcturus PixCell II LCM System (Molecular Devices) and a total of 700 to 800 α-motoneuron cross-sections per animal (selected based on size and location) were collected on CapSure HS LCM caps. To limit RNA degradation, samples were collected for up to 30 min per slide, after which the LCM caps were placed on a sterile microcentrifuge tube containing 10 ul of lysis buffer (RNAqueous Micro Kit, Ambion) which was inverted to wet the cap, vortexed, and stored upside down for 30 min at 42 °C to aid in tissue digestion.