Prostaglandin E2 Modulates TTX-R $I_{\text{Na}}$ in Rat Colonic Sensory Neurons

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This study was performed to determine the impact of the inflammatory mediator prostaglandin E$_2$ (PGE$_2$) on the biophysical properties of tetrodotoxin resistant voltage-gated Na$^+$ currents (TTX-R $I_{\text{Na}}$) in colonic dorsal root ganglion (DRG) neurons. TTX-R $I_{\text{Na}}$ was studied in DRG neurons from thoracolumbar (TL: T$_{13}$–L$_2$) and lumbarosacral (LS: L$_4$–S$_2$) DRG retrogradely labeled following the injection of DiIC$_{18}$ (DiI) into the wall of the descending colon of adult male rats.

TTX-R $I_{\text{Na}}$ in colonic DRG neurons had a high threshold for activation ($V_{\text{th}}$ of conductance-voltage (G-V) curve = −3.1 ± 1.0 (SE) mV) and steady-state availability ($V_{\text{th}}$ for H-infinity curve = −18.4 ± 1.4 mV), was slowly inactivating (10.6 ± 1.4 ms at 0 mV), and recovered rapidly from inactivation (83.5 ± 5.0% of the current recovered with a time constant of 1.3 ± 0.1 ms at −80 mV). TTX-R $I_{\text{Na}}$ was present in every colonic DRG neuron studied (n = 62). PGE$_2$ induced a rapid (<15 s) increase in TTX-R $I_{\text{Na}}$ that was associated with a hyperpolarizing shift in the G-V curve (3.4 ± 0.7 mV), an increase in the rate of inactivation (4.21 ± 0.7 ms at 0 mV), and no change in steady-state availability. There was no statistically significant difference (P > 0.05) between TL and LS colonic DRG neurons with respect to the biophysical properties of TTX-R $I_{\text{Na}}$, the current density or the magnitude of PGE$_2$-induced changes in the current. However, both the proportion of TL and LS neurons in which TTX-R $I_{\text{Na}}$ was modulated by PGE$_2$ (16 of 16 TL neurons and 12 of 14 LS neurons) as well as the magnitude of PGE$_2$-induced changes in the current were significantly larger in colonic DRG neurons than in the total population of DRG neurons. These results suggest that changes in nociceptive processing associated with inflammation of the colon do not reflect differences between TL and LS neurons with respect to the properties of TTX-R $I_{\text{Na}}$, distribution of current, or magnitude of inflammatory mediator-induced changes in the current. However, these results do suggest modulation of TTX-R $I_{\text{Na}}$ in colonic afferents is an underlying mechanism of hyperalgesia and pain associated with inflammation of the colon and that this current constitutes a novel target for therapeutic relief of visceral inflammatory pain.

INTRODUCTION

Two general classes of voltage-gated Na$^+$ current ($I_{\text{Na}}$) have been described in sensory neurons based on the sensitivity to tetrodotoxin (TTX): TTX-sensitive voltage-gated Na$^+$ currents (TTX-S $I_{\text{Na}}$) and TTX-resistant voltage-gated Na$^+$ currents (TTX-R $I_{\text{Na}}$) (Elliott and Elliott 1993; Gold et al. 1996b; Kostyuk et al. 1981; Ogata and Tatebayashi 1992; Roy and Narahashi 1992). While both currents are present in nociceptive sensory neurons, evidence from clinical studies and animal models suggests that changes in the biophysical properties, expression, and/or distribution of TTX-R $I_{\text{Na}}$ are an underlying mechanism of both inflammatory and neuropathic pain (see Gold 2000 for review). Evidence in support of a role for TTX-R $I_{\text{Na}}$ in various pain states has been obtained following injury to somatic tissue (Khasar et al. 1998; Novakovic et al. 1998; Porreca et al. 1999; Tanaka et al. 1998), the dura (Strassman and Raymond 1999), and more recently, the urinary bladder (Yoshimura et al., 2001). While recent results with antisense oligodeoxynucleotides provided compelling evidence in support of a role for TTX-R $I_{\text{Na}}$ in urinary bladder hyperactivity observed following intravesical infusion of acetic acid, at least two observations suggest TTX-R $I_{\text{Na}}$ may not contribute to injury-induced hyperexcitability of visceral structures. First, Yoshimura and de Groat observed that spinal-injury-induced urinary bladder hyper-reflexia is associated with an increase in the excitability of urinary bladder afferents that appears to reflect an increase in TTX-S $I_{\text{Na}}$ and a decrease in the density of TTX-R $I_{\text{Na}}$ (Yoshimura and de Groat 1997). Second, Su and colleagues observed that inflammatory mediators, such as prostaglandin E$_2$ (PGE$_2$), serotonin, and adenosine, fail to influence the properties of voltage-gated Na$^+$ currents present on colonic dorsal root ganglion (DRG) neurons (Su et al. 1999).

TTX-R $I_{\text{Na}}$ has been further subdivided into several different classes of ionic current on the basis of unique biophysical properties. These include a high-threshold slowly inactivating current referred to as TTX-R1 (Rush et al. 1998) or the slow TTX-R current (Scholz et al. 1998) and a low-threshold, rapidly activating TTX-R current referred to as TTX-R2 (Rush et al. 1998) or fast TTX-R currents (Scholz et al. 1998). There also is evidence for very-low-threshold TTX-R currents [i.e., TTX-R3 and 4 (Rush et al. 1998)]. Finally, there is evidence for a low-threshold, persistent TTX-R current (Cummins et al. 1999). While TTX-R currents in colonic DRG neurons have been described previously (Su et al. 1999; Yoshimura and de Groat 1997), a biophysical characterization of TTX-R currents was not the focus of either of these previous studies, and therefore the possibility that subtypes of TTX-R currents might...
be present in colonic DRG neurons was not investigated. That there may be differences between subpopulations of DRG with respect to the expression of TTX-R $I_{\text{Na}}$ is suggested by the observations that there are differences between subpopulations of DRG nociceptive afferents with respect to the level of TTX-R $I_{\text{Na}}$ expression (Stucky and Lewin 1999), and there are differences between muscle and cutaneous afferents with respect to the expression of TTX-R $I_{\text{Na}}$ (Rizzo et al. 1994). However, it is unknown whether the biophysical properties of TTX-R $I_{\text{Na}}$ vary among specific subpopulations of sensory neurons and, more specifically, which of the previously described TTX-R currents are present in visceral afferents.

There is a growing body of evidence indicating that there are important electrophysiological differences between visceral and somatic afferents. For example, the high prevalence of low-threshold visceral afferents with nociceptive properties (Sengupta and Gebhart 1994a,b) is not observed in somatic afferents (Lynn and Carpenter 1982). Furthermore, unlike most somatic structures, visceral structures receive innervation via at least two distinct nerves (2 different spinal nerves or a spinal nerve and the vagus). The rat colon receives sensory innervation via the pelvic nerve [arising from lumbosacral (LS) DRG] and the hypogastric/lumbar colonic nerves [arising from the thoracolumbar (TL) DRG]. In the absence of inflammation, noxious stimulation of the colon results in referred pain and dorsal horn activation that appears to reflect pelvic but not hypogastric/lumbar colonic nerve activation. However, in the presence of colonic inflammation, noxious stimulation of the colon results in referred pain and dorsal horn activation that appears to reflect activation of both colonic nerves (Mayer and Gebhart 1994; Mayer et al. 2000; Traub and Murphy 2002). This difference in nociceptive processing may reflect differences in the electrophysiological properties of the afferents traveling in the two nerves.

In an effort to address the dearth of information about the ionic mechanisms controlling the excitability of colonic afferents while beginning to address potential mechanisms underlying inflammation-induced changes in visceral nociceptive processing, we attempted to answer three questions in the present study: 1) is the heterogeneity in biophysical properties observed in the total population of sensory neurons present in a unique population of neurons defined by target of innervation or, more specifically, what are the biophysical properties of TTX-R $I_{\text{Na}}$ present in colonic DRG neurons; 2) is TTX-R $I_{\text{Na}}$ present in colonic DRG neurons a target for modulation by inflammatory mediators; and 3) are there differences between LS and TL colonic DRG neurons with respect to the biophysical properties and/or expression pattern of TTX-R $I_{\text{Na}}$ present in these neurons that could account for inflammation-induced changes in nociceptive processing. To address these questions, we have used patch-clamp electrophysiological techniques to record $I_{\text{Na}}$ from retrogradely labeled colonic DRG neurons in vitro. Our results indicate that TTX-R $I_{\text{Na}}$ is present in virtually all colonic DRG neurons and that the biophysical properties of this current are relatively homogeneous. PGE$_2$ (1 μM) enhanced TTX-R $I_{\text{Na}}$ in almost all neurons tested (16/16 TL neurons and 12/14 LS neurons). The PGE$_2$-induced change in TTX-R $I_{\text{Na}}$ is consistent with an underlying mechanism of nociceptor sensitization. Finally, while the magnitude of PGE$_2$-induced modulation was larger than that previously observed in the total population of DRG neurons, there was no difference between TL and LS neurons with respect to the degree and magnitude of modulation.

**Methods**

Adult male Sprague Dawley rats (Harlan Sprague Dawley) were used for this study. Rats were housed in the University of Maryland Dental School Animal Facility in groups of three prior to colonic labeling and then individually thereafter. Food and water was available ad lib. All experiments were approved by the University of Maryland Institutional Animal Care and Use Committee.

**Identification of colonic afferents**

Colonic DRG neurons were identified by retrograde labeling following injection of retrograde tracer DiIC$_{18}$ [DiI (3)] into the descending colon. Labeling was performed as described previously (Traub et al. 1999), except that DiI (25 mg/ml in methanol) was injected rather than fluoro gold. Briefly, rats were anesthetized with pentobarbital sodium (60 mg/kg ip). The descending colon was exposed by a midline laparotomy, and a total volume of 20 μl DiI was injected over 10–15 sites into the ventral and lateral colon wall with the aid of a dissecting microscope. DiI that leaked from the injection site was wiped away with cotton swabs. The surgical wound was sutured in layers, and rats were allowed to recover from anesthesia. Neurons were studied 10–21 days after labeling. DiI-labeled neurons were easily identified under epifluorescence illumination with a Texas-red/ rhodamine filter set (Fig. 1).

**Immunohistochemistry**

TL and LS ganglia from four rats were used to assess the percentage of colonic DRG neurons giving rise to myelinated axons. The presence of a 200-kDa neurofilament protein (NF200) was used to distinguish myelinated from unmyelinated neurons as this protein is only present in myelinated neurons (Lawson et al. 1984, 1993). Indirect immunohistochemistry with a combination of commercially available antibodies was used to assess the presence of NF200. The primary antibody was a monoclonal (clone N52, Sigma Chemical, St. Louis, MO) that recognizes phosphorylated and unphosphorylated forms of the protein. Cy2-conjugated secondary antibody was used to visualize the presence of N52-like immunoactivity. TL and LS ganglia were harvested from anesthetized animals following transcardiac perfusion with 60 ml of 1 X phosphate-buffered saline (PBS) followed by 500 ml of cold fixative solution (4% paraformaldehyde in 1 X PBS). Ganglia were postfixed for 3 h in the fixative solution, equilibrated in 30% sucrose, frozen, and then sectioned serially at 16 μm on a cryostat.

One slide of sections from each ganglia was processed for immunohistochemistry. Tissue was preincubated at room temperature for 30 min with a solution consisting of 1 X PBS, 5% normal goat serum, and 0.03% Triton X prior to addition of primary antibody (1:500 in the same solution) and then incubated in a humidified chamber at 4°C overnight. The slides were then washed in PBS for 30 min. Secondary antibody (1:200), was applied for 2 h at room temperature. Slides were washed again in 1 X PBS and a cover slip applied with PBS and glycerol. No immunoreactivity was observed when the primary antibody was omitted (data not shown).

Sections were inspected for the presence of DiI-labeled neurons and one to two images were obtained from each slide. To avoid counting the same neuron twice, no images were obtained of adjacent sections. The images were acquired on a Fluoview personal confocal microscope (Olympus Instruments, New York) fitted with krypton/argon lasers and filters for the detection of Cy2/FITC/DTAF and Cy3/TRITC/DiI. The monochrome confocal digital images were pseudo-

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colored green (Cy2) or red (DiI) in the FluoView confocal software. The individual color images were then superimposed, contrast balanced, and assembled into double montages (Merge). The number of neurons positively labeled with N52 was determined with a combination of Photoshop 5.0 photo-editing software (Adobe Systems) and National Institutes of Health imaging software (Scion, Fredrick, MD). Images were converted to gray-scale and auto-contrasted using Photoshop 5.0 photo-editing software. National Institutes of Health imaging software was used to analyze cell body size and immunofluorescence intensity. The nadir between modes of the bi-modal distribution for the fluorescence intensity plot of all neurons was used as the cutoff point between neurons considered N52 positive (N52+/H11001) and neurons considered N52 negative (N52–).

**Cell dissociation**

The colon receives innervation from two spinal nerves: the pelvic and the hypogastric/lumbar colonic. These nerves arise from lumbar-sacral (LS: L6–S 2 ) and thoracolumbar (TL: T 13–L 2 ) DRG, respectively. DRG neurons were prepared for recording as described previously (Gold et al. 1996a). Briefly, rats were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg ip); TL and LS DRG were removed, and rats were subsequently killed by an overdose of pentobarbital sodium (100 mg/kg ic). DRG were desheathed in ice-cold MEM-BS composed of: 90% minimal-essential-medium (MEM; Gibco BRL, Gaithersburg, MD), 10% heat-inactivated fetal bovine serum (BS, Gibco BRL), and 1000 U/ml each of penicillin and streptomycin (Sigma). DRGs were then incubated 45 min at 37°C in 5 ml MEM, to which collagenase P (Boehringer Mannheim, Indianapolis, IN) had been added to a final concentration of 0.125% and bubbled with carbogen (95% O 2 -5% CO 2 ). DRGs were then incubated 5 min at 37°C in Ca 2+ - and Mg 2+ -free Hanks balanced salt solution (GIBCO BRL) containing 0.25% trypsin (Worthington, Bristol, UK) and 0.025% EDTA (Sigma). Trypsin activity was inhibited by the addition of MEM-BS containing 0.125% MgSO 4 , and DRG were dissociated by trituration with a fire-polished Pasteur pipette. DRG cells were plated onto glass cover slips, previously coated by a solution of 5 μg/ml mouse laminin (GIBCO BRL) and 0.1 mg/ml poly-L-ornithine (Sigma). The cells were incubated in MEM-BS at 37°C, 3% CO 2 and 90% humidity for 2 h at which point they were transferred to a HEPES-buffered L-15 media containing 10% BS and 5 mM glucose and stored at room temperature. TL and LS DRG were processed in parallel. Neurons were studied between 2 and 7 h after removal from the animal.

**FIG. 1.** Retrograde labeling of thoracolumbar (TL) and lumbosacral (LS) dorsal root ganglion (DRG) neurons. Neurons were retrogradely labeled with DiIC 18 (DiI). Ten to 15 injections (20 μl total) of a 2.5% solution (wt/vol in methanol) of DiI were made in the wall of the colon. Ten to 21 days later, DRG were surgically removed and either processed for immunohistochemistry or enzymatically treated and dissociated as described in METHODS. A: confocal photomicrograph of DiI-labeled neuron (arrow) in an S1 DRG. B: indirect immunohistochemistry was used to assess the presence of a marker for myelinated neurons: neurofilament 200 (NF200). A monoclonal antibody (clone N52) was used to detect the presence of NF200. Neurons with N52-like immunoreactivity (arrowheads) were larger then N52 negative neurons. C: colonic DRG neurons were typically N52-negative as illustrated by the lack of double labeling in the merged image. The scale bar for A–C = 50 μm. D: histogram showing the cell body diameter of all neurons (open bars) counted on 20 sections of TL and LS DRG. The distribution of N52-negative neurons (black bars) was similar to that of all neurons while that of N52-positive neurons tended to be larger (green bars). DiI-labeled neurons (red bars) are plotted as a function of the number of neurons in each size bin. E: bright-field photomicrograph of dissociated LS (L 6–S 2 ) DRG neurons. F: Dil-labeled neuron (arrow) is visualized under epifluorescence illumination. Scale bar for E and F = 50 μm.
Electrophysiology

Voltage-clamp recordings were performed using a HEKA EPC9 (HEKA Electonik, Lambrecht/Pfalz Germany) or an Axopatch 200B (Axon Instruments, Union City, CA). Data were low-pass filtered at 5–10 kHz with a 4-pole Bessel filter and digitally sampled at 25–100 kHz. Capacity transients were cancelled and series resistance was compensated (>80%); a P/4 protocol was used for leak subtraction. Electrodes (0.7–3 MΩ) were filled with (in mM) 100 Cs-methanesulfonate, 40 tetraethylammonium-Cl, 5 Na-methanesulfonate, 1 CaCl₂, 2 MgCl₂, 11 EGTA, 10 HEPES, 2 Mg-ATP, and 1 Li-GTP; pH was adjusted to 7.2 with Tris-base, osmolality was adjusted to 310 mOsm with sucrose. Bath solution used to record whole cell Na⁺ currents in isolation contained (in mM) 35 NaCl, 30 tetraethylammonium-Cl, 65 choline-Cl, 0.1 CaCl₂, 5 MgCl₂, 10 HEPES, and 10 glucose, pH adjusted to 7.4 with Tris-base, osmolality adjusted to 325 mOsm with sucrose. All salts were obtained from Sigma.

Experimental protocol

After formation of a tight seal (>5 GΩ) and compensation of pipette capacitance with amplifier circuitry, whole cell access was established. Cell capacitance was determined with five hyperpolarizing pulses (10 ms) from −50 to −80 mV. Whole cell capacitance and series resistance were compensated with the amplifier circuitry. The membrane potential was then stepped to 0 mV for 15 ms every 5 s for 5 min to monitor the stability of evoked currents and the recording configuration. To assess PGE₂-induced changes in the current-voltage (I-V) relationships, data were collected for an I-V curve every 2 min. Membrane potential was held at −80 mV. Current was evoked following a 500-ms prepulse to either −100 or −50 mV with a 15-ms step to potentials between −60 and +40 mV in 5-mV increments. To unequivocally determine the reversal potential for I_{Na}, 10 additional neurons were studied with steps to potentials between −60 and +80 mV. At least three complete I-V curves were collected prior to the application of PGE₂ (1 μM, Sigma). These I-V curves were used to establish the baseline response from which PGE₂-induced changes were compared. A prepulse to −50 mV was used to inactivate low-threshold rapidly activating currents. Neurons were also studied in the presence and absence of TTX (1 μM, Sigma) to determine which currents were TTX resistant. With membrane depolarization, voltage-gated Na⁺ channels undergo a transition from a closed to an inactivated state that is distinct from the transition from an open to an inactivated state. Because the voltage-clamp protocol was used to assess the former transition involves the determination of the fraction of channels available for activation from a given membrane potential, we refer to the measurement of channels in the closed-to-inactivated state as “steady-state availability.” Steady-state availability was assessed for TTX-R I_{Na} with a 1-s prepulse varying between −100 and +10 mV followed by a voltage step to 0 mV. This 1-s prepulse was also used to assess the presence of the persistent TTX-R current as this current appears to have a low threshold for activation but should be available for activation from a holding potential of −80 mV (Cummins et al. 1999). Current density was determined by dividing the peak current evoked at 0 mV by the cell capacitance.

Data analysis

Conductance-voltage (G-V) curves were constructed from I-V curves by dividing the evoked current by the driving force on the current, such that \( G = I/(V_m - V_{rev}) \) where \( V_m \) is the potential at which current was evoked and \( V_{rev} \) is the reversal potential for the current determined by extrapolating the linear portion of the I-V curve through 0 current. The validity of this approach was assessed empirically by analyzing I-V curves from 10 neurons in which outward currents had been evoked at membrane potentials between +55 and +80 mV; there was no statistically significant difference between the reversal potential determined by extrapolation (i.e., 47.4 ± 2.7 mV for TTX-R I_{Na}) as described above or by interpolation (i.e., 45.8 ± 1.8 mV for TTX-R I_{Na}) between inward and outward currents (\( P > 0.05, n = 10 \)). Linear regression of current evoked at potentials between +5 and +80 mV (\( R^2 = 0.995 \) for TTX-S I_{Na} and 0.992 for TTX-R I_{Na}) normalized to peak inward current suggested that there is little rectification of I_{Na} in colonic DRG neurons. Activation and steady state availability data were fitted with a Boltzmann equation of the form:

\[
G = G_{max}/[1 + \exp((V_{0.5} - V_m)/k)]
\]

where \( G \) = observed conductance, \( G_{max} \) = the fitted maximal conductance, \( V_{0.5} \) = the potential for half activation or availability, \( V_m \) = command potential and \( k \) = the slope factor. Once \( G_{max} \) was determined, data were normalized with respect to \( G_{max} \).

Drugs

PGE₂ was the single inflammatory mediator used in the present study both because of its clinical importance (Vane 1971) and because PGE₂ causes little activation of nociceptors [depolarization and generation of action potentials (Birrell et al. 1991)], thereby avoiding potential confounds associated with changes in membrane conductance associated with nociceptor activation. PGE₂ was dissolved in 100% ethanol, stored at a 10 mM stock solution at −20°C, and diluted in bath solution immediately prior to use.
capacitance as a way of estimating cell body size as such a measure is not biased by membrane folds and enables normalization of current measurements. The average cell body capacitance of colonic DRG neurons was 63.7 ± 16.3 (SD) pF (n = 60, range 30.2–93.5 pF). To estimate the cell body diameter of these neurons, post hoc, we used data from a separate experiment with DiI-labeled neurons innervating the glabrous skin of the rat hindpaw in which we established the relationship between cell body size (determined with a calibrated eye-piece reticle) and membrane capacitance. Based on a conversion factor derived from a linear regression of these data, we estimate our capacitance measurements translate to an average cell body diameter of 33.3 ± 3.1 μm with a range of 27–39 μm. There was no statistically significant difference between the cell body diameter of TL and LS neurons, although LS neurons tended to be larger (Table 1).

Because the average cell body capacitance of colonic DRG neurons was considerably larger than that previously reported (Yoshimura and de Groat 1997), we sought to determine whether the intercalation of the lipophilic tracer, DiI, into the plasma membrane, influenced our measurements of cell body capacitance. Data from the hindpaw experiment described in the preceding text was compared with the capacitance measurements obtained from 20 unlabeled DRG neurons. The average cell body diameter was the same for both groups (~30 ± 0.3 μm). However, the membrane capacitance of DiI-labeled neurons was 16% larger than that of unlabeled neurons. Importantly, DiI had no significant influence on passive or active electrophysiological properties of DRG neurons.

Low-threshold rapidly activating Na⁺ currents in colonic DRG neurons are TTX sensitive

Voltage steps to potentials between −60 and +40 mV following a 500-ms prepulse to −100 mV (holding potential was −80 mV) resulted in the activation of an inward current that had at least two components: a low-threshold, rapidly activating, rapidly inactivating component and a more slowly activating and inactivating component (Fig. 2A). The rapidly activating component was completely inactivated when the prepulse amplitude was reduced to 20 mV (Fig. 2B), enabling this component to be studied in isolation following digital subtraction (Fig. 2C). There was a difference between the rapid current and the more slowly activating current with respect to the reversal potential for the current (Fig. 2, E and F), suggesting that there is a difference in the ion selectivity of the channels underlying these two classes of current. In 10 of 10 neurons tested, the rapid component was completely blocked by 1 μM TTX (Fig. 2G), indicating that it was TTX sensitive. Thus colonic DRG neurons did not appear to express low-threshold, rapidly activating TTX-R currents similar to those previously described (Rush et al. 1998; Scholz et al. 1998). The high-threshold, slowly inactivating TTX-R current was present in every colonic DRG neuron studied (n = 62).

Persistent TTX-R current in colonic DRG neurons has a high threshold for activation

Cummins and colleagues described a TTX-R current in DRG neurons that was fully expressed in Naᵥ1.8 null mutant mice (Cummins et al. 1999). Naᵥ1.8, formerly SNS (Akopian et al. 1996) and PN3 (Sangameswaran et al. 1996) encodes the α subunit of a TTX-R Na⁺ channel with biophysical properties similar to the high-threshold current described in Fig. 2. The TTX-R current present in Naᵥ1.8 null mice inactivated extremely slowly and thus was referred to as a persistent TTX-R current. This current was not described in the original characterization of the Naᵥ1.8 null mice because it is completely inactivated at holding potentials at least −60 mV (Cummins et al. 1999). Because this current may be evoked from a holding potential of −80 mV, we used our steady-state availability protocol, which involved 1-s conditioning voltage steps to potentials between −100 and +10 mV, to assess for the presence of the low-threshold persistent current. We failed to detect the presence of the low-threshold persistent current in any of the 38 neurons in which its presence was assessed. However, a high-threshold persistent current >5% of the peak inward current was present in 31 of the 38 neurons studied. Current-voltage relationship for the persistent current indicated that the current activated at a potential between 5 and 10 mV more hyperpolarized than that of TTX-R I₅Na (Fig. 3, A and B). Peak inward current occurred at 0 mV and was 384 ± 26 pA (n = 38), constituting 10.5 ± 1.1% (range 23.5–2.4%) of the peak inward current. The current demonstrated little inactivation over the voltage range tested. While we utilized bath and electrode solutions that were constructed to minimize contamination of voltage-gated Na⁺ currents with voltage-gated Ca²⁺ currents, we believe the persistent current present in colonic DRG neurons reflects Ca²⁺ current flowing through voltage-gated Ca²⁺ channels. Consistent with this suggestion, the current was unaffected in the presence of a bath solution in which all Na⁺ had been replaced by choline (n = 4, data not shown) and was blocked following the addition of 50 μM Cd²⁺ to the bath solution (Fig. 3C). While detectable in the majority of

### TABLE 1. Biophysical properties of TTX-R I₅Na in colonic DRG neurons

<table>
<thead>
<tr>
<th>Ganglia</th>
<th>Membrane Capacitance, pF</th>
<th>Density, pA/pF</th>
<th>Activation</th>
<th>SS Availability</th>
<th>Recovery</th>
<th>Inactivation @ 0 mV, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled</td>
<td>62.8 ± 2.1 (62)</td>
<td>62.7 ± 5.4 (62)</td>
<td>113.2 ± 8.1 (35)</td>
<td>−2.8 ± 0.9 (35)</td>
<td>4.5 ± 0.4 (35)</td>
<td>2403 ± 491 (25)</td>
</tr>
<tr>
<td>LS²</td>
<td>69.2 ± 3.6 (22)</td>
<td>76.6 ± 11.3 (22)</td>
<td>129.4 ± 12.8 (15)</td>
<td>−3.6 ± 1.2 (15)</td>
<td>4.8 ± 0.4 (15)</td>
<td>5252 ± 892 (12)</td>
</tr>
<tr>
<td>TL²</td>
<td>59.7 ± 3.5 (22)</td>
<td>52.7 ± 6.3 (22)</td>
<td>100.7 ± 8.6 (20)</td>
<td>−2.3 ± 1.4 (20)</td>
<td>4.4 ± 0.6 (20)</td>
<td>3154± 289 (13)</td>
</tr>
</tbody>
</table>

Values under activation and steady state (SS) Availability were obtained from Boltzmann fits of activation and availability data, respectively. TTX-R I₅Na, TTX-resistant voltage-gated Na⁺ current. N.A, not available. *F(r1)= the fraction of recovery from inactivation accounted for by r1, where r1 is the time constant in the first exponential and r2 is the time constant in the second exponential term in the equation used for fitting recovery from inactivation data. † Inactivation @ 0 mV is the time constant for current inactivation at 0 mV. ‡ LS = lumbar sacral dorsal root ganglia (DRG) (L₅–S₂), TL = thoracolumbar DRG (T₁₂–L₄). § reflects significant difference between TL and LS neurons (P < 0.05).
colonic DRG neurons, activation of this current was too slow to contaminate voltage-gated Na⁺ currents studied with a 15-ms voltage step (Fig. 3D). Furthermore, the depolarizing voltage steps used to determine steady-state availability of Na⁺ currents did not decrease the magnitude of the high-threshold persistent current (data not shown). Nor was the voltage dependence of activation or magnitude of the current influenced by PGE₂ (data not shown). The presence of relatively large inward voltage-gated Ca²⁺ currents observed in the presence of a bath solution contain only 0.1 mM Ca²⁺ raises the possibility that colonic DRG neurons have an exceptionally high density of high-threshold voltage-gated Ca²⁺ channels.

**TTX-R I\textsubscript{Na} in colonic DRG neurons has relatively homogenous steady-state properties**

The conductance-voltage relationship associated with TTX-R I\textsubscript{Na} activation was well fitted by a single Boltzmann equation (Fig. 4). There was little variability in the voltage dependence of TTX-R I\textsubscript{Na} activation between colonic DRG neurons as illustrated by the small variance associated with the membrane potential resulting in a half-maximal activation of current (\(V_{0.5} = -2.8 \pm 0.9\) mV, \(n = 35\), range -12.9 to +8.3 mV, Table 1). Similarly, there was little variability in the steady-state availability of TTX-R I\textsubscript{Na}. The potential at which 50% of the current was available for activation was \(-18.6 \pm\)
0.9 mV, range −29 to −11 mV (Table 1). Even with a relatively long (1 s) conditioning voltage step, TTX-R $I_{\text{Na}}$ was almost fully available for activation at −40 mV, suggesting that the availability of TTX-R $I_{\text{Na}}$ in colonic DRG neurons was not greatly influenced by slow inactivation. Recovery from inactivation occurred very rapidly at −80 mV (Fig. 4). The majority (83.5 ± 4.3%) of the current recovered with a time constant of 1.3 ± 0.2 ms. Finally, TTX-R $I_{\text{Na}}$ in DRG neurons was subject to little activity-dependent block. Activity-dependent block was assessed by stepping the membrane potential to 0 mV for 15 ms 20 times at 1 Hz. The ratio of current evoked on the last pulse (P20) to that on the first pulse (P1) was used as a measure of activity-dependent block. P20:P1 was 0.91 ± 0.03 (n = 6).

**PGE$_2$ modulates TTX-R $I_{\text{Na}}$ in colonic DRG neurons**

Application of PGE$_2$ (1 μM) to colonic DRG neurons resulted in a rapid increase in the magnitude of TTX-R $I_{\text{Na}}$ evoked at 0 mV (Fig. 5A). This increase in current was detectable within 15 s of PGE$_2$ application and generally reached a steady state within 3–5 min. The majority (28 of 30) of colonic neurons were responsive to PGE$_2$. A neuron was considered responsive to PGE$_2$ if, following drug application, there was a change in conductance at the potential for half-maximal activation that was more than two times the SD from the mean of three baseline measurements taken prior to the application of PGE$_2$ (Gold et al. 1998). The percentage of responsive colonic DRG neurons was significantly higher than the percentage (50% (Gold et al. 1996b, 1998)) of responsive neurons observed in the population of unlabeled DRG neurons. PGE$_2$ evoked an increase in maximal conductance (Fig. 5, Table 2) that was associated with a small but significant hyperpolarizing shift in the potential for half-maximal activation (Table 2) and an increase in the rate of current inactivation (Fig. 5). These changes were not associated with any change in properties describing steady-state availability (Table 2).

**TL and LS colonic DRG neurons are similar with respect to TTX-R $I_{\text{Na}}$ and its modulation by PGE$_2$**

TTX-R $I_{\text{Na}}$ present in TL and LS colonic neurons were similar with respect to steady-state and kinetic properties (Table 2). LS neurons tended to have more TTX-R $I_{\text{Na}}$ than TL neurons.
The high prevalence of TTX-R $I_{Na}$ in colonic DRG neurons (5.3 ± 0.9 vs. 3.2 ± 0.3 nA; $P < 0.05$), although this difference was not significant when current was normalized with respect to cell body size ($P = 0.06$, Table 1).

There was also no statistically significant difference between TL and LS neurons with respect to the proportion of neurons responsive to PGE$_2$ or the magnitude of PGE$_2$-induced modulation of TTX-R $I_{Na}$ (Fig. 6, Table 2). Strikingly, TTX-R $I_{Na}$ was modulated in 16 of 16 TL neurons tested and 12 of 14 LS neurons tested. Both of these proportions are significantly larger than the proportion of neurons responsive to PGE$_2$ in the unlabeled population of DRG neurons. It was also striking that the magnitude of PGE$_2$-induced modulation of TTX-R $I_{Na}$ in colonic DRG neurons (72 ± 12%) was larger than that observed in unlabeled DRG neurons (46 ± 5%, (Gold et al. 1998)).

**Discussion**

TTX-R $I_{Na}$ was present in all colonic DRG neurons studied. The high prevalence of TTX-R $I_{Na}$ among colonic DRG neurons is consistent with the association between this current and putative nociceptive afferents. TTX-R $I_{Na}$ in colonic DRG neurons appears to have relatively homogenous properties with a high-threshold for activation and steady-state availability, a relatively slow rate of inactivation, and a rapid recovery from inactivation. We found no evidence for a low-threshold rapidly inactivating TTX-R current among colonic DRG neurons, nor did we find evidence for a low-threshold persistent current. In contrast to a previous report on the effects of inflammatory mediators on $I_{Na}$ in colonic DRG neurons (Su et al. 1999), TTX-R $I_{Na}$ was subject to modulation following application of PGE$_2$ in the majority of colonic DRG neurons. Finally, we observed no statistically significant differences between TL and LS colonic DRG neurons with respect to either TTX-R $I_{Na}$ properties or the magnitude of PGE$_2$-induced modulation of the current.

The cell body diameter of colonic DRG neurons in the present study fell within the range of what is generally considered to be a medium-diameter DRG neuron (Scroggs and Fox 1992). This cell body size is larger than that of neurons generally believed to give rise to unmyelinated axons (Harper and Lawson 1985a; Lawson et al. 1993). These results are in general agreement with our previous observations in situ in which the mean DRG cell body diameter for neuropeptide
containing colonic afferents fell within the range of 27–32 μm in fixed tissue in which no correction for cell shrinkage was employed (Traub et al. 1999). They are also in general agreement with previous results from a study involving labeling of the entire splanchnic nerve. In this study, it was observed that visceral afferents had cell body diameters distributed in a unimodal fashion over a range encompassed by medium diameter neurons (Perry and Lawson 1998). In these previous studies, fluoro-gold and fast blue were used as retrograde tracers arguing against the possibility that the lipophilic DiI used in the present study, preferentially labeled neurons with more rapidly conducting axons and therefore a larger cell body diameter. We directly tested this latter possibility by assessing the co-incidence DiI labeling in TL and LS DRG neurons with that of NF200 (a marker for myelinated neurons): only 6 of 45 DiI labeled neurons were neurofilament positive and 5 of these were barely so. Thus over 85% of DiI labeled neurons in situ were unmyelinated. That DiI-labeled DRG neurons innervated the colon is supported by the observation that sectioning the pelvic nerve prior to DiI injection resulted in the complete loss of labeled neurons in LS ganglia (data not shown). Importantly, both our observed cell size distribution and NF200 staining rate are consistent with results obtained following labeling of the entire splanchnic nerve (Perry and Lawson 1998), suggesting our observations are applicable to other visceral structures. Given that data from single-unit studies indicate that the colon is innervated by afferents with unmyelinated and thinly myelinated axons, our results suggest that predictions about afferent function based on the size of the cell body diameter should be made cautiously.

However, our observations that colonic DRG neurons have relatively large-diameter cell bodies is in contrast to observations reported in three previous studies (Keast and de Groat 1992; Su et al. 1999; Yoshimura and de Groat 1997). In each of these studies, cell body diameter of colonic DRG neurons was relatively small. The basis for the difference between our results and those reported in these previous studies is unclear. We feel that it is unlikely that neurons labeled in the present study somehow reflect nonspecific labeling, given that the labeling procedure was performed under a dissecting microscope where it was possible to visually detect and remove leaked dye, post hoc analysis of the abdominal cavity revealed clear labeling in the colon wall with no detectable labeling on any other visceral structure, and dye injections into the lumen of the colon (n = 2) resulted in a large number of weakly labeled neurons, a pattern markedly different from that observed following DiI injection in to the colon wall. We suggest that variations in labeling procedures is a likely explanation for the differences between our results and those of previous studies, although not completely satisfying. That is, while the two earlier studies utilized different tracers (Keast and de Groat 1992; Yoshimura and de Groat 1997), the latter, by Su and colleagues, employed DiI albeit at twice the concentration used in the present study. Furthermore, Su and colleagues injected 70 μl of DiI, while we only injected 20 μl of tracer. Thus it is possible that different tracers and/or a larger injection volume results in the labeling of a smaller population of colonic neurons than labeled with our injection procedure. Whatever the basis for the difference between our results and those of previous investigators, based on the results of our efforts to ensure specificity of labeling and our immunohistochemical results, we suggest that we have studied a subpopulation of colonic DRG neurons reflective of the total population of colonic neurons.

TTX-R \(I_{Na}\) present in colonic DRG neurons was similar to currents described by Rush and colleagues (1998) as TTX-R1 and by Scholz and colleagues (1998) as the slow TTX-R current. Although the values we obtained for membrane po-

![Graph showing modulation of TTX-R current by PGE2](image-url)
The only rapidly activating and rapidly inactivating voltage-gated current that we observed was TTX sensitive. The only likely explanation re the use of a low Cl\textsuperscript{−} electrode solution accounts for much of the differences in observations. We did not detect the presence of the low-threshold persistent \(*I_{Na}^*\) described by Cummins and colleagues (1999) as the only persistent current detectable in colonic DRG neurons appeared to reflect activation of a voltage-gated Ca\textsuperscript{2+} current. The only rapidly activating and rapidly inactivating voltage-gated current that we observed was TTX sensitive.

It is not clear why we were able to detect a PGE\textsubscript{2}-induced increase in voltage-gated \(Na^+\) current while Su and colleagues failed to do so (Su et al. 1999). Similar labeling protocols and recording configurations were utilized in both studies. Given that Su and colleagues only studied colonic neurons from S1 ganglia, it is possible that colonic neurons from the S1 ganglia are unresponsive to PGE\textsubscript{2}, while colonic neurons from other ganglia are responsive. However, this is an unlikely possibility given the proportion of LS neurons that responded to PGE\textsubscript{2}. A more likely explanation reflects the fact that Su and colleagues did not study the effect of inflammatory mediators on \(I_{Na}\) in isolation. TTX-S \(I_{Na}\) present in several excitable tissues is inhibited by compounds, such as inflammatory mediators, that increase the intracellular concentration of cAMP (Cantrell et al. 1997). Thus if the TTX-S current in colonic DRG neurons is inhibited by cAMP while TTX-R \(I_{Na}\) is augmented, Su and colleagues may have failed to detect the influence of inflammatory mediators in TTX-R \(I_{Na}\).

We have previously reported that acute colorectal pain is processed in the lumbosacral spinal cord, while inflammatory colorectal pain is processed in the thoracolumbar and lumbosacral spinal cord segments (Traub 2000; Traub and Murphy 2002). These results from animal studies are consistent with clinical reports indicating that there is an increase in the area of referred pain associated with colorectal hypersensitivity (Bernstein et al. 1996; Mayer et al. 2000; Naliboff et al. 2000). Results from the present study do not enable us to determine the relative contribution of primary afferent neurons and CNS circuitry to the inflammation-induced changes in nociceptive processing of colonic stimuli. However, our results do suggest that differences in the level of expression or biophysical properties of TTX-R \(I_{Na}\) are unlikely to contribute to differences between LS and TL colonic afferents that may underlie inflammation-induced changes in nociceptive processing. Furthermore, differences between LS and TL neurons are unlikely to reflect differences in the proportion of neurons responsive to inflammatory mediators or the magnitude of inflammation-induced changes in TTX-R \(I_{Na}\).

In a preliminary study in our laboratories, we observed that PGE\textsubscript{2} increases the excitability of 92% (33 of 36) of colonic DRG neurons in vitro (Traub and Gold 2000). This percentage is similar to the proportion of colonic neurons sensitized by an inflammatory stimulus in vivo (Sengupta et al. 1999; Su et al. 1997) and suggests that changes intrinsic to colonic afferents are likely to contribute to an inflammation-induced increase in excitability. Given that the nature of stimulus transduction in the colon is likely to involve prolonged membrane depolarization associated with mechanical stimuli that most effectively activate colonic afferents, we would suggest that TTX-R \(I_{Na}\) is likely to be the current primarily responsible for spike initiation in this population of afferents. A TTX-R \(I_{Na}\) has been shown to be involved in spike initiation in the cornea (Brock et al. 1998) and dura (Strassman and Raymond 1999). Thus given the potential role of TTX-R \(I_{Na}\) in spike initiation and our results indicating that inflammatory mediator-induced modulation of this current should contribute to sensitization of colonic afferents, blocking the inflammation-induced increase in TTX-R \(I_{Na}\) may be an effective treatment for visceral pain.

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