European Journal of Neuroscience, Vol. 13, pp. 1078–1084, 2001

Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle

F. Gómez-Pinilla, Z. Ying, P. Opazo, R. R. Roy and V. R. Edgerton
Department of Physiological Science and Brain Research Institute, UCLA, Los Angeles, CA 90095-1527, USA

Keywords: activity-dependency, exercise, neuromuscular system, neurotrophins, rat, rehabilitation, trophic factors

Abstract

We have investigated the impact of neuromuscular activity on the expression of neurotrophins in the lumbar spinal cord region and innervating skeletal muscle of adult rats. Rats were exercised on a treadmill for 1 day or 5 consecutive days and euthanized at 0, 2 or 6 h after the last bout of exercise. By Day 1, there was no clear evidence of an increase in brain-derived neurotrophic factor (BDNF) mRNA in the spinal cord or the soleus muscle. By Day 5, there was a significant increase in BDNF mRNA in the spinal cord at 2 h post-training, and the soleus muscle showed a robust increase between 0 and 6 h post-training. Immunoassays showed significant increases in BDNF protein in the soleus muscle by training Day 5. Immunohistochemical analyses showed elevated BDNF levels in motoneuron cell bodies and axons in the ventral horn. Neurotrophin-3 (NT-3) mRNA was measured to determine whether selected neurotrophins respond with a selective pattern of induction to neuromuscular activity. In the spinal cord, there was a progressive post-training decrease in NT-3 mRNA following a single bout of training, while there was a significant increase in NT-3 mRNA at 2 h post-training by Day 5. The soleus muscle showed a progressive increase in NT-3 mRNA by Days 1 and 5 following training. These results show that neuromuscular activity has specific effects on the BDNF and NT-3 systems, and that repetitive exercise affects the magnitude and stability of these responses.

Introduction

A large body of evidence indicates that locomotor activity can be beneficial in maintaining and even improving neural function following insult or disease (Gleser & Mendelberg, 1990; Edgerton *et al*., 1991; Wernig *et al*., 1999). The mechanisms for how exercise impacts neural function at the cellular and molecular levels are largely unexplored. It is generally recognized that trophic factors are critical modifiers of the structure and function of neural networks. Physical activity can induce the expression of trophic factors in the hippocampus and other brain regions (Neeper *et al*., 1995; Neeper *et al*., 1996; Gómez-Pinilla *et al*., 1997; Gómez-Pinilla *et al*., 1998). It has been assumed that the activation of specific neural networks by exercise leads to the expression of trophic factors. Yet evaluation of this possibility has been difficult, because of the complexity of the pathways involved with the activation of suprasegmental centres such as the hippocampus. The neuromuscular system is a more suitable model to evaluate the effects of exercise and trophic factors on specific neural pathways, because the connectivity of the spinal cord with select skeletal muscle groups is well defined.

We have shown previously that exercise induces the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is not directly related to motor function (Neeper *et al*., 1995; Neeper *et al*., 1996). In the present article, we evaluate the impact of exercise on BDNF production in a system in which motor activity seems to be the primary stimulus. The spinal cord and skeletal muscle form an anatomical and functional unit, such that the motoneuron pools innervating the hindlimb muscle groups are located within the ventral horn of the lumbosacral region of the cord. Therefore, the question of how neuromuscular activity affects the expression of neurotrophins in select structures involved with the activity can be experimentally scrutinized. BDNF and NT-3 are suitable for this study as BDNF (Koliatsos *et al*., 1993; Friedman *et al*., 1995; Xu *et al*., 1995; Ye & Houle, 1997) and NT-3 (Koliatsos *et al*., 1993; Grill *et al*., 1997) promote survival and growth of spinal cord cells affected by several types of insults.

The role of physical activity as an intrinsic modifier of neuronal health and plasticity is becoming evident. For example, a recent study has shown that physical activity stimulates neurogenesis in the adult hippocampus (van Praag *et al*., 1999). Repetitive neuromuscular activity also has therapeutic value for improving locomotor ability following trauma in animals (Burgess & Villablanca, 1986; Barbeau & Rossignol, 1987; Edgerton *et al*., 1991; Stummer *et al*., 1995), and humans (Perry, 1983; Wernig *et al*., 1995; Harkema *et al*., 1997). Therefore, it is possible that managed physical activity can be used to induce trophic factors in selective networks with beneficial effects for neuronal plasticity. It is therefore our objective to link exercise with neurotrophin expression in skeletal muscle and associated spinal cord regions, optimizing exercise protocols to maximize endogenous trophic factor production. Such physiological induction of trophic factors, using the pharmacology that is intrinsic to the neuromuscular system, can be a pivotal mechanisms by which select experiences translate into structural changes in the central nervous system.

Materials and methods

We have determined the effects of controlled amounts of locomotor activity provided by treadmill training on the expression of BDNF and its mRNA in the lumbar spinal cord and innervating soleus

Correspondence: Dr F. Gómez-Pinilla, as above.
E-mail: fgomezpi@ucla.edu

Received 28 September 2000, revised 11 January 2001, accepted 12 January 2001

muscle of rats. We have also evaluated the effects of elevated neuromuscular activity on the expression of neurotrophin-3 (NT-3). NT-3 belongs to the same neurotrophin family as BDNF, and the comparative results of BDNF and NT-3 may provide insight into the mechanism of regulation of neurotrophins by neuromuscular activity levels.

Experimental design

Adult Sprague–Dawley male rats ≈ 3 months of age were assigned randomly to a control ($n = 29$) or exercise ($n = 56$) group. The exercised rats were given 30 min of training per day at a speed of 27 m/min at a 3% incline using a motor-driven treadmill belt, conditions that are commonly used for physiological studies related to locomotion in the rat. The rats in the exercise group were trained for 1 or 5 days and killed immediately (0), or 2 or 6 h after the last bout of exercise. All rats were acclimated to the treadmill for 3 days by exposing them to the apparatus moving at low speed. All animals were kept singly in standard polyethylene cages with food and water given *ad libitum* and with a 12 : 12 h light : dark cycle. Animal procedures were approved by the Animal Use Committee at UCLA and followed the American Physiological Society Animal Care Guidelines.

At the end of the experimental period, the rats to be used for biochemical assays ($n = 6$ or 7 at each time-point) were decapitated, and the spinal cord and the soleus muscle were removed. The lumbar spinal cord region was selected for this study as the motoneuron pools which innervate the hindlimb musculature are located in this region. The soleus muscle was selected because of its high motoneuron recruitment level during locomotion at the intensity used in the present study (Roy *et al*., 1991). All tissues were stored at –70 °C until processed.

Nuclease protection assays

RNA isolation

Total cellular RNA was isolated by guanidine thiocyanate extraction according to Chomczynski & Sacchi (1987), and nuclease protection assay was performed according to described protocols (Gómez-Pinilla *et al*., 1998). The resulting gel was exposed to a Storage Phosphor Screen and data digitized with a Phosphor Imager System using Image Quant software (Molecular Dynamics, Piscataway, NJ, USA).

Transcription reactions were performed using a transcription kit (Promega, Madison, WI, USA) and α-^{32}P-CTP (Amersham Labs, Piscataway, NJ, USA; 800 Ci/mmol). A 900 base ^{32}P-labelled antisense probe which contained a 750 base corresponding to the entire amino acid coding region of rat BDNF cDNA (provided by Dr Paul Isackson) was used to assess the relative levels of BDNF mRNA. A pBluescript 900-bp fragment cDNA template was linearized with PvU II and transcribed with T3 RNA polymerase to generate BDNF cRNA with the antisense orientation. The rat NT-3 probe was prepared from templates provided by Dr D. Valenzuela, Regeneron Pharmaceuticals (Tarrytown, NY, USA). An 800-base cDNA fragment was linearized with *Eco*RI and transcribed with T3 RNA polymerase to obtain a 540-bp NT-3 cRNA with antisense orientation (Maisonpierre *et al*., 1993). In all experiments, hybridization was performed with a 356-base glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Ambion, TX, USA) cRNA probe to ensure that samples used contained an equivalent amount of RNA. All of the BDNF and NT-3 values were corrected against GAPDH values.

Protein immunoassay

Lumbar spinal cord and soleus muscle samples were homogenized in 3 volumes of homogenization buffer (50 mM Tris-HCl pH 8.0, 600 mM NaCl, 1% BSA, 0.1 mM PMSF, 220 TIUs/L aprotinin, 0.1 mM benzethonium chloride, 1 mM benzamidine HC, 4% triton X-100). Homogenates were centrifuged, supernatants were collected and protein concentrations were estimated with the MicroBCA procedure (Pierce, Rockford, IL, USA). BDNF protein was quantified using an enzyme-linked immunosorbent assay and standard protocols (ELISA; BDNF Emax ImmunoAssay System kit, Promega).

Immunohistochemistry

Separate groups of rats ($n = 7$ per group) to be used for histological analyses were injected with a lethal dose of Nembutal (75 mg/kg i.p.), then intracardially perfused with phosphate-buffered saline (PBS; pH = 7.3) followed by 4% paraformaldehyde in 0.1 M Sorensen buffer (pH = 7.3). Serial coronal sections (25 μm) of the spinal cord lumbar enlargement were cut on a cryostat, collected free floating in PBS and processed for immunohistochemistry, as previously described (Gómez-Pinilla *et al*., 1992). Tissue sections were incubated at 25 °C overnight in a solution containing an anti-BDNF antibody (diluted 1 : 1000 in PBS, 2% BSA, and 0.1% Triton X-100). We used a rabbit polyclonal anti-BDNF antisera (AB 1779 Chemicon, Temecula, CA, USA), which specifically recognizes rat BDNF and does not cross-react with other neurotrophins. Immunohistochemistry controls consisted of exclusion of the primary antibody or incubation with a secondary antibody against a different species. The results of these immunohistochemistry controls were negative as no staining was observed in cell structures. Some sections were stained with cresyl violet to visualize the general cytoarchitecture of the areas of interest.

Statistical analyses

Multiple comparisons among groups were performed using analysis of variance (ANOVA) and Statview software (Abacus Concepts, Berkeley, CA, USA). The mean values for the mRNA and protein levels were computed for the control and experimental groups. Overall differences were determined using a two-way ANOVA and individual group differences were detected with two-tailed *t*-test adjusted for multiple comparisons. The mean values for BDNF and NT-3 mRNAs were expressed as a percentage of control. The mean values for BDNF protein measurements were expressed as picograms of BDNF per milligram of total protein.

Results

BDNF mRNA

After 1 day of training, the levels of BDNF mRNA in the spinal cord were 113%, 156% ($P > 0.05$) and 111% of control immediately (0 h), and 2 h and 6 h post-training, respectively (Fig. 1A). After 5 days of training, the levels of BDNF mRNA were similar to control immediately post-training and 160% ($P < 0.05$) of control 2 h post-training (Fig. 1B). By 6 h post-training, the mRNA levels showed were 87% of control ($P > 0.05$).

There were no detectable changes in the levels of BDNF mRNA in the soleus muscle at any post-training time-point after 1 day of training (Fig. 1C). However, after 5 days of training the levels of BDNF mRNA were significantly increased at each post-training time-point: 310% ($P < 0.001$), 203% ($P < 0.05$) and 180% ($P < 0.01$) of control immediately, and 2 h and 6 h post-training, respectively (Fig. 1D).

BDNF protein

We performed ELISA to quantify the levels of BDNF protein after 5 days of treadmill training. This training duration elicited the

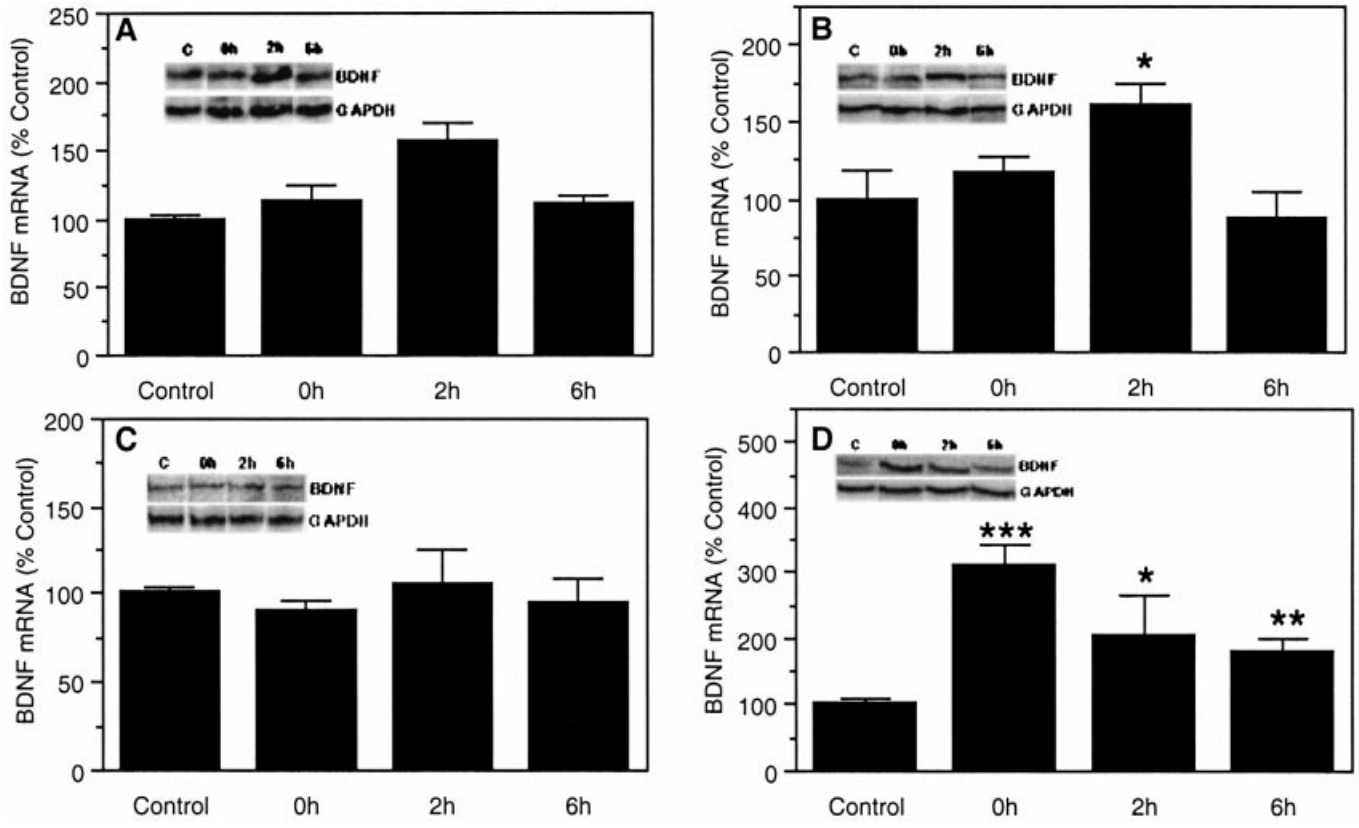

FIG. 1. Relative levels of brain-derived neurotrophic factor (BDNF) mRNA were measured in the lumbar region of the spinal cord (A and B) and soleus muscle (C and D) following 1 day and 5 consecutive days of treadmill training. The values are shown for rats killed 0, 2 and 6 h following a single training session. In the spinal cord, BDNF mRNA levels showed a tendency for an increase 2 h post-training at Day 1 (A), and a significant increase ($P < 0.05$) 2 h post-training at Day 5. In the soleus muscle, there were no detectable changes in the levels of BDNF mRNA for any of the post-trained periods at Day 1 (C), and at Day 5 (D) levels reached a peak immediately after training ($P < 0.001$) and lasted up to 6 h ($P < 0.01$) post-training. A 900-base ^{32}P-labelled antisense probe which contained a 780-base BDNF gene coding region was used to assess the relative levels of BDNF mRNA using nuclease protection assays. All of the BDNF mRNA values were corrected for equivalent levels of total mRNA using a GAPDH cRNA probe in the same assay solution. Values are expressed as a percentage of control. The top lanes of the gel display the protected BDNF cRNA fragment, and the bottom lanes display the protected GAPDH cRNA fragment for sedentary control and trained rats. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ANOVA, *t*-test, values are mean ± SEM.

relatively largest induction of BDNF mRNA in the spinal cord and skeletal muscle observed in our experiments (discussed later). BDNF values in the spinal cord of trained rats increased to 170% ($P < 0.05$) of control values at this time-point (Fig. 2A). Mean BDNF protein levels were 138% ($P > 0.05$) of control in the soleus muscle of trained rats (Fig. 2B).

We performed BDNF immunohistochemistry in a separate group of animals to determine the cellular location for changes in BDNF protein in the lumbar spinal cord, using the same training protocol used for ELISA experiments. Motoneurons, motor axons and associated astrocytes were the only elements stained in the ventral horn of the spinal cord, as reported recently (Buck *et al*., 2000). Cell bodies of motoneurons located in the ventral horn showed moderate BDNF immunoreactivity in sedentary rats. There also was moderate BDNF immunoreactivity in small round cellular elements located in the white matter which resembled astrocytes (Fig. 2C). Animals that exercised for 5 days showed an overall increase in the BDNF staining intensity in motoneuron elements and astrocyte-like cells (Fig. 2D). BDNF staining appeared strong in the cell bodies and axons of motoneurons, such that the course of emerging axons could be traced throughout the white matter up to the ventral root exit. There also was a qualitative increase in the BDNF staining intensity in astrocyte-like cells in the white matter, and this increase helped to identify their distinctive pattern of distribution along BDNF-stained axonal elements coursing through the white matter.

NT-3 mRNA

To investigate whether locomotor activity has a differential impact on the expression of selective neurotrophins, the levels of NT-3 mRNA were measured in parallel with BDNF mRNA in the spinal cord and soleus muscle. In the spinal cord, there was a progressive decrease in NT-3 mRNA after 1 day of exercise, reaching a value of 53% ($P < 0.05$) of control 6 h postexercise (Fig. 3A). After 5 days of training, the levels of NT-3 mRNA were 205% ($P < 0.05$) at 2 h and 150% ($P > 0.05$) 6 h post-training, relative to control (Fig. 3B). In the soleus muscle, NT-3 mRNA levels were 140% ($P > 0.05$), 181% ($P < 0.01$) and 215% ($P < 0.01$) of control (0 h), and 2 and 6 h after 1 day of exercise, respectively (Fig. 3C). After 5 days of training, the levels of NT-3 mRNA were 176% ($P < 0.01$), 152% ($P < 0.05$) and 250% ($P < 0.01$) higher than control at 0, 2 and 6 h post-training, respectively (Fig. 3D).

Discussion

The present results demonstrate that treadmill training can modulate the expression of BDNF and NT-3 in the lumbar region of the spinal

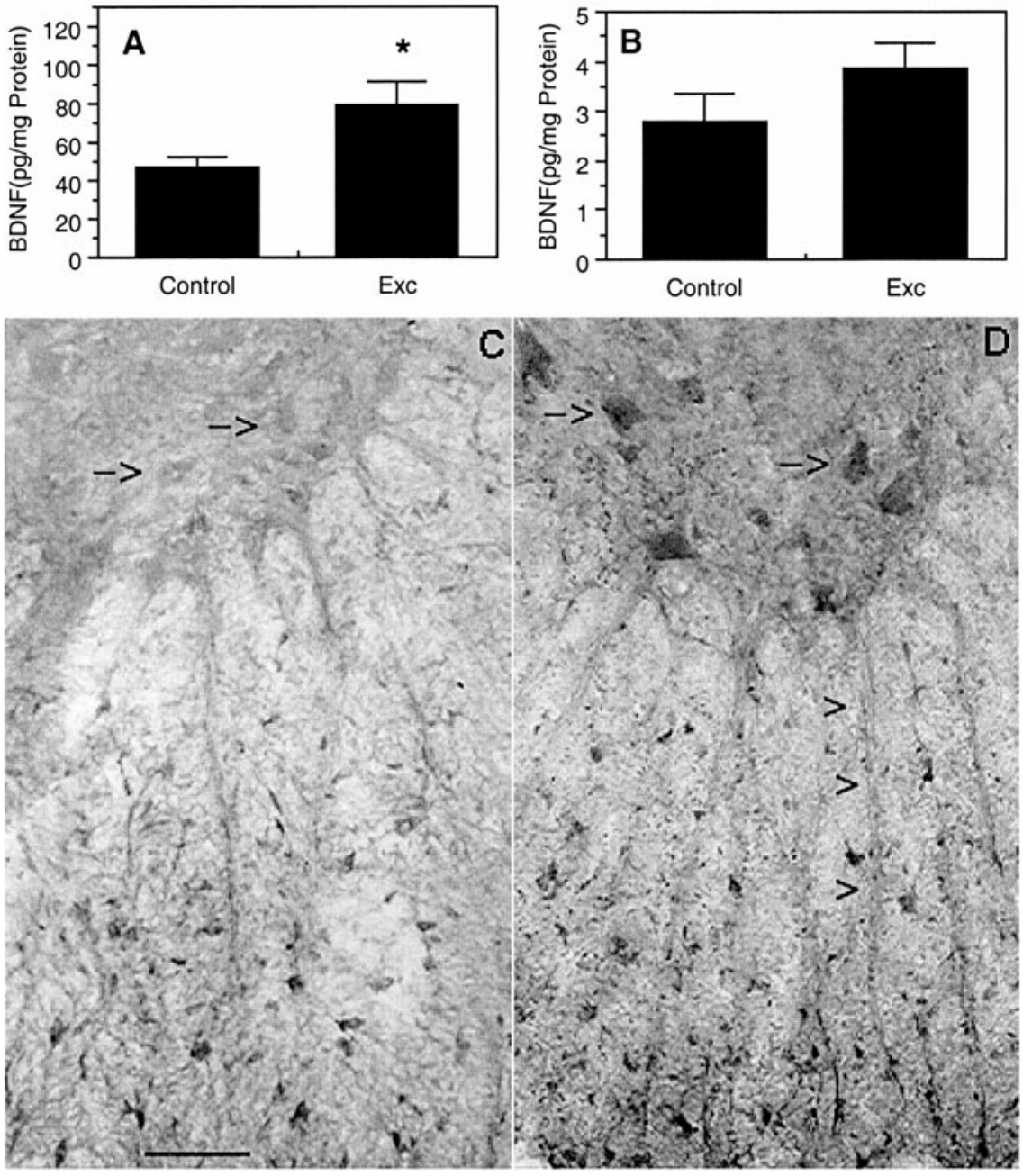

FIG. 2. Levels of brain-derived neurotrophic factor (BDNF) protein were measured in the lumbar region of the spinal cord (A) and in the soleus muscle (B) in the group of rats trained for 5 consecutive days and killed 2 h post-training. BDNF protein was detected and quantified using ELISA. (A) There was a significant increase in the level of BDNF ($P < 0.05$) in the spinal cord in the group of rats that received exercise (Exc) compared with sedentary control rats. (B) BDNF values in the soleus muscle of rats that exercised (Exc) showed an increasing trend. BDNF immunohistochemistry was performed in coronal sections of the lumbar spinal cord of rats trained for 5 days and killed 2 h post-training. (C) In sedentary control rats, light BDNF immunostaining was observed in motoneuron cell bodies in the ventral horn (arrows) and astrocyte-like cells located in the white matter. (D) In trained rats, BDNF staining appeared strong relative to sedentary rats, particularly in the cell bodies (arrows) and axons (arrow heads) of motoneurons. Astrocyte-like cells distributed along BDNF-labelled axons also showed strong BDNF labelling in rats that received exercise. Calibration bar, 20 μm (shown in A). *$P < 0.05$; ANOVA, *t*-test, values are mean ± SEM.

cord and in the soleus muscle. The motoneurons which innervate the soleus muscle are located within the lumbar spinal cord region. Each neurotrophin showed a specific response pattern in the spinal cord and muscle, suggesting a specific effect of neuromuscular activity on select neurotrophins. Because of the powerful effects of BDNF and NT-3 on neuroplasticity, these results suggest that BDNF, NT-3, and perhaps other neurotrophins, may mediate some of the effects of neuromuscular activity on the spinal cord and skeletal muscle.

Time-course of the induction of BDNF mRNA

In the spinal cord, there was a nonsignificant increase in the levels of BDNF mRNA after one bout of treadmill exercise, but after 5 days of training, BDNF mRNA levels at 2 h post-training reached statistical significance. The relatively lower interanimal variability after 5 days of training suggests that training repetition may have resulted in a more consistent locomotor performance and therefore a more

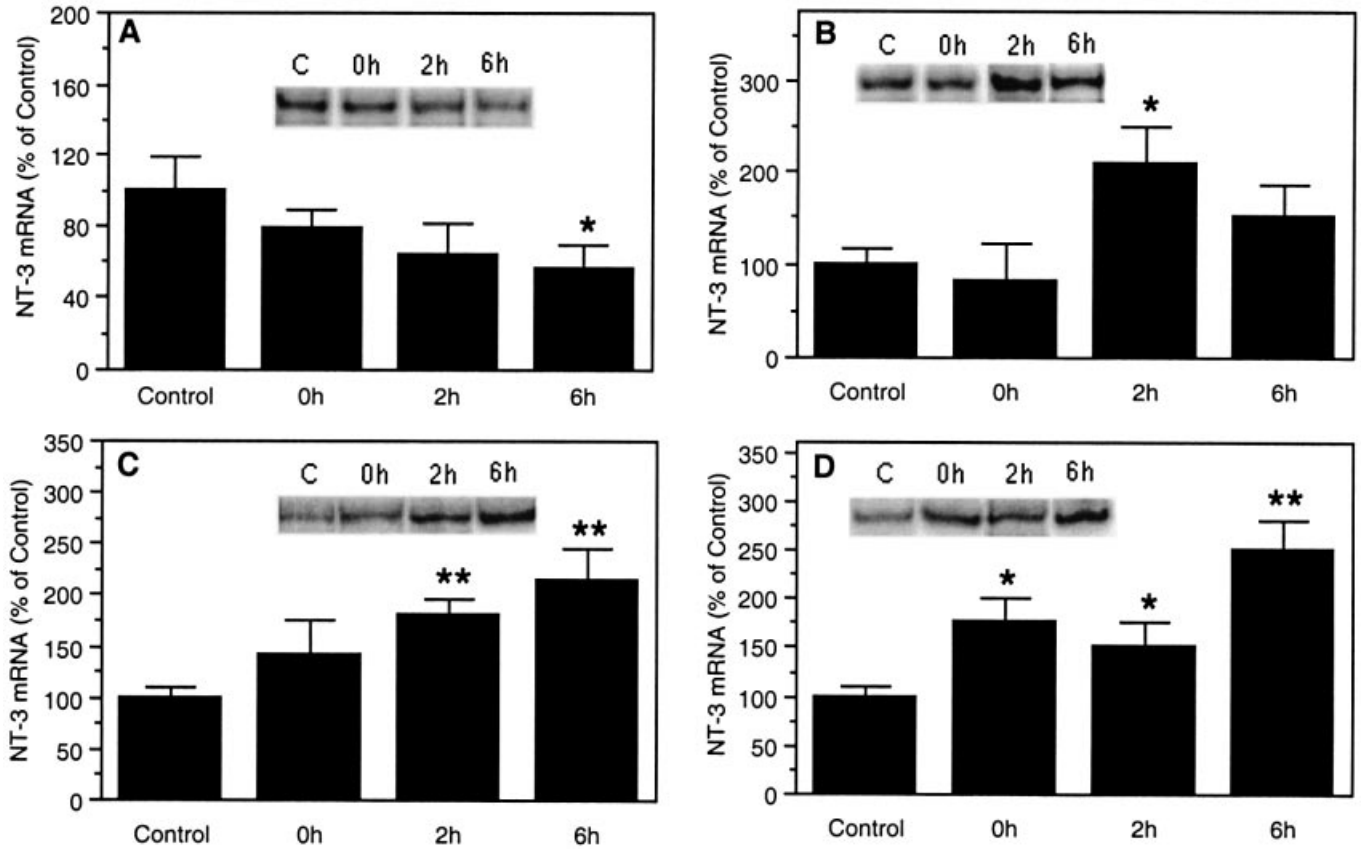

FIG. 3. Levels of neurotrophin-3 (NT-3) mRNA were measured in the lumbar spinal cord region (A and B) and soleus muscle (C and D) at 0, 2 and 6 h after 1 day or 5 consecutive days of training using nuclease protection assays. (A) At Day 1, there was a progressive decrease in NT-3 mRNA levels in the spinal cord that reached significance at 6 h ($P < 0.05$). (B) At Day 5, there was an increase in NT-3 mRNA 2 h post-training ($P < 0.05$). There was a progressive increase in NT-3 mRNA values in the spinal cord (C) and soleus muscle (D) following exercise. All of the NT-3 mRNA values were corrected for equivalent levels of total mRNA using a GAPDH cRNA probe. The gel displays the protected NT-3 cRNA fragment. *$P < 0.05$, **$P < 0.01$; ANOVA, *t*-test, values are mean ± SEM.

consistent upregulation of BDNF mRNA in response to training. A single bout of exercise was not sufficient to elevate soleus muscle BDNF mRNA, however, a robust increase in BDNF mRNA was observed after completing the fifth training day and these levels remained elevated for at least 6 h. Therefore, repetitive training over a period of days apparently can sustain elevated levels of BDNF mRNA for a longer period of time than that elicited by a single bout of exercise.

Regulation of neurotrophins in the spinal cord vs. muscle

Rats trained for 5 days showed a significant increase in BDNF protein (170% of control) in the spinal cord but not in the soleus muscle. It is puzzling that the increases in BDNF protein in the spinal cord were associated with relatively small increases in BDNF mRNA following 5 days of training. It is also striking that the robust increase in BDNF mRNA (310%) in the soleus muscle was not paralleled by a significant increase in BDNF protein. It is possible that elevated levels of BDNF mRNA in the spinal cord are sufficient to support translation processes leading to elevated BDNF protein. The combined results from the spinal cord and muscle, however, suggest the interesting possibility that neuromuscular activity might increase retrograde transport of BDNF from the muscle. Motoneuron axonal elements of trained rats showed stronger BDNF immunostaining relative to sedentary controls, as it would be expected with an increased axonal transport of BDNF. It is known that motoneurons can retrogradely transport BDNF that has been injected into skeletal muscle (Koliatsos *et al*., 1993; Sagot *et al*., 1998) or the sciatic nerve (Yan *et al*., 1992; Curtis *et al*., 1998), and that administration of BDNF can prevent the degeneration of motoneurons (Yan *et al*., 1992; Koliatsos *et al*., 1993; Sagot *et al*., 1998). Therefore, it is possible that BDNF synthesized in muscle as a result of increased neuromuscular activity may have an effect on spinal cord cells.

The pattern of expression of BDNF in response to exercise differed from that of NT-3 in the spinal cord and muscle, suggesting that neuromuscular activity has a differential effect on the expression of select neurotrophins. For example, at the time that there was a progressive decrease in NT-3 mRNA in the spinal cord (after 1 day of exercise), the spinal cord or muscle showed relatively uniform levels of BDNF mRNA. This differential modulation of neurotrophins may be a significant factor in defining a potential interactive role of neurotrophins on activity-dependent plasticity.

Possible functions of BDNF and NT-3 in the neuromuscular system

It is becoming well-established that BDNF and NT-3 have a role in the development of functional connectivity between skeletal muscle and the spinal cord. For example, applications of BDNF or NT-3 result in modifications in the latency and amplitude of Ia muscle afferents in the spinal cord of neonatal rats (Seebach *et al*., 1999). BDNF and NT-3 also appear to promote development of adult

electrophysiological properties of the neuromuscular synapse, as studied in *Xenopus* nerve-muscle cocultures (Wang *et al*., 1995). BDNF and NT-3 also may impact the structure of the neuromuscular synapse (Braun *et al*., 1996). Thus, BDNF, NT-3 and other neurotrophins produced in skeletal muscle (Funakoshi *et al*., 1995), may enhance the potential of innervation of motoneurons and play a critical role in the plasticity of the neuromuscular synapse. It is noteworthy that the observed modulations in neurotrophins in the soleus may not be representative of the response of all other hindlimb muscles.

BDNF administration into the spinal cord can stimulate the growth of severed axons and enhance the survival of damaged cells (Koliatsos *et al*., 1993; Friedman *et al*., 1995; Xu *et al*., 1995; Ye & Houle, 1997), and can promote functional recovery (Jakeman *et al*., 1998). Similarly, NT-3 administration promotes the survival and growth of spinal cord cells (Nakahara *et al*., 1996; Grill *et al*., 1997) after spinal cord injury in adult animals. It is possible that BDNF and NT-3 play complementary actions in response to increased neuromuscular activity. Indeed, there is evidence that the actions of BDNF and NT-3 on promoting supraspinal axonal regeneration may be complementary (Xu *et al*., 1995).

Exercise-induced neurotrophins may help functional recovery following injury

The effects of exogenous applications of BDNF and NT-3 on spinal motoneurons suggest that neurotrophins supplied by endogenous sources may have an even greater effect on compromised cells. Repetitive performance of a motor task is currently used as one form of therapy to promote functional recovery following spinal cord injury (for reviews see Bohannon, 1993; Edgerton *et al*., 1997). The positive effects of motor training have been documented in animals (Burgess & Villablanca, 1986; Barbeau & Rossignol, 1987; Edgerton *et al*., 1991; Hodgson *et al*., 1994; de Leon *et al*., 1998) and humans (Hurwitz, 1989; Wernig *et al*., 1995; Harkema *et al*., 1997; Kramer *et al*., 1999). It appears that the success of rehabilitative strategies is highly task specific, in which strategies which closely simulate the functional situation of walking are the most effective in promoting the restoration of locomotion (Barbeau & Rossignol, 1994; Edgerton *et al*., 1997; de Leon *et al*., 1998).

The present results in the neuromuscular system, combined with those reported in the brain (Neeper *et al*., 1995; Neeper *et al*., 1996), are consistent with the view that activity-dependent induction of neurotrophins has a significant effect on motor pathways during development and during the functional reorganization associated with trauma or disease. But perhaps more importantly, the present results suggest that in normal adult animals, modest levels of locomotor exercise can induce marked modulation in the expression of BDNF protein and BDNF and NT-3 mRNA in the spinal cord and skeletal muscle. These findings raise the question as to whether these neurotrophic factors may play a role in the routine maintenance and plasticity of the neuromuscular system.

Acknowledgements

This work was supported by the NIH Awards NS38978 and NS39522. We thank Philip Yen for assistance with the immunohistochemical experiments.

Abbreviations

BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NT-3, neurotrophin-3; PBS, phosphate-buffered saline.

References

Barbeau, H. & Rossignol, S. (1987) Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.*, **412**, 84–95.

Barbeau, H. & Rossignol, S. (1994) Enhancement of locomotor recovery following spinal cord injury. *Curr. Opin. Neurol.*, **7**, 517–524.

Bohannon, R.W. (1993) Physical rehabilitation in neurologic diseases. *Curr. Opin. Neurol.*, **6**, 765–772.

Braun, S., Croizat, B., Lagrange, M.C., Warter, J.M. & Poindron, P. (1996) Neurotrophins increase motoneurons' ability to innervate skeletal muscle fibers in rat spinal cord – human muscle cocultures. *J. Neurol. Sci.*, **136**, 17–23.

Buck, C.R., Seburn, K.L. & Cope, T.C. (2000) Neurotrophin expression by spinal motoneurons in adult and developing rats. *J. Comp. Neurol.*, **416**, 309–318.

Burgess, J.W. & Villablanca, J.R. (1986) Recovery of function after neonatal or adult hemispherectomy in cats. II. Limb bias and development, paw usage, locomotion and rehabilitative effects of exercise. *Behav. Brain Res.*, **20**, 1–17.

Chomczynski, P. & Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159.

Curtis, R., Tonra, J.R., Stark, J.L., Adryan, K.M., Park, J.S., Cliffer, K.D., Lindsay, R.M. & DiStefano, P.S. (1998) Neuronal injury increases retrograde axonal transport of the neurotrophins to spinal sensory neurons and motor neurons via multiple receptor mechanisms. *Mol. Cell. Neurosci.*, **12**, 105–118.

Edgerton, V.R., de Guzman, C.P., Gregor, R.J., Roy, R.R., Hodgson, J.A. & Lovely, R.J. (1991) Trainability of the spinal cord to generate hindlimb stepping patterns in adult spinalized cats. In Shimamura, M., Grillner, S., Edgerton, V.R. (eds), *Neurobiological Basis of Human Locomotion*. Japan Scientific Society Press, Tokyo, pp. 411–423.

Edgerton, V.R., Roy, R.R., Leon, R., Tillakaratne, N. & Hodgson, J.A. (1997) Does motor learning occur in the spinal cord? *Neuroscientist*, **3**, 287–294.

Friedman, B., Kleinfeld, D., Ip, N.Y., Verge, V.M., Moulton, R., Boland, P., Zlotchenko, E., Lindsay, R.M. & Liu, L. (1995) BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons. *J. Neurosci.*, **15**, 1044–1056.

Funakoshi, H., Belluardo, N., Arenas, E., Yamamoto, Y., Casabona, A., Persson, H. & Ibáñez, C.F. (1995) Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons. *Science*, **268**, 1495–1499.

Gleser, J. & Mendelberg, H. (1990) Exercise and sport in mental health: a review of the literature. *Isr. J. Psychiatry Relat. Sci.*, **27**, 99–112.

Gómez-Pinilla, F., Dao, L. & So, V. (1997) Physical exercise induces FGF-2 and its mRNA in the hippocampus. *Brain Res.*, **764**, 1–8.

Gómez-Pinilla, F., Lee, J.W. & Cotman, C.W. (1992) Basic FGF in adult rat brain: cellular distribution and response to entorhinal lesion and fimbria–fornix transection. *J. Neurosci.*, **12**, 345–355.

Gómez-Pinilla, F., So, V. & Kesslak, J.P. (1998) Spatial learning and physical activity contribute to the induction of FGF: Neural substrates for increased cognition associated with exercise. *Neuroscience*, **85**, 53–61.

Grill, R., Murai, K., Blesch, A., Gage, F.H. & Tuszynski, M.H. (1997) Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. *J. Neurosci.*, **17**, 5560–5572.

Harkema, S.J., Hurley, S.L., Patel, U.K., Requejo, P.S., Dobkin, B.H. & Edgerton, V.R. (1997) Human lumbosacral spinal cord interprets loading during stepping. *J. Neurophysiol.*, **77**, 797–811.

Hodgson, J.A., Roy, R.R., Leon, R., Dobkin, B. & Edgerton, V.R. (1994) Can the mammalian lumbar spinal cord learn a motor task? *Med. Sci. Sports Exerc.*, **26**, 1491–1497.

Hurwitz, A. (1989) The benefit of a home exercise regimen for ambulatory Parkinson's disease patients. *J. Neurosci Nurs.*, **21**, 180–184.

Jakeman, L.B., Wei, P., Guan, Z. & Stokes, B.T. (1998) Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury. *Exp Neurol.*, **154**, 170–184.

Koliatsos, V.E., Clatterbuck, R.E., Winslow, J.W., Cayouette, M.H. & Price, D.L. (1993) Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons *in vivo*. *Neuron*, **10**, 359–367.

Kramer, A.F., Hahn, S., Cohen, N.J., Banich, M.T., McAuley, E., Harrison, C.R., Chason, J., Vakil, E., Bardell, L., Boileau, R.A. & Colcombe, A. (1999) Ageing, fitness and neurocognitive function. *Nature*, **400**, 418–419.

de Leon, R.D., Hodgson, J.A., Roy, R.R. & Edgerton, V.R. (1998) Locomotor capacity attributable to step training versus spontaneous recovery following spinalization in adult cats. *J. Neurophysiol.*, **79**, 1329–1340.

Maisonpierre, P.C., Belluscio, L., Squinto, S., Ip, N.Y., Furth, M.E., Lindsay,

R.M. & Yancopoulos, G.D. (1993) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science*, **247**, 1446–1451.
Nakahara, Y., Gage, F.H. & Tuszynski, M.H. (1996) Grafts of fibroblasts genetically modified to secrete NGF, BDNF, NT-3, or basic FGF elicit differential responses in the adult spinal cord. *Cell Transplant.*, **5**, 191–204.
Neeper, S.A., Gómez-Pinilla, F., Choi, J. & Cotman, C. (1995) Exercise and brain neurotrophins. *Nature*, **373**, 109.
Neeper, S.A., Gómez-Pinilla, F., Choi, J. & Cotman, C.W. (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res.*, **726**, 49–56.
Perry, J. (1983) Rehabilitation of the neurologically disabled patient: principles, practice, and scientific basis. *J. Neurosurg.*, **58**, 799–816.
van Praag, H., Kempermann, G. & Gage, F.H. (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neurosci.*, **2**, 266–270.
Roy, R.R., Hutchison, D.L., Pierotti, D.J., Hodgson, J.A. & Edgerton, V.R. (1991) EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. *J. Appl. Physiol.*, **70**, 2522–2529.
Sagot, Y., Rosse, T., Vejsada, R., Perrelet, D. & Kato, A.C. (1998) Differential effects of neurotrophic factors on motoneuron retrograde labeling in a murine model of motoneuron disease. *J. Neurosci.*, **18**, 1132–1141.
Seebach, B.S., Arvanov, V. & Mendell, L.M. (1999) Effects of BDNF and NT-3 on development of Ia/motoneuron functional connectivity in neonatal rats. *J. Neurophysiol.*, **81**, 2398–2405.
Stummer, W., Baethmann, A., Murr, R., Schürer, L. & Kempski, O.S. (1995) Cerebral protection against ischemia by locomotor activity in gerbils. Underlying mechanisms. *Stroke*, **26**, 1423–1429.
Wang, T., Xie, K. & Lu, B. (1995) Neurotrophins promote maturation of developing neuromuscular synapses. *J. Neurosci.*, **15**, 4796–4805.
Wernig, A., Muller, S., Nanassy, A. & Cagol, E. (1995) Laufband therapy based on 'rules of spinal locomotion' is effective in spinal cord injured persons. *Eur. J. Neurosci.*, **7**, 823–829.
Wernig, A., Nanassy, A. & Muller, S. (1999) Laufband treadmill therapy in incomplete paraplegia and tetraplegia. *J. Neurotrauma*, **16**, 719–726.
Xu, X.M., Guénard, V., Kleitman, N., Aebischer, P. & Bunge, M.B. (1995) A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Exp. Neurol.*, **134**, 261–272.
Yan, Q., Elliott, J. & Snider, W.D. (1992) Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature*, **360**, 753–755.
Ye, J.H. & Houle, J.D. (1997) Treatment of the chronically injured spinal cord with neurotrophic factors can promote axonal regeneration from supraspinal neurons. *Exp. Neurol.*, **143**, 70–81.