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RESEARCH ARTICLES

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Reinnervation of the rat musculocutaneous nerve stump after its direct reconnection with the C5 spinal cord segment by the nerve graft following avulsion of the ventral spinal roots: a comparison of intrathecal administration of brain-derived neurotrophic factor and Cerebrolysin

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Abstract Experimental model based on the C5 ventral root avulsion was used to evaluate the efficacy of brain-derived neurotrophic factor (BDNF) and Cerebrolysin treatment on motor neuron maintenance and survival resulted in the functional reinnervation of the nerve stump. In contrast to vehicle, BDNF treatment reduced the loss and atrophy of motor neurons and enhanced the regrowth axon sprouts into the distal stump of musculocutaneous nerve. However, the axon diameter of the myelinated fibers was smaller than those of control rats. The morphometric results were related to a low score in behavioral test similar to vehicle-treated rats. Cerebrolysin treatment greatly protected the motor neurons against cell death. Moreover, morphometric features of myelinated axons were better than those of rats treated with vehicle or BDNF. The mean score of grooming test suggested better results of the functional motor reinnervation than after BDNF administration. The majority of rescued motor neurons regenerating their axons through nerve graft in both BDNF- and Cerebrolysin-treated rats expressed choline acetyltransferase immunostaining. The results demonstrate that BDNF has more modest effects in preventing the death of motor neurons and functional recovery of injured motor nerve after root avulsion than Cerebrolysin.

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Introduction

Traction injuries of the spinal nerve followed frequently by avulsion of the spinal roots are very difficult for surgical management and functional recovery. Alternative methods for functional reinnervation of avulsed ventral root have been investigated in various experimental animal models including direct reimplantation of the roots back to the spinal cord (Carlstedt et al. 1986, 1995; Horvat et al. 1988). There are experimental models of surgical reconnection of the motor neuron pool with the distal stump of motor nerves by the way of implanted nerve grafts (Bertelli and Mira 1993; Haninec et al. 1996, 1997, 2000). The experiments with adult rats have also demonstrated that avulsion of the ventral spinal roots leads to cell death of motor neurons in the affected spinal cord segment (Koliatsos et al. 1994; Wu 1993). The loss of motor neurons may significantly restrict the achievement of the muscle reinnervation after a surgical repair of the ventral root connection. There are lines of evidence that the administration of various neurotrophic factors in vivo may prevent cell death of adult rat spinal motor neurons not only after nerve injury (Chiu et al. 1994; Li et al. 1994; Yan et al. 1992) but also following ventral root avulsion (Haninec et al. 2003; Kishino et al. 1997; Novikov et al. 1995).

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and interacts with the cell surface transmembrane receptor tyrosine receptor kinase B to activate intracellular signaling pathways leading to the transcription of regenerative associated genes and the inhibition of apoptosis (reviewed in Heumann 1994; Klocker et al. 2000; Segal and Greenberg 1996). Cerebrolysin (EBEWE, Austria) is a brain tissue hydrolysate containing a mixture of 85% free amino acids and

15% small peptides (MW <10,000) with neurotrophic and neuroprotective activity. Cerebrolysin has been reported to stimulate a neuron survival and neurite growth in vitro (Hutter-Paier et al. 1998; Satou 1993; Sugita et al. 1993) and in vivo (Akai et al. 1992). The effects of Cerebrolysin have been also investigated and confirmed in neurodegeneration and ischemia by clinical trials (Ruther et al. 1994; Windisch et al. 1998). In addition, Cerebrolysin is able to protect the neurons from neurodegeneration induced by distal axotomy (Cruz et al. 1998) and ventral root avulsion (Haninec et al. 2003). Our experimental model is based on the C5 ventral root avulsion and reconnection of the distal stump of the musculocutaneous nerve with motor neuron pool of the C5 spinal cord segment by the nerve graft from saphenous nerve (Haninec et al. 2000, 2003). The model allows us to evaluate the efficacy of neurotrophic agents on motor neuron death in adult rats simultaneously with functional reinnervation by means of morphological and electrophysiological analysis as well as behavioral (grooming) test (Bertelli and Mira 1993).

The aim of the present study was to evaluate and compare an amelioration of the motor neuron rescue and functional reinnervation of the rat biceps muscle following intrathecal administration of BDNF and Cerebrolysin in an experimental model of the ventral root reconnection.

Materials and methods

Twenty-four adult female rats (Wistar, Charles River, 200–250 g) were divided randomly into four experimental groups (n=6). All surgical experiments and treatments were carried out according to protocols approved by the Ethics Committee of the Faculty of Medicine, Brno, Czech Republic. The rats were housed in an animal facility at temperature 20°C and with a natural day-night cycle. Food and water were available ad libitum.

Surgical procedures and experimental groups

The first group of intact rats (n=6) was used to obtain numbers of motor neurons and morphometric features of myelinated axons in the musculocutaneous nerve. The animals in the experimental groups were anesthetized with a mixture of ketamine (40 mg/ml) and xylazine (4 mg/ml) administered intraperitoneally (0.2 ml/100 g body weight). The right C5 ventral root of 24 rats was avulsed by traction following posterolateral hemilaminectomy. On the same side the musculocutaneous nerve was sectioned, and the proximal stump was turned, ligated, and sutured to the large pectoral muscle. The fresh nerve graft prepared from the contralateral saphenous nerve was implanted into the C5 spinal cord segment at the position of the ventral horn and fixed in place by fibrin glue. The opposite end of the graft was anastomozed by 10.0 Ethicon suture to the distal stump of the musculocutaneous nerve.

The brain infusion cannula (Brain Infusion Kit 3–5 mm, ALZA, Palo Alto, Calif., USA) was introduced into the right lateral ventricle following a cut in the scalp and drill of opening 1 mm rostral and 1 mm right of the coronal suture. The cannula was introduced to the depth of 3.8–4 mm and connected by a polyethylene catheter tube with an ALZET 2002 osmotic minipump (rate 0.5 µl/h, 2 weeks) implanted subcutaneously in the back of the rat.

The osmotic minipumps of the second rat group were filled with a vehicle solution (phosphate-buffered saline, PBS). The third group contained the rats infused with BDNF (R&D Systems) diluted in PBS for final concentration 375 µg/ml, while the osmotic minipumps of the forth group of animals were filled with a commercially available and nondiluted Cerebrolysin. The infusion speed corresponded to 5 µg/day of BDNF and 12 µl/day of Cerebrolysin. The osmotic minipumps were replaced by second ones containing the same solution after 2 weeks, and the total time of intrathecal administration was therefore 4 weeks. All animals were left to survive for 4 months.

Behavioral analysis

Behavioral analysis of active elbow flexion in the right forelimb was evaluated and scored in the cages using the grooming test (Bertelli and Mira 1993) 4 months after surgery. Analysis of variance followed by appropriate post hoc tests (Tukey's multiple comparison and Dunnett's) by STATISTICA 5.5 software (StatSoft, Tulsa, Okla., USA) were used for statistical comparisons of behavioral data. Statistical significance was accepted at the 5% level (P<0.05).

Motor neurons of the musculocutaneous nerve and their cross-sectional soma area

The motor neuron pool of intact and grafted musculocutaneous nerves was labeled retrogradely by tracer after behavioral analysis. Intact rats (n=6) were anesthetized (see above), their right musculocutaneous nerve was exposed, and a segment (2 mm) was removed approximately at the level of graft suture in the experimental groups. The proximal stump of musculocutaneous nerve was inserted into the yellow pipette tips filled with 10 µl 10% fluoro-ruby (Molecular Probes). The stump was gently washed with PBS after the tracer exposure for 20 min, and the wound was closed with 5/0 sutures. The animals were left to survive for 6 days, deeply anesthetized (sodium pentobarbital), and perfused with PBS followed Zamboni's fixative (Zamboni and De Martino 1967).

Animals of the experimental groups were reanesthetized (see above), the distal stump of their musculocutaneous nerve was reexposed, and a segment (2 mm) was removed 5 mm distal to suture with graft. The motor neurons regenerating of their axons into the stump of musculocu-

taneous nerve were retrogradely labeled by fluoro-ruby as in the intact rats. The spinal cord segments in the range of C4-C6 were removed from all perfused rats and immersed in Zamboni's fixative overnight. The samples were washed in 10% and 20% sucrose overnight. Serial longitudinal cryostat sections (50 μm) through individual segments were collected onto chrome-alum coated slides and mounted into VectaShield medium (Vector Laboratories, Burlingame, Calif., USA). The sections were viewed and digitalized in a Leica DMLB fluorescence microscope equipped with the Leica DC-100 digital camera using N2.1 filter to identify labeled neurons. The number of motor neurons with distinct nucleoli was counted in the right ventral horn of C4–C6 segments and cross-sectional soma area was measured using an image analyzing system Lucia 4.5 (Imaging Laboratory, Prague, Czech Republic).

Simultaneous retrograde labeling of neurons by fluoro-ruby and their immunostaining for choline acetyltransferase

A part of cryostat sections prepared from fluoro-ruby labeled spinal cord segments was incubated for choline acetyltransferase (ChAT) immunofluorescence staining. Sections were rinsed in PBS containing 0.05% Tween 20 (PBS-TW20) and 1% bovine serum albumin. After being treated with 5% normal goat serum in PBS-TW20 for 30 min the sections were incubated with the mouse monoclonal anti-ChAT antibody (Chemicon, Temecula, Calif., USA; dilution 1:250) in a humid chamber at room temperature (20–23°C) overnight (18 h) followed by PBS-TW20 washes. Sections were then incubated in a humid chamber at room temperature for 2 h with fluorescein isothiocyanate conjugated goat anti-mouse secondary antibody (Chemicon; dilution 1:200). Control sections were treated by the whole immunohistochemical staining procedure excluding incubation with primary antibody. After final washes sections were coverslipped with VectaShield mounting medium (Vector).

Immunofluorescence staining for ChAT and fluorochrome distribution (fluoro-ruby) were observed and analyzed by a Leica DMLB epifluorescence microscope equipped with appropriate filter combinations for simultaneous fluorescein isothiocyanate and tetramethylrhodamine isothiocyanate excitation (G/R) and HCX PL Fluotar objective, x20 (Leica Microsystems, Wetzlar, Germany).

Morphometric evaluation of myelinated axons in the musculocutaneous nerves

Segments removed from intact and operated musculocutaneous nerves were fixed overnight by immersion in the fixative solution containing 4% depolymerized paraformaldehyde, 1.5% glutaraldehyde, and 10% sucrose in cacodylate buffer (0.1 M, pH 7.2). The samples were then postfixed in 1% osmium tetroxide following washing in cacodylate buffer (0.1 M, pH 7.2), and embedded into Durcupan (Durcupan ACM, Fluka) by standard procedure.

The transverse semithin sections, 80 nm thick, were stained with toluidine blue. Six randomly selected sections were digitalized under a Leica DMBL light microscope equipped with a DC-100 digital camera at a final

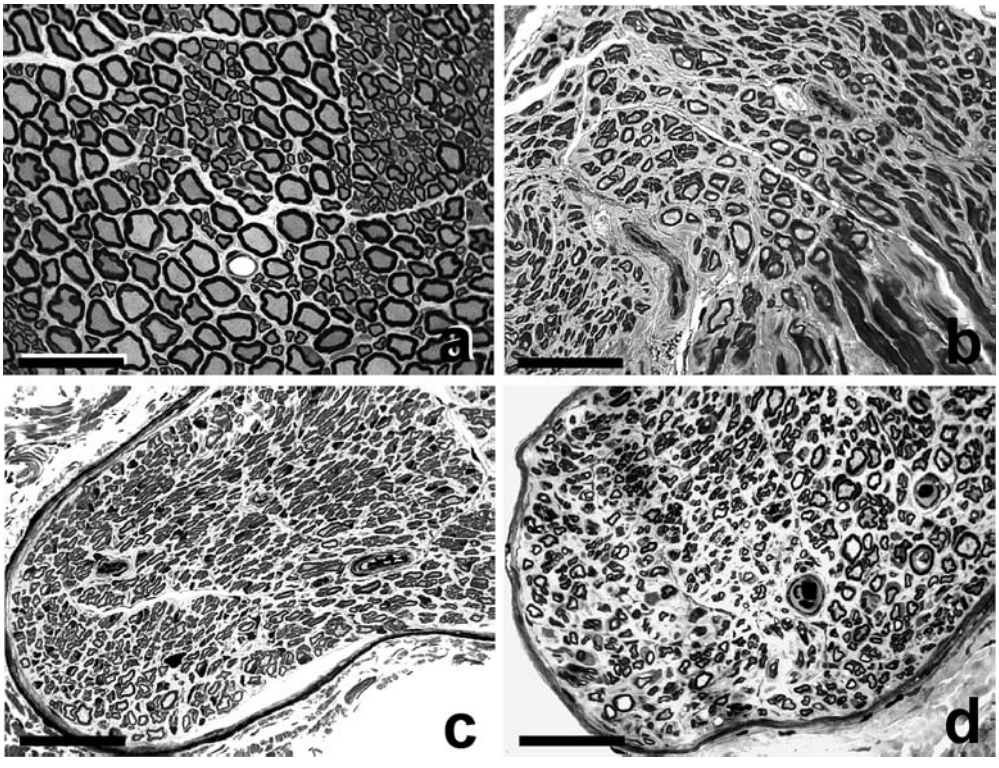

Fig. 1 Representative transverse sections through the intact musculocutaneous nerve (**a**) and the stumps of musculocutaneous nerves 4 months after their reconnection with the C5 spinal cord segment by the saphenous nerve graft of the rats treated with PBS (**b**), BDNF (**c**), and Cerebrolysin (**d**). *Scale bars* 40 μm

magnification of ×600. Representative transverse sections through the intact musculocutaneous nerve and the stumps of musculocutaneous nerves 4 months after their reconnection with the C5 spinal cord segment by the saphenous nerve graft of the rats treated with PBS, BDNF, and Cerebrolysin are illustrated in Fig. 1. The total number and diameter of myelinated axons as well as the myelin sheath thickness were counted and measured by means of a computer-assisted image analysis system Lucia-G (Laboratory Imaging, Prague, Czeck Republic) from digitalized pictures in BMP format.

The total number of myelinated axons, the axon diameters and myelin thickness were compared among the musculocutaneous nerves of intact and operated rats supported by intrathecal administration of BDNF and Cerebrolysin for 4 weeks. The data were statistically evaluated by a one-way analysis of variance with post hoc comparisons of means using Statistica 5.5 software (StatSoft). Statistical significance was accepted at the 5% level ($P<0.05$).

Results

Fluoro-ruby labeled motor neurons of intact musculocutaneous nerve were found in sections through C4-C6 but most were located in C5 spinal cord segment. The range of motor neuron pool for the musculocutaneous nerve was in agreement with previously published results (Bertelli and Mira 1995). However, labeled motor neurons were restricted only to C5 spinal cord segment of operated and treated animals. Therefore the C5 spinal cord segment was used to compare the influence of vehicle, BDNF, and Cerebrolysin on motor neurons regenerating their axons through the nerve graft into the musculocutaneous nerve stump (Fig. 2).

Following vehicle (PBS) treatment, only a very low number of motor neurons (about 6% of intact rat) were retrogradely labeled, indicating a reduced regeneration of their axons into the musculocutaneous nerve stump. In addition, the motor neurons displayed strong atrophy (Fig. 2b). The low number of labeled motor neurons was

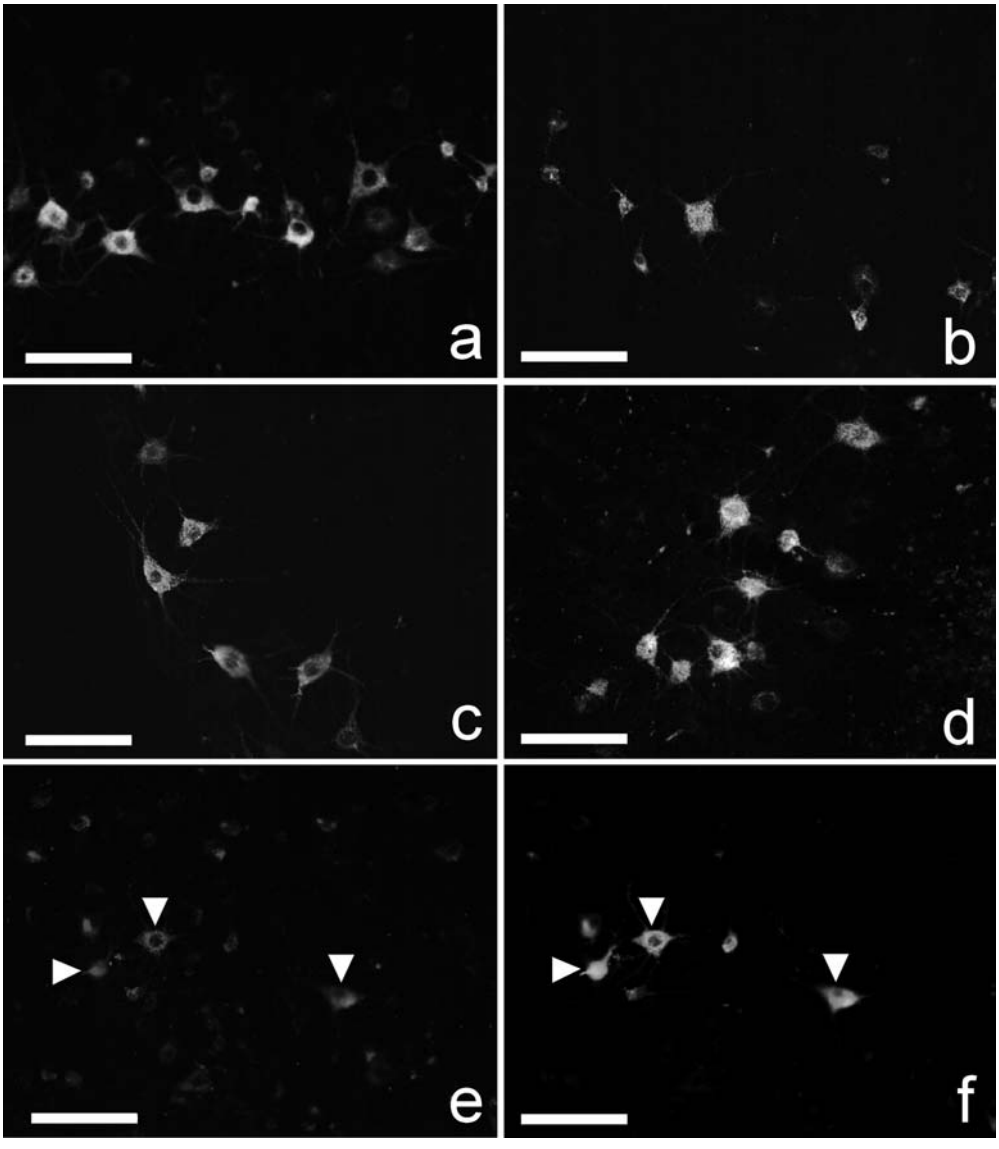

Fig. 2 Longitudinal sections of the C5 spinal cord segments from intact rat (**a**) and PBS- (**b**), BDNF- (**c**) and Cerebrolysin-treated (**d**–**f**) rats. Fluoro-ruby labeled motor neurons illustrated that BDNF and Cerebrolysin treatment reduced atrophy and supported motor neuron survival. Spinal motor neurons labeled with fluoro-ruby of intact and treated rats displayed ChAT immunoreactivity as illustrated in representative sections of the C5 spinal cord segments from Cerebrolysin-treated rat (**e**, **f**). *Scale bars* 135 μm

correlated with very low density of myelinated axons regenerated into the stump of musculocutaneous nerve (Table 1).

Intrathecal administration with BDNF or Cerebrolysin for 4 weeks and survival for 4 months after surgery resulted in a significant increased number of retrogradely labeled motor neurons, their cross-sectional soma areas (Fig. 2c, d; Table 1), and the density of regenerated axons in comparison with vehicle treatment. However, the mean diameter of the myelinated axons regenerated into the musculocutaneous nerve stump of BDNF-treated animals was even smaller than those of rats treated with vehicle (PBS). The mean thickness of myelin sheaths of the axons was very similar in the group of BDNF and vehicle-treated rats. These morphometric features of regenerated axons correlated with similarities between mean scores of behavioral (grooming) tests comparing BDNF- and vehicle-treated animals (Table 1). We need to note that not all nerve fibers regenerated into the musculocutaneous nerve stump are motor fibers. It is known that sensory fibers even in the muscular peripheral nerve can constitute at least half of the myelinated fibers (Bertelli and Mira 1995)

Intrathecal administration with Cerebrolysin for 4 weeks and next survival up to 4 months after surgery significantly increased the number of motor neurons that regenerated axons into the musculocutaneous nerve stump after its reconnection with the spinal cord segment by the nerve graft. The number of the motor neurons was more than three times higher in Cerebrolysin- than in BDNF-treated rats. The density of regenerated axons was also significantly higher in rats treated with Cerebrolysin than in those treated with BDNF (Table 1). In addition, the mean diameter of the axons regenerated through the nerve graft into the musculocutaneous nerve stump and their myelin thickness were greater in Cerebrolysin- than BDNF-treated rats. Better morphometric features of regenerated axons in the rats treated with Cerebrolysin were related to a significantly higher score of behavioral test than in the BDNF group of rats (Table 1).

The motor neurons of C5 spinal cord segment of intact and treated animals expressed immunofluorescence staining for ChAT without visible differences. Control sections incubated with omission of primary antibody were free of the green immunofluorescence staining. The majority of fluoro-ruby labeled motor neurons in both Cerebrolysin- and BDNF-treated rats simultaneously displayed immunofluorescence staining for ChAT (for illustration see Fig. 2e, f).

Discussion

Experimental and rare clinical results (Bertelli and Mira 1993; Carlstedt et al. 1986, 1995; Horvat et al. 1988) have suggested that avulsed ventral root can be reinnervated by motor neurons following direct insertion of ventral root stump back to the spinal cord. The encouraging results were extended by experimental model of direct reconnection of the distal stump of musculocutaneous nerve with the C5 spinal cord segment by cellular or acellular graft prepared from the saphenous nerve (Haninec et al. 1996, 1997, 2000). However, in contrast to peripheral nerve axotomy, the ventral root avulsion of adult rats leads to loss of most of the corresponding spinal cord motor neurons within 2–6 weeks (Koliatsos et al. 1994; Wu 1993). Therefore the rescue of motor neurons after the ventral root avulsion is an important prerequisite for further approaches to reconnect the spinal motor neurons with their target muscle.

The axotomy-induced loss of neurons can be interrupted by provision of neurotrophic factors (Friedman et al. 1995; Li et al. 1995) or antiapoptotic molecules, for example, BCl-2 (Shibata et al. 2000), which block the apoptotic process. Injured neurons that are rescued from retrograde degeneration can potentially continue to contribute to functional spinal circuits. An increasing number of neurotrophic agents have so far been found to support the motor neuron survival in a variety of experimental paradigms (reviewed by Oppenheim 1996). For example, BDNF, neurotrophins 3 and 4, and insulin-like growth factor I are candidates for target-derived trophic factors that are able to prevent lesion-induced death of the motor neurons (Kishino et al. 1997; Novikov et al. 1995; Oppenheim et al. 1993; Yan et al. 1992). Several experimental strategies of delivery of the neurotrophic factors have been employed to minimize tissue damage and to enhance axonal regrowth after spinal cord injury (see for review Murray et al. 2002).

The experimental ventral root avulsion is considered to be suitable and reproducible model for assessing mechan-

Table 1 A comparison of motor neuron numbers, their atrophy, and morphometric characteristics of the myelinated axons and results of the grooming test in the intact rats and the rats treated with PBS, BDNF, and Cerebrolysin for 4 weeks (six animals in each group). The experimental rats were allowed to survive for 4 months after the ventral root avulsion and the nerve reconnection with the C5 spinal cord segments by the saphenous nerve grafts

	Intact	PBS	BDNF	Cerebrolysin
Number of stained motor neurons	215±26	12±6	51±17*	173±50*,**
Cross-sectional soma area (μm^2)	1226±55	854±68	1281±78*	1175±61*
Number of myelinated axons per 10,000 μm^2	156±8	57±13	102±15*	146±34*,**
Mean diameter of myelinated axons (μm)	4.08±0.06	2.07±0.04	1.85±0.02*	2.31±0.05*,**
Mean score of grooming test	5±0	3±0.31	3±0.45	4±0.33*,**

*P<0.05 vs. PBS, **P<0.05 vs. BDNF

isms of motor neuron death and testing the ability of neurotrophic factors to promote their survival in adult mammals (Haninec et al. 2003; Koliatsos et al. 1994; Li et al. 1995; Novikov et al. 1995). Our previous experiments have confirmed that avulsion of the C5 ventral roots in adult rat results in significant loss of spinal motor neurons due mainly to apoptosis. In comparison to the untreated rats, the amount of motor neuron survival in avulsed C5 ventral horn was significantly higher after 4 weeks intrathecal administration of Cerebrolysin or insulin-like growth factor I (Haninec et al. 2003).

It has been shown that spinal motor neurons are progressively altered after their regenerated axons have reestablished functional synapses with their peripheral targets. Therefore the degeneration of axotomized motor neurons is a progressive process and includes a "second wave" after extended survival intervals (Bowe et al. 1992). Moreover, transmitter markers (e.g., ChAT) are reduced in various types of CNS neurons through postlesion periods of time (Koliatsos et al. 1989). In our experimental paradigm we analyzed surviving neurons extending regenerated axons after 8 weeks from postlesion; therefore we evaluated only the final effects of intrathecal administration of BDNF and Cerebrolysin.

There are contrary reports about BDNF effect in the prevention of cell death and atrophy (Li et al. 1995; Novikov et al. 1995) as well as the reduction in ChAT immunoreactivity in axotomized adult motor neurons (Clatterbuck et al. 1994; Kishino et al. 1997), perhaps depending on differences in the type or site of spinal cord injury, differences in the quality, and extent of BDNF delivery. In the root avulsion model intrathecal administration of BDNF prevented the cell death of severely damaged motor neurons and their atrophy (Novikov et al. 1995) and promoted axonal sprouting from the rescued neurons (Kishino et al. 1997). In contrast to experiments by Novikov et al. (1995) and Kishimo et al. (1997), we counted motor neurons not only surviving ventral root avulsion but also regenerating their axons through nerve graft into the distal nerve stump. In addition, we administered only a one-half dose of BDNF for 4 weeks to rescue motor neurons from their avulsion-induced loss and to induce axon regeneration. Our present results confirm that intrathecal administration of BDNF significantly rescues motor neurons from avulsion-induced cell death and attenuates the atrophy of surviving motor neurons on the lesioned side. In addition, the motor neurons regenerating their axons into the musculocutaneous nerve demonstrated no visible reduction in ChAT immunoreactivity in comparison to the intact or contralateral motor neurons. The results also support previous conclusions indicating a superior availability and effect of neuron-promoting agents after their continuous intrathecal rather than single or intravenous administration even if their concentrations were lower (Haninec et al. 2003; Kishino et al. 1997).

It has been suggested that the significant motor neuron loss after spinal root avulsion in adult rats is owing to the deprivation of trophic support from Schwann cells associated with the peripheral nerve (Wu 1993, 1996). Our experiments indicate that when the motor neurons are stimulated to regrow their axons immediately after injury, the axons reach to the Schwann cells of the graft or distal stump and the neurotrophic molecules may be retrogradely transported to promote motor neuron survival. In addition, the Schwann cells of nerve graft implanted into the spinal cord are able to stimulate a spontaneous growth of axonal sprouts (Haninec et al. 2000) and induce rescue of small amount of motor neurons as shown results of control (vehicle-treated) animals. Exogenous neurotrophin administration may last only within the period when regenerating axons need a support to reach the Schwann cells or other peripheral source of neurotrophins. Then the axons are able to protect the neurons from their atrophy or death. Consistent with this speculation are our results that intrathecal treatment with BDNF in our experiments promoted significant reduction in the loss of motor neurons in the C5 spinal cord segment and enhanced the axon regrowth into the distal stump of musculocutaneous nerve.

The axonal sprouts in the distal stump of musculocutaneous nerve were numbered after treatment with BDNF, however thinner than those of control (vehicle-treated) animals, indicating promotion of axonal sprouting without their further maturation. The results illustrate that BDNF promotes axonal sprouting from the rescued neuron (Kishino et al. 1997). However, the mean score of the behavioral (grooming) test confirmed that many but morphologically immature axons do not increase conduction velocity of peripheral nerve (Gillespie and Stein 1983) and functional reinnervation (De Medinaceli 1995). Here we show that intrathecal administration of BDNF immediately after ventral root avulsion may reduce the loss of motor neurons and stimulate them to regrowth their axon sprouts, but without their morphological maturation and final functional benefit.

In comparison to BDNF, intrathecal administration of Cerebrolysin provided not only the rescue of motor neurons and enhanced regrowth of their axons but also a higher maturation of the regenerated axons. The morphometric quality of regenerated axons following Cerebrolysin administration was related to better results in functional (grooming) test. Cerebrolysin is an extract from pig brain obtained after enzymatic digestion containing free amino acids and biologically active peptides showing neurotrophic efficiency (Sugita et al. 1993). Results of our previous experiments revealed that continuous intrathecal administration of Cerebrolysin may prevent the loss of motor neurons in the C5 spinal cord segment from avulsion-induced death in the adult rats. The neuroprotective effect of Cerebrolysin is comparable to that with administration of insulin-like growth factor I (Haninec et al. 2003) or BDNF, as shown by the present results. The fact of better results with Cerebrolysin than with BDNF treatment in our experiment supports conclusions that a combination of neurotrophic factors would have better survival-promoting or axonal growth effects than indivi-

dual neurotrophic factors used alone (Cao and Shoichet 2003; Mitsumoto et al. 1994).

Experimental research has produced exciting progress in understanding the neurotrophic factors that affect neuronal survival. Our experimental results in adult rats indicate that Cerebrolysin has a very powerful effect on the survival of motor neurons as well as the results of functional reinnervation following the ventral root avulsion. This can encourage the initiation of clinical trials for neurodegenerative consequence of the peripheral nerve traumas.

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References

Akai F, Hiruma S, Sato T, Iwamoto N, Fujimoto M, Ioku M, Hashimoto S (1992) Neurotrophic factor like effect of FPF1070 on septal cholinergic neurons after transection of fimbria-fornix in the rat brain. Histol Histopathol 7:213–221

Bertelli JA, Mira JC (1993) Behavioral evaluating methods in the objective clinical assessment of motor function after experimental brachial plexus reconstruction in the rat. J Neurosci Methods 46:203–208

Bertelli JA, Mira JC (1995) The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods 58:1–2

Bowe CM, Evans NH, Vlacha V (1992) Progressive morphological abnormalities observed in rat spinal motor neurons at extended intervals after axonal regeneration. J Comp Neurol 321:576–590

Cao X, Shoichet MS (2003) Investigating the synergistic effect of combined neurotrophic factor concentracion gradients to guide axonal growth. Neuroscience 122:381–389

Carlstedt T, Linda H, Cullheim S, Risling M (1986) Reinnervation of hind limb muscles after ventral root avulsion and implantation in the lumbar spinal cord. Acta Physiol Scand 128:645–646

Carlstedt T, Grane P, Hallin RG, Norén G (1995) Return of function after spinal cord implantation of avulsed spinal nerve roots. Lancet 346:1323–1325

Chiu AY, Chen EW, Loera S (1994) Distinct neurotrophic responses of axotomized motor neurons to BDNF and CNTF in adult rats. Neuroreport 5:693–696

Clatterbuck RE, Price DL, Koliatsos VE (1994) Further characterization of the effects of brain-derived neurotrophic factor and ciliary neurotrophic factor on axotomized neonatal and adult mammalian motor neurons. J Comp Neurol 342:45–56

Cruz R, Francis L, Diaz-Suarez CM, Gonzalez-Fraguela ME (1998) Short-term effects of septo-hippocampal pathway transection and Cerebrolysin effects on glutathione-related enzymes in the rat brain. Rev Neurol (Paris) 26:551–554

De Medinaceli L (1995) Interpreting nerve morphometry data after experimental traumatic lesions. J Neurosci Methods 58:29–37

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

Gillespie MJ, Stein RB (1983) The relationship between axon diameter, myelin thickness and conduction velocity during atrophy of mammalian peripheral nerves. Brain Res 259:41–56

Haninec P, Dubový P, Houšt'ava L, Stejskal L (1996) Alternative methods of the brachial plexus reconstruction-An experimental study. Scripta Med 69:221–226

Haninec P, Dubový P, Houšt'ava L, Stejskal L (1997) Reconstructive operations of brachial plexus injury and the experimental model of their management. Intern Proc Div, Centennial perspective. Monduzzi, Amsterdam, pp 2103–2107

Haninec P, Dubový P, Houšt'ava L, Stejskal L (2000) Acellular nerve graft re-seeded by Schwann cells migrating from the nerve stump can stimulate spinal motor neurons for functional reinnervation of the rat muscle. Ann Anat 182:123–131

Haninec P, Houšt'ava L, Stejskal L, Dubový P (2003) Rescue of rat spinal motor neurons from avulsion-induced cell death by intrathecal administration of IGF-I and Cerebrolysin. Ann Anat 185:233–238

Heumann R (1994) Neurotrophin signaling. Curr Opin Neurobiol 4:668–679

Horvat JC, Pecot-Deachavassine M, Mira JC (1988) Functional reinnervation of a denervated skeletal muscle of the adult rat by axons regenerating from the spinal cord through a peripheral nervous system graft. Progr Brain Res 78:219–224

Hutter-Paier B, Steiner E, Windisch M (1998) Cerebrolysin protects isolated cortical neurons from neurodegeneration after brief histotoxic hypoxia. J Neural Transm 53:351–361

Kishino A, Ishige Y, Tatsuno T, Nakayama C, Noguchi H (1997) BDNF prevents and reverses adult rat motor neuron degeneration and induces axonal outgrowth. Exp Neurol 144:273–286

Klocker N, Kermer P, Weishaupt JH, Labes M, Ankerhold R, Bahr M (2000) Brain-derived neurotrophic factor-mediated neuroprotection of adult rat retinal ganglion cells in vivo does not exclusively depend on phosphatidyl-inositol-3′-kinase/protein kinase B signaling. J Neurosci 20:6962–6967

Koliatsos VE, Applegate MD, Kitt CA, Walker LC, DeLong MR, Price DL (1989) Aberrant phosphorylation of neurofilaments accompanies transmitter-related changes in rat septal neurons following transection of the fimbria-fornix. Brain Res 482:205–218

Koliatsos VE, Price WL, Pardo CA, Price DL (1994) Ventral root avulsion-an experimental model of death of adult motor neurons. J Comp Neurol 342:35–44

Li L, Oppenheim RW, Lei M, Houenou J (1994) Neurotrophic agents prevent motor neuron death following sciatic nerve section in the neonatal mouse. J Neurobiol 25:759–766

Li LX, Wu WT, Lin LFH, Lei M, Oppenheim RW, Houenou LJ (1995) Rescue of adult mouse motor neurons from injury-induced cell death by glial cell line-derived neurotrophic factor. Proc Natl Acad Sci USA 92:9771–9775

Mitsumoto H, Ikeda K, Klinkosz B, Cedarbaum JM, Wong V, Lindsay RM (1994) Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. Science 265:1107–1110

Murray M, Kim D, Liu Y, Tobias C, Tessler A, Fischer I (2002) Transplantation of genetically modified cells contributes to repair and recovery from spinal injury. Brain Res Brain Res Rev 40:292–300

Novikov L, Novikova L, Kellerth JO (1995) Brain-derived neurotrophic factor promotes survival and blocks nitric oxide synthase expression in adult rat spinal motor neurons after ventral root avulsion. Neurosci Lett 200:45–48

Oppenheim RW (1996) Neurotrophic survival molecules for motor neurons: an embarrassment of riches. Neuron 17:195–197

Oppenheim RW, Prevette D, Haverkamp LJ, Houenou L, Yin QW, McManaman J (1993) Biological studies of a putative avian muscle-derived neurotrophic factor that prevents naturally occurring motor neuron death in vivo. J Neurobiol 24:1065–1079

Ruther E, Ritter R, Apecechea M, Freytag S, Windish M (1994) Efficacy of peptidergic nootropic drug Cerebrolysin in patients with senile dementia of Alzheimer type. Pharmacopsychiatry 27:32–40

Satou T, Imano M, Akai F, Hashimoto S, Itoh T, Fujimoto M (1993) Morphological observation of effects of Cerebrolysin on cultured neuronal cells. In: Nicolini, Zatta, Coraine (eds) Alzheimer's disease and related disorders. Pergamon, Oxford, pp 195–196

Segal RA, Greenberg ME (1996) Intracellular signaling pathways activated by neurotrophic factors. Annu Rev Neurosci 19:463–489

Shibata M, Murray M, Tessler A, Ljubetic C, Connors T, Saavedra RA (2000) Single injections of a DNA plasmid that contains the human Bcl-2 gene prevent loss and atrophy of distinct neuronal populations after spinal cord injury. Neurorehabil Neural Repair 14:319–330

Sugita Y, Kondo T, Kanazawa A, Itou T, Mizuno Y (1993) Protective effect of FPF 1070 (Cerebrolysin) on delayed neuronal death in the gerbil-detection of hydroxyl radicals with salicylic acid. No To Shinkei 45:325–331

Windisch M, Gschanes A, Hutter-Paier B (1998) Neurotrophic activities and therapeutic experience with a brain derived peptide preparation. J Neural Transm 53:289–298

Wu W (1993) Expression of nitric-oxide synthase (NOS) in injured CNS neurons as shown by NADPH diaphorase histochemistry. Exp Neurol 120:153–159

Wu W (1996) Potential roles of gene expression change in adult rat spinal motor neurons following axonal injury: a comparison among c-jun, low-affinity nerve growth factor receptor (LNGFR), and nitric oxide synthase (NOS). Exp Neurol 141:190–200

Yan Q, Elliott J, Snider WD (1992) Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. Nature 360:755–777

Zamboni L, De Martino C (1967) Buffered picric acid-formaldehyde: a new, rapid fixative for electron microscopy. J Cell Biol 35:14