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Short communication

Up-regulation of the TrkB receptor in mice injured by the partial ligation of the sciatic nerve

Minoru Narita [a], Yoshinori Yajima [a], Takeshi Aoki [a], Satoru Ozaki [a], Michiko Narita [b], Hirokazu Mizoguchi [b], Leon F. Tseng [b], Tsutomu Suzuki [a,*]

[a] *Department of Toxicology, School of Pharmacy, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA*

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Abstract

Partial nerve injury induced by tying a tight ligature around the sciatic nerve induced a marked hyperalgesia, and this persistent painful state lasted for 14 days in mice. Under these conditions, the nerve injury induced a significant increase in protein level of protein kinase Cγ isoform in plasma membranes in the spinal cord. We report here for the first time that protein level of TrkB receptor located in plasma membranes was clearly up-regulated in the spinal cord obtained from the nerve-injured mice. These findings suggest that the up-regulation of protein kinase Cγ associated with activated TrkB receptors following partial sciatic nerve ligation may induce sensitization of synaptic transmission and may in turn cause the persistent pain in mice. © 2000 Published by Elsevier Science B.V.

Keywords: Nerve ligation; Chronic pain; Protein kinase C; TrkB receptor; Spinal cord; (Mouse)

1. Introduction

The family of neurotrophic factors collectively termed the neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, neurotrophin-4/5 and neurotrophin-6 (Thoenen, 1991). The neurotrophins have been shown to play important roles in the regulation of persistent pain (Lewin and Mendell, 1993; Theodosiou et al., 1999). The evidence to date suggests that BDNF, which is an endogenous neurotransmitter/neuromodulator in small-diameter nociceptive neurons, may be released in the spinal cord in an activity-dependent fashion, thereby regulating post-synaptic excitability within the spinal dorsal horn (Theodosiou et al., 1999; Thompson et al., 1999).

The Trk family (TrkA, TrkB and TrkC) of receptor tyrosine kinases constitutes high-affinity receptors for the neurotrophins. BDNF has been shown to possess higher affinity for the TrkB receptor than TrkA and TrkC receptors (Maness et al., 1994). Recent work on inflammatory hyperalgesia has shown that behavioral nociceptive responses in inflamed rats evoked by the injection of formalin into the hind paw are reduced by intrathecal administration of TrkB-immunoglobulin G (IgG) (Thompson et al., 1999), indicating the implication of the BDNF-TrkB pathway in the inflammatory hyperalgesia.

Injury to a peripheral nerve often results in a persistent neuropathic pain condition that is characterized by spontaneous pain, allodynia and hyperalgesia. There is considerable evidence that protein kinase C contributes to the development of the long-term changes that underlie injury-associated allodynia and hyperalgesia (Coderre, 1992; Mao et al., 1993; Meller et al., 1996). A recent report using the transgenic mice has provided direct evidence that activation of the gamma isoform of protein kinase is critical for the development of neuropathic pain (Malmberg et al., 1997). With respect to the intracellular mechanisms of the BDNF-TrkB pathway, the stimulation of TrkB receptor by BDNF leads to the activation of phospholipase Cγ as well as tyrosine kinases and subsequently activates protein kinase C (Kaplan and Stephens, 1994; Stephens et al., 1994). Thus, these findings suggest the possibility that functional changes in TrkB receptors are implicated in the long-term hyperalgesia associated with activation of protein kinase Cγ after nerve injury. The present study was then designed to investigate the

* Corresponding author. Tel.: +81-3-5498-5831; fax: +81-3-5498-5831.
E-mail address: suzuki@hoshi.ac.jp (T. Suzuki).

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change in protein levels of the TrkB receptor and protein kinase Cγ isoform in the spinal cord following partial sciatic nerve ligation in the mouse.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

2.1. Animals

Male ICR mice weighing 23–30 g were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed at a room temperature of 22 ± 1°C with a 12-h light-dark cycle (light on 8:00 am to 8:00 pm). Food and water were available ad libitum during pre-experimental period.

2.2. Nerve injury pain models

The mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) with ether. We produced a partial nerve injury model by tying a tight ligature with 7-0 silk suture around approximately 1/3 to 1/2 the diameter of the sciatic nerve located in the right-paw side (ipsilateral site), similar to the approach described in rats by Seltzer et al. (1990) and in mice by Malmberg et al. (1997). In sham-operated mice, the nerve was exposed, but not ligated.

2.3. Withdrawal latencies from the thermal stimulus

Prior to the testing of the behavioral responses to thermal stimuli, mice were habituated to the test environment for 60 min. To assess heat thermal sensitivity, each of the hind paws of the mice was measured individually using a thermal stimulus apparatus (Biological Research Apparatus Type 7370, UGO BASILE, Varese, Italy). Paw withdrawal latency was determined as the average of two measurements per paw. Only quick hind paw movements (with or without licking of the hind paw) away from the stimulus were considered a withdrawal response. Paw movements associated with locomotion or weight shifting were not counted as a response, and mice were re-tested for that trial. Just before the ligation, and 7 and 14 days after the surgery, withdrawal latencies of the operated paw from the thermal stimulus were measured.

2.4. Tissue dissection and preparation

To investigate the functional changes in protein levels of the TrkB receptor and protein kinase Cγ isoform, the mice were decapitated at 14 days after the ligation. The area of L4 to L6 from the spinal cord was dissected, and was divided along the midline into ipsilateral and contralateral sides. The ipsilateral tissue was immediately homogenized in ice-cold buffer containing 20 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml of leupeptin, 0.1 mg/ml of aprotinin and 0.32 M sucrose. The homogenate was then centrifuged at $1000 \times g$ for 10 min and the supernatant was ultracentrifuged at $20{,}000 \times g$ (for TrkB) or $100{,}000 \times g$ (for protein kinase Cγ) for 30 min at 4°C. The pellets were then re-homogenated and re-centrifuged at $20{,}000 \times g$ (for TrkB) or $100{,}000 \times g$ (for protein kinase Cγ) for 30 min at 4°C. The resulting pellets were resuspended and retained as membranous fractions. An aliquot of tissue sample was diluted with electrophoresis sample buffer (Protein Gel Loading Dye 2×; AMRESCO, Solon, OH) containing 2% sodium dodecyl sulfate (SDS) and 10% glycerol with 0.2 M dithiothreitol. Proteins (10 μg/lane) were separated by size on 4-20% SDS-polyacrylamide gradient gel and transferred to nitrocellulose membranes in Tris-glycine buffer. After blocking with 5% non-fat dried milk, the membrane was incubated with polyclonal IgG against TrkB or protein kinase Cγ diluted 1:1000 (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. The membrane was then washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates, Birmingham, AL) diluted 1:10,000 for 2 h. After this incubation, the antigen–antibody peroxidase complex was then finally detected by enhanced chemiluminescence (PIERCE,

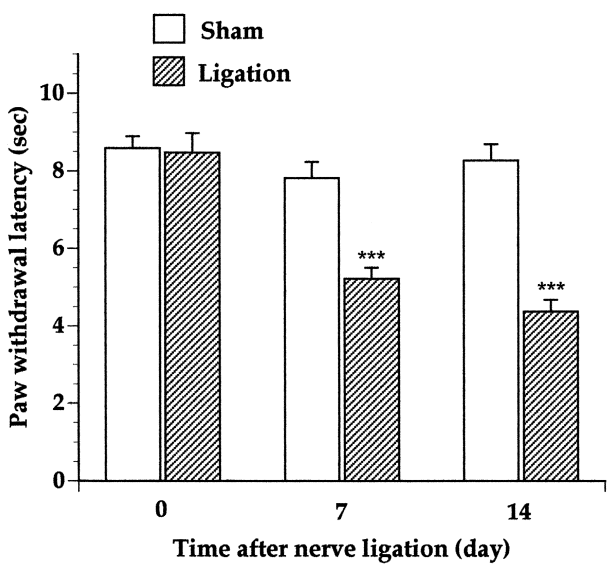

Fig. 1. Changes in paw withdrawal thresholds to thermal stimulation on the ipsilateral side in sham-operated or nerve-injured mice. Just before the ligation, and 7 and 14 days after the surgery, withdrawal latencies of the operated paw from the heat thermal stimulus were measured. Each column represents the mean ± S.E.M. for 15 mice. $^{***}P < 0.001$ vs. sham.

Rockford, IL) and visualized by exposure to Hyperfilm (Amersham Life Sciences, Arlington Heights, IL). Film autoradiograms were analyzed and quantified by computer-assisted densitometry using an NIH image.

2.5. Statistical analysis

The data were expressed as mean ± S.E.M. and the statistical analysis was performed using Student's *t*-test.

3. Results

The hyperalgesic response after the nerve injury is shown in Fig. 1. The withdrawal latencies of the ipsilateral paw to the heat thermal stimulus were significantly decreased at 7 days after the partial ligation of the sciatic nerve. The hyperalgesia lasted, at least, for 14 days after the ligation (Fig. 1). Paw withdrawal latencies in sham-operated mice on the contrary were not changed.

The changes in protein levels of protein kinase Cγ isoform and TrkB receptor after the nerve injury are shown in Fig. 2. Western blots showed that protein levels of either protein kinase Cγ isoform or TrkB receptor in the ipsilateral site of spinal cords were significantly up-regulated at 14 days after the nerve ligation as compared to the sham-operated group (Fig. 2a and b). In contrast, protein levels of protein kinase Cγ isoform and TrkB receptor in the contralateral site of spinal cords obtained from nerve-injured mice was not altered at 14 days (102.1 ± 1.0% and 104.1 ± 2.6% of sham-operated control, respectively).

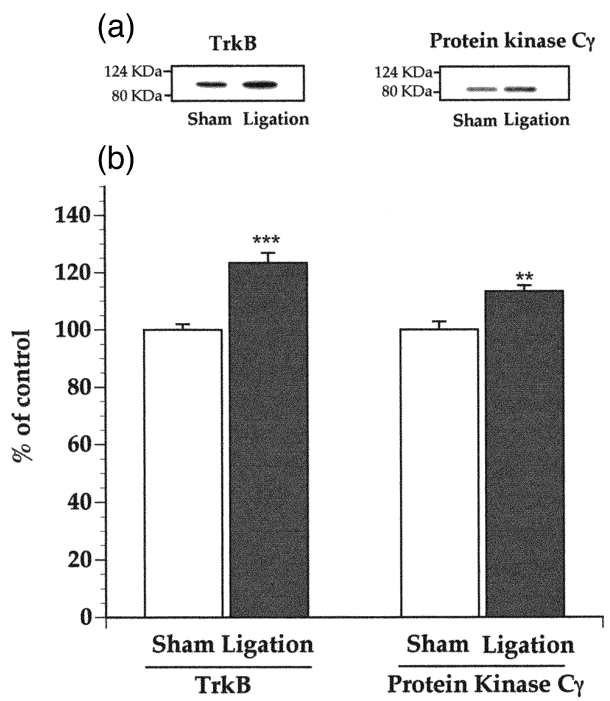

Fig. 2. (a) Representative Western blot of TrkB receptor and protein kinase Cγ isoform proteins. (b) Changes in the membrane-located protein levels of the TrkB receptor and protein kinase Cγ isoform in the ipsilateral side of spinal cords (L4–L6) obtained from sham-operated or nerve-injured mice. The membranes were prepared at 14 days after the ligation. Each column represents the mean ± S.E.M. for five samples. $^{***}P < 0.001$, $^{**}P < 0.01$ vs. sham.

4. Discussion

In the present study, mice with the sciatic nerve injury displayed a marked hyperalgesia, and this persistent painful state lasted for 14 days. The present study confirmed that the nerve injury induced a significant increase in protein level of protein kinase Cγ isoform in plasma membranes in the spinal cord, as reported by Malmberg et al. (1997). To further ascertain the mechanisms that underlie the development of this persistent pain, the level of TrkB receptors after the nerve injury was analyzed using the specific polyclonal antibody. We report here for the first time that protein level of TrkB receptor located in plasma membranes was clearly up-regulated in ipsilateral spinal cords of nerve-injured mice.

There is a considerable evidence that BDNF is present in the terminals of small- and medium-sized sensory neurons (Zhou and Rush, 1996) and thus may be released with constitutively noxious stimulation following the nerve injury. BDNF placed on the spinal cord can produce the post-synaptic excitability mediated by TrkB receptors (Thompson et al., 1999). It is likely that the increased excitability of post-synaptic neurons in the spinal cord induced by the released BDNF following the nerve injury may lead to translocation of TrkB receptors to the cell surface and subsequently activate protein kinase Cγ isoform via phospholipase Cγ (Kaplan and Stephens, 1994; Stephens et al., 1994; Malmberg et al., 1997; Thompson et al., 1999; Woolf and Costigan, 1999). The up-regulation of protein kinase Cγ associated with activated TrkB receptors in turn may induce long-lasting sensitization of synaptic transmission to maintain the persistent neuropathic pain.

In conclusion, the sciatic nerve injury induced the up-regulation of both protein kinase Cγ isoform and TrkB receptor in the spinal cord. These neurochemical changes in the spinal cord may lead to the synaptic plasticity and thus contribute to the persistent pain.

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