In Vivo NGF Deprivation Reduces SNS Expression and TTX-R Sodium Currents in IB4-Negative DRG Neurons

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Fjell, Jenny, Theodore R. Cummins, Kaj Fried, Joel A. Black, and Stephen G. Waxman. In vivo NGF deprivation reduces SNS expression and TTX-R sodium currents in IB4-negative DRG neurons. J. Neurophysiol. 81: 803–810, 1999. Recent evidence suggests that changes in sodium channel expression and localization may be involved in some pathological pain syndromes. SNS, a tetrodotoxin-resistant (TTX-R) sodium channel, is preferentially expressed in small dorsal root ganglion (DRG) neurons, many of which are nociceptive. TTX-R sodium currents and SNS mRNA expression have been shown to be modulated by nerve growth factor (NGF) in vitro and in vivo. To determine whether SNS expression and TTX-R currents in DRG neurons are affected by reduced levels of systemic NGF, we immunized adult rats with NGF, which causes thermal hypoalgesia in rats with high antibody titers to NGF. DRG neurons cultured from rats with high antibody titers to NGF, which do not bind the isoelectin IB4 (IB4−) but do express TrkA, were studied with whole cell patch-clamp and in situ hybridization. Mean TTX-R sodium current density was decreased from 504 ± 77 pA/pF to 307 ± 61 pA/pF in control versus NGF-deprived neurons, respectively. In comparison, the mean TTX-sensitive sodium current density was not significantly different between control and NGF-deprived neurons. Quantification of SNS mRNA hybridization signal showed a significant decrease in the signal in NGF-deprived neurons compared with the control neurons. The data suggest that NGF has a major role in the maintenance of steady-state levels of TTX-R sodium currents and SNS mRNA in IB4− DRG neurons in adult rats in vivo.

INTRODUCTION

Changes in sodium channel expression and distribution in primary sensory neurons have been suggested to be involved in several models of neuropathic and inflammatory pain, including neuronomas (Matzner and Devor 1994), axonal transection (Dib-Hajj et al. 1996), chronic constriction of the sciatic nerve (Novakovic et al. 1998) and inflammatory pain (Tanaka et al. 1998). Adult rat dorsal root ganglion (DRG) neurons have been shown to express multiple sodium channel mRNAs (Black et al. 1996). One of the sodium channel isoforms, αSNS/PN3, has received considerable attention with respect to possible involvement in pain syndromes because it is preferentially expressed in small-diameter DRG neurons, which include neurons involved in nociception and thermoreception. When expressed in Xenopus oocytes, αSNS/PN3 produces a slowly inactivating, tetrodotoxin-resistant (TTX-R) sodium current (Akopian et al. 1996; Sangameswaran et al. 1996). Sensory neurons that express TTX-R sodium currents have broader action potentials and characteristic inflections on the descending limb of the action potentials; these characteristics are seen in virtually all C-type neurons as well as in some Aδ and high-threshold Aβ neurons (see Koerber and Mendell 1992).

Recently, the expression of αSNS/PN3 and TTX-R sodium currents was examined in several models of neuropathic and inflammatory pain. After transection of the sciatic nerve, there is a reduction in TTX-R currents in small- and medium-diameter DRG neurons (Cummins and Waxman 1997; Rizzo et al. 1995), and concurrently there is a decrease in αSNS/PN3 mRNA in these neurons (Dib-Hajj et al. 1996). Tight ligature of L5/6 dorsal roots is also accompanied by a substantial reduction in the expression of αSNS/PN3 mRNA (Okuse et al. 1997). In contrast to the reduction in TTX-R currents and αSNS/PN3 mRNA expression after axonal transection, TTX-R currents and αSNS/PN3 mRNA expression are increased in small DRG neurons after carrageenan-induced inflammation (Tanaka et al. 1998). Although the mechanism for alterations of αSNS/PN3 mRNA levels is not understood, it is noteworthy that nerve growth factor (NGF) is thought to be a mediator of inflammatory pain (Lewin and Mendell 1993; Lewin et al. 1993, 1994; Woolf et al. 1994, 1996).

Although the mechanism has not been clearly identified, NGF appears to play an important role in the regulation of αSNS/PN3 expression. In an in vitro model of axotomy, supplementing the growth medium with NGF inhibited a substantial reduction of αSNS/PN3 mRNA expression in small DRG neurons (Black et al. 1997). Moreover, exogenous delivery of NGF to the transected sciatic nerve stump resulted in an up-regulation of TTX-R sodium currents and αSNS/PN3 mRNA in small DRG neurons (Dib-Hajj et al. 1998). NGF has also been shown to be crucial for the maintenance of the TTX-R component of the action potential in adult DRG neurons (Aguayo and White 1992; Lewin et al. 1992; Oyalese et al. 1997; Ritter and Mendell 1992). More recently, however, Wood and coworkers (Okuse et al. 1997) questioned the importance of NGF on the regulation of SNS mRNA.

In adult animals, NGF is produced in small quantities in the epidermis and is thought to act directly on some sensory neurons through the neurotrophin receptors TrkA and p75. Despite the fact that most sensory neurons are dependent on NGF during embryogenesis, less than one-half of small DRG neurons in the adult have receptors for NGF (McMahon et al.
1994). The NGF-responsive DRG neurons constitute a population of peptidergic nociceptors, which are characterized by their production of the neuropeptide CGRP. In contrast, non-peptidergic small DRG neurons are characterized by their ability to bind the lectin IB4 and are nonresponsive to NGF in the adult (Molliver et al. 1997; Petruska et al. 1997; Plenderleith and Snow 1993; Plenderleith et al. 1988). Thus most small neurons that are not labeled by IB4 would be expected to be responsive to NGF.

Previous studies that examined the modulation of the expression of αSNS/PN3 mRNA and TTX-R sodium currents in DRG neurons by NGF utilized models that involve axonal transection or inflammatory responses, making the role of NGF per se unclear. To study the role of NGF on uninjured adult DRG neurons that have receptors for NGF, we depleted adult rats of systemic NGF through autoimmunization (Chudler et al. 1997; Doubleday and Robinson 1994, 1995; Gorin and Johnson 1979, 1980; Otten et al. 1979; Schwartz et al. 1982) and examined the IB4-negative (IB4-) neurons. Our results demonstrate that small, IB4- DRG neurons from NGF-depleted rats exhibit a significant decrease in the expression of TTX-R sodium currents and αSNS/PN3 mRNA. These results are consistent with a direct action of NGF in the regulation of αSNS/PN3 in vivo.

METHODS

Animals

Twenty adult (100–125 g) male Sprague-Dawley rats were immunized with 50 μg 7S mouse NGF in 100 μl normal saline solution emulsified with 100 μl complete Freund’s adjuvant (Sigma). The animals were boosted with a second injection of NGF in incomplete Freund’s adjuvant (Sigma) 2 wk later. Untreated animals served as controls. Animals were group housed and had access to food and water ad libidum. All immunized animals gained weight, and they did not show signs of discomfort. Their weights ranged from 350 to 405 g by the time of killing.

Two and 4 wk after the second immunization, peripheral blood was collected, and the antibody titers were analyzed with enzyme-linked immunosorbert assay. Multwell plates (Nalge-Nunc) were coated by the time of killing.

Controls. Animals were group housed and had access to food and animals were boosted with a second injection of NGF in incomplete saline solution (CSS) (pH 7.2). The DRGs were then dissected with collagenase A (1 mg/ml) for 20 min at 37°C in CSS and then in collagenase D (1 mg/ml) containing papain (30 units/ml) for 15 min at 37°C in CSS. The DRGs were gently centrifuged (100 g for 3 min), and the pellet was dissolved and triturated in DRG media (DMEM:F12, 10% FCS) with 1 mg/ml trypsin inhibitor (Sigma). The cells were then plated on poly-ornithine lamin-coated glass coverslips and incubated at 37°C in a humidified 95% air-5% CO2 incubator.

Whole cell recordings

DRG neurons were recorded from in the whole cell patch-clamp configuration 18–30 h after dissociation and plating. The cells were incubated for 30–60 min with FITC-labeled isolectin B4 (40 μg/ml Sigma) just before being moved to the recording chamber. Cells that did not exhibit IB4 fluorescence were chosen for recording. All recordings were made with an EPC-9 amplifier, a Macintosh Quadra 950, and the Pulse program (v. 7.52, HEKA Electronic, Germany). Recording electrodes (0.8–1.5 MΩ) were fabricated from 1.65-mm capillary glass (WPI) with a Sutter P-87 puller. Cells were not considered for analysis if the initial seal resistance was <2 GΩ or if they had high leakage currents (holding current > 1 nA at ~80 mV) or an access resistance >5 MΩ. The average access resistance was 2.5 ± 0.9 MΩ (mean ± SD, n = 117). Voltage errors were minimized with 70–80% series resistance compensation. Linear leak subtraction and capacitance artifact cancellation were used for all recordings.

Membrane currents were filtered at 2.5 kHz and sampled at 10 kHz. The pipette solution contained (in mM) 140 CsF, 2 MgCl2, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, and 10 Na-N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES; pH 7.3). The standard extracellular solution contained (in mM) 140 NaCl, 3 KCl, 2 MgCl2, 1 CaCl2, 0.1 CdCl2, and 10 HEPES (pH 7.3). Cadmium was included to block calcium currents. The osmolarity of the solutions was adjusted to 310 mmosm (Wescor 5550 osmometer). The liquid junction potential for these solutions was <7 mV; data were not corrected to account for this offset. The offset potential was zeroed before patching the cells and checked after each recording for drift. All recordings were conducted at room temperature (~21°C).

In situ hybridization

Before in situ hybridization, the culturing medium was supplemented with biotin labeled-isoelectin IB4 (Sigma) at 40 μg/ml and incubated for 30 min at 37°C. Coverslips with cells from NGF-deprived and control animals were washed with CSS and then fixed for 10 min in 4% formaldehyde in 0.14 M Sorensen’s buffer, pH 7.2. SNS probe construction and in situ hybridization were performed as previously described (Black et al. 1996) with minor modifications. After hybridization and stringent washes, the coverslips were incubated with 40 μg/ml streptavidin-CY2 (Amersham) and alkaline phosphatase conjugated anti-digoxigenin antibody (1:500) (Boehringer-Mannheim) in Tris-buffered blocking solution (1% BSA, 2% normal sheep serum) at 4°C overnight. For each experiment, cells from control and the NGF-deprived animals were incubated in the chromagen solution for the same length of time, and the reaction stopped before the NBT-reaction reached saturation.

Quantification

Coverslips were examined with a BioRad MRC-600 confocal microscope equipped with brightfield and BHS filter. Cells were randomly selected from six to eight coverslips from each DRG culture for each condition and captured with COMOS image acquisition program. IB4- neurons were characterized by the lack of fluorescent signal above background levels (e.g., Fig. 3). Quantification of the SNS hybridization signal was performed as previously described (Black et al. 1997). Briefly, OD measurements of the neurons were obtained with the NIH Image program. The brightfield gray levels were linearly calibrated to OD (R² > 0.99) with optical filters with OD = 0.1, 0.3, and 0.6. The OD of randomly selected IB4- neurons was obtained by outlining the cell body and then measuring average
density and surface area. Within each experiment, the mean OD of IB4<sup>−</sup> cells in the NGF-immunized rat was compared with the mean OD of DRG neurons from control animals with the Student’s t-test. A total of 193 IB4<sup>−</sup> neurons was analyzed from 5 high antibody titer animals and 4 age-matched controls.

**RESULTS**

**Animals**

Adult male Sprague-Dawley rats were immunized to 7S NGF, and the effect on TTX-R sodium current and SNS mRNA expression in IB4<sup>−</sup> DRG neurons was examined. The NGF autoimmunization resulted in antibody titers ranging from 1:4000 to 1:96000. DRG neurons from animals with the highest anti-NGF antibody titers (>1:48000; n = 5) were used for whole cell patch-clamp and in situ hybridization studies. Age-matched control rats showed no titers to NGF.

**Whole cell patch clamp**

**Effect of NGF deprivation on sodium current amplitude**

Sodium currents were recorded in the whole cell patch-clamp configuration from small (14- to 28-μm diam) DRG neurons that did not exhibit IB4 fluorescence (IB4<sup>−</sup> neurons). We recorded 47 control cells (cultured from 4 control animals) and 70 NGF-deprived cells (cultured from 5 NGF-deprived animals). For the majority of cells, we used prepulse inactivation (Cummins and Waxman 1997; McLean et al. 1988; Roy and Narahashi 1992) to distinguish between the fast inactivating, TTX-sensitive (TTX-S) and the slow inactivating, TTX-R sodium currents. This allowed simultaneous measurement of both TTX-R and TTX-S current amplitudes in every cell. In a previous study, without IB4 labeling, we observed that ~85% of small cells exhibited both TTX-R and TTX-S currents (Cummins and Waxman 1997). In our control group, the average TTX-R current density in the IB4<sup>−</sup> cells was 504 ± 77 pA/pF (mean ± SE, n = 47), where current density is estimated by dividing the peak current amplitude by the cell capacitance. Thirty-eight percent of the control IB4<sup>−</sup> neurons expressed low levels (<200 pA/pF) of TTX-R current. By contrast, the average TTX-R current density in the IB4<sup>−</sup> cells from NGF-deprived animals was 307 ± 61 pA/pF (n = 70), and 67% of these cells exhibited low levels of TTX-R currents (Fig. 1A). Both the peak TTX-R current amplitude (measured with a test pulse to −10 mV) and the TTX-R current density were significantly (P < 0.05) lower for the NGF-deprived cells than for the control cells. However, we did not find a significant difference between control and NGF-deprived animals in terms of TTX-S peak current amplitude (Fig. 1B) or TTX-S current density (1,111 ± 131 pA/pF for control cells; 1,312 ± 135 pA/pF for anti-NGF cells).

When we compared the amplitude of sodium currents in IB4<sup>−</sup> cells from control and NGF-deprived animals, we did not find a significant difference in the peak current density for TTX-R currents (1,129 ± 161 pA/pF, n = 25 for control IB4<sup>−</sup> cells; 1,103 ± 136 pA/pF, n = 23 for anti-NGF IB4<sup>−</sup> cells) or TTX-S currents (1,083 ± 152 pA/pF, n = 25 for control IB4<sup>−</sup> cells; 1,024 ± 145 pA/pF, n = 23 for anti-NGF IB4<sup>−</sup> cells). These observations are consistent with a selective reduction in TTX-R currents in IB4<sup>−</sup> neurons, in contrast to a lack of effect on TTX-S currents, or on TTX-R sodium currents in IB4<sup>+</sup> neurons.

**TTX-R current properties in control and NGF-deprived cells**

We examined the basic properties of TTX-R sodium currents in IB4<sup>−</sup> neurons to determine if they were altered by NGF deprivation. Figure 2A shows TTX-R currents recorded from a typical control cell and a typical NGF-deprived cell. The voltage dependence of activation and steady-state inactivation were similar for neurons in both groups (Fig. 2, B and C). The mean midpoint of activation obtained by prepulse subtraction (Cummins and Waxman 1997) was near −19 mV, and the mean midpoint of steady-state inactivation (V<sub>1/2</sub>) was near −30 mV for both groups. Thus, although NGF deprivation decreases the amplitude of the TTX-R current, it does not alter the properties of the TTX-R currents in small IB4<sup>−</sup> DRG neurons.

![Graph showing TTX-R current density](http://jn.physiology.org/DownloadedFrom/10.22033.6)
Rizzo and coworkers (1994) observed a large degree of interneuronal variation in the properties of the TTX-R sodium current in small DRG neurons. In our study, the amount of variability in the voltage dependence of activation and steady-state inactivation of the TTX-R currents was similar for control and NGF-deprivation groups. Recently, Rush and Elliott (1997) proposed the existence of two distinct populations of TTX-R currents based on differences in drug sensitivity and midpoints of steady-state inactivation. By their classification, type I and type II TTX-R currents had average $V_h$ of $-29$ and $-46$ mV, respectively. If $-37.5$ mV is used as the dividing value between type I and type II TTX-R currents, the majority of cells in both our control and NGF-deprived IB4$^+$ groups would be classified as displaying type I currents. Only two cells in each group might be considered as displaying TTX-R type II currents.

**In situ hybridization**

The SNS hybridization signal in control and NGF-deprived IB4$^+$ DRG neurons was quantified by microdensitometry. The confocal laser microscope was operated in dual-channel mode (brightfield and FITC fluorescence), which allowed simultaneous visualization of neurons and their IB4-reactivity (Fig. 3). Images of DRG neurons were captured and subsequently analyzed in terms of IB4 fluorescence, size (expressed as surface area) and hybridization signal (OD) with NIH-Image software. The neuronal size distributions were similar in control and autoimmune animals (Fig. 4). The mean OD of NGF-deprived, IB4$^+$ neurons with a surface area $<1,200 \, \mu m^2$ ($\sim 39 \, \mu m$ in diameter) was $0.115 \pm 0.70$ compared with $0.22 \pm 0.108$ for control IB4$^+$ neurons; the difference in hybridization signal between control and NGF-deprived neurons was significant ($P < 0.05$) in four of five separate experiments (Table 1). The relative hybridization signal of IB4-reactive (IB4$^+$) and IB4$^-$ neurons from NGF-deprived rats is shown in Fig. 5. Although IB4$^+$ neurons from NGF-deprived rats exhibit a significant decrease of hybridization signal compared with control neu-
rons, no significant change of the hybridization signal is seen in IB4− neurons. As a measure of the number of IB4− neurons expressing significant levels of SNS mRNA, the number of IB4− neurons with an OD twofold greater than background in situ hybridization signal was determined. With this threshold, 25 ± 20% of neurons from NGF-deprived animals expressed significant levels of SNS mRNA (Table 2), compared with 57 ± 4.1% of neurons from control animals. The difference between the number of cells positive for SNS mRNA in the autoimmune (27/110) versus control group (46/83) was significant at $P < 0.0005$ (odds ratio = 0.26, $\chi^2$ with Yates correction).

DISCUSSION

To study the effect of NGF deprivation on the expression of sodium currents and sodium channel SNS mRNA in uninjured DRG neurons in vivo, we immunized adult Sprague-Dawley rats to NGF according to the protocol of Chudler and coworkers (1997). With this immunization protocol, approximately one-third of the immunized rats developed high antibody titers (1:48,000) to NGF, which are associated with decreased levels of systemic NGF and thermal hypoalgesia (Chudler et al. 1997). In the present study, IB4− DRG neurons cultured from high titer rats exhibited a significant reduction in TTX-R sodium currents and in the levels of SNS mRNA. In contrast, no significant changes in the amplitude of TTX-R currents or in the levels of SNS mRNA in IB4+ neurons were detected.

Adult DRG neurons are heterogeneous with respect to size, sensory modalities, and expression of neurotrophin receptors (Averill et al. 1995). Small-diameter neurons constitute 70–80% of neurons in L4 and L5 DRG and are principally nociceptors and thermoreceptors. Approximately one-half of these small DRG neurons express neurotrophin receptors TrkA and p75 and synthesize neuropeptides, whereas the other one-half do not express TrkA or p75 receptors but bind isolectin B4 from *Griffonia simplicifolia* (Averill et al. 1995; McMahon et al. 1994; Molliver et al. 1995; Wright and Snider 1995). IB4 provides an especially tractable label for the discrimination between small neurons that express TrkA, and thus respond to NGF, and those small neurons that lack TrkA receptors because the expression of many other possible workers, such as CGRP, is modulated by NGF and may be down-regulated in NGF-depleted neurons. Because IB4 labeling predominately is found on neurons lacking NGF receptors, it is unlikely that NGF depletion would affect the IB4 labeling. In support of this notion, our studies showed no difference in the intensity or distribution of IB4− reactivity in neurons from NGF-depleted versus control animals (data not shown).

Previous studies have implicated NGF in the up-regulation of TTX-R sodium currents (Dib-Hajj et al. 1998; Oyalese et al. 1997) and aSNS/PN3 mRNA (Black et al. 1997; Dib-Hajj et al. 1998) in DRG neurons after transection of their peripheral

![FIG. 4. Cell size distribution. The surface area of IB4− cells was measured by outlining the cells in NIH image. The percentage of cells in different size groups is shown. No significant difference was seen in surface area in NGF-deprived vs. control cells.](http://jn.physiology.org/)

![TABLE 1. SNS in situ hybridization signal in IB4-negative cells](http://jn.physiology.org/)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>NGF-Deprived Animals</th>
<th>Control Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean OD</td>
<td>IB4− Cells, n</td>
</tr>
<tr>
<td>1</td>
<td>0.222</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>0.075</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>0.080</td>
<td>19</td>
</tr>
<tr>
<td>4a*</td>
<td>0.052</td>
<td>27</td>
</tr>
<tr>
<td>4b*</td>
<td>0.148</td>
<td>24</td>
</tr>
</tbody>
</table>

Mean optical density (OD) of small to medium ($<1.200 \, \mu m^2$) IB4-negative dorsal root ganglion neurons after in situ hybridization. NGF, nerve growth factor. NS, not significant. *Cells from two NGF-deprived animals were processed for in situ hybridization simultaneously with a single control. n, number of neurons. $P$ values refer to two-tailed Student’s t-test.
TABLE 2. SNS-positive population in IB4-negative cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>IB4− Cells, n</th>
<th>IB4− Cells, n</th>
<th>SNS-Positive Population, %</th>
<th>SNS-Labeled Cells, n</th>
<th>SNS-Positive Population, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>7</td>
<td>24</td>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>2</td>
<td>18</td>
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<td>60</td>
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<td>3</td>
<td>19</td>
<td>5</td>
<td>28</td>
<td>18</td>
<td>56</td>
</tr>
<tr>
<td>4a</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>24</td>
<td>13</td>
<td>54</td>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td>All cells</td>
<td>110</td>
<td>27</td>
<td>26</td>
<td>83</td>
<td>57</td>
</tr>
</tbody>
</table>

Number (n) and percentage of IB4− cells expressing significant levels of SNS mRNA. Cells with a mean optical density more than two times background hybridization signal were considered to be SNS positive. The percentage of cells with significant SNS mRNA levels was significantly (χ², P < 0.0005) reduced in nerve growth factor (NGF)-deprived animals.

axons. However, little information has been available concerning the role of NGF in the maintenance of TTX-R sodium channels or αSNS/PN3 transcript levels in intact adult DRG neurons. Here we show that TTX-R sodium currents and αSNS/PN3 mRNA are decreased in IB4− neurons from NGF-autoimmunized rats compared with age-matched adult control rats. Previous work has demonstrated that rats immunized with NGF develop characteristics indicative of systemic NGF deprivation, including loss of substance P immunoreactivity (Schwartz et al. 1982) and a reduction of the size and protein content of adrenergic neurons in the superior cervical ganglia (Otten et al. 1979). Our observations are consistent with an important modulatory role by NGF in the expression of αSNS/PN3 mRNA and TTX-R sodium currents in the adult nervous system. This hypothesis is supported by several recent studies. A reduction in TTX-R sodium currents and αSNS/PN3 mRNA was observed after sciatic nerve transection (Cummins and Waxman 1997; Dib-Hajj et al. 1996; Okuse et al. 1997), and infusion of exogenous NGF to the transected nerve stump partially rescued TTX-R sodium currents and expression of αSNS/PN3 mRNA (Dib-Hajj et al. 1998). These observations suggest that the loss of SNS mRNA and TTX-R currents in small DRG neurons after axonal transection might, at least in part, depend on a reduction of retrogradely transported NGF from the periphery. Furthermore, although induction of inflammation with Freund’s adjuvant might not result in changes in the levels in SNS mRNA (Okuse et al. 1997), it has been shown that experimentally induced inflammation of the rat hind paw by carrageenan increases cutaneous levels of NGF (Donnerer et al. 1992) and, concomitantly, TTX-R sodium currents and αSNS/PN3 mRNA in small DRG neurons (Tanaka et al. 1998). These observations are consistent with a direct role for peripherally derived NGF in the regulation of αSNS/PN3 expression in small DRG neurons.

Rats that develop high antibody titers to NGF through autoimmunization show thermal hypoalgesia (Chudler et al. 1997). Therefore NGF deprivation could affect neuronal excitability by selectively influencing the levels of TTX-S sodium channels at nerve terminals or by altering the properties of TTX-S currents. Third, NGF deprivation through autoimmunization has been shown to decrease the levels of the neuropeptide substance P, a sensitizing mediator of acute inflammation, in sensory ganglia, spinal cord, and hind paw skin (Schwartz et al. 1982). However, the role, if any, for substance P in noninflamed tissue is not well known. Fourth, although Chudler and coworkers (Toledo-Aral et al. 1997) did not see a difference in somatic TTX-S sodium current density, this does not rule out the possibility that NGF deprivation causes hypoalgesia by altering TTX-S sodium currents. Mandel and coworkers (Toledo-Aral et al. 1997) have shown that PN1 TTX-S channels, which are modulated by NGF in PC12 cells, are preferentially targeted to the neurite terminals of cultured DRG neurons. Moreover, PN1 channels have been shown to respond to slow depolarizing inputs close to resting potential, consistent with a role in signal amplification or transduction close to sensory terminals (Cummins et al. 1998). Therefore NGF deprivation could affect neuronal excitability by selectively influencing the levels of TTX-S sodium channels at nerve terminals or by altering the properties of TTX-S currents.

TTX-R currents play in the firing patterns of DRG neurons is not known, Elliott (1997) proposed that slow sodium channel inactivation (as seen in αSNS/PN3) may be a major factor in the generation and/or maintenance of spontaneous bursts of action potentials. Therefore a decrease in αSNS/PN3 channels might be expected to affect the excitability of DRG neurons.

Several alternatives might account for the thermal hypoalgesia associated with NGF deprivation, in addition to a reduction of TTX-R currents. First, NGF might have a general trophic effect that maintains or enhances protein synthesis in a nonselective manner, although such an effect seems unlikely because the amplitude and density of the TTX-S currents were unaffected in the NGF-deprived animals. Second, although we did not see a difference in somatic TTX-S sodium current density, this does not rule out the possibility that NGF deprivation causes hypoalgesia by altering TTX-S sodium currents. Mandel and coworkers (Toledo-Aral et al. 1997) did not detect any antibodies to NGF in cerebrospinal fluid, the possibility that peripheral NGF depletion might cause central desensitization through indirect mechanisms cannot be excluded. Fifth, the sympathetic nervous system, which has been suggested to be involved in some pain syndromes, is dependent on NGF (Andreev et al. 1995); however, sympathectomy only produces a very transient reduction of TTX-R sodium currents. However, the role, if any, for substance P in noninflamed tissue is not well known. Fourth, although Chudler and coworkers (1997) did not detect any antibodies to NGF in cerebrospinal fluid, the possibility that peripheral NGF depletion might cause central desensitization through indirect mechanisms cannot be excluded. Fifth, the sympathetic nervous system, which has been suggested to be involved in some pain syndromes, is dependent on NGF (Andreev et al. 1995); however, sympathectomy only produces a very transient reduction of TTX-R sodium currents. Therefore NGF deprivation might cause central desensitization through indirect mechanisms cannot be excluded. Fifth, the sympathetic nervous system, which has been suggested to be involved in some pain syndromes, is dependent on NGF (Andreev et al. 1995); however, sympathectomy only produces a very transient reduction of TTX-R sodium currents. Therefore NGF deprivation might cause central desensitization through indirect mechanisms cannot be excluded. Fifth, the sympathetic nervous system, which has been suggested to be involved in some pain syndromes, is dependent on NGF (Andreev et al. 1995); however, sympathectomy only produces a very transient reduction of TTX-R sodium currents.
produce and release NGF (Leon et al. 1994), which enables an autocrine amplification of the mast cell response. Experimental degranulation of mast cells reduces and delays thermal hyperalgesia after systemic NGF injection (Lewin et al. 1994), and mast cells are therefore thought to be mediators of inflammatory hyperalgesia. Although NGF can activate mast cells as part of the inflammatory response, it is not clear if mast cells have a role in maintaining the sensitivity of sensory neurons in noninflamed tissue. Arguing against this possibility, Lewin and coworkers (1994) did not detect a significant difference in heat algesia between animals whose mast cells had been degranulated and control animals before NGF injection. This suggests that the thermal hypoalgesia seen in the NGF-depletion model may not be explained solely by a reduction in mast cell activity. Finally, the expression of bradykinin receptors was found to be under NGF control and might be involved in the regulation of sensitivity in NGF-deprived animals (Bennett et al. 1998; Petersen et al. 1998).

Our observations suggest that NGF is involved in the regulation of SNS/PN3 sodium channel expression in the DRG of adult rats. Why is there a need for tonic regulation of the electrical properties of sensory neurons in the adult animal? One possible role of this phenomenon could be to recruit nociceptors after tissue-damaging injury. Several mechanisms, such as sensitization of peripheral nerves through local mediators and central sensitization, play a role in ensuring that injured and damaged tissue is guarded from further injury. It has also been suggested that the activation of “silent nociceptors” might be involved in hyperalgesia and allodynia. These silent or “very-high-threshold” nociceptors were detected in viscera, joint capsule, and skin and are characterized by being evokable only in inflamed tissue (McMahon and Koltzugen 1990; Michaelis et al. 1996; Schmidt et al. 1995). The levels of NGF increase dramatically in experimentally induced inflammation of the skin (Donnerer et al. 1992; Woolf et al. 1994), and high levels of NGF are found in synovial fluid from rheumatic arthritis patients (Aloe et al. 1992). Furthermore, some of the algic actions of NGF in inflammatory models have a delay of onset consistent with an effect on gene transcription (Woolf et al. 1994). Thus the sensitivity of some nociceptors to NGF in the adult animal may provide a mechanism for lowering the threshold of silent nociceptors as an additional mechanism to ensure guarding behavior to protect an inflamed limb or joint. Regardless of the physiological role for tonic regulation of nociceptors, the finding that NGF affects the electrical properties of uninjured small sensory neurons suggests that NGF may play a role in pain associated with chronic inflammatory conditions by modulating the sensitivity of some nociceptive neurons.

Our findings support the conclusion that in adult rats, the expression of αSNS/PN3 is tonically regulated by NGF in IB4+ DRG neurons, many of which are nociceptive. These observations add to the body of data indicating that NGF participates in maintaining the phenotypic properties of some neuronal populations in the adult nervous system and suggest that NGF can modulate the electrophysiological properties of DRG neurons, thereby affecting pain perception.

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