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Decreased expression of glial cell line-derived neurotrophic factor signaling in rat models of neuropathic pain

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1 In an attempt to clarify whether glial cell line-derived neurotrophic factor (GDNF), a survival factor for subpopulations of primary afferent neurons, is involved in the states of neuropathic pain, we observed changes in the expressions of GDNF and its signal-transducing receptor Ret after nerve injury in two rat models of neuropathic pain.

2 In the rats treated with sciatic nerve ligation (chronic constrictive injury (CCI) model) or spinal nerve ligation at L5 (SNL model), the thresholds of paw withdrawal in response to mechanical or heat stimuli began to decrease on the injured side within the first week after the operation and the decreases in the thresholds persisted for more than 2 weeks.

3 In CCI-treated rats, the GDNF contents in L4 and L5 dorsal root ganglia (DRGs) on the injured side were markedly decreased at day 7 after the operation and stayed at low levels at day 14. In SNL-treated rats, comparable reductions of GDNF levels in L4 and L5 DRGs on the injured side were observed at 14 postoperative days.

4 Significant decreases of the percentages of DRG neurons expressing Ret were also observed at L4 DRGs in CCI-treated rats at 7 and 14 postoperative days and in SNL-treated rats at 14 days.

5 In CCI- or SNL-treated rats, continuous intrathecal administration of GDNF ($12\,\mu g\,day^{-1}$) using an osmotic pump suppressed the increased sensitivities to nociceptive stimuli to control levels.

6 The present results suggested that the dysfunction of GDNF signaling in the nociceptive afferent system may contribute to the development and/or maintenance of neuropathic pain states.
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Keywords: Chronic constrictive injury; dorsal root ganglion; glial cell line-derived neurotrophic factor; neuropathic pain; Ret; spinal nerve ligation; two-site enzyme immunoassay

Abbreviations: BDNF, brain-derived neurotrophic factor; CCI, chronic constrictive injury; DRG, the dorsal root ganglion; GDNF, glial cell line-derived neurotrophic factor; NGF, nerve growth factor; SNL, spinal nerve ligation

Introduction

Persistent pain causes suffering and distress with no apparent biological advantages. Such maladaptive pain typically results from damages to the nervous system, including the peripheral nerve, dorsal root ganglion (DRG) or central nervous system and is known as neuropathic pain (Woolf & Mannion, 1999). Since neuropathic pain is resistant to conventional analgesics such as opiates and nonsteroidal anti-inflammatory drugs, development of new types of drugs for its treatment has been awaited. Several key molecules associated with signal processing of nociception have been suggested as potential targets for new analgesics (Woolf & Mannion, 1999; Scholz & Woolf, 2002).

Neurotrophic factors control the survival, growth and differentiation of distinct populations of neurons. Glial cell line-derived neurotrophic factor (GDNF) was originally purified as a potent neurotrophic factor that enhances the survival of midbrain dopaminergic neurons (Lin *et al*., 1993). Subsequent studies revealed that GDNF supports the survival of neurons of other types including the motor and primary sensory neurons (Airaksinen & Saarma, 2002). GDNF signaling is mediated through a multicomponent receptor consisting of a glycosylphosphatidylinositol (GPI)-linked receptor termed GFRα-1 that binds to GDNF (Jing *et al*., 1996; Treanor *et al*., 1996) and the receptor tyrosine kinase Ret, which is an essential component to transduce the signals intracellularly (Durbec *et al*., 1996).

In the DRG, about one-half of small-diameter primary afferents are dependent in their survival on GDNF (Molliver *et al*., 1997), and the other half are on nerve growth factor (NGF) (Averill *et al*., 1995; Molliver *et al*., 1995). These unmyelinated fibers are known to transmit nociceptive signals from the periphery to the dorsal horn of the spinal cord. Therefore, these neurotrophic factors may be involved in the etiology of neuropathic pain, and thus can be target molecules for its treatment (Airaksinen & Saarma, 2002; Thoenen &

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Sendtner, 2002). Whereas there has been accumulating evidence suggesting the involvement of NGF in neuropathic pain (Mendell *et al*., 1999), it is not clear whether the expression of the GDNF signaling system changes in the state of neuropathic pain and whether GDNF contributes to the development and/or maintenance of the pain. Solving these questions may lead us to develop GDNF-related drugs for relieving the pain. As the first step toward this goal, in this study we used two different models of the rat for neuropathic pain and observed changes in the expressions of GDNF and its receptor Ret in DRGs. Furthermore, the effects of intrathecal administration of GDNF on nociceptive responses in these models were examined.

Methods

Experimental animals and production of neuropathic pain models

Experimental procedures were approved by the institutional committee on laboratory animals (approved number 13–16) and were carried out under the guidelines of the International Association for the Study of Pain (Covino *et al*., 1980). Rats were individually housed in plastic cages with a soft bedding under a 12 h light cycle.

Young adult male Sprague–Dawley rats (5 weeks old and 140–160 g weight at the time of surgery) were used for all experiments. All surgical procedures were carried out on rats deeply anesthetized with sodium pentobarbital (50 mg kg^{-1} intraperitoneally). For a chronic constrictive injury (CCI) model (Bennett & Xie, 1988), the left common sciatic nerve was exposed in the left mid-thigh and loosely ligated with 4-0 silk thread in four regions at about 1-mm interval. The right sciatic nerve was left intact for control. For a spinal nerve ligation (SNL) model (Kim & Chung, 1992), the left fifth lumbar (L5) spinal nerve was isolated and tightly ligated with 4-0 silk thread in two regions at about 1-mm interval. The right sciatic nerve was left intact for control. Control rats were also individually housed without the operation until euthanasia for tissue sampling.

Behavioral tests

Mechanical allodynia was examined before the surgery and at days 3, 5, 7, 10, 12 and 14 after the surgery ($n=6$ for CCI and 12 for SNL). The paw withdrawal in response to mechanical stimuli was measured using a set of von Frey filaments (Muromachi kikai, Japan) with bending forces ranging from 2.0 to 44.0 g. Each rat was placed on the metallic mesh floor covered with a plastic box and a von Frey monofilament was applied from under the mesh floor to the plantar surface of either the right or left hind paw. Each paw was stimulated with each filament five times at 10-s intervals in the individual trial. The weakest force (g) inducing withdrawal of the stimulated paw at least three times in each trial was referred to as the paw withdrawal threshold. Plantar Test (Ugo Basile, Italy) was used to examine heat hypersensitivity. Each rat was placed on the glass plate with a radiant heat equipment underneath. After the acclimating period, radiation heat was applied onto either the right or left hind paw pad independently. The latency of paw withdrawal from heat stimuli was measured three times at 5-min intervals, and its average value was used as the latency of the response.

Two-site EIA for GDNF

The concentration of GDNF proteins were measured using a two-site EIA as previously described (Nagano & Suzuki, 2003). The fourth lumbar (L4) and fifth lumbar (L5) DRGs were dissected out, immediately frozen in liquid nitrogen and stored at −80°C. Each DRG was homogenized with a Polytron homogenizer with 450 μl of homogenizing buffer (50 mM Tris, 0.5 M NaCl, 0.3% Triton X-100, pH 7.5) containing a premade cocktail of protease inhibitors (Complete mini, Roche Diagnostics, Germany). The homogenate was centrifuged at 15,000 r.p.m. for 20 min at 4°C, and the supernatant was used for the measurement of GDNF. EIA titer plates (FluoroNunc plate, Nalgen Nunc International, U.S.A.) were coated with primary polyclonal antibodies against GDNF (100 ng well^{-1}, Promega, U.S.A.) for 18 h and then blocked with EIA buffer (50 mM Tris, 0.5 M NaCl, 0.3% Triton X-100, 1% bovine albumin and 1% gelatin, pH 7.5) at 4°C for more than 3 h. A measure of 100 μl of DRG extracts (in duplicates) or a GDNF standard (1–1000 pg; in triplicate) in EIA buffer was placed into each well and the plates were incubated at room temperature for 12–18 h. After three washes with Wash-buffer (EIA buffer without bovine serum albumin), 100 μl of biotinylated anti-GDNF antibody (300 ng ml^{-1}) in EIA buffer was added to wells and the plates were incubated for 12–18 h at room temperature. The biotinylated secondary antibody bound to GDNF was detected by incubation with streptavidin-β-galactosidase (1 : 5000 dilution) for 3 h. The unbound enzyme was removed by extensive washes with Wash buffer followed by phosphate-buffered saline (PBS, pH 7.3). Then the β-galactosidase activity in each well was measured by incubation with a substrate, 200 mM 4-methylumbelliferyl-β-galactoside (MUG) in 50 mM sodium phosphate (pH 7.3) and 10 mM $MgCl_2$. The reaction was carried out in the dark at room temperature for 3–5 h, and the amount of fluorescent products was monitored using a Spectraflour Plus microplate reader (Tecan, Austria) with excitation at 360 nm and emission at 465 nm. A standard curve was drawn for each assay in the range of 1–1000 pg of recombinant GDNF.

Immunohistochemistry

For immunohistochemistry, four rats were examined in each model, receiving SNL or CCI, and control. At 1 or 2 weeks after the surgery, the rats were deeply anesthetized with sodium pentobarbital (50 mg kg^{-1} intraperitoneally) and perfused transcardially with 250–300 ml of PBS (pH 7.2), followed by 250–300 ml of 4% paraformaldehyde in PBS (pH 7.2). The L4 and L5 DRGs were dissected out and postfixed in 4% paraformaldehyde in PBS overnight at 4°C, and then immersed in 20% sucrose in PBS at 4°C overnight. The DRG was frozen in acetone chilled with dry ice. The DRG was cut on a cryostat at a thickness of 14 μm and thaw-mounted onto the slide. Sections were preincubated in PBS containing 5% normal donkey serum and 0.02% Triton X-100 for 30 min, then incubated with anti-Ret polyclonal antibody (1 : 100 dilution) and anti-TrkA polyclonal antibody (1 : 1000 dilution) at 4°C overnight. The sections were washed in PBS

and then incubated with the secondary antibody labeled with Alexor Fluor 594 (against anti-Ret antibody) and that labeled with Alexor Fluor 488 (against anti-TrkA antibody). For image analysis of Ret, images of three sections from each DRG were captured using a high-resolution digital camera and a computer (Olympus, Japan). At least 100 neurons were examined in each image in a blind manner. The signal intensity of each neuron was determined by using Photoshop (Adobe Systems, Japan) and the frequency histogram of the intensity was constructed. The histogram comprised of two separate peaks and the population of Ret-positive neurons was identified as the group with higher intensities. The number of labeled cells was expressed as a percentage of the total cell count.

Intrathecal administration of recombinant human GDNF

Before the day of the operations for producing neuropathic pain, 5-week-old male Sprague–Dawley rats (140–160 g at the time of surgery) were deeply anesthetized with sodium pentobarbital (50 mg kg^{-1}, intraperitoneally), and a polyethylene catheter (PE-10) filled with saline was slowly inserted through an opening of the cisterna magna into the spinal subarachnoid space so that its tip lay at the level of lumbar enlargement of the spinal cord (Yaksh *et al*., 1985). The catheter was tied with a loose knot at the point of an exit from the cisterna magna, closed firmly at the outer end and then was implanted subcutaneously. On the next day, the CCI or SNL operation was performed as described above after confirming that the catheter-implanted rats showed no obvious movement disturbances such as paralysis. On the fifth day after the CCI or SNL operation, the catheter was cleared with 10 μl of saline and then attached to an Alzet miniosmotic pump (Alzet, U.S.A.) delivering solutions at a rate of 0.5 μl h^{-1} in the rats that developed hyperalgesia. Animals received either saline (control, $n=3$ for CCI and 6 for SNL) alone or the recombinant human GDNF (rhGDNF, $n=4$ for CCI and 6 for SNL) dissolved in saline at 12 μg day^{-1} (Boucher *et al*., 2000). After the surgery, kanamycin sulfate (50 mg kg^{-1}) was injected intraperitoneally to prevent infection.

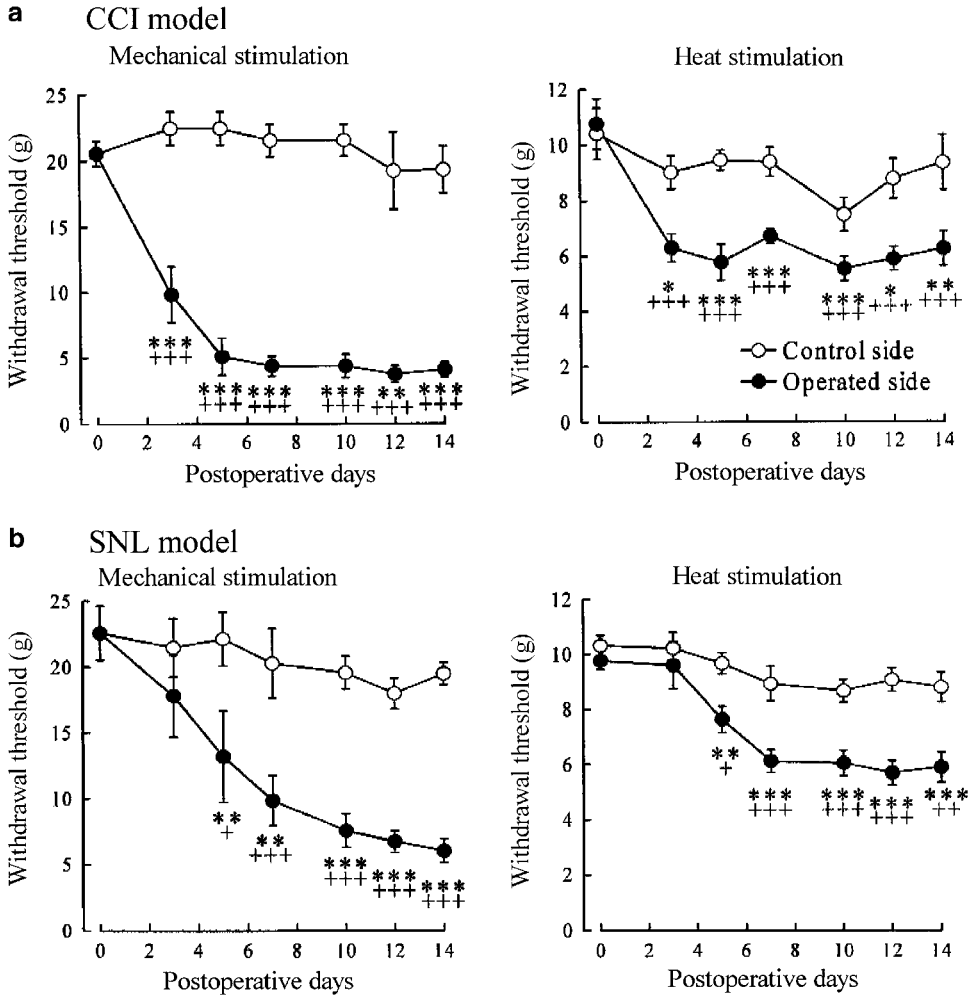

Figure 1 Thresholds of foot withdrawal of the operated left side (closed circles) and intact right side (open circles) in response to mechanical or thermal stimuli applied to the corresponding hind paw pad in the rats treated with CCI (a) or SNL (b) procedure. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared with the values on control side at corresponding days by paired *t*-test; $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ compared with the values at the preoperative day by Tukey's multiple comparisons. $n=6$ (CCI) and $n=12$ (SNL).

Drugs

The primary polyclonal anti-human GDNF antibody for EIA was obtained from Promega. Streptavidin-conjugated β-galactosidase was from Roche Molecular Biochemicals (U.S.A.). The secondary polyclonal anti-human GDNF antibody for EIA and the polyclonal goat anti-mouse Ret antibody for immunohistochemistry were from Genzyme/Techne (U.S.A.). The rhGDNF for EIA and the polyclonal rabbit anti-rat TrkA antibody were from Chemicon (U.S.A.). MUG and Triton X-100 were from Sigma (U.S.A.). EIA-grade gelatin was from BioRad (U.S.A.). NHS-LC-biotin was from Pierce (U.S.A.). Globulin-free EIA/RIA-grade bovine serum albumin was from Nakalai Tesque (Japan). Alexor Fluor 594 donkey anti-goat antibody and Alexor Fluor 488 donkey anti-rabbit antibody were from Molecular Probe (U.S.A.). The recombinant human GDNF used for the intrathecal administration was in part a gift from Amgen and in part purchased from R & D systems (U.S.A.).

Statistical analysis

Values are expressed as mean$\pm$s.e.m. Paired *t*-test was used to compare the threshold or latency values between the control and operated sides in the behavioral tests. Unpaired *t*-test was used to compare the levels of GDNF contents or Ret expression between the operated and control rats. Tukey's test for multiple comparisons was used to compare the threshold or latency values obtained before and after the operations in the behavioral tests. $P<0.05$ was considered to be statistically significant.

Results

Behavioral studies

The paw withdrawal thresholds of unoperated 5-week-old rats in response to stimulation with von Frey filaments were 20.5 ± 0.9 and 20.5 ± 0.9 g on the left and right sides of the paw, respectively. The latencies of withdrawal to heat stimulation in unoperated rats were 8.5 ± 1.2 and 8.5 ± 0.9 s on the left and right sides of the paw, respectively. These values were almost unchanged in the control rats during the period examined (5–7 weeks old). In the rats treated with the CCI or SNL procedure, the threshold of paw withdrawal in response to mechanical or heat stimulus began to decrease on the injured left side, but not the intact right side, within the first week after the operation (Figure 1). Statistically significant decreases in the thresholds of both tests were observed at day 3 in the CCI and day 5 in the SNL procedure. The increased sensitivities to stimuli persisted in the second week after the operation.

Changes in GDNF expression in the DRG

We examined the levels of GDNF expression in the DRG following the surgical operations. In unoperated 5-week-old rats, the GDNF contents were 539.1 ± 105.0 and 592.4 ± 99.2 pg mg total protein^{-1} ($n=9$) on the left L4 and L5 DRGs, respectively. These values were not significantly changed for the subsequent 2 weeks (Figure 2). In the CCI-treated rats, a remarkable reduction of the GDNF contents, to approximately 40% of the control values, were observed in both the L4 and L5 DRGs of the injured side at postoperative day 7 (L4, 227.2 ± 79.6 pg mg total protein^{-1}, $n=5$, $P<0.05$; L5, 184.4 ± 63.7 pg mg total protein^{-1}, $n=5$, $P<0.01$). The low levels of GDNF persisted at day 14. In the SNL-treated rats, the GDNF contents in the left DRG at L5, at which level the spinal nerve was ligated, were not altered at days 3 and 7, but reduced at day 14 to approximately the same levels of the CCI-treated rats (317.5 ± 11.6 pg mg total protein^{-1}, $n=6$, $P<0.05$). A similar degree of reduction of GDNF contents was observed at L4 DRG at day 14 (280.5 ± 13.0 pg mg total protein^{-1}, $n=6$, $P<0.05$).

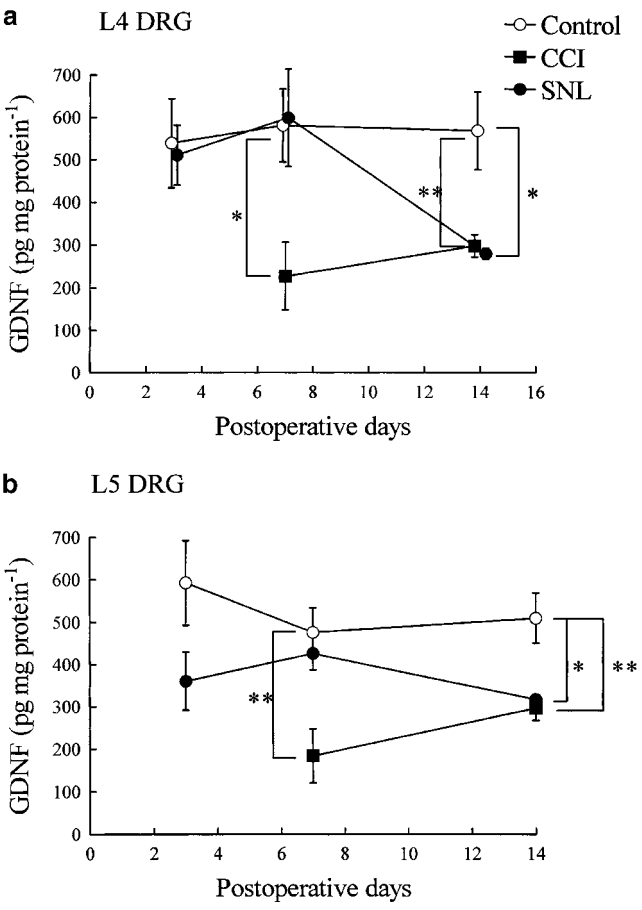

Figure 2 Changes in GDNF contents in the L4 (a) and L5 (b) DRGs of control (open circles), CCI-treated (closed squares) and SNL-treated rats (closed circles). *$P<0.05$ and **$P<0.01$ compared with the values of control rats by unpaired *t*-test. $n=5$–14.

Changes in the proportions of RET-expressing neurons in the DRG

It was of interest to explore whether there are alterations in the receptor components of the GDNF signaling system in the neuropathic pain states. We therefore examined the changes in the expression of Ret, a receptor component transducing signal for GDNF, in the DRG by immunohistochemical examinations. In 6-week-old intact rats, Ret-positive cells were 52.9 ± 2.9% of total DRG neurons at left L4, 51.2 ± 1.2% at right L4, 48.0 ± 2.1% at left L5 and 52.3 ± 2.0% at right L5,

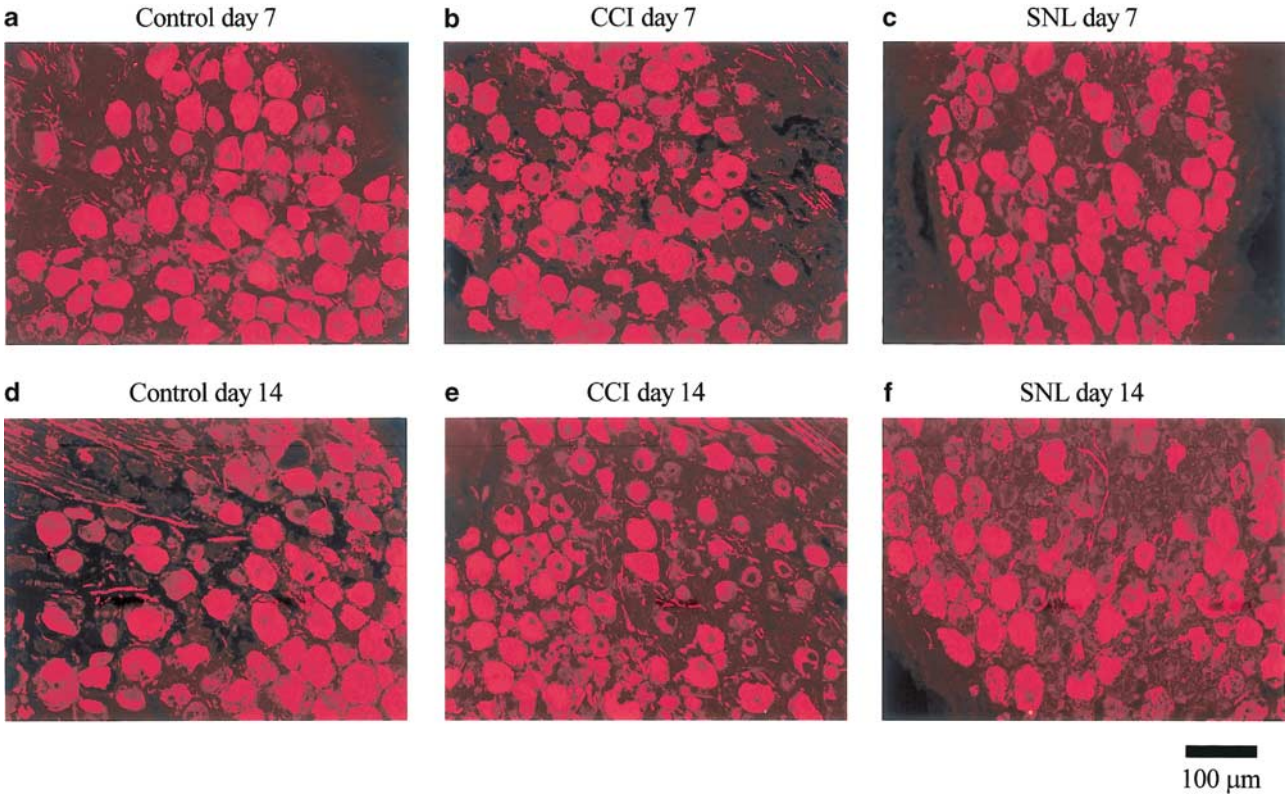

Figure 3 Immunohistochemistry of Ret expression in the L4 DRGs at day 7 (a–c) and day 14 after the operation (d–f) of control, CCI-treated and SNL-treated rats. Ret-positive cells are stained in red with Alexor Fluor 594-labeled secondary antibody.

respectively ($n=4$; see Figures 3a and 4a, b). The percentages of Ret-expressing DRG cells in 7-week-old rats were similar to those in 6-week-old rats ($n=4$; Figures 3d and 4a, b). In CCI-treated rats, significant reductions in the proportions of Ret-positive cells at L4 on the injured side were observed at postoperative day 7 ($40.6\pm2.7\%$, Figures 3b and 4a, $n=4$, $P<0.05$ compared with control rats) and day 14 ($40.1\pm1.9\%$, Figures 3e and 4a, $P<0.01$). The SNL procedure did not alter the level of Ret expression at day 7 (Figures 3c and 4a), but reduced to $42.5\pm2.1\%$ ($n=4$, $P<0.05$) at day 14 at L4 on the injured side (Figures 3f and 4a). No significant changes were observed in the Ret expression at L5 for 1 or 2 weeks after the SNL or the CCI operation (Figure 4b, $n=4$). As a comparison, we also examined the expression of TrkA, a high-affinity receptor for NGF, in the DRG. Both nerve injury procedures did not cause any changes in the percentages of TrkA-bearing neurons at least first 2 weeks after the operation (data not shown).

Effects of intrathecal administration of GDNF

The significant reduction in the GDNF signaling system, that is, reduction in GDNF level and its receptor Ret, in injured sensory neurons, suggested that it might be associated with the sustained state of hyperalgesia in the severed rats and prompted us to examine the effects of replacement of GDNF to these animals. Rats were treated with the CCI or SNL procedure and at day 5 after the operation they were intrathecally infused with saline containing GDNF ($12\,\mu g\,day^{-1}$) or saline alone (for control) using osmotic pumps. The intrathecal catheter and the connecting tube outside the subarachnoid space were filled with saline (about $25\,\mu l$) and attached before the SNL or CCI operation to preclude the rats injured by catheter insertion from the subsequent experiment. It took about 2 days for GDNF to reach the spinal cord after starting the administration by the pump. In the CCI model, the reduced thresholds of responses to both the mechanical and thermal stimuli applied to the operated side returned to the control levels within 5 days after commencement of GDNF administration (Figure 5a, $n=4$). GDNF did not affect the threshold levels on the intact side. As shown in Figure 5b, saline infusion, instead of GDNF, had no effect on allodynia and hyperalgesia caused by the CCI procedure ($n=4$). In the SNL model, GDNF administration also suppressed the nociceptive responses in a similar manner in the CCI model (Figure 6a and b, $n=6$).

Discussion

We found that the levels of GDNF expression in DRGs decreased following nerve injury procedures in two rat models of neuropathic pain. Thus after CCI operation, the GDNF contents in both L4 and L5 DRGs of the injured side were reduced to about one-third of the control levels at 7 postoperative days. After the SNL procedure, the GDNF levels were not significantly changed at 3 and 7 days. However, at 14 postoperative days, the GDNF levels after SNL, as well as CCI, were reduced to about a half of the control levels (Figure 2). We also observed a similar pattern of reductions in

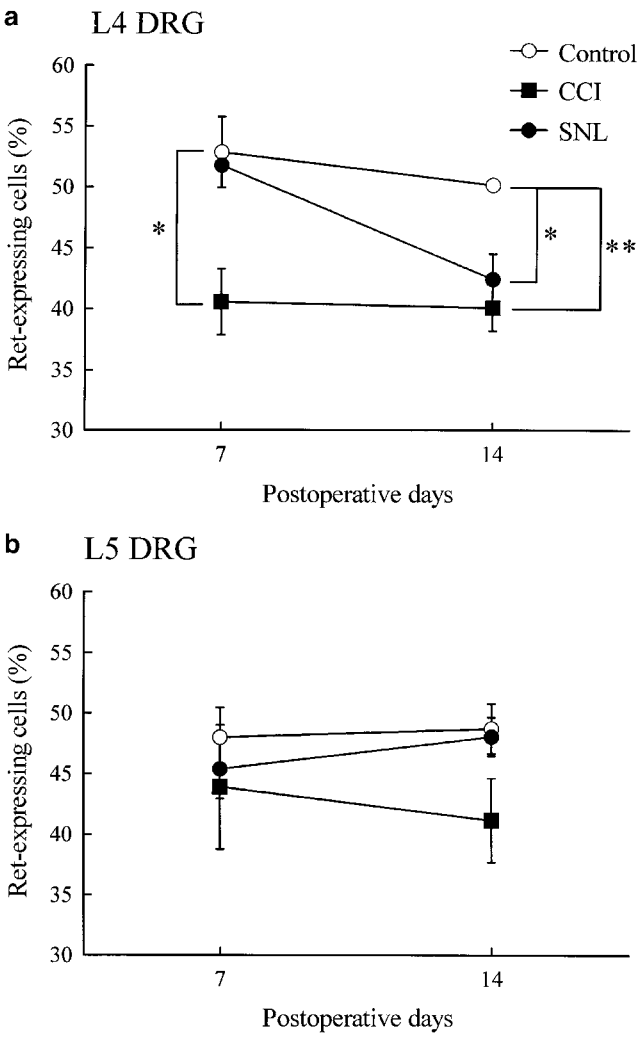

Figure 4 Changes in the percentages of Ret-expressing neurons in the L4 (a) and L5 (b) DRGs of control (open circles), CCI-treated (closed squares) and SNL-treated rats (closed circles). $*P<0.05$ and $**P<0.01$ compared with the values of control rats by unpaired t-test. $n=4$.

the proportions of DRG neurons bearing Ret, a signal-transducing component for GDNF, at L4 DRG in both procedures, although the degrees of reduction were smaller than the GDNF levels (Figures 3 and 4). CCI and SNL models of neuropathic pain are reported to have differences in the magnitudes of pain components (Kim *et al*., 1997) and inflammatory responses, including increased skin blood flow (Daemen *et al*., 1998) and the distribution of the intact and the severed neurons in DRGs. In spite of these differences, reductions of GDNF signaling were observed in animals treated with either procedure. Furthermore, the changes in GDNF signaling were almost in parallel with the development of hyperalgesia after the operations (Figure 1). These results therefore suggest a close relationship between the functional deficit in GDNF signaling associated with primary afferents and neuropathic pain states.

The changes in GDNF signaling after the nerve injury procedures of this study are different from those observed after axotomy of the sciatic nerve, in which case mRNAs of GDNF and its receptors Ret and GFR-α were increased in Schwann cells and DRGs (Hammarberg *et al*., 1996; Naveilhan *et al*., 1997; Bennett *et al*., 2000). The cause of the opposite changes in the GDNF signaling system after nerve ligation and axotomy procedures is unknown, but may be associated with differences in activities of primary afferents between both procedures.

Using rat pain models, Boucher *et al*. (2000) reported that exogenously applied GDNF has potent analgesic effects. As shown in Figures 5 and 6, both in CCI and SNL models, intrathecal administration of GDNF clearly suppressed the behavioral responses associated with neuropathic pain, confirming the analgesic action of GDNF demonstrated by Boucher *et al*. (2000). The mechanisms of the action of the GDNF signaling system are not clear, but it may act by enhancing the activities of pain-inhibitory systems. For example, some of the GDNF-dependent DRG neurons express somatostatin, an inhibitory neuropeptide (Bennett *et al*., 1998) and intrathecal administration of GDNF has been shown to produce a release of somatostatin upon electrical stimulation in the dorsal horn (Issa *et al*., 2001). In addition, GDNF may affect synaptic formation in the central terminals of the primary neurons, since it is observed that GDNF abolishes axotomy-induced sprouting of the larger-diameter primary afferents into the lamina I and II in the dorsal horn of the spinal cord (Bennett *et al*., 2000). From these considerations it may be speculated as follows. The GDNF signaling system in nociceptive sensory neurons functions to suppress pain sensation and/or associated nociceptive responses. Nerve injury, such as loose ligation but not axotomy, of these neurons causes a reduction in the GDNF signaling and this may contribute to the development and/or maintenance of the hyperalgesia observed in neuropathic pain states.

In view of the above hypothesis, it is of interest to note that the changes after nerve injuries to the sensory NGF signaling system and its actions appear to be opposite to those of the GDNF signaling system. Thus Herzberg *et al*. (1997) found that after CCI procedure NGF protein and mRNA were increased in DRGs on the injured side at 14 days following the operation. With regard to the SNL procedure, Fukuoka *et al*. (2001) also showed an increase in NGF protein at L4 DRG following SNL at L5, whereas Oh *et al*. (2000) reported that NGF protein levels of L3 to L6 DRGs were unchanged after ligating spinal nerves at L5 and L6. In contrast to the decrease in the degree of Ret expression found in this study, Fukuoka *et al*. (2001) found that the proportion of the cells expressing TrkA, a high-affinity receptor for NGF, was unchanged at L4 DRG after the SNL procedure. In addition, in the ipsilateral L4 DRG after L5 SNL, they found an increase in the percentage of cells coexpressing TrkA and brain-derived neurotrophic factor (BDNF), another member of the neurotrophin family, which is thought to be involved in central sensitization in the spinal cord during neuropathic pain state (Woolf & Salter, 2000). In contrast to the analgesic action of GDNF, as demonstrated in the study of Boucher *et al*. (2000) and in this study, NGF may act to sustain or enhance pain transmission (Mendell *et al*., 1999). For example, treatment with anti-NGF antibody abolishes the sprouting of sympathetic nerves into DRGs following nerve injury (Ramer & Bisby, 1999; Zhou *et al*., 1999), and prevents the development of hyperalgesia (Fukuoka *et al*., 2001). Furthermore, exogenous application of NGF increases the release of substance P

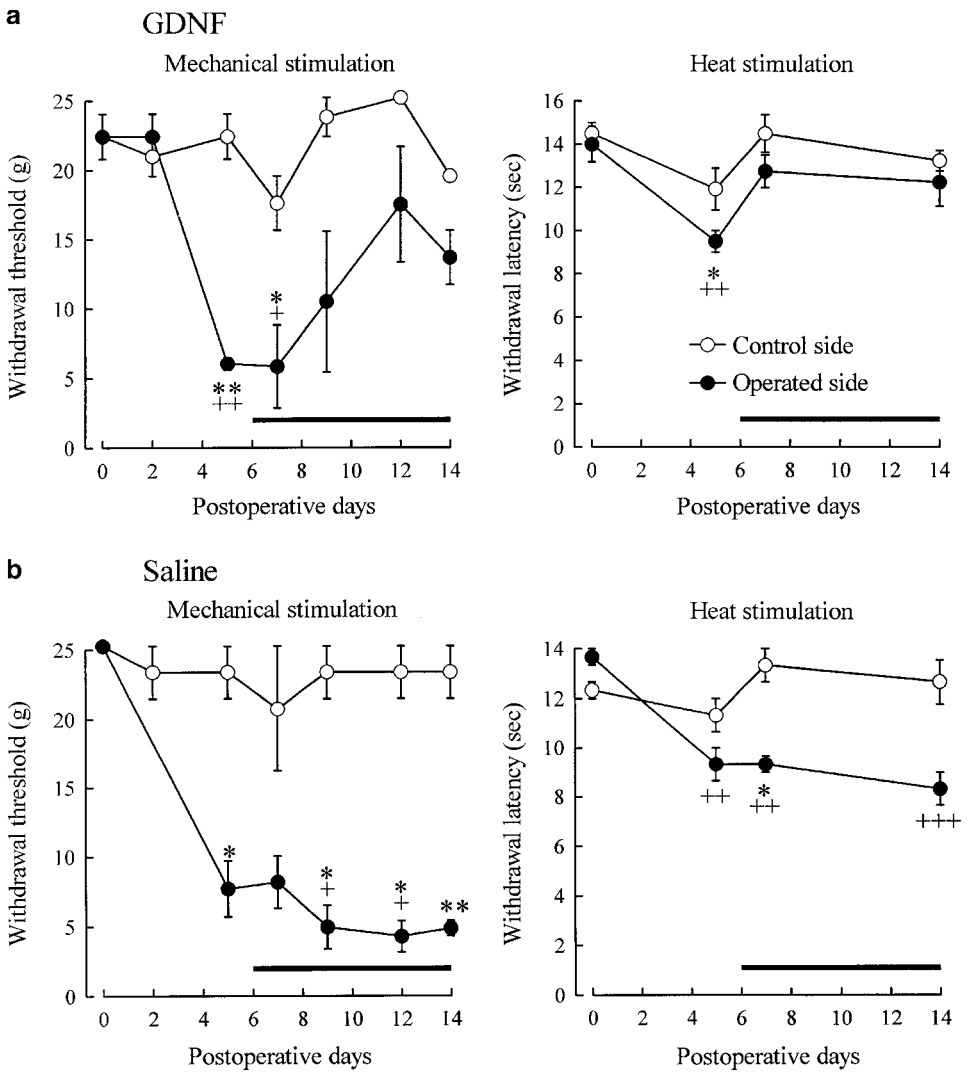

Figure 5 Effect of GDNF administration in the rats treated with CCI procedure. Thresholds of foot withdrawal were examined as in Figure 1. GDNF-containing saline (a; $n=4$) or saline alone (b; $n=3$) was applied intrathecally by an osmotic pump at day 5 as indicated by the horizontal bars. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the values on the control side at the corresponding days by paired t-test; $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ compared with the values at the preoperative day by Tukey's multiple comparisons.

from the spinal cord (Malcangio *et al*., 2000), a major neurotransmitter mediating nociception.

GDNF- and NGF-dependent neurons constitute distinct subpopulations of unmyelinated primary afferents. GDNF-dependent afferents terminate their central axons at the internal part of lamina II, a different part from the one that NGF-dependent fibers project to (Snider & McMahon, 1998). Although both types of sensory neurons may be associated with pain transmission, their roles may well be quite different. An interesting possibility is that the NGF-dependent sensory system may be activated by nociceptive stimuli and transmit the signal to the central nervous system to evoke the sensation of pain, whereas the GDNF-dependent sensory system may also perceive nociceptive stimuli, but rather act to regulate the activities of the nociceptive pathway.

In the present study, we found that the GDNF signaling pathway was impaired in the injured sensory neurons in the rat models of neuropathic pain and both allodynia and hyperalgesia were attenuated by intrathecal administration of GDNF. These results suggest distinctive roles of GDNF in the pathology of the neuropathic pain states and encourage us to search for the development of treatment with GDNF-related compounds to relieve intractable neuropathic pain.

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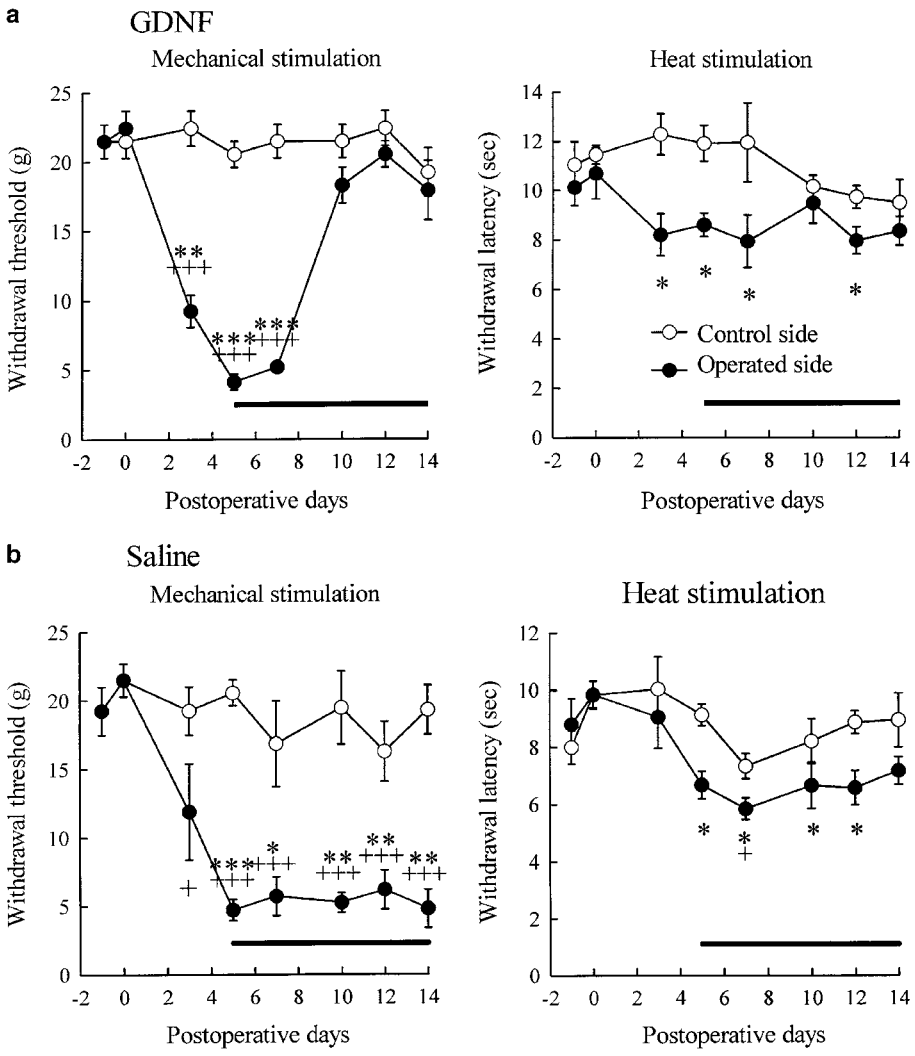

Figure 6 Effect of GDNF administration in the rats treated with SNL procedure. Thresholds of foot withdrawal were examined as in Figure 1. GDNF-containing saline (a) or saline alone (b) was applied intrathecally by an osmotic pump at day 5 as indicated by the horizontal bars. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared with the values on the control side at the corresponding days by paired *t*-test; $^{+}P<0.05$ and $^{+++}P<0.001$ compared with the values at the preoperative day by Tukey's multiple comparisons. $n=6$.

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