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BDNF–EXERCISE INTERACTIONS IN THE RECOVERY OF SYMMETRICAL STEPPING AFTER A CERVICAL HEMISECTION IN RATS

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Abstract—Clinical evidence indicates that motor training facilitates functional recovery after a spinal cord injury (SCI). Brain-derived neurotrophic factor (BDNF) is a powerful synaptic facilitator and likely plays a key role in motor and sensory functions. Spinal cord hemisection decreases the levels of BDNF below the injury site, and exercise can counteract this decrease [Ying Z, Roy RR, Edgerton VR, Gomez-Pinilla F (2005) Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. Exp Neurol 193:411–419]. It is not clear, however, whether the exercise-induced increases in BDNF play a role in mediating the recovery of locomotion after a SCI. We performed a lateral cervical (~C4) hemisection in adult rats. Seven days after hemisection, the BDNF inhibitor trkB IgG was injected into the cervical spinal cord below the lesion (~C5–C6). Half of the rats were exposed to voluntary running wheels for 14 days. Locomotor ability was assessed by determining the symmetry between the contralateral (unaffected) vs. the ipsilateral (affected) forelimb at the most optimum treadmill speed for each rat. Sedentary and exercised rats with BDNF inhibition showed a higher level of asymmetry during the treadmill locomotion test than rats not treated with the BDNF inhibitor. In hemisected rats, exercise normalized the levels of molecules important for synaptic function, such as cyclic AMP response element binding protein (CREB) and synapsin I, in the ipsilateral cervical enlargement, whereas the BDNF blocker lessened these exercise-associated effects. The results indicate that BDNF levels play an important role in shaping the synaptic plasticity and in defining the level of recovery of locomotor performance after a SCI.

Key words: spinal cord injury, locomotion, TrkB IgG, synaptic plasticity, neurotrophin.

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Abbreviations: BDNF, brain-derived neurotrophic factor; Con, sedentary control group; CREB, cyclic AMP response element binding protein; Ex, exercised hemisected group; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NT-3, neurotrophin-3; Sal, saline injection hemisected group; SCI, spinal cord injury; Sed, sedentary hemisected group; UNG, uracil glycosylase.

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Although neurotrophins have been identified as molecular systems with the potential to enhance spinal cord repair, most of the strategies to induce motor recovery after a spinal cord injury (SCI) have involved the addition of exogenous neurotrophins into the CNS. These strategies, however, do not address the intrinsic potential of the neural system to produce neurotrophins that could have an important effect on the recovery of stepping after a SCI. Given the capacity of voluntary exercise to induce endogenous neurotrophins in the spinal cord (Ying et al., 2003), we hypothesized that neurotrophins play a crucial role in the effects of exercise on the recovery of motor function after a SCI.

A large number of studies have shown the potential of motor training to promote functional recovery after a SCI (Edgerton et al., 2004; Frigon and Rossignol, 2006), yet the specific mechanisms and molecular systems involved remain largely unidentified. It is known that physical activity increases the expression of select neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), in the intact and injured spinal cord (Gomez-Pinilla et al., 2002; Ying et al., 2003). The role of these neurotrophins in promoting synaptic plasticity or functional recovery after a SCI, however, remains unclear. In particular, BDNF is a powerful modulator of neuronal excitability and synaptic transmission (Lu and Figurov, 1997; Kafitz et al., 1999), two processes that are crucial for functional recovery after an injury. The well-described involvement of BDNF in promoting neuronal excitability and synaptic modification suggests that it could play a supporting role in determining the level of functional recovery after a SCI. For example, in addition to promoting axonal growth (Bregman et al., 1997), BDNF delivered to the injured spinal cord can facilitate step-like oscillations in adult rats after a mid-thoracic contusion or complete spinal cord transection when the hind limbs are suspended. In addition, when BDNF was administered continuously over a 4-week period, an open field motor score in complete spinal rats was significantly higher in treated than non-treated rats during weekly testing (Jakeman et al., 1998).

A further question is, through what mechanisms might BDNF operate to alter the course of functional recovery after a SCI? Several of the downstream molecular systems that can mediate the action of BDNF on synaptic plasticity have been identified. BDNF affects the synthesis (Wang et al., 1995) and phosphorylation (Jovanovic et al., 1996) of synapsin I. Synapsin I is a member of a family of nerve terminal–specific phosphoproteins involved in neurotransmitter release (Jovanovic et al., 2000), 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 insult 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). GAP-43 is present in growing axon terminals and has an important role in axonal growth, neurotransmitter release (Oestreicher et al., 1997), and learning and memory (Routtenberg et al., 2000).

In the present study, we investigated the role of BDNF and some of its downstream molecular regulators in mediating the effects of exercise on the recovery of motor function after a SCI. We used a specific molecule, i.e., trkB IgG, to block the function of BDNF during voluntary exercise in rats hemisected at a cervical level. We hypothesized that BDNF modulation induced by exercise plays a critical role in facilitating the recovery of motor function following a SCI. The results, in general, support this hypothesis.

EXPERIMENTAL PROCEDURES

Animals and general procedures

Male Sprague–Dawley rats at 2 months of age (n=34, Charles River, San Diego, CA, USA) were housed singly in standard polyethylene cages. After 1 week of acclimation, the animals were assigned randomly to either a sedentary control group (Con, n=5) or one of four hemisected groups, i.e. sedentary (Sed) or exercised (Ex) with saline (Sal) injection (Sed/Sal, n=8, Ex/Sal, n=8), Sed or Ex with BDNF-inhibitor (IgG) injection (Sed/IgG, n=8, Ex/IgG, n=5). All rats were housed in standard polycarbonate cages (10.25-inch×18.75-inch×8-inch) individually during the experimental period. Rats in the exercise groups were housed in standard cages equipped with running wheels beginning 1 week after the cervical hemisection. The vivarium room was maintained at 26±1 °C, with 40% humidity and a 12-h light/dark cycle. All rats were supplied with rat chow and water *ad libitum*. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the University of California at Los Angeles Chancellor's Animal Research Committee. The suffering and number of animals used were minimized.

Cervical hemisection procedures

All surgeries and injections were performed under aseptic conditions. The rats received an analgesic (Buprenex, 1.0 mg/kg, s.c.; Reckitt Benckiser, Pharmaceuticals Inc., Richmond, VA, USA) 45 min prior to surgery. Surgery was performed with the rats anesthetized deeply with isoflurane gas (1.0–2.5%) via facemask and placed on a water-circulating heating pad maintained at 37 °C to prevent hypothermia. A longitudinal dorsal midline skin incision was made over the spinal column from ~C2 to C6 and the muscles overlying the vertebral column were reflected. A partial laminectomy was performed approximately between vertebral levels C4 and C6. The dura mater was incised longitudinally and lidocaine hydrochloride (1%; two or three drops) was applied at the hemisection site (~C4). The right one-half of the spinal cord was isolated using a specifically designed probe and then transected using microscissors. Small cotton balls were used to separate the cut ends of the hemicord to assure a complete hemisection. Gelfoam was inserted between the cut ends of the hemicord. The paravertebral muscles and fascia surrounding the spinal column were sutured using 4–0 Dexon and the skin incision was sutured using 4–0 Ethilon. The rats were allowed to fully recover from anesthesia in an incubator (27 °C) and were given lactated Ringers solution (5 ml, s.c.). PolyFlex (G.C. Hanford Manufacturing Co., Syracuse, NY, USA), a general antibiotic, was administered (100 mg/kg, s.c., twice daily) during the first 3 days of recovery.

Inhibitor preparation and cervical spinal cord injection procedures

Recombinant Human TrkB/Fc Chimera (TrkB IgG) was used to block the BDNF action by subtracting it from the medium. It was in powder form when purchased from R&D Systems, Inc., Minneapolis, MN, USA. Sterile PBS containing 0.1% of bovine serum albumin (BSA) was added to the vial to prepare a stock solution of 100 μg/ml. Saline was used as a standard control for microspheres injection. Microspheres were used as the vehicle for drug insertion to spinal cord. We prepared microspheres (Lumafluor, New York, NY, USA) using methods described (Riddle et al., 1997) and used in our laboratory (Vaynman et al., 2004). These procedures consisted of coating the microspheres with each solution via passive absorbency by incubating overnight at 4 °C with a 1:5 mix of microspheres to trkB IgG or saline. The morning after coating the microspheres, the solution was centrifuged at 14,000×g for 30 min and the microspheres were re-suspended in sterile water at a 10% concentration. After 1 week of recovery from the surgery, the hemisected rats were assigned randomly into either a sedentary or an exercise group. Under the same surgical conditions as described above, a partial laminectomy was performed between C5 and C7 to expose the dorsal spinal cord. One half of the rats in each group were injected with TrkB IgG (Sed/IgG and Ex/IgG groups) and the other one-half with the same volume of a standard control saline solution (Sed/Sal and Ex/Sal groups). For these injections, the vertebral column was stabilized on a stereotaxic apparatus using clamps on the spinal processes at C2 and C9. TrkB IgG (5 μg/ul) or saline coated with microspheres was injected bilaterally at C5–C6 (0.6 mm lateral to midline, 1.5 mm below the dura mater for the first injection, and 0.8 mm below the dura mater for the second injection) using a Hamilton syringe in a volume of 2 μl over a 15 min period. For the next 14 days, the rats in the exercised groups were housed in cages with voluntary running wheels, whereas the non-exercised rats were housed in same type of cages but without running wheels. A fifth group of rats served as a Con, i.e. no surgery, no exercise, and no injections. TrkB IgG fusion proteins mimic the TrkB receptor and abolish BDNF function by sequestering free BDNF. We have successfully used TrkB IgG embedded in microspheres to block BDNF action for up to 14 days after the injection (Vaynman et al., 2004).

Voluntary exercise procedures

The rats in the exercise groups (Ex/Sal and Ex/IgG) were placed in standard cages equipped with running wheels that rotated against a resistance of 100 g (Ying et al., 2005). Sedentary rats (Con, Sed/Sal, and Sed/IgG) were left undisturbed in their home cages. The numbers of wheel revolutions were monitored and recorded by computer.

Biochemical analyses

The day after the last exercise session (14 days after the BDNF inhibitor or saline treatment), all rats were decapitated in the early morning. The cervical spinal cord enlargement (C3–C6) ipsilateral to the lesion was dissected rapidly, frozen on dry ice, and stored at −70 °C until processed. We used the RNA STAT-60 kit (TEL-TEST, Friendswood, TX, USA) and the manufacturer's protocol for total RNA isolation. The mRNAs for BDNF, NT-3, synapsin I,