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Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury

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Abstract

We have conducted studies to determine the potential of exercise to benefit the injured spinal cord using neurotrophins. Adult rats were randomly assigned to one of three groups: (1) intact control (Con); (2) sedentary, hemisected at a mid-thoracic level (Sed-Hx), or (3) exercised, hemisected (Ex-Hx). One week after surgery, the Ex-Hx rats were exposed to voluntary running wheels for 3, 7, or 28 days. BDNF mRNA levels on the lesioned side of the spinal cord lumbar region of Sed-Hx rats were ~80% of Con values at all time points and BDNF protein levels were ~40% of Con at 28 days. Exercise compensated for the reductions in BDNF after hemisection, such that BDNF mRNA levels in the Ex-Hx rats were similar to Con after 3 days and higher than Con after 7 (17%) and 28 (27%) days of exercise. After 28 days of exercise, BDNF protein levels were 33% higher in Ex-Hx than Con rats and were highly correlated ($r = 0.86$) to running distance. The levels of the downstream effectors for the action of BDNF on synaptic plasticity synapsin I and CREB were lower in Sed-Hx than Con rats at all time points. Synapsin I mRNA and protein levels were higher in Ex-Hx rats than Sed-Hx rats and similar to Con rats at 28 days. CREB mRNA values were higher in Ex-Hx than Sed-Hx rats at all time points. Hemisection had no significant effects on the levels of NT-3 mRNA or protein; however, voluntary exercise resulted in an increase in NT-3 mRNA levels after 28 days (145%). These results are consistent with the concept that synaptic pathways under the regulatory role of BDNF induced by exercise can play a role in facilitating recovery of locomotion following spinal cord injury.

Keywords: Neuromuscular activity; BDNF; Exercise; Spinal cord injury; Synaptic plasticity

Introduction

The capacity of repetitive locomotor activity to promote functional restoration after CNS injury is well recognized (Edgerton and Roy, 2002; Edgerton et al., 2004). It seems that the outcome of recovery is task specific such that rehabilitative strategies that simulate the action of walking are particularly effective in promoting the recovery of locomotion. Although neurotrophins have been identified as a molecular system with the potential to enhance spinal cord repair, most of the strategies to induce motor recovery after SCI have involved the addition of exogenous neurotrophins into the CNS. These strategies, however, ignore the intrinsic capabilities of the neural system to produce neurotrophins. Our experimental approach is to modulate the synthesis of endogenous neurotrophins in the CNS via a systemic stimulus, i.e., physical activity. This approach infers that those specific neural pathways that are activated during an exercise may have access to the highest concentration of brain-derived neurotrophic factor (BDNF) and, therefore, able to show the most synaptic plasticity.

Recent studies illustrate that physical activity increases the expression of BDNF and neurotrophin-3 (NT-3) in the intact spinal cord (Gomez-Pinilla et al., 2002a,b; Ying et al., 2003). It is our contention that a better understanding of the variables involved in the regulation of neurotrophins by

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exercise in the injured spinal cord will enable us to optimize the impact of this process on CNS function. BDNF delivered exogenously to the injured spinal cord can increase axonal growth (Bregman et al., 1997) and stimulate hindlimb stepping (Jakeman et al., 1998). These observations, together with evidence that BDNF is a powerful modifier of neuronal excitability and synaptic transmission (Kafitz et al., 1999; Lu and Figurov, 1997), suggest that BDNF is a crucial effector of experience-dependent plasticity. The spinal cord expresses NT-3 and its TrkC receptor (McAllister et al., 1999; Scarisbrick et al., 1999), and NT-3 plays a role in mediating synaptic transmission (Xie et al., 1997) and regeneration in the spinal cord (Ramer et al., 2000; Schecterson and Bothwell, 1992; Shibayama et al., 1998). NT-3 also is involved in the survival and function of sensory neurons, such that mice lacking the NT-3 gene show a severe loss of sensory neurons and concomitant gait abnormalities (Tessarollo et al., 1994).

BDNF affects the synthesis (Wang et al., 1995) and phosphorylation (Jovanovic et al., 1996) of synapsin I, resulting in elevated neurotransmitter release (Jovanovic et al., 2000). Therefore, synapsin I can be used as a marker to evaluate the role of BDNF on synaptic adaptation and function (Vaynman et al., 2003). Synapsin I is a member of a family of nerve terminal-specific phosphoproteins and is involved in neurotransmitter release, axonal elongation, and maintenance of synaptic contacts (Brock and O'Callaghan, 1987). The transcription factor cyclic AMP response element binding protein (CREB) is required for various forms of memory including spatial learning (Silva et al., 1998) and appears to play a role in neuronal resistance to trauma in conjunction with BDNF (Walton et al., 1999). CREB is characterized by its ability to modulate gene expression encoding BDNF and cell survival in the CNS (Tao et al., 1998; Ying et al., 2002). Growth associated protein 43 (GAP-43) is present in growing axon terminals and has important roles in axonal growth, neurotransmitter release (Oestreicher et al., 1997), and learning and memory (Routtenberg et al., 2000). In the present study, we investigate the capacity of exercise to counteract the reduced expression of BDNF and NT-3 and their effects on molecular systems related to synaptic plasticity and axonal growth in regions of the spinal cord distal to an injury. The results are consistent with the view that neurotrophin modulation induced by neuromuscular activity can play a role in facilitating functional recovery following spinal cord injury.

Materials and methods

Seventy-six male Sprague–Dawley rats of 2 months of age (Charles River, San Diego, CA) were housed singly in standard polyethylene cages with food and water ad libitum, and exposed to alternate light and dark periods of 12 h. After 1 week of acclimation, the animals were randomly assigned to a sedentary control (Con) or a hemisected (Hx) group.

A spinal cord hemisection at a mid-thoracic level (T7–T9) was performed under aseptic conditions. Briefly, the rats were pre-sedated with buprenorphine (0.03 mg/kg body weight, s.c.) and anesthetized with a mixture of ketamine hydrochloride (70 mg/kg body weight) and xylazine (10 mg/kg body weight) given i.p. and supplemented as needed (25% of original dose). A laminectomy was made in the vertebral column in the mid-thoracic region (~T7–T9), the dura opened and the spinal cord exposed. The midline of the spinal cord was identified and one half (the right side) of the cord was transected completely at ~T7–T9 using fine scissors and surgical forceps. Lidocaine, a local anesthetic, was applied at the transection site just prior to cutting the cord to minimize discomfort. Gelfoam was packed at the transection site to minimize bleeding. The surgical areas were lavaged and the wounds were closed.

Spinal cord hemisected rats were divided into an exercise (Ex-Hx) and a sedentary (Sed-Hx) group. Beginning 1 week after hemisection, the Ex-Hx group was exposed to running wheels for 0, 3, 7, or 28 days. Con and Sed-Hx rats were maintained in single cages without running wheels, and were sacrificed at the same time points after hemisection as the Ex-Hx group, i.e., 7, 10, 14, and 35 days after surgery.

The exercise apparatus included a polyethylene cage (30 × 40 × 20 cm) equipped with a running wheel (diameter, 31.8 cm; width, 10 cm) with adjustable resistance. The wheel rotates on the shaft whenever the rat moves in either direction and the number of revolutions is recorded continuously by a computer. On the first day of exercise, the rats were exposed to freely moving running wheels (no load) as an adaptation period. The load then was increased to 100 g for all subsequent days. The lumbar region of the spinal cord was selected for study because the motoneuron pools that innervate the hindlimb musculature are located in this region.

All rats used for biochemical assays (n = 6 /group at each time point) were killed around 8:00 AM. The lumbar enlargement of the spinal cord was identified, separated longitudinally at the midline, frozen on dry ice and stored at −70°C until processed. The entire lumbar enlargement on the lesion side of the Hx rats was used for analysis and compared with data from the entire lumbar region of Con rats.

Separate groups of Con and Hx-Ex rats were used for histological analyses (n = 5/group) at the 28-day time point. The rats were treated as described above. After the last day of running, the rats were anesthetized deeply with sodium pentobarbital (75 mg/kg, i.p.) and perfused intracardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M Sorensen's phosphate buffer, and 20% sucrose in PBS. The perfused lumbar region of each spinal cord was stored at −70°C until sectioned in a cryostat.

Taqman RT-PCR

The RNA STAT-60 kit (TEL-TEST, Inc., Friendswood, TX) was used for total RNA isolation according to the

manufacturer's instructions. The mRNAs for BDNF, synapsin I, GAP-43, NT-3, and CREB were measured by TaqMan real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Atlanta). The technique is based on the ability to directly detect the RT-PCR product with no downstream processing. This is accomplished with the monitoring of the increase in fluorescence of a dye-labeled DNA probe specific for each factor under study plus a probe specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene used as an endogenous control for the assay. Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer, Branchburg, NJ). The sequences of probes, forward and reverse primers (Integrated DNA Technologies, Coralville, IA) were: BDNF: probe (5′-AGTCATTTGCGCACAACTTTAAAAGTCTGCATT-3′), forward (5′-GGACATATCCATGACCAGAAAGAAA-3′), reverse (5′-GCAACAAACCACAACATTATCGAG-3′); NT-3 probe (5′-TGACCGACAAGTCCTCAGCCATTGAC-3′), forward (5′-TGTGACAGTGAGAGCCTGTGG-3′), reverse (5′-TGTAACCTGGTGTCCCCGAA-3′); synapsin I: probe (5′-CATGGCACGTAATGGAGACTACCGCA-3′), forward (5′-CCGCCAGCTGCCTTC-3′), reverse (5′-TGCAGCCCAATGACCAAA-3′); GAP-43: probe (5′-CTCATAAGGCTGCAACCAAAATTCAGGCT-3′), forward (5′-GATGGTGTCAAACCGGAGGAT-3′), reverse (5′-CTTGTTATGTGTCCACGGAAGC-3′); CREB: probe (5′-CATGGCACGTAATGGAGACTACCGCA-3′), forward (5′-CCGCCAGCATGCCTTC-3′), reverse (5′-TGCAGCCCAATGACCAAA-3′). An oligonucleotide probe specific for the rat GAPDH gene was used as an endogenous control to standardize the amount of sample RNA. The RT-reaction conditions were 2 min at 50°C as the initial step to activate uracil glycosylase (UNG), followed by 30 min at 60°C as the reverse transcription and completed by a UNG-deactivation at 95°C for 5 min. The 40 cycles of the two-step PCR-reaction conditions were 20 s at 94°C and 1 min at 62°C.

Protein immunoassay measurements

Lumbar spinal cord 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). The supernatants were collected after the homogenates were centrifuged for 30 min. The protein concentrations were estimated with the MicroBCA procedure (Pierce, Rockford,IL) using bovine serum albumin (BSA) as the standard. BDNF and NT-3 proteins were quantified using an enzyme-linked immunosorbent assay (ELISA, BDNF, and NT-3 Emax ImmunoAssay System Kits, Promega Inc., Madison, WI) according to the manufacturer's instructions. Unknown BDNF or NT-3 concentrations were compared to known BDNF or NT-3 concentrations using a calibration curve. "Synapsin I, phospho-synapsin I, CREB, and phospho-CREB proteins were analyzed by Western blot, quantified by densitometric scanning of the film under linear exposure conditions and normalized for actin levels. Membranes were incubated with the following primary antibodies: anti-synapsin I (1:2000; Santa Cruz Biotechnology Inc., Sta Cruz, CA), anti-phospho-synapsin I (1:2000; Santa Cruz Biotechnology), anti-CREB (1:1000; Cell Signaling Technology, Inc., Beverly, MA, USA), anti-phospho-CREB (1:1000; Cell Signaling Technology, Inc.), anti-actin (1:2000; Santa Cruz Biotechnology) followed by anti-goat IgG horseradish peroxidase conjugate for synapsin, phospho-synapsin and actin or anti-rabbit IgG horseradish peroxidase conjugate for CREB and phospho-CREB (Santa Cruz Biotechnology)." Immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer's instructions.

Immunohistochemistry

Spinal cord tissues were sliced in the transverse plane (30 μm), collected free floating in PBS, and processed for BDNF immunohistochemistry as previously described (Gomez-Pinilla et al., 2001). A 1:500 dilution was used for the rabbit polyclonal anti-BDNF antisera (Chemicon International Inc., Temecula, CA).

Statistical analyses

GAPDH and actin were employed as internal standards for real-time RT-PCR and for Western blots, respectively. For quantification of TaqMan RT-PCR results, fluorescent signal intensities were plotted against the number of PCR cycles on a semilogarithmic scale. The amplification cycle at which the first significant increase of fluorescence occurred was designated as the threshold cycle (C_T). The C_T value of each sample then was compared to those of the internal standard. This process is fully automated and carried out with ABI sequence detector software version 1.6.3 (PE Biosystem, Foster City, CA). Taqman EZ RT-PCR values for GAPDH were subtracted from BDNF, synapsin I, NT-3, CREB, or GAP-43 values. The resulting corrected values were used to make comparisons across the different experimental groups. The mean mRNA or protein levels were computed for the control and experimental rats for each time point. An ANOVA and Fisher's test (Statview software, Abacus Concepts, CA) were used to determine any significant differences among the groups at $P < 0.05$ or higher. The results were expressed as mean percent of Con values for graphic clarity and represent the mean ± standard error of the mean (SEM) of 6 independent determinations. Regression analyses were used to determine the relationship between the level of BDNF protein and the distance that the Ex-Hx rats ran daily in the running wheel.

Results

BDNF levels after spinal cord injury with and without exercise (Figs. 1 and 2)

The levels of BDNF mRNA in the lumbar hemicord ipsilateral to the hemisection in Sed-Hx rats decreased to 83% (day 7, $P < 0.05$), 84% (day 10, $P < 0.05$), 84% (day 14, $P < 0.05$), and 85% (day 35, $P < 0.05$) of Con values (Fig. 1A) relative to the injury onset. The effects of injury were more dramatic at the protein level: ELISA analyses showed that BDNF levels were ~40% of Con values 35 days after the hemisection (Fig. 1B). In the Ex-Hx rats, 3 days of exercise were sufficient to normalize the levels of BDNF mRNA (110%, $P > 0.05$), and longer exercise periods increased the levels of BDNF mRNA beyond Con values (7 days, 117%, $P < 0.05$; 28 days, 127%, $P < 0.05$; Fig. 1A). After 28 days of exercise, BDNF protein had increased to 133% ($P < 0.05$) of the levels in Con rats (Fig. 1B). BDNF protein levels were increased in proportion to the amount of voluntary exercise performed after the spinal cord hemisection (Fig. 1C).

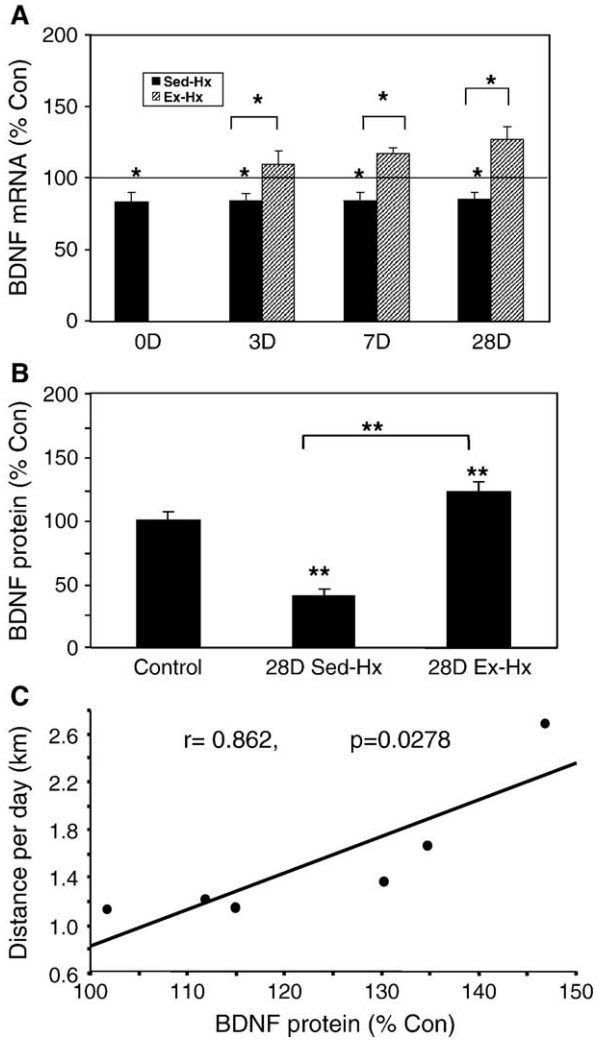

Fig. 1. BDNF mRNA (A) and protein (B) in the lumbar region of the ipsilateral hemicord of rats that received a spinal cord hemisection. All rats were maintained in sedentary cages for 7 days. Thereafter, one group of hemisected rats remained in the sedentary cages (Sed-Hx) and one group was exposed to 0, 3, 7, or 28 consecutive days of voluntary wheel running exercise (Ex-Hx). Data are presented as a percent of sedentary control rats. BDNF mRNA (A) and protein (B) levels were lower in Sed-Hx rats than in control or Ex-Hx rats at all time points. BDNF mRNA levels were similar in control and Ex-Hx rats at all time points (A), and BDNF protein levels were higher in Ex-Hx than control rats at 28 days (B). (C) Relationship between the levels of BDNF protein and the average distance run per day showing that the levels of BDNF were increased in proportion to the amount of exercise performed after spinal cord hemisection. *Significantly different at $P < 0.05$, **significantly different at $P < 0.001$ (ANOVA, Fisher's test).

Based on immunohistochemical analyses, BDNF expression was localized to motoneurons in the ventral horn and their axonal processes coursing through the white matter in both Sed-Hx and Ex-Hx rats (Fig. 2). Glial-like cells surrounding the axons coursing through the white matter also were BDNF immunopositive. There was a relative decrease in BDNF immunostaining in the side ipsilateral to the lesion (Fig. 2A) while exercise appeared to preserve the immunostaining (Figs. 2B and C).

Synapsin I levels after spinal cord injury with and without exercise (Fig. 3)

Synapsin I, a downstream effector for the action of BDNF on synaptic plasticity, was evaluated to provide information regarding the mechanisms by which exercise can affect the injured spinal cord. Synapsin I mRNA levels were significantly lower in Sed-Hx than Con rats at the 7-day (87%, $P < 0.05$), 10-day (76%, $P < 0.05$), 14-day (85%, $P < 0.05$), and 35-day (87%, $P < 0.05$) time points relative to the injury onset (Fig. 3A). The synapsin I mRNA levels progressively increased in the Ex-Hx rats, such that the levels were similar to Con values after 28 days of exercise. Based on Western blot analyses, the level of synapsin I was 75% ($P < 0.05$) of Con values after 35 days of hemisection. In contrast, these values in the Ex-Hx rats were 120% of Con at the same time point (Fig. 3B). The level of phosphorylated synapsin I (the activated state) in Sed-Hx rats was 53% ($P < 0.01$) of Con and this level in the Ex-Hx rats was 138% of Con ($P < 0.01$; Fig. 3C), after 35 days of hemisection.

NT-3 levels after spinal cord injury with and without exercise (Fig. 4)

To evaluate the specificity of the spinal cord injury with and without exercise on select neurotrophins, we assessed the levels of another member of the neurotrophin family, i.e., NT-3, that has an important role in spinal cord plasticity. Hemisection had no significant effects on the levels of NT-3 mRNA (Fig. 4A) or protein (Fig. 4B). However, there was a significant increase in NT-3 mRNA levels after 28 days of exercise (145%, $P < 0.05$) (Fig. 4A).

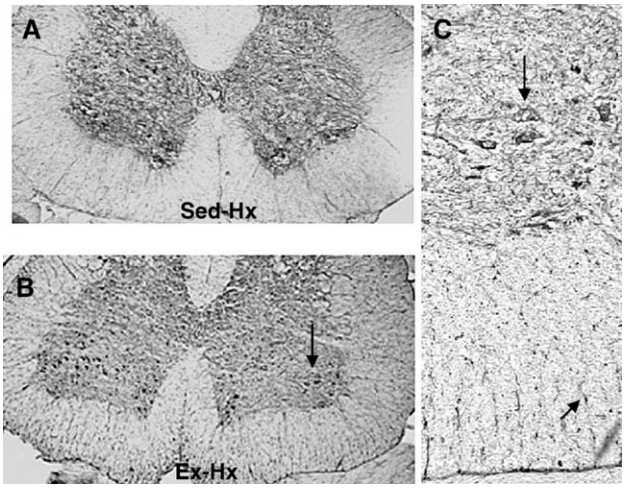

Fig. 2. Representative photomicrographs showing BDNF immunostaining in the lumbar spinal cord region of a sedentary rat (A) and an exercised rat (B) after receiving a right side spinal cord hemisection. (A) There was a relative decrease in BDNF immunostaining in motoneuron cell bodies on the side affected by the hemisection (right side). (B) Exercise preserved BDNF immunostaining on the hemisected side. (C) Higher magnification of the lesion side hemicord (shown in B) displays neuronal elements in the ventral horn (dark arrow) and neurites coursing the white matter (small arrow).

CREB levels after spinal cord injury with and without exercise (Figs. 5A–C)

CREB mRNA levels were determined because it is involved in several intracellular events associated with the action of BDNF on neuronal plasticity (Barde, 1994; Hardingham and Bading, 2002; Lo, 1995). The levels of CREB mRNA in the Sed-Hx rats were 78% (day 7, $P < 0.01$), 68% (day 10, $P < 0.01$), 71% (day 14, $P < 0.01$), and 75% (day 35, $P < 0.01$) of Con values (Fig. 5A) after the injury onset. In contrast, CREB mRNA levels in the Ex-Hx rats were maintained near Con values, i.e., 89%, 90%, and 88% of Con at the 3-, 7-, and 28-day exercise time points. Based on Western blot analyses, the level of CREB was 75% ($P < 0.05$) of Con values after 35 days of hemisection. In contrast, these values in the Ex-Hx rats were similar to Con at the same time point (Fig. 5B). The level of phosphorylated CREB (the activated state) in Sed-Hx rats was 79% ($P < 0.05$) of Con and the level in the Ex-Hx rats was similar to Con (Fig. 5C) after 35 days of hemisection.

GAP-43 levels after spinal cord injury with and without exercise (Fig. 5D)

The levels of GAP-43 mRNA in Sed-Hx rats were 70% (day 7, $P < 0.01$), 62% (day 10, $P < 0.01$), 68% (day 14, $P < 0.01$), and 73% (day 35, $P < 0.01$) of Con values (Fig. 5D) after the injury onset. Exposure of the hemisected animals to wheel running resulted in a significant increase in GAP-43 mRNA after 28 (from 73% to 96% of Con values) but not 7 or 14 days of exercise.

Discussion

Abundant evidence indicates that exercise can facilitate the recovery of locomotion after spinal cord injury (Edgerton and Roy, 2002; Edgerton et al., 2004). The present studies were performed to help better understand the molecular mechanism by which exercise can modulate the plasticity of the injured spinal cord. After receiving a

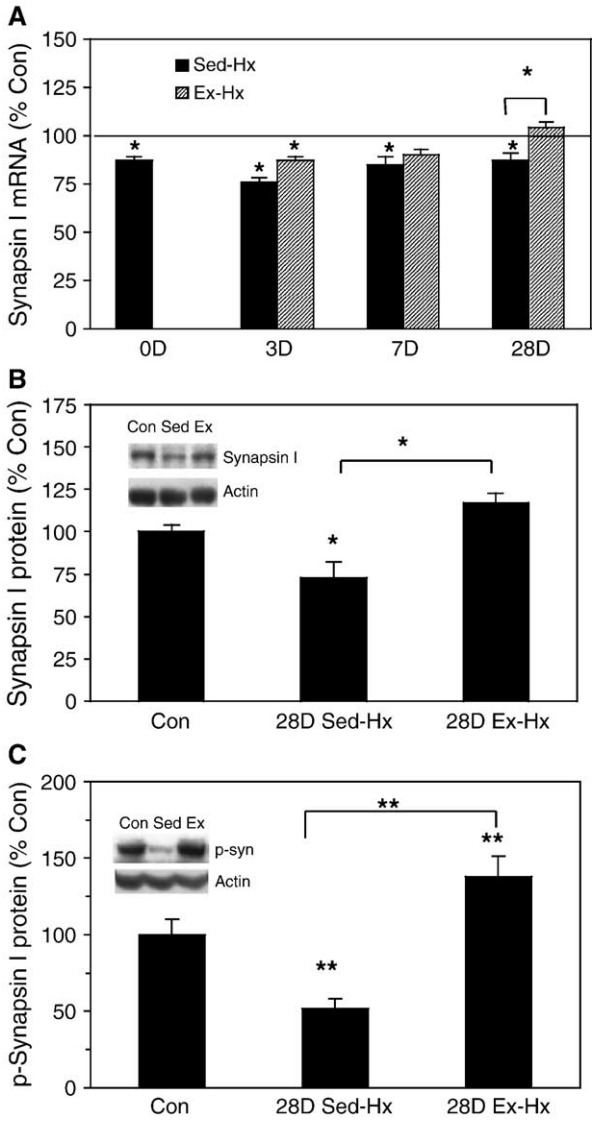

Fig. 3. Relative levels of synapsin I mRNA (A), synapsin I (B), and phospho-synapsin I protein (p-Synapsin I) (C) in the lumbar region of the ipsilateral hemicord in sedentary (Sed-Hx) and exercised (Ex-Hx) hemisected rats. Data are presented as a percent of sedentary control rats. Compared to control, the hemisected rats had lower levels of synapsin I mRNA and protein, whereas exercised rats had similar levels to control rats. Synapsin I mRNA levels were measured using real-time RT-PCR and corrected for equivalent levels of total mRNA using a GAPDH mRNA probe in the same assay solution. Synapsin I and phospho-synapsin I proteins were assessed by Western blots using actin as a standard control. Values are means ± SEM for rats used for mRNA ($n = 6$/group) or protein ($n = 6$/group) analyzed at each time point. * and **, Significantly different at $P < 0.05$ and $P < 0.01$, respectively (ANOVA, Fisher's test).

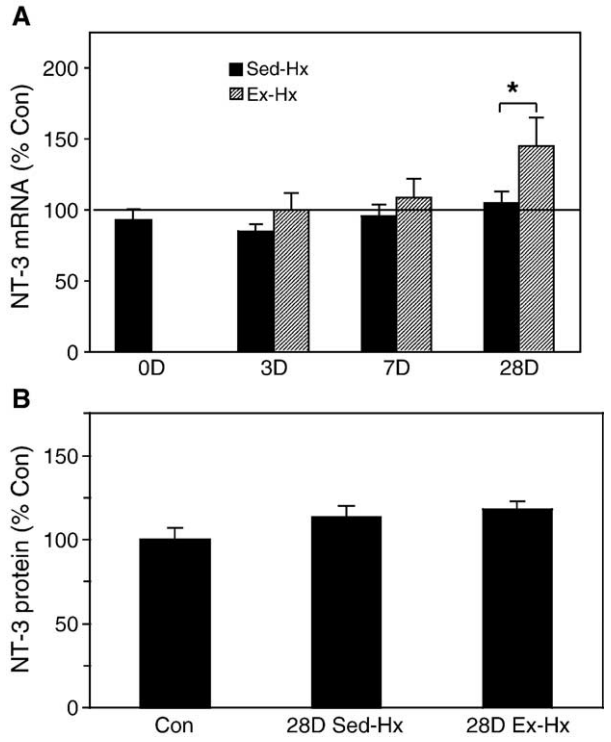

Fig. 4. (A) Levels of NT-3 mRNA in the lumbar spinal cord of hemisected rats that were subsequently exposed to 3, 7, or 28 consecutive days of voluntary running exercise. There was a decrease in NT-3 mRNA after hemisection, and exposure to voluntary wheel running increased the levels of NT-3 mRNA. (B) ELISA showed no changes in NT-3 protein after 28 days of exercise. Values are mean ± SEM for 6 rats per group and are expressed as a percent of sedentary control values. *$P < 0.05$ (ANOVA, Fisher's test).

spinal cord hemisection, rodents were exposed to voluntary running wheel exercise for up to 28 days. Compared to controls, hemisected rats had lower levels of BDNF and of the synaptic plasticity-related molecules synapsin I and CREB in the lumbar region of the hemicord ipsilateral to the lesion. Voluntary exercise significantly attenuated these lesion-related decreases, indicating that physical activity can effectively maintain normal levels of substrates that are important for synaptic plasticity after a spinal cord injury.

Differential effects of hemisection on BDNF and NT-3 levels in the spinal cord

The levels of BDNF mRNA in the lumbar region of the spinal cord ipsilateral to the lesion were lower than in control rats beginning 1 week after injury and lasting up to 35 days after injury (last time point examined). These effects were even more dramatic for BDNF protein, suggesting that the injury affected BDNF translational mechanisms or may have increased BDNF degradation. In contrast, there were no significant effects of hemisection on the levels of NT-3 mRNA or protein. These results are partially consistent with our recent findings that a complete elimination of supraspinal and sensory inputs to the lumbar spinal cord region decreased the levels of both BDNF and NT-3 (Gomez-Pinilla et al., 2002a,b). It is possible that the reduction in suprasegmental input involved with the hemisection in the present study may not have been sufficient to produce a decrease in the levels of NT-3. An alternative explanation for the differential effect of hemisection on the expression of BDNF and NT-3 may be that these two neurotrophins present different mechanisms of regulation in response to a spinal cord lesion and/or a change in neuromuscular activity level. It is well recognized that neural activity can induce production and release of BDNF (Lessmann et al., 2003), but current knowledge for NT-3 is less abundant and inconclusive (Lessmann et al., 2003). It is also likely that other signals intrinsic to the lesion and exercise, such as hormones and neurotransmitters, can provide additional modulation for the observed differential changes in the neurotrophins.

Exposure to exercise completely abrogated the decrease in BDNF mRNA and protein levels observed after hemisection. It is noteworthy that the exercise regime was initiated 1 week after the injury. The rationale behind this time delay was based on studies suggesting that exercise can have a better outcome on neuroplasticity when provided with a short delay after the injury (Griesbach et al., 2004). Although NT-3 mRNA/protein did not decrease relative to Con values after injury, NT-3 mRNA was increased in injured animals that were exposed to 28 days of exercise. We have previously shown that a few days of exercise are sufficient to upregulate NT-3 mRNA and protein in the intact spinal cord (Ying et al., 2003). The current results, in turn, indicate that the injured spinal cord requires more than 7 days of exercise to upregulate the levels of NT-3 mRNA. It is somewhat puzzling as to why the levels of NT-3 protein were not increased with exercise in the spinal cord of the hemisected rats. Possible explanations may be related to the presence of some effects of the spinal cord injury on promoting degradation, depletion, or translocation of NT-3 protein. The levels of BDNF increased in proportion to the average distance run by individual injured rats, implying that exercise can be delivered in doses for specific applications.

The impact of exercise on synaptic plasticity in the injured spinal cord

To evaluate possible mechanisms by which exercise and BDNF can benefit the injured spinal cord, we measured the levels of various molecules that have a recognized interaction with BDNF and are important for synaptic plasticity. It is notable that mRNA levels for all the molecular systems under study (except for NT-3) remained below control levels up to 28 days after the lesion. Interestingly, there was a hint for an increase with time in all the molecules except BDNF, suggesting that some

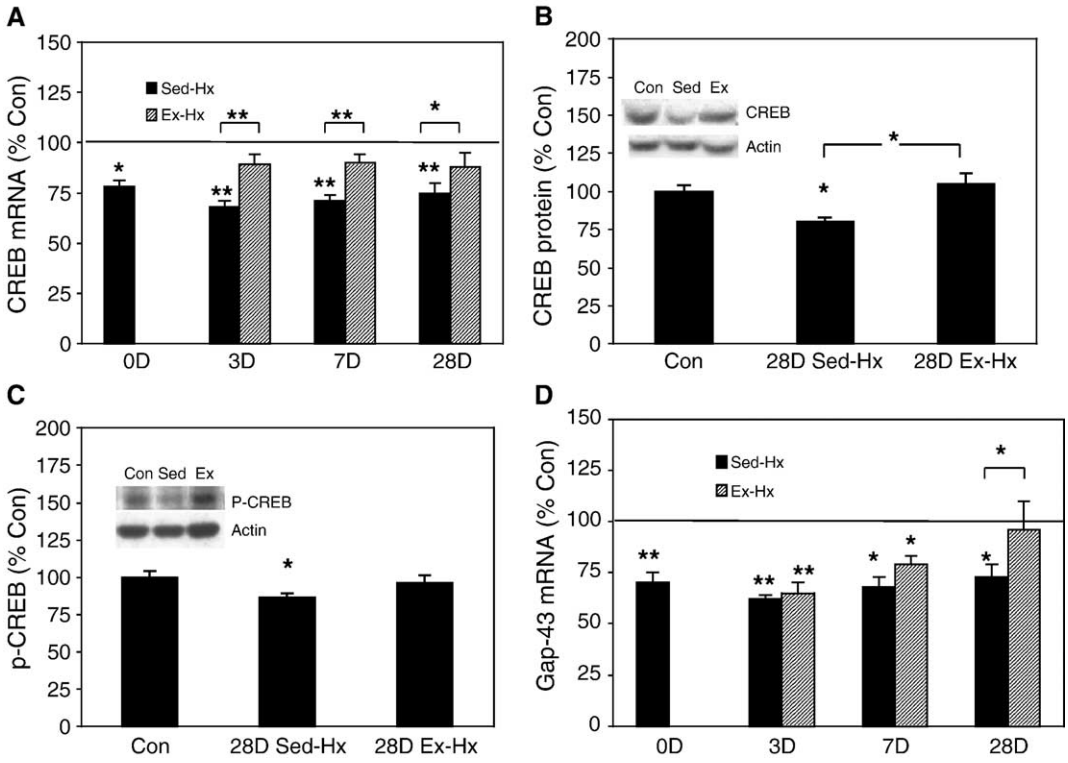

Fig. 5. Relative levels of CREB mRNA (A), CREB (B), phospho-CREB (C), and GAP-43 mRNA (D) were measured in the lumbar region of the spinal cord in hemisected rats that were exposed to exercise for 0, 3, 7, and 28 days. Hemisection decreased the levels of CREB (mRNA and protein) and GAP-43 mRNA, whereas exercise counteracted the effects of the lesion. mRNA levels were measured using real-time RT-PCR and corrected for equivalent levels of total mRNA using a GAPDH mRNA probe in the same assay solution. Proteins were assessed by Western blots using actin as a standard control. Values are mean ± SEM for 6 rats per group and are expressed as a percent of sedentary control values. *$P < 0.05$, **$P < 0.01$ (ANOVA, Fisher's test).

spontaneous recovery was occurring very slowly with time after the injury. Indeed, the animals showed an obvious locomotor deficit after the hemisection lesion. This deficit was reduced 1 week after the injury and the animals were able to freely run in the wheels at that time. Given the preponderant action of BDNF, synapsin I, CREB, and GAP-43 in synaptic function, it is likely that reductions in their levels after the injury may be associated with protracted synaptic function and/or plasticity.

Voluntary exercise maintained the levels of synapsin I mRNA and protein at Con values after injury. We also have observed that in the spinal cord, the modulation of synapsin I mRNA is associated closely with changes in the levels of trkB mRNA in response to exercise (Gomez-Pinilla et al., 2002a,b). BDNF phosphorylates synapsin I primarily through the trkB receptor to induce the mitogen-activated protein kinase signaling pathway which modulates neurotransmitter release (Jovanovic et al., 2000). Therefore, It is likely that exercise-induced BDNF may account for the observed increase in synapsin I phosphorylation in the exercised injured rats. The levels of CREB mRNA were decreased after hemisection, and voluntary exercise restored the values to near normal. CREB is one of the best-characterized transcription factors in the brain and can be modulated by BDNF (Finkbeiner et al., 1997). CREB is phosphorylated by BDNF at the transcription regulatory site, and CREB can feedback on BDNF by regulating its gene transcription via a calcium-dependent mechanism. CREB is required for various forms of memory (Silva et al., 1998), and appears to play a role in neuronal resistance to insult (Walton et al., 1999). It is also notable that exercise elevated the levels of GAP-43 mRNA that was decreased after the hemisection. GAP-43 is present in growing axon terminals and has important roles in axonal growth (Oestreicher et al., 1997), neurotransmitter release (Oestreicher et al., 1997), and learning and memory (Routtenberg et al., 2000). We have previously shown that GAP-43 and synapsin I appear to act in concert under the influence of exercise (Gomez-Pinilla et al., 2002a,b).

Given the preponderant effects of BDNF, synapsin I, and CREB on synaptic plasticity and function, it is highly likely that their reduced levels after injury and subsequent increase after exercise are involved in the cellular dynamics by which exercise enhances spinal cord plasticity. It is notable that there was a tendency for an additional increase in most molecules studied after 28 compared to 3 days of exercise. It is possible that larger increases were not observed because spinal cord injury may have limited the effectiveness of the exercise. It is remarkable, however, that exercise seems to have cumulative effects for longer running periods, such that BDNF and synapsin I values after 28 days of exercise were

greater than those after 3 days. It is worth mentioning that adaptations on the side contralateral to the lesion may have influenced plasticity on the ipsilateral side. Although the lesion or exercise may have promoted sprouting from the contralateral side, this sprouting may have had only an indirect effect on the changes in mRNAs on the ipsilateral side—although BDNF mRNA has been found in dendrites (Tongiorgi et al., 2004), it has not been reported in axonal terminals.

The present results demonstrate that voluntary exercise can maintain the levels of specific neurotrophins within a normal range after a spinal cord injury. In addition, the results show that BDNF plays a central role in synaptic plasticity. All of these results suggest that the therapeutic efficacy of exercise after a spinal cord injury may be closely related to BDNF modulation. Further studies are required to directly relate the changes in neurotrophins after exercise with the functional recovery observed after spinal cord injury.

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