TNBS ileitis evokes hyperexcitability and changes in ionic membrane properties of nociceptive DRG neurons

BEVERLEY A. MOORE, TIMOTHY M. R. STEWART, CEREDWYN HILL, AND STEPHEN J. VANNER
Gastrointestinal Diseases Research Unit, Queen’s University, Kingston, Ontario, Canada K7L 5G2
Received 17 September 2001; accepted in final form 23 January 2002

Moore, Beverley A., Timothy M. R. Stewart, Ceredwyn Hill, and Stephen J. Vanner. TNBS ileitis evokes hyperexcitability and changes in ionic membrane properties of nociceptive DRG neurons. Am J Physiol Gastrointest Liver Physiol 282: G1045–G1051, 2002. First published February 20, 2002; 10.1152/ajpgi.00406.2001.—This study examines whether intestinal inflammation leads to changes in the properties of ion channels in dorsal root ganglia (DRG) neurons. Ileitis was induced by injection of trinitrobenzene sulfonic acid (TNBS), and DRG neurons innervating the ileum were labeled using fast blue. Intracellular recording techniques were used to measure electrophysiological properties of acutely dissociated neurons 12–24 h after dissection. Nociceptive neurons were identified by sensitivity to capsaicin, tetrodotoxin resistance, and size (<30 μm). The action potential threshold in neurons from TNBS-treated animals was reduced by >70% compared with controls (P < 0.001), but the resting membrane potential was unchanged. Cell diameter, input resistance (67%), and action potential upstroke velocity (22%) increased in the TNBS group (P < 0.05). The number of action potentials discharged increased in the TNBS group (P < 0.001), whereas application of 4-aminopyridine to control cells mimicked this effect. This study demonstrates that ileitis induces hyperexcitability in nociceptive DRG neurons and changes in the properties of Na+ and K+ channels at the soma, which persist after removal from the inflamed environment.

sensory neurons; small intestine; abdominal pain; inflammation; peripheral sensitization; trinitrobenzene sulfonic acid; dorsal root ganglia

ABDOMINAL PAIN IS A MAJOR cause of morbidity in patients suffering from inflammatory bowel diseases such as Crohn’s ileitis. The neurons that relay sensory information from the intestine to the spinal cord are the dorsal root ganglia (DRG) neurons (18). These pseudounipolar neurons have a peripheral axon that travels to the intestine and a central axon that projects to sensory neurons in the dorsal horn of the spinal cord. Aδ- and small unmyelinated C fiber neurons in the DRG respond to nociceptive stimuli and play a major role in the transmission of painful stimuli. Many C fibers are high-threshold neurons, and during inflammation, these cells undergo peripheral sensitization. This alteration leads to a decreased threshold for activation and increased excitability in response to stimulation. These changes help to explain increased pain in response to noxious stimuli (hyperalgesia) or painful sensation in response to nonnoxious stimuli (allodynia) (17).

In studies in the somatic nervous system, such as in the skin, peripheral sensitization appears to involve both acute and chronic mechanisms (40). The release of a broad range of neuroactive agents occurs during inflammation. Whereas many of these, such as histamine, prostaglandins, and serotonin, can depolarize nerve endings, they can also act by changing the properties of the sensory neurons. Several agents, such as adenosine, serotonin, and prostaglandins, which acutely sensitize DRG neurons, have been shown to enhance sodium currents and/or reduce potassium currents (19, 40). These actions occur within minutes and appear to be confined to mechanisms at the nerve terminal (37, 40). There are, however, later and longer lasting transcription-dependent changes, which are evoked by either signaling molecules, such as nerve growth factor (NGF), and/or activity-dependent (i.e., Ca2+ influx) second-messenger cascades (40). These transcription-dependent events can result in altered expression of voltage-gated ion channels, such as increased expression of TTX-resistant (TTX-R) sodium channels (37, 40).

There is considerably less known about the cellular events evoked in visceral DRG neurons during intestinal inflammation. Single-fiber recording studies examining the properties of DRG neurons innervating the intestine have shown that inflammatory mediators can sensitize peripheral nerve terminals (4, 30, 32). However, these studies do not examine longer term changes that can occur at the level of the cell body. Thus the aim of the present study was to characterize long-term changes in the ionic membrane properties associated with hyperexcitability of DRG neurons innervating the inflamed intestine. Studies were performed on cell bodies of acutely dissociated DRG neurons to isolate events from those confined to the nerve terminals. DRG neurons innervating the inflamed intestine were identified using the retrograde label fast blue (FB).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIALS AND METHODS

Guinea pigs (140–225 g) of either sex were obtained from Charles River Laboratories (Montreal, Quebec, Canada). Experiments were performed according to the guidelines of the Canadian Council of Animal Care and approved by the Queen's University animal care committee.

**FB injections and induction of trinitrobenzene sulfonic acid ileitis.** Animals were anesthetized with a Hypnorm-midazolam combination. The abdomen was opened by midline laparotomy, and the cecum and terminal ileum were gently exteriorized. Small volumes (1–2 µl) of the retrograde tracer FB (Sigma; -20 µl, 3% wt/vol in sterile water) were injected into the wall of the ileum, commencing 4–5 cm proximal to the ileocecal junction. Multiple injections were made on both sides of the intestine over a distance of ~5 cm of terminal ileum using a 30-gauge needle fitted to a Hamilton syringe (total volume 15 µl). The exterior of the ileum was swabbed after each injection to remove residual tracer to avoid erroneous labeling. Persistently leaking sites were sealed with tissue adhesive (cyanoacrylate; Vetbond; 3 M). Trinitrobenzene sulfonic acid (TNBS) ileitis was induced by injecting 0.5 ml TNBS (25 mg/ml in 25% ethanol-sterile water) into the ileal lumen 7–10 cm proximal to the ileocecal junction (20).

**Measure of inflammation.** After the 7- to 10-day recovery period, the terminal ileum was removed from each animal to establish the presence or absence of TNBS-induced ileitis. The ileum was opened along the mesenteric border and pinned flat with the mucosal surface uppermost. Mucosal damage was assessed using a macroscopic damage score. A score of 0 or 1 was applied for the absence or presence, respectively, of each of four parameters: erosions, adhesions, hyperemia, and petechial hemorrhage (maximum damage score of 4). The wet weight per unit length of the whole gut tissue was then determined to provide a measure of tissue edema and wall thickening, as previously described (36).

**Isolation and identification of ileal projecting DRG neurons.** The distribution of FB-labeled DRG neurons was examined in animals killed 4–6 days after injection. Animals were anesthetized with isoflurane (IsoVet, Schering-Plough) and killed by cervical transection. The vertebral column was removed en bloc from the first thoracic vertebra to the lumbar-sacral junction and transferred to chilled PBS (0.1 M, pH 7.4). The spinal cord and DRG were exposed by laminectomy. DRG were dissected bilaterally from thoracic vertebra 6 (T6) to lumbar vertebra 4 (L4) and transferred to fresh PBS. Ganglia were fixed at 4°C in 4% paraformaldehyde in PBS, equilibrated in 30% sucrose-PBS, placed in embedding medium (TissueTek, Fisher Scientific), and rapidly frozen. Frozen serial sections 15–20 µm thick were collected on 3-aminopropylmethoxysilane (APTEX; Fisher Scientific)-coated glass slides and mounted with 30% glycerol-PBS. Sections were viewed by fluorescence microscopy (excitation wavelength 350–390 nm; barrier filter with a 460-nm barrier filter) to identify perikarya containing bright blue fluorescent label. Labeled cells of all sizes were counted for all sections collected for each ganglion, and the mean number of labeled cells per section was determined. Only those cell bodies having a clearly defined nucleus were included in the counts to minimize the possibility that individual neurons were counted more than once in adjacent serial sections.

**Ectrophysiological experiments.** Electrophysiological experiments were conducted on DRG neurons isolated from three groups: unoperated controls and animals killed 7–10 days after injection of FB (FB controls) or FB and TNBS. The spinal column from thoracic vertebra six to lumbar vertebra four was placed in sterile calcium and magnesium-free Hanks' balanced salt solution (HBSS; GIBCO-BRL). DRG were dissected bilaterally from spinal levels T15-T12 and transferred to fresh chilled HBSS. Connective tissue was dissected from ganglia, but they were not desheathed. Whole ganglia were incubated for 10 min at 37°C with HBSS containing 0.2 mg/ml papain (Worthington) activated with 0.4 mg/ml cysteine (Sigma). The ganglia were washed twice with L-15 medium (GIBCO-BRL) containing 10% heat-inactivated fetal bovine serum (FBS; GIBCO-BRL) and once with HBSS. This was followed by a 10-min incubation with HBSS containing 1 mg/ml collagenase type I (Worthington) and 4 mg/ml dispase II (Boehringer-Mannheim). Ganglia were triturated 10 times through a flame-polished Pasteur pipette and returned to the incubator for an additional 7–10 min. Trituration was repeated to obtain a single-cell suspension. Cells were washed twice in L-15-10% FBS, resuspended in HBSS, and layered onto L-15-10% FBS. The final pellet was relatively free of myelin debris and was resuspended in MEM culture medium (with Earle’s salts and NaHCO3; GIBCO-BRL) containing 2.5% by volume guinea pig serum (collected in house), 1% penicillin-streptomycin, 2% sodium pyruvate, and 0.25% fetal bovine serum. Cells were plated onto collagen-coated coverslips (rat tail collagen) in 24-well culture plates (~5,000 cells/well). The cells were maintained at 37°C in a humidified atmosphere of 95% air-5% CO2 until they were retrieved for use in electrophysiological experiments.

Previous studies suggest that acutely dissociated neurons retain FB fluorescence in short-term culture (<24 h) without altering the passive or active electrical properties of the membrane. Furthermore, short-duration exposure to ultraviolet light (<30 s) allows identification of labeled cell bodies without inducing phototoxic injury (6, 44). Therefore, all experiments were performed within ~24 h of isolation.

**Ectrophysiological recordings.** Coverslips containing adherent DRG neurons were placed in small (1 ml) recording chambers and mounted on an inverted microscope (Zeiss Axiovert 100) fitted for both fluorescence and bright-field microscopy. Chambers were continuously superfused at room temperature at 2 ml/min with external medium containing (in mM) 140 NaCl, 5 KCl, 1 MgSO4, 1 CaCl2, 5 HEPES, and 5 glucose (pH 7.4). Pharmacological agents were added to the bath by superfusion or by pressure pulse application (Picospritzer, General Valve).

Conventional intracellular and, in one later experiment, whole cell current-clamp recording techniques were used to examine the electrophysiological properties of cultured DRG neurons. FB-labeled neurons were identified by their blue fluorescence following brief (<15 s) exposure to ultraviolet light, after which cells were viewed under bright field. Stable intracellular impalements were obtained using glass microelectrodes filled with 2 M KCl with tip resistances of 70–120 MΩ. In one set of experiments, whole cell current-clamp recordings were obtained using glass microelectrodes filled with (in mM) 140 KCl, 5 HEPS, 1 MgSO4, and 1 EGTA (pH 7.2). Tip resistances in normal solutions were between 1.5 and 4 MΩ. Changes in membrane potential were recorded with an Axoclamp 2A amplifier (intracellular recording) or an Axopatch 2B (current-clamp recording), displayed on a Gould T240 chart recorder or digitized at 10–20 kHz using a Digidata 1200A acquisition board and analyzed using Axoscope and Clampex software (Axon Instruments). Input resistances were calculated from the slope of the linear portion of the voltage-current relationship generated in response to hyperpolarizing current steps from 0.05 to 0.6 nA.

**Statistics.** Results are expressed as means ± SE. Student’s t-tests were applied to demonstrate statistical differences between two groups. ANOVA with a Tukey’s post hoc test
was used to assess differences among the three groups (unoperated controls, FB controls, and FB and TNBS).

RESULTS

Assessment of ileitis. The presence of ileitis was established using a damage score (see MATERIALS AND METHODS) and tissue weights. Wet weights per unit length and damage scores did not differ between animals injected with FB alone (n = 6) and unoperated controls (n = 6; Fig. 1). After TNBS treatment (n = 8), ileal weight was significantly greater than that of both the FB control and unoperated control groups. Visual inspection of the ileal mucosa revealed erosions, adhesions, hyperemia, and occasional petechial hemorrhage in all animals treated with TNBS. Damage scores in the ileitis group compared with FB, and unoperated controls were markedly increased (Fig. 1).

Segmental distribution of DRG neurons projecting to the terminal ileum. Four to six days following FB injection into the terminal ileum, frozen sections from DRG ganglia at T6–L4 were examined for FB immunofluorescence (Fig. 2A). FB fluorescent cells were found in DRG from spinal levels T6–L3 (Fig. 2B; n = 3 animals). Increased numbers of labeled cells were observed at spinal levels T10–T12, with the peak density found at T11. In subsequent electrophysiological experiments, ganglia were dissected bilaterally from T10 to T12 to maximize the proportion of labeled neurons in acute dissociations of the ganglia.

Identification of nociceptive sensory afferent neurons. Neuronal properties characteristic of nociceptive DRG neurons were used to identify neurons: small size, sensitivity to capsaicin, presence of long duration action potentials, and resistance of the action potential to TTX (2, 3, 5, 8, 9, 12, 15, 28, 35). In current-clamp recordings of DRG neurons from unoperated control animals, 95% (18 of 19) small-diameter (23–30 μm) neurons exhibited long-duration action potentials (6.7 ± 0.6 ms, measured at half-maximum amplitude) with a prominent shoulder on the falling phase in response to depolarizing current pulses (Fig. 3A). All neurons tested exhibited action potentials that were resistant to TTX (2–5 μM; n = 10) and depolarized an average of 8 ± 2 mV in response to pressure-pulse application of capsaicin (10 μM; n = 6). In two neurons, depolarization in response to capsaicin reached firing threshold, and the neuron responded with a burst of action potentials. In contrast, 87% (7 of 8) neurons having diameters >35 μm exhibited short-duration (3.2 ± 0.5 ms) action potentials that lacked a shoulder.
Table 1. Membrane characteristics of ileal nociceptive afferent neurons in control and TNBS-treated guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>Unoperated Control</th>
<th>Fast Blue Control</th>
<th>Fast Blue-TNBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>$-49.8 \pm 0.6$</td>
<td>$-49.2 \pm 1.6$</td>
<td>$-49.9 \pm 1.5$</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>83.4 ± 2.9</td>
<td>77.1 ± 3.7</td>
<td>80.0 ± 3.9</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>5.9 ± 0.3</td>
<td>7.1 ± 1.3</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>AP upstroke velocity, mV/ms</td>
<td>44.5 ± 2.6</td>
<td>43.2 ± 1.3</td>
<td>55.0 ± 3.1</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>13.0 ± 1.2</td>
<td>15.2 ± 3.2</td>
<td>15.9 ± 1.8</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>75.5 ± 8.2 (n = 11)</td>
<td>69.8 ± 13.4 (n = 8)</td>
<td>116.6 ± 14.1 (n = 8)*</td>
</tr>
<tr>
<td>Somal diameter, µm</td>
<td>23.9 ± 0.3 (n = 32)</td>
<td>24.8 ± 0.3 (n = 37)</td>
<td>29.7 ± 0.3 (n = 43)*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of cells. TNBS, trinitrobenzene sulfonic acid; RMP, resting membrane potential; AP, action potential; AHP, after hyperpolarizing potential. *Statistical difference, P < 0.05.

(Fig. 3B) were abolished in the presence of TTX (1 µM; n = 8), and were insensitive to capsaicin (n = 8). In all subsequent experiments, only those neurons having diameters <30 µm and that exhibited long-duration action potentials were selected for electrophysiological analysis.

Effects of inflammation on the action potential and passive membrane properties. The passive membrane properties of nociceptive neurons in the three experimental groups are summarized in Table 1. The resting membrane potential of neurons obtained from the TNBS-ileitis group did not differ from the other two groups. In the TNBS-ileitis group, however, the mean input resistance of these cells was increased by 67% (n = 8) compared with the FB and unoperated control groups (P < 0.05). The mean cell diameter was measured in all FB and unoperated control neurons in which electrophysiological studies were conducted and those with similar morphological characteristics that could not be impaled (i.e., associated connective tissue). The mean cell diameter was increased (20%) in the neurons innervating the TNBS-inflamed intestine compared with controls. This could represent selection bias, although other studies have reported an increase in somal diameter of DRG neurons following chronic inflammation (8, 13, 39, 43).

The amplitude of the action potential after hyperpolarization, however, did not differ between groups (Table 1). Significant differences in membrane properties were not observed between unoperated and FB control neurons (Table 1). Although acute exposure to alcohol is not known to have long-term effects on neurons, an alcohol group was not included in these studies and, as a result, a possible contribution from an alcohol effect on the findings cannot be entirely excluded.

The mean minimum current injection required to elicit a single action potential (rheobase) was markedly decreased (74%) in the TNBS-ileitis group compared with unoperated and FB controls (P < 0.001; Fig. 4). Depolarizations (500 ms) at twice the rheobase elicited a significantly greater number of action potentials in neurons from the TNBS-ileitis group compared with controls (mean increase 4.3 spikes over controls; P < 0.001; Fig. 4B).

The effects of 4-aminopyridine (4-AP) on action potential discharge are shown in Fig. 5A. Whole cell current-clamp recordings of action potentials elicited by current injections of 1 × rheobase and 2 × rheobase from FB-labeled control neurons were compared in the presence and absence of 4-AP (0.5 mM; n = 3). 4-AP...
had no effect on action potential discharge at the rheobase. However, at 2× rheobase, there was an increase in action potential discharge in the presence of 4-AP compared with FB controls (mean increase 3.3 spikes over controls; P < 0.05).

Analysis of the action potential waveform revealed that action potential amplitude and half-width were unchanged among experimental groups (Table 1). The velocity of the action potential upstroke was increased (mean increase = 22%; P < 0.05) in neurons from the TNBS-ileitis group compared with unoperated and FB controls (Fig. 5B). The increase in upstroke velocity was not altered in the presence of TTX (2 μM; n = 7).

**DISCUSSION**

This study demonstrates that chronic intestinal inflammation increases the excitability of nociceptive DRG neurons innervating the inflamed intestine. The membrane properties were examined in cell bodies located in DRG outside the intestine to isolate changes from acute events confined to the nerve terminals. Because changes in cell diameter as well as passive and active membrane properties persist 12–24 h after the cells have been isolated, this hyperexcitability is not due to local effects of inflammatory mediators. Rather, this hyperexcitability reflects more sustained changes in cellular events occurring at the soma.

**C fiber nociceptive DRG neurons characteristically have a small somal diameter, respond to capsaicin, and have TTX-insensitive action potentials. Both TTX-sensitive and TTX-R Na⁺ currents have been identified in guinea pig (22, 25), rat (3, 10, 11, 23, 42), and human (29) DRG neurons, in which it was shown that neurons with large TTX-R Na⁺ currents had TTX-insensitive action potentials. In rats, the presence of long-duration action potentials in DRG neurons were found to correlate with the presence of TTX-R Na⁺ currents, because the broad, inflected action potentials of C-fiber DRG neurons were resistant to TTX, whereas narrow spikes were sensitive to TTX (35). TTX-R Na⁺ channels have been cloned in the rat, and their expression is confined to small-diameter DRG neurons that are sensitive to capsaicin (2, 3, 5, 8, 12, 28, 34). In the present study, all capsaicin-sensitive neurons tested also had TTX-insensitive action potentials. In some studies in the rat, however, not all DRG neurons containing TTX-R Na⁺ currents responded to capsaicin (33). In rat, 95% of the capsaicin-sensitive afferents are unmyelinated C fibers, whereas only a small proportion of Aδ fibers are sensitive to capsaicin (15). In the guinea pig, long-duration action potentials were recorded almost exclusively in C-fiber afferent neurons that responded to nociceptive stimuli (9). Thus, in the present study, small-diameter, capsaicin-sensitive neurons with TTX-insensitive action potentials were classified as nociceptive neurons.

Nociceptive neurons from animals with TNBS ileitis were considered hyperexcitable, because they 1) had lower current thresholds for action potential activation compared with controls and 2) exhibited enhanced spike discharge in response to prolonged stimulation. These changes would increase the responsiveness of these neurons to low-threshold stimuli in vivo in the inflamed intestine. Under inflammatory conditions, sustained depolarization of the nerve terminals by inflammatory mediators would prolong firing, resulting in increased afferent traffic to the central nervous system. Ultimately, this would lead to increased perception of visceral pain. This concept is supported by studies in the rat, in which it was demonstrated that graded colorectal distention evoked increased afferent discharge to spinal neurons (24, 31). The threshold for activation of excitatory bursts of discharge in response to distension was lowered, and the rate of spontaneous discharge in the absence of distension was increased when the colon was inflamed (24). In awake rats, increased afferent discharge elicited by noxious colorectal distension was perceived as painful (21, 24, 26, 31).
Furthermore, the perception of pain was relieved after ablation of nociceptive afferents with capsaicin (21). Similar findings in response to distention and inflammation were reported in studies of the urinary bladder (14), in which cystitis led to lowered threshold of activation and enhanced spike discharge in nociceptive sensory afferent neurons projecting to the bladder (43). Together, these findings suggest that visceral inflammation alters the intrinsic firing pattern of nociceptive neurons projecting to their visceral organs. This leads to increased excitability of nociceptive DRG neurons and, ultimately, enhanced perception of painful abdominal sensations.

Our data suggest that Na\(^+\) and/or K\(^+\) currents are altered in inflammation and may underlie the inflammation-induced increase in excitability. The increase in the upstroke velocity of the action potential in TNBS compared with control animals suggests an increase in Na\(^+\) currents. Moreover, TTX did not block this effect, implying TTX-R Na\(^+\) channels underlie the increase in Na\(^+\) current. This result is consistent with another inflammation model, in which increased TTX-R Na\(^+\) current density and TTX-R Na\(^+\)-channel mRNA levels were found in DRG neurons 4 days after carrageenan injection into the rat hindlimb (34). Increases in Na\(^+\)-channel density would tend to lower the threshold for action potential discharge (16). Furthermore, Rizzo and co-workers (27) indicate that coexpression of abnormal types of Na\(^+\) channels can cause destabilization of the neuronal membrane, with TTX-R Na\(^+\) channels cross-activating other Na\(^+\) channels, thus causing spontaneous activity. Therefore, increased expression of TTX-R Na\(^+\) channels may underlie the lowered threshold for action potential discharge and/or other changes in somal membrane properties observed in TNBS ileitis.

Our data also suggest K\(^+\) currents were altered in ileal DRG neurons following inflammation. The significant increase in input resistance in cells from TNBS animals implies K\(^+\) currents were suppressed. Furthermore, the nonselective K\(^+\)-channel blocker 4-AP enhanced spike discharge in response to prolonged stimulation in control neurons. Thus pharmacological suppression of K\(^+\) currents with 4-AP partially mimics the effects of TNBS-induced inflammation. A previous study of afferent sensory neurons indicated that inflammation suppressed rapidly inactivating K\(^+\) currents (I\(_A\)) (43). Indeed, in DRG neurons innervating the urinary bladder, application of 4-AP inhibits I\(_A\) and mimics the alterations caused by chronic bladder inflammation (43). Nevertheless, the pharmacology of K\(^+\) channels in ileal-projecting guinea pig DRG neurons remains undefined. Thus further study is necessary to conclude which subtype(s) of K\(^+\) channels are altered following TNBS-induced ileitis.

Together, our data demonstrate that neuronal excitability is associated with significant changes in the Na\(^+\) and K\(^+\) ionic properties of the somal membrane. These changes in ionic membrane properties are likely to be important determinants in the genesis of inflammation-induced peripheral sensitization. The mechanisms that initiate these inflammation-induced changes remain to be elucidated, but studies in other organ systems outside the intestine suggest that transcriptional events lead to altered expression of ion channels (40). The mediators that signal this change are not completely known, but it has been proposed that neurotrophins may affect Na\(^+\) -channel expression in DRG neurons during chronic inflammation (37). In fact, increased NGF concentrations have been found in tissues following application of inflammatory agents (38, 41), and the regulation of TTX-R Na\(^+\) channels by NGF is well established in cell culture (1, 45) and in vivo axotomy models (7). Whereas NGF is not thought to regulate K\(^+\) currents, other activity-dependent mechanisms have been proposed to suppress K\(^+\) currents in inflammation (40). Future electrophysiological and molecular studies will characterize the altered currents fully and determine the mechanism of inflammation-induced changes.

This work was supported by the Canadian Institute of Health Research (to C. Hill and S. J. Vanner) and the Canadian Association of Gastroenterology/AstraZeneca Research Award (to B. Moore).

REFERENCES


13. Goodness TP, Albers KM, Davis FE, and Davis BM. Over-expression of nerve growth factor in skin increases sensory


