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Changes in the expression of tetrodotoxin-sensitive sodium channels within dorsal root ganglia neurons in inflammatory pain

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Abstract

Nociceptive neurons within dorsal root ganglia (DRG) express multiple voltage-gated sodium channels, of which the tetrodotoxin-resistant (TTX-R) channel $Na_v1.8$ has been suggested to play a major role in inflammatory pain. Previous work has shown that acute administration of inflammatory mediators, including prostaglandin E_2 (PGE_2), serotonin, and adenosine, modulates TTX-R current in DRG neurons, producing increased current amplitude and a hyperpolarizing shift of its activation curve. In addition, 4 days following injection of carrageenan into the hind paw, an established model of inflammatory pain, $Na_v1.8$ mRNA and slowly-inactivating TTX-R current are increased in DRG neurons projecting to the affected paw. In the present study, the expression of sodium channels $Na_v1.1$–$Na_v1.9$ in small (≤ 25 μm diameter) DRG neurons was examined with in situ hybridization, immunocytochemistry, Western blot and whole-cell patch-clamp methods following carrageenan injection into the peripheral projection fields of these cells. The results demonstrate that, following carrageenan injection, there is increased expression of TTX-S channels $Na_v1.3$ and $Na_v1.7$ and a parallel increase in TTX-S currents. The previously reported upregulation of $Na_v1.8$ and slowly-inactivating TTX-R current is not accompanied by upregulation of mRNA or protein for $Na_v1.9$, an additional TTX-R channel that is expressed in some DRG neurons. These observations demonstrate that chronic inflammation results in an upregulation in the expression of both TTX-S and TTX-R sodium channels, and suggest that TTX-S sodium channels may also contribute, at least in part, to pain associated with inflammation.

Keywords: Carrageenan; Inflammatory pain; Sodium channel; Tetrodotoxin-resistant-sensitive Na current; Dorsal root ganglia

1. Introduction

Dorsal root ganglia (DRG) neurons are unique in comparison to most neurons within the CNS, in expressing tetrodotoxin-resistant (TTX-R) sodium currents, in addition to tetrodotoxin-sensitive (TTX-S) currents (Rush et al., 1998). The TTX-R currents are preferentially expressed in small DRG neurons, which give rise to unmyelinated C- and thinly-myelinated Aδ-fibers, many of which are nociceptive (Akopian et al., 1999; Djouhri et al., 2003; Fang et al., 2002). Five sodium channels, $Na_v1.1$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$, are expressed at substantial levels in DRG of adult rats, and most, if not all, DRG neurons express multiple sodium channel isoforms (Black et al., 1996), with the repetoire of TTX-S and TTX-R channels expressed influencing their electroresponsive properties.

The expression of sodium channels in DRG neurons is dynamic. For example, the TTX-S sodium channel $Na_v1.3$ is upregulated in DRG neurons in several models of neuropathic pain, including sciatic nerve transection (Black et al., 1999a; Waxman et al., 1994), chronic constriction injury (Dib-Hajj et al. 1999) and tight spinal nerve ligation (Kim et al., 2001), while TTX-S channels $Na_v1.1$, $Na_v1.6$ and $Na_v1.7$ are downregulated following tight spinal nerve ligation (Kim et al., 2001, 2002). In addition, neurotrophic factors can affect the expression of specific sodium

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channels. Nerve growth factor (NGF) and/or glial cell neurotrophic factor (GDNF) alter the expression of $Na_v1.3$, $Na_v1.8$ and $Na_v1.9$ (Boucher et al., 2000; Cummins et al., 2000; Dib-Hajj et al., 1998a; Fjell et al., 1999a,b; Leffler et al., 2001).

Inflammatory pain results, in part, from sensitization of primary afferent nociceptive fibers innervating the inflamed tissue, and is mediated by a complex series of events that includes activation of multiple signal transduction pathways and activity-dependent alterations in neurons (see Woolf and Costigan, 1999). In particular, modulation of a slowly-inactivating TTX-R sodium current has been implicated as playing a prominent role in inflammatory pain. Acute administration of inflammatory mediators to DRG neurons results in an increase in the TTX-R current amplitude, a hyperpolarizing shift in the activation curve and an increase in activation and inactivation rates of slowly-inactivating TTX-R current in these cells (Cardenas et al., 1997; England et al., 1996; Gold et al., 1996), which would be expected to increase excitability of the neuron by decreasing threshold and increasing action potential electrogenesis, thus producing an increased response to a fixed stimulus.

In the present study, we have utilized in situ hybridization, immunocytochemical, Western blot and whole-cell patch clamp methods to examine the expression of neuronal sodium channels, i.e. $Na_v1.1$–$Na_v1.3$ and $Na_v1.6$–$Na_v1.9$, in small DRG neurons following carrageenan injection in the hind paw, an established model of inflammatory pain (Iadarola et al., 1988). Our results demonstrate that, in addition to the upregulation of $Na_v1.8$ mRNA and slowly-inactivating TTX-R current previously reported (Tanaka et al., 1998), there is increased expression of $Na_v1.3$ and $Na_v1.7$ and a parallel increase in TTX-S currents in DRG neurons following carrageenan injection. These observations suggest that TTX-S sodium channels may also contribute, at least in part, to pain associated with inflammation.

2. Methods

2.1. Animal model

Sprague–Dawley male rats (250–275 g) were housed under a 12 h light–12 h dark cycle and given free access to water and food. Under anesthesia (ketamine/xylazine, 80/5 mg/kg, i.p.), 4% carrageenan (λ carrageenan, Sigma type IV) in 150 μl sterile saline was injected into the plantar surface of the right hind paw (carrageenan ipsilateral; Iadarola et al., 1988), and 150 μl of sterile saline was injected in the left hind paw (carrageenan contralateral). In addition, in a group of separate naive rats not injected with carrageenan, 150 μl of sterile saline was injected in the left hind paw (saline), to serve as an additional control in the event of contralateral effects of carrageenan-induced inflammation on sodium channel expression. The experimental procedures described here were approved by the institutional animal use and care committee and meet all NIH guidelines for the humane treatment of animals.

2.2. In situ hybridization histochemistry

Four days after carrageenan injection, rats had marked swelling of the right hind paw and ankle and were deeply anesthetized with ketamine/xylazine and transcardially perfused first with 150 ml of phosphate-buffered saline and then with 250 ml of ice-cold 4% paraformaldehyde in 0.14 M Sorensen's phosphate buffer (pH 7.4). Ipsilateral (carrageenan-injected) and contralateral (saline-injected) L4/L5 DRG were removed and immersion-fixed for 3–4 h in the same fixative and then cryoprotected with 25% (w/v) sucrose in 0.14% Sorensen's phosphate buffer for 2 days at 4 °C. L4/5 DRG were also obtained 4 days following saline injection into naive rats, and were processed in the same manner as described for carrageenan-injected rats. Twelve micrometer cryosections of carrageenan-injected (ipsilateral), saline-injected contralateral to carrageenan-injection (contralateral), and saline-injected naive (saline) DRG were mounted on Fischer Superfrost Plus glass slides and processed for in situ hybridization histochemistry with digoxigenin-labeled probes specific for sodium channels $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ mRNA as previously described (Black et al., 1996; Dib-Hajj et al., 1998b). Images were captured using a Nikon Eclipse E800 microscope with IPLab software (Scanalytics, Fairfax, VA). Signal intensity was obtained by manually outlining individual cells and using Scion Image software (Scanalytics) to calculate the mean signal intensity for the selected area. Cells were sampled only if the nucleus was visible within the plane of section. Background levels of signal were subtracted, and control and experimental conditions were evaluated in identical manners. The area value was converted to diameter by assuming a circular shape for the neurons. DRG from 4 carrageenan-injected; 3 saline-injected contralateral to carrageenan-injected; and 3 saline-injected naive rats were examined, and the mean signal intensity from a minimum of 169 neurons was quantified for each sodium channel isoform.

2.3. Immunocytochemistry

To examine the abundance of sodium channel proteins, 4 days following carrageenan (ipsilateral), saline contralateral to carrageenan (contralateral), or saline in naive (saline) injections, a set of rats separate from those utilized for in situ hybridization studies were anesthestized, perfused with 4% paraformaldehyde, and ipsilateral, contralateral, and saline L4/L5 DRG were obtained. Tissue was immersion fixed for an additional 20 min (total fixation time 30 min), cryoprotected in 30% sucrose in 0.14 M phosphate buffer, and 12 μm sections cut and mounted on Fischer Superfrost Plus glass slides. Sections were processed for immunocytochemistry as previously described (Black et al., 1999b).

Primary antibodies utilized were: pan sodium channel (monoclonal, 1:300, Sigma), $Na_v1.1$ (1:100, Alomone, Jerusalem), $Na_v1.2$ (1:300, Upstate Biotechnology, Lake Placid, NY), $Na_v1.3$ (1:2000, Hains et al., 2002), $Na_v1.6$ (1:200, Alomone), $Na_v1.7$ (1:100, Alomone), $Na_v1.8$ (Black et al., 1999b) and $Na_v1.9$ (Fjell et al., 2000). Secondary antibodies used were goat anti-mouse IgG–Cy3 (for pan and $Na_v1.2$ primary antibodies; 1:2000, Amersham) and goat-anti-rabbit IgG–Cy3 (for other isoform-specific antibodies; 1:2000, Amersham). Sections were examined with a Nikon E800 light microscope equipped with a Dage DC330 camera. Images were collected with IPLab software, and optical intensity of the fluorescent signal was quantified by microdensitometry using Scion Image software (Scanalytics) in a manner similar to that employed for quantification of the hybridization signal, except that signal was inverted in order to have a greater fluorescent signal correspond to a higher value (Black et al., 2003). For immunocytochemical studies, DRG from 4 carrageenan-injected; 3 saline-injected contralateral to carrageenan-injection; and 3 saline-injected naive rats were examined. The mean signal intensity from a minimum of 146 neurons was quantified for each sodium channel isoform.

For both in situ hybridization and immunocytochemistry experiments, statistical comparisons between saline- and carrageenan-injected, saline and contralateral to carrageenan and contralateral to carrageenan and carrageenan groups were performed with a two-tailed Student's *t*-test utilizing Excel software.

2.4. Membrane fraction preparation

Four days following injections, L4/L5 DRG from carrageenan-injected and saline-injected contralateral to carrageenan injection of Sprague–Dawley rats, and also from saline-injected naive Sprague–Dawley rats, were dissected and immediately processed in ice cold lysis buffer (0.3 M sucrose, 10 mM Tris (pH 8.1) and 2 mM EDTA) supplemented with protease inhibitor. Tissues were then homogenized in a glass tissue grinder in ice-cold lysis buffer. Homogenates were kept on ice for 1 h before centrifugation at $1000 \times g$ (low-speed spin) for 7 min at 4 °C to remove nuclei and intact cells. The pellet was re-homogenized and spun again under the same conditions. The supernatants from the two low-speed spins were combined and centrifuged at $120{,}000 \times g$ for 1 h at 4 °C. The pellet, containing the total membrane fraction, was suspended in 0.2 M KCl and 10 mM HEPES, pH 7.4. An equal volume of 5% Triton X-100 and 10 mM HEPES, pH 7.4, was added to the sample to solubilize the membrane fraction, and the suspension was kept on ice for 1 h. The insoluble material was pelleted by centrifugation at $10{,}000 \times g$ for 10 min at 4 °C, and the supernatant was collected. Protein content was determined using Protein *dot*METRIC Assay (Geno Technology, St Lous, MO).

2.5. Immunoblot assay

Samples (20–30 μg) were denatured in Laemmli's sample buffer for 20 min at 37 °C. Proteins were fractionated by SDS-PAGE using 4–15% gradient Tris–HCl Ready gels (Bio-Rad) and then electrotransferred at 22 mV to Immun-Blot PVDF membrane (Bio-Rad) overnight at 4 °C. Membranes were blocked in TBST containing 5% non-fat dried milk for 1 h at room temperature (for $Na_v1.6$, blocked overnight at 4 °C) before incubation for 2 h at room temperature with the primary antibody diluted in TBST containing 5% BSA. Blots were washed extensively in TBST and incubated with goat anti-rabbit IgG (for isoform-specfic primary antibodies) or goat anti-mouse IgG (for pan primary antibody) conjugated to horseradish peroxidase (1:10,000; Dako, Glostrup, Denmark) in TBST/1.25% BSA for 1 h at room temperature. The signal was detected by Renaissance Chemiluminescence according to the recommendations of the manufacturer (NEN, Boston, MA). The intensity of the selected bands was captured and analyzed using Kodak1D Image Analysis Software (Eastman Kodak, Rochester NY). Carrageenan-injected (ipsilateral), saline-injected contralateral to carrageenan (contralateral), and saline-injected in naive rats conditions were evaluated. The data obtained with isoform-specific antibodies for carrageenan-injected DRG were plotted as a percent change from the contralateral value. In addition, mean intensities of the bands obtained with the pan antibody for carrageenan-injected, saline-injected contralateral to carrageenan and saline-injected in naive rats were determined.

2.6. Tissue culture

Cultures of DRG neurons were established as described previously (Sleeper et al., 2000). Briefly, L4/L5 DRG from saline-injected (contralateral) and carrageenan-injected (ipsilateral) rats were freed from their connective sheaths and incubated sequentially in enzyme solutions containing collagenase and then papain. The tissue was triturated in culture medium containing 1:1 Dulbecco's modified Eagle's medium and Hank's F-12, 10% fetal calf serum, 1.5 mg/ml bovine serum albumin, 100 U/ml penicillin and 0.1 mg/ml mg streptomycin and plated (500–1000 cells/mm^2) on poly-ornithine/laminin-coated coverslips. The cells were maintained at 37 °C in a humidified 95% air/5% CO_2 incubator overnight, and studied with patch-clamp techniques after short-term culture (12–24 h).

2.7. Whole-cell patch-clamp recordings

Patch-clamp recordings of small diameter ($\leq$25 μm) neurons were carried out as described previously (Cummins and Waxman, 1997) in the whole-cell configuration using 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) using a Sutter P-87 puller. Cells were not considered for analysis if the initial seal resistance was <5 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 2.4 ± 0.6 mV (mean ± SD, $n = 108$). Voltage errors were minimized using 70–80% series resistance compensation. Linear leak subtraction and capacitance artifact cancellation were used for all recordings. Membrane currents were filtered at 5 KHz and sampled at 20 KHz. TTX-S and TTX-R sodium currents were separated using prepulse-inactivation (500 ms prepulses to -50 mV) and digital subtraction (Cummins and Waxman, 1997). The TTX-S current density of was obtained by dividing the estimated peak current by the whole-cell capacitance. The pipette solution contained (in mM): 140 CsF, 2 $MgCl_2$, 1 EGTA, and 10 Na-HEPES (pH 7.3). The bathing solution contained (in mM): 140 NaCl, 3 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 0.1 $CdCl_2$, and 10 HEPES (pH 7.3), adjusted to 310 mosM. The liquid junction potential was <5 mV; data were not corrected to account for this offset. The offset potential was zeroed before and checked after each recording for drift. All recordings were conducted at room temperature (21 °C).

3. Results

As previously described (Tanaka et al. 1999), carrageenan rapidly produces edema, hyperthermia and hyperalgesia within the injected hind paw, and this has been used as a model of inflammatory pain (Iadarala et al. 1988). The edema within the carrageenan-injected (ipsilateral) paw continued until sacrifice at 4 days post-injection, and the mean (± SEM) mid-metatarsal thickness of ipsilateral paws was significantly greater than the contralateral (saline-injected) paws (8.7 ± 1.1 vs 3.2 ± 0.3 mm, respectively; $n = 9$; $P < 0.05$).

To examine whether the expression of specific sodium channels within DRG neurons, in addition to $Na_v1.8$ (Tanaka et al. 1999), is altered by hind paw injection of carrageenan, we hybridized sections of carrageenan-injected (ipsilateral), saline-injected contralateral to carrageenan-injected (contralateral) and saline-injected in naive rats (saline) DRG with subtype-specific riboprobes to $Na_v1.1$– $Na_v1.3$ and $Na_v1.6$–$Na_v1.9$. In agreement with previous descriptions of the expression of sodium channel mRNAs within control DRG (Akopian et al., 1996; Beckh 1990; Black et al., 1996; Dib-Hajj et al., 1998b; Sangameswaran et al., 1996), hybridization signals for $Na_v1.1$ and $Na_v1.6$–$Na_v1.9$ were detected in saline (Fig. 1A) and contralateral (data not shown) DRG neurons, while $Na_v1.2$ and $Na_v1.3$ hybridization signals were not above background levels; there was not an apparent difference in the expression of specific sodium channel transcripts in contralateral versus saline DRG neurons.

3.1. Nav1.3 and Nav1.7 mRNA are upregulated after carrageenan injection

Four days following injection of carrageenan, there was not a qualitative difference in the hybridization signals in ipsilateral, contralateral and saline DRG neurons for sodium channel mRNAs $Na_v1.1$, $Na_v1.6$ and $Na_v1.9$; $Na_v1.2$ hybridization signal was not detected in ipsilateral, contralateral or saline DRG. In contrast, hybridization signals for $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ were substantially greater in ipsilateral DRG neurons compared to contralateral or saline DRG neurons (Fig. 1A). Fig. 1B shows quantification of hybridization signals in small (≤ 25 μm diameter) neurons from ipsilateral, contralateral and saline DRG for $Na_v1.1$–$Na_v1.3$ and $Na_v1.6$–$Na_v1.9$. Hybridization signals for $Na_v1.1$, $Na_v1.2$, $Na_v1.6$ and $Na_v1.9$ were not significantly different between contralateral, ipsilateral and saline DRG neurons. In contrast, the hybridization signal for $Na_v1.3$ mRNA in small DRG neurons was increased approximately 2-fold following carrageenan injection, while signals for $Na_v1.7$ and $Na_v1.8$ transcripts increased about one-third in ipsilateral DRG neurons compared to contralateral or saline neurons. For specific sodium channel mRNAs, there was not a significant difference in hybridization signal between contralateral and saline DRG.

3.2. Nav1.3 and Nav1.7 protein are upregulated after carrageenan injection

We also reacted DRG sections with subtype-specific antibodies to examine the levels of sodium channel proteins in these neurons following carrageenan injection. In parallel with the results obtained by in situ hybridization, increased immunofluorescent signals were observed for sodium channels $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ in neurons from ipsilateral (carrageenan) compared to saline (Fig. 2A) or to contralateral to carrageenan injection (data not shown) DRG neurons. Levels of immunoreactivity for $Na_v1.1$, $Na_v1.2$, Na_v6 and $Na_v1.9$ sodium channel were not qualitatively different between carrageenan-injected and saline-injected DRG neurons. In addition, immunofluorescent signals for specific sodium channel proteins were similar in contralateral and saline DRG neurons. To confirm that carrageenan injection induced an overall upregulation of sodium channels in DRG, we also reacted ipsilateral, contralateral and saline DRG sections with an anti-pan sodium channel antibody (Fig. 2A). Consistent with the results obtained with isoform-specific antibodies, there was increased immunofluorescent signal in carrageenan compared to saline DRG neurons (Fig. 2A); qualitative differences in signals for the sodium channel isoforms between contralateral and saline DRG neurons were not observed (data not shown).

Fig. 2B shows microdensitometric quantitative analyses of fluorescent signals obtained with pan and isoform-specific

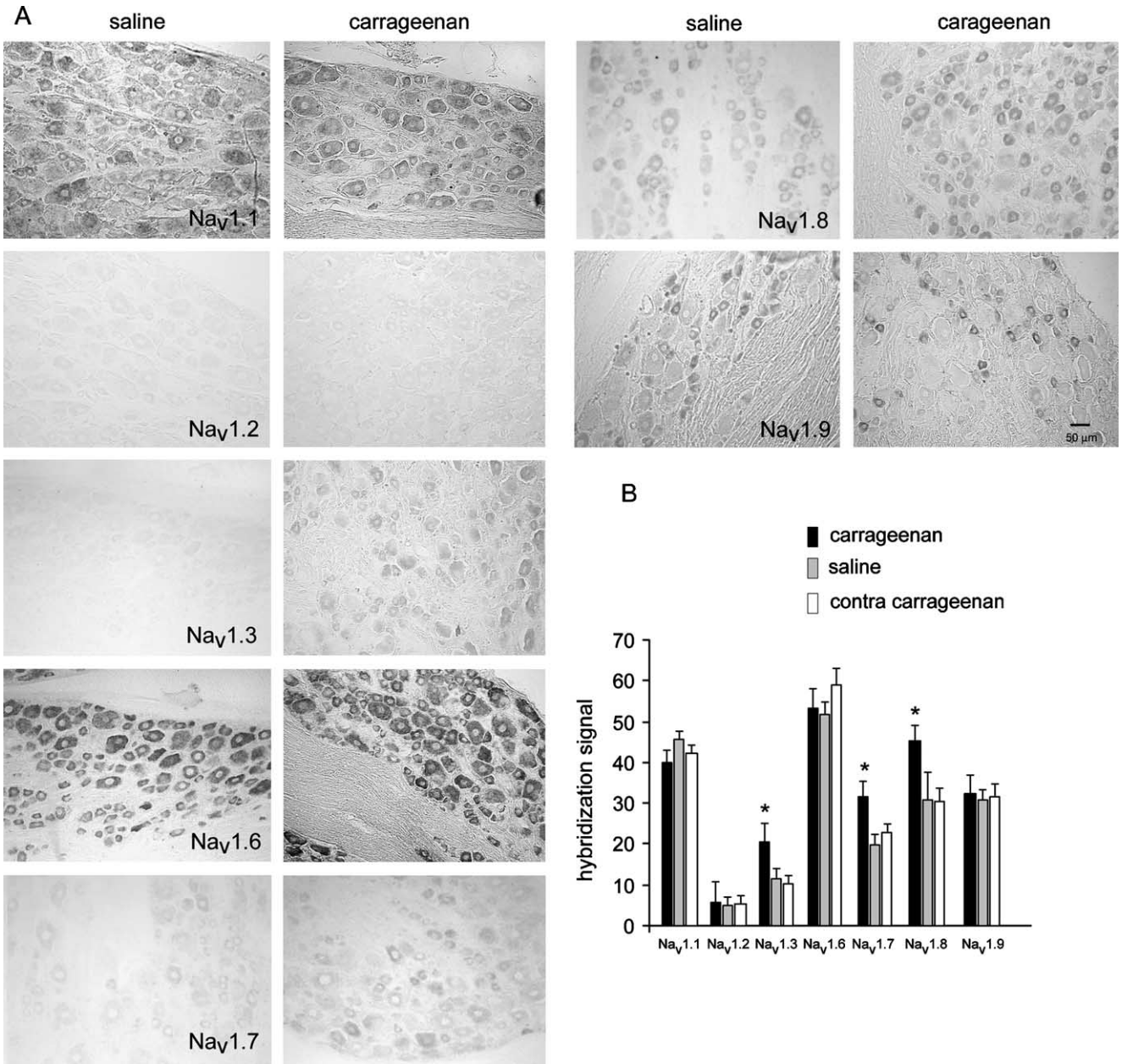

Fig. 1. Sodium channel mRNA expression in DRG neurons from saline-injected naive and carrageenan-injected rats. (A) Sections of DRG from saline and carrageenan-injected rats were probed with isoform-specific riboprobes for sodium channels $Na_v1.1$–$Na_v1.3$ and $Na_v1.6$–$Na_v1.9$. Similar levels of in situ hybridization signals are present for $Na_v1.1$, $Na_v1.6$ and $Na_v1.9$ in neurons from saline and carrageenan DRG, while signals for $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ are substantially greater in carrageenan DRG neurons compared to saline neurons. (B) Microdensitometry quantitative analysis demonstrates that the hybridization signals for $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ are significantly ($^*P < 0.05$) upregulated in small (<25 μm) DRG neurons from carrageenan-injected DRG compared to saline-injected contralateral to carrageenan-injected (contra carrageenan) and saline-injected naive rat (saline) DRG. The signal for $Na_v1.3$ is approximately 2-fold greater in carrageenan DRG neurons than in control neurons, and the signals for $Na_v1.7$ and $Na_v1.8$ are about one-third greater in carrageenan neurons than in control neurons. Hybridization signals for $Na_v1.1$, $Na_v1.2$, $Na_v1.6$ and $Na_v1.9$ are not significantly different between contra carrrageenan and saline DRG and carrageenan-injected DRG. Scale bar, 50 μm.

antibodies in small neurons within carrageenan, saline contralateral to carrageenan, and saline DRG. In parallel with results obtained with in situ hybridization, there were significantly ($P < 0.05$) greater signals for pan, $Na_v1.3$, $Na_v1.7$, and $Na_v1.8$ in neurons from carrageenan compared to saline or contralateral DRG (Fig. 2B).

To further evaluate the change in sodium channel expression in DRG following carrageenan injection, we performed Western blot analyses on contralateral and ipsilateral L4/5 DRG pooled from six rats with isoform-specific antibodies. Antibodies for $Na_v1.1$ and $Na_v1.3$ were not sufficiently robust for Western blot analyses, and blots were not probed with the $Na_v1.2$ antibody since this channel was not detected by in situ hybridization or immunocytochemistry in contralateral, ipsilateral or saline DRG. As illustrated in Fig. 3A, bands representing contralateral

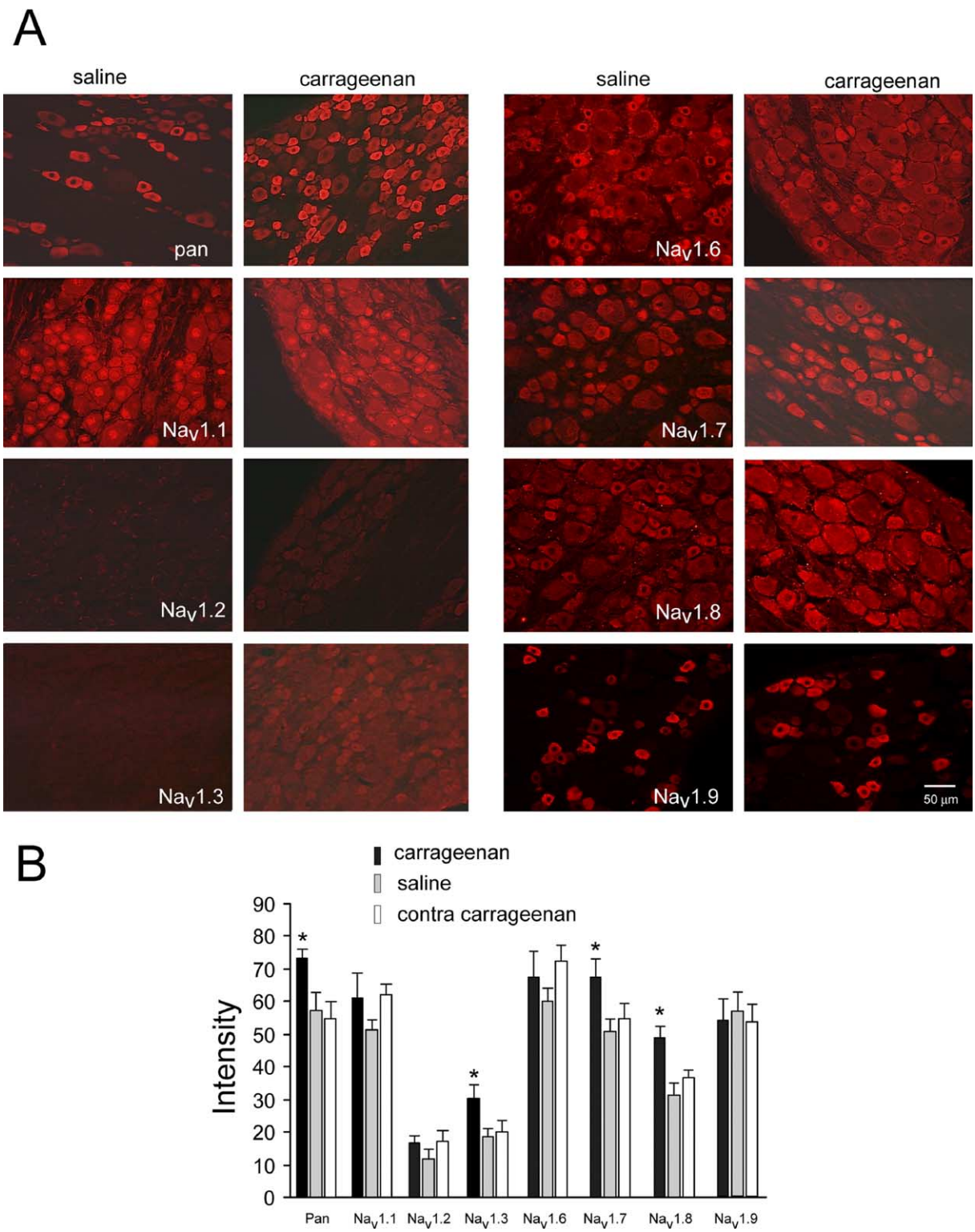

Fig. 2. Sodium channel protein expression in DRG neurons from saline-injected naive and carrageenan-injected rats. (A) Sections from saline and carrageenan-injected DRG were reacted with isoform-specific antibodies to $Na_v1.1$–$Na_v1.3$ and $Na_v1.6$–$Na_v1.9$, and also a pan sodium channel antibody. The pan sodium channel antibody demonstrates an overall increase in immunofluorescent signal in carrageenan compared to saline DRG neurons. In parallel to results obtained by in situ hybridization, sodium channels $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ exhibit greater immunolabeling in carrageenan neurons compared to saline neurons. Sodium channels $Na_v1.1$, $Na_v1.6$ and $Na_v1.9$ display similar levels of signal in control and carrageenan DRG. (B) Microdensitometry quantitative analysis demonstrates that the immunofluorescent signals for $Na_v1.3$, $Na_v1.7$ and $Na_v1.8$ are significantly ($^*P < 0.05$) upregulated in small (<25 μm) DRG neurons from carrageenan-injected DRG compared to saline-injected contralateral to carrageenan-injected (contra carrageenan) and saline-injected naive rat (saline) DRG. immunofluorescent signals for $Na_v1.1$, $Na_v1.2$, $Na_v1.6$ and $Na_v1.9$ are not significantly different between contra carrrageenan and saline DRG and carrageenan-injected DRG. Scale bar 50 μm.

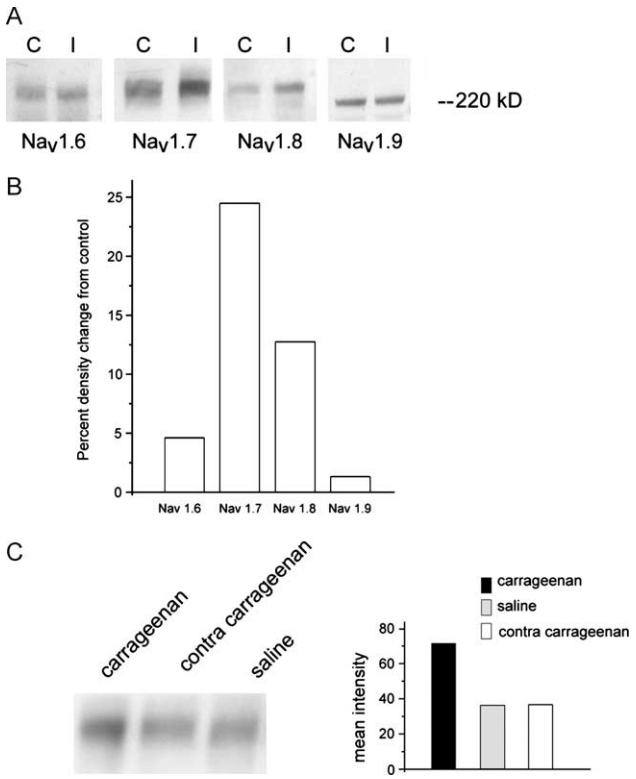

Fig. 3. Western blot analyses of sodium channel expression in control and carrageenan-injected DRG. (A) Blots from contralateral (saline-injected; C) and ipsilateral (carrageenan-injected; I) DRG were probed with isoform specific antibodies to Na$_v$1.6–Na$_v$1.9. Blots for Na$_v$1.7 and Na$_v$1.8 exhibit substantially increased signal in ipsilateral compared to contralateral DRG. Na$_v$1.6 and Na$_v$1.9 display similar signals for ipsi- and contralateral DRG. (B) Quantification of band densities demonstrates similar levels of signal for Na$_v$1.6 and Na$_v$1.9 channels, while Na$_v$1.7 and Na$_v$1.8 exhibit 12–25% increase in signal of ipsilateral DRG compared to contralateral DRG. (C) Blots from carrageenan-injected (carrageenan), saline-injected contralateral to carrageenan injected (contra carrageenan), and saline-injected naive rat (saline) DRG were probed with a pan sodium channel antibody. Quantification of band signals demonstrates that contra carrageenan and saline DRG yield similar band intensities, which are approximately one-half of the carrageenan band.

and ipsilateral DRG exhibited similar densities for Na$_v$1.6, and also for Na$_v$1.9. In contrast, both Na$_v$1.7 and Na$_v$1.8 exhibited substantially greater band densities for ipsilateral compared to contralateral DRG. Densimetric analysis of immunoblot band intensities is illustrated in Fig. 3B, and demonstrates approximately 25 and 12% increases in band intensities of ipsilateral compared to contralateral DRG for Na$_v$1.7 and Na$_v$1.8, respectively. We also examined band intensities of L4/L5 DRG samples obtained from 3 carrageenan-injected and saline-injected contralateral to carrageenan (contralateral) rats and 3 saline-injected naive (saline) rats and reacted with pan antibody. Fig. 3C shows that carrageenan DRG yield a substantially greater signal than either contralateral or saline DRG; densimetric analysis of mean intensities for the bands demonstrates similar signals for contralateral and saline samples, which are significantly lower than that for carrageenan.

3.3. TTX-S currents are increased after carrageenan injection

We have previously shown that carrageenan injection in the hind paw increases the amplitude of TTX-R sodium current (which is expected to be primarily Na$_v$1.8, and not Na$_v$1.9, current due to the recording protocol enlisted) in ipsilateral compared to contralateral DRG neurons (Tanaka et al., 1998). Because our in situ hybridization and immunocytochemical experiments indicated upregulation of Na$_v$1.3 and Na$_v$1.7, both of which are TTX-S, we used whole-cell patch-clamp to measure the amplitude and density of TTX-S sodium currents in ipsilateral and contralateral DRG neurons. Representative recordings of TTX-S currents in small neurons from contralateral (saline-injected) and ipsilateral (carrageenan-injected) DRG are shown in Fig. 4A. Peak TTX-S sodium currents were significantly ($P < 0.01$) larger in ipsilateral (35 ± 2.3 nA, $n = 54$) compared to contralateral (25.4 ± 2.1 nA, $n = 54$) DRG neurons. The TTX-S current density, which can be estimated by dividing peak sodium current by whole-cell capacitance, was approximately 40% larger (($P < 0.01$) in neurons in the carrageenan-injected group compared to the saline-injected group (Fig. 4B). We also examined voltage-dependence of inactivation in carrageenan-injected and saline-injected DRG neurons (Fig. 4C). Steady-state inactivation curves for saline- and carrageenan-injected DRG neurons were nearly identical, with V_h values of -73.8 ± 0.9 and -73.8 ± 1.1 mV, respectively. Because previous studies reported that acute application of inflammatory mediators can cause an ~ 5 mV hyperpolarizing shift in the voltage-dependence of activation of TTX-R currents (Gold et al., 1996), we examined the effect of chronic inflammation on the activation of sodium currents in DRG neurons. For this measurement, TTX-S and TTX-R currents were not separated; however, only data from cells in which the TTX-S current amplitude was larger than the TTX-R current amplitude (determined from the steady-state inactivation data) are presented. No difference was observed in the voltage-dependence of activation of sodium currents in neurons from contralateral (saline-injected) and ipsilateral (carrageenan-injected) DRG (Fig. 4D). These data, and our previous data on the lack of an effect of carrageenan injection on the voltage-dependent properties of TTX-R currents (Tanaka et al., 1998), indicate that chronic inflammation does not induce sustained shifts in the voltage-dependence of DRG sodium currents.

4. Discussion

Our results demonstrate that inflammation, produced by injection of carrageenan, is accompanied by upregulated expression of both TTX-S and TTX-R sodium channels within small (≤ 25 μm dia.) DRG neurons. The present work confirms our previous study which showed that

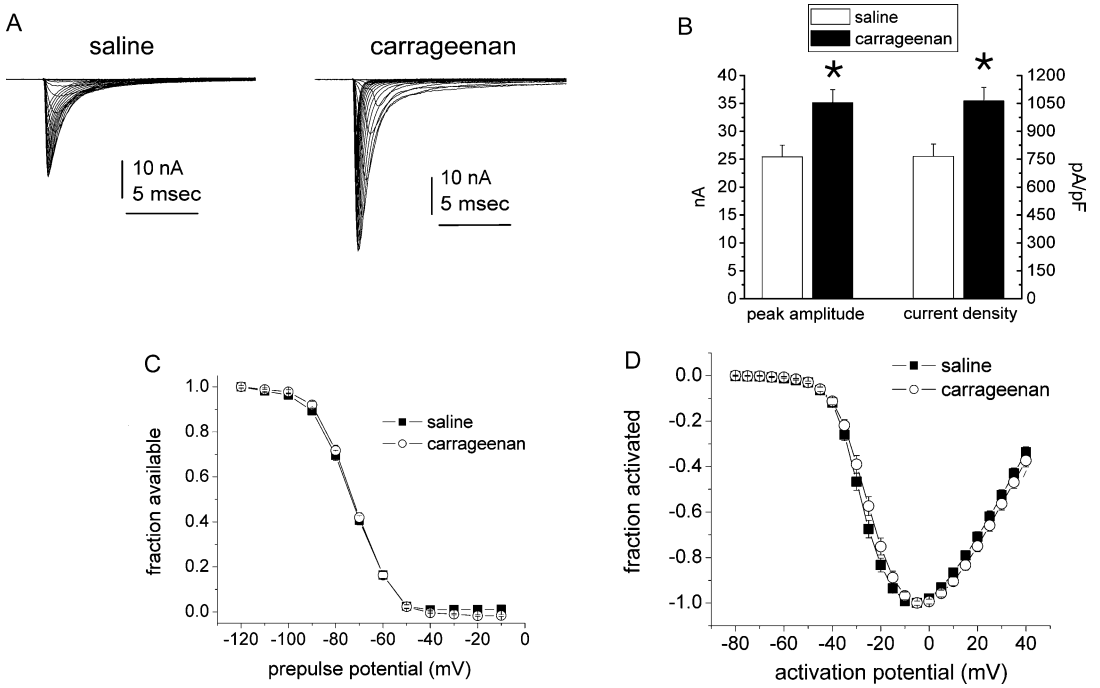

Fig. 4. Whole-cell patch-clamp of small neurons obtained from saline- or carrageenan-injected DRG. (A) Representative families of voltage-activated TTX-S current traces for small neurons cultured from saline (left) and carrageenan (right) DRG are shown. The currents were elicited by 40 ms test pulses ranging from −80 to +40 mV in 5 mV steps. Cells were held at −100 mV. (B) Sodium currents are significantly larger in carragenan neurons (*$P < 0.01$). The TTX-S peak current amplitude and current density were estimated in saline-injected ($n = 54$), carrageenan-injected ($n = 54$) small DRG neurons. TTX-S and TTX-R sodium currents were separated using prepulse-inactivation (500 ms prepulses to −50 mV) and digital subtraction. The TTX-S current density was obtained by dividing the estimated peak current by the whole-cell capacitance (*$P < 0.01$). The average whole-cell capacitance was 35.8 ± 1.5 for saline-injected neurons and 35.2 ± 2.3 for carrageenan-injected neurons. (C) Comparison of steady-state inactivation of TTX-S currents in small DRG neurons from saline- (open circles, $n = 54$) and carrageenan (filled squares, $n = 54$) injected rats; the midpoints of steady-state inactivation were −73.8 ± 0.9 and −73.8 ± 1.1 mV, respectively. Steady-state inactivation was estimated by measuring the peak current amplitude elicited by 20 ms test pulses to −10 mV after 500 ms prepulses to potentials over the range of −120 to −10 mV. Current is plotted as a fraction of the maximum peak current. (D) Comparison of voltage-dependence of activation of total sodium currents in DRG neurons from saline (open circles, $n = 27$) and carrageenan (filled squares, $n = 29$) injected rats; the midpoints of steady-state activation −21.0 ± 1.2 and −23.2 ± 0.9 mV, respectively, were not significantly different. Only cells where the TTX-S currents were larger than the TTX-R currents were used for this comparison. Currents were activated as described in (A).

sodium channel $Na_v1.8$ mRNA and slowly inactivating TTX-R current are increased following carrageenan injection (Tanaka et al., 1998), but also extends these observations to demonstrate that the expression of the TTX-R channel $Na_v1.9$ is not significantly up- or down-regulated in this model of inflammatory pain at 4 days following injection. Moreover, the results demonstrate that transcripts and protein of two TTX-S sodium channels, $Na_v1.3$ and $Na_v1.7$, are upregulated following carrageenan injection, while the expression of two additional TTX-S sodium channels, $Na_v1.1$ and $Na_v1.6$, is not altered under these experimental conditions. In parallel with the upregulation of $Na_v1.3$ and $Na_v1.7$, there is a significant increase in the level of TTX-S sodium current in small DRG neurons following carrageenan injection.

Our results failed to detect a contralateral effect on the expression of sodium channel isoforms in small DRG neurons following carrageenan injection. In situ hybridization, immunocytochemistry and Western blot studies consistently demonstrated similar hybridization and immunoreactive signals for each of the respective sodium channels in small neurons within saline-injected contralateral to carrrageenan injection and saline-injected naive DRG. These observations are in agreement with our previous study examining the expression of $Na_v1.8$ following carrageenan injection, in which contralateral and naive DRG yielded similar results (Tanaka et al., 1998). In contrast, peripheral nerve injury has been associated with an affect on numerous non-lesioned contralateral structures (for review see Koltzenburg et al. 1999), including a single report of downregulation of $Na_v1.8$ following ipsilateral sciatic nerve transection (Oaklander and Belzberg, 1997). Since we only examined small (≤25 μm dia.) DRG neurons in the present study, as this population of cells is principally nociceptive (Djouhri et al., 2003), we cannot rule out the possibility that contralateral effects occur in larger diameter neurons.

Considerable attention has been devoted to the role of TTX-R sodium channels in inflammatory hyperalgesia (see Baker and Wood, 2001; Gold, 1999 for reviews). Numerous mediators are generated during inflammation, including bradykinin, serotonin, adenosine, histamine, prostaglandins (Dray et al. 1995) and NGF (Woolf et al.,

1994), and an increase in the magnitude of slowly-inactivating TTX-R current in some DRG neurons has been demonstrated following administration of prostaglandin E_2 (PGE_2), adenosine and serotonin (Cardenas et al., 1997; England et al., 1996; Gold et al., 1996). Subsequent studies have implicated the TTX-R sodium channel $Na_v1.8$ as playing an important role in inflammatory hyperalgesia (Khasar et al., 1998), and our studies (Tanaka et al., 1998; present study) demonstrating an increase in $Na_v1.8$ mRNA and protein and $Na_v1.8$ current following carrageenan injection are consistent with this conclusion.

The mechanisms that govern the upregulation of $Na_v1.8$ channels following inflammation are not entirely understood. Acute administration of inflammatory mediators to DRG neurons in vitro increases the amplitude of slowly-inactivating TTX-R current (presumably largely due to $Na_v1.8$ channels) and triggers a hyperpolarizing shift in its activation curve (Cardenas et al., 1997; England et al., 1996; Gold et al., 1996, 2001). Likewise, acute application of NGF has been reported to enhance the amplitude of peak TTX-R currents and to shift the activation to more hyperpolarized voltages (Zhang et al. 2002). However, these effects occur within seconds of application and attain maximum levels within minutes, and thus do not reflect upregulation of $Na_v1.8$ gene transcription, but are suggested to involve protein kinase A and/or protein kinase C (England et al., 1996; Gold et al., 1998; but see Cardenas et al., 1997) or sphingomyelin signalling (Zhang et al. 2002) pathways. On the basis of GTP-induced upregulation of low-threshold, persistent sodium current and a parallel increase in excitability of small DRG neurons, Baker et al. (2003) have suggested that inflammatory mediators may also act acutely through G-protein-coupled receptors to upregulate $Na_v1.9$ currents, contributing to the induction of inflammatory pain.

In contrast to the actions of inflammatory mediators on TTX-R sodium currents, few studies have examined the effects of inflammation on TTX-S channels within primary sensory neurons. TTX-S sodium channel isoforms $Na_v1.1$, $Na_v1.6$ and $Na_v1.7$ are expressed in normal adult rat DRG (Black et al., 1996; Kim et al., 2001, 2002); $Na_v1.3$ is re-expressed in adult DRG neurons following peripheral axotomy (Black et al., 1999a; Kim et al., 2001; Waxman et al., 1994). Previous immunocytochemical work utilizing a pan sodium channel antibody (which does not distinguish between sodium channel isoforms) has demonstrated an upregulation of sodium channel immunoreactivity in DRG neurons following complete Freund's adjuvant (CFA) injection into the hind paw, which is maximal at 24 h post-injection and persists in some neurons for at least 2 months (Gould et al., 1998, 1999). While the upregulation of $Na_v1.8$ is responsible for at least some of the observed increase in sodium channel signal following CFA injection, our results indicate that expression of TTX-S sodium channels $Na_v1.3$ and $Na_v1.7$ is upregulated also. The upregulation of $Na_v1.7$ in DRG neurons following carrageenan injection is consistent with the reported increase in $Na_v1.7$ immunoreactivity in DRG neurons 1 week following injection of NGF in the hind paw, albeit at a time when hyperalgesia was not present (Gould et al., 2000).

It has been suggested (Tanaka et al., 1998) that NGF, which is known to be elevated in the skin for at least 5 days following inflammation (Woolf et al., 1994), may provide a sustained trophic influence that upregulates $Na_v1.8$. Several lines of evidence support the proposal that NGF effects the expression of $Na_v1.8$. Both in vitro (Black et al., 1997) and in vivo (Dib-Hajj et al., 1998a) administration of exogeneous NGF rescues the downregulation of $Na_v1.8$ following axotomy in DRG neurons. It is also well-established that exposure to NGF can upregulate the expression of some TTX-S channels, including $Na_v1.2$ and $Na_v1.7$ in PC-12 cells (Fanger et al., 1993; Mandel et al., 1988; Toledo-Aral et al., 1997), $Na_v1.3$ in pancreatic β cells (Vidaltamayo et al., 2002) and TTX-S currents in NB69 human neuroblastoma cells (Urbano and Buno, 2000). In conjunction with these observations and the report of elevated levels of NGF in skin following carrageenan injection (Woolf et al., 1994), the present results are consistent with the hypothesis that NGF initiates and sustains upregulation of one or more TTX-S channels and the TTX-R sodium channel $Na_v1.8$ in DRG neurons projecting to carrageenan-injected hind paws.

Unlike the upregulation of TTX-R $Na_v1.8$, a role for the enhanced expression of TTX-S channels in inflammatory hyperalgesia has yet to be definitively established. In this regard, $Na_v1.7$ has been shown to be targeted to nerve terminals of cultured DRG neurons (Toledo-Aral et al., 1997), while $Na_v1.3$ is localized within the tips of DRG neurites in vitro and also within transected DRG axons in vivo (Black et al., 1999a). It has been suggested that the biophysical properties of $Na_v1.7$ sodium channels position these channels to amplify excitatory inputs (Cummins et al., 1998). Moreover, the slow closed-state inactivation kinetics and relatively rapid recovery from inactivation attributed to $Na_v1.3$ suggest that this channel may support a reduced threshold and/or relatively high frequency of firing (Cummins et al., 2001). Thus, we suggest that the upregulation and selective deployment of $Na_v1.3$ and $Na_v1.7$ TTX-S channels may contribute to the maintenance of pain syndromes in inflamed tissues.

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References

Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 1996;379: 258–62.

Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat Neurosci 1999;2:541–8.

Baker MD, Wood JN. Involvement of Na^+ channels in pain pathways. Trends Pharmacol Sci 2001;22:27–31.

Baker MD, Chandra SY, Ding Y, Waxman SG, Wood JN. GTP-induced tetrotoxin-resistant Na^+ current regulates excitability in mouse and rat small diameter sensory neurones. J Physiol 2003;548:373–82.

Black JA, Dib-Hajj S, McNabola K, Jeste S, Rizzo MA, Kocsis JD, Waxman SG. Spinal sensory neurons express multiple sodium channel α-subunit mRNAs. Brain Res 1996;43:117–31.

Black JA, Langworthy K, Hinson AW, Dib-Hajj SD, Waxman SG. NGF has opposing effects on Na^+ channel III and SNS gene expression in spinal sensory neurons. NeuroReport 1997;8:2331–5.

Black JA, Cummins TR, Plumpton C, Chen Y, Clare J, Waxman SG. Upregulation of a previously silent sodium channel in axotomized DRG neurons. J Neurophysiol 1999a;82:2776–85.

Black JA, Fjell J, Dib-Hajj S, Duncan ID, O'Connor LT, Fried K, Gladwell A, Tate S, Waxman SG. Abnormal expression of SNS/PN3 sodium channel in cerebellar Purkinje cells following loss of myelin in the taiep rat. NeuroReport 1999b;10:913–8.

Black JA, Cummins TR, Yoshimura N, de Groat WC, Waxman SG. Tetrodotoxin-resistant sodium channels $Na_v1.8$/SNS and $Na_v1.9$/NaN in afferent neurons innervating urinary bladder in control and spinal cord injured rats. Brain Res 2003;963:132–8.

Boucher TJ, Okuse K, Bennett DLH, Munson JB, Wood JN, McMahon SB. Potent analgesic effects of GDNF in neuropathic pain states. Science 2000;290:124–7.

Cardenas CG, Del Mar LP, Cooper BY, Scroggs RS. $5HT_4$ receptors couple positively to tetrodotoxin-insensitive sodium channels in a subpopulation of capsaicin-sensitive rat sensory neurons. J Neurosci 1997;17: 7181–9.

Cummins TR, Waxman SG. Downregulation of tetrodotoxin-resistant sodium currents and up-regulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. J Neurosci 1997;17:3503–14.

Cummins TR, Howe JR, Waxman SG. Slow closed-state inactivation: a novel mechanism underlying ramp currents in cells expressing the hNE/PN1 sodium channel. J Neurosci 1998;18:9607–19.

Cummins TR, Black JA, Dib-Hajj SD, Waxman SG. Glial-derived neurotrophic factor upregulates expression of function SNS and NaN sodium channels and their currents in axotomized dorsal root ganglion neurons. J Neurosci 2000;20:8754–61.

Cummins TR, Alieco F, Renganathan M, Herzog RI, Dib-Hajj SD, Waxman SG. $Na_v1.3$ sodium channels: rapid repriming and slow closed-state inactivation display quantiative differences after expression in a mammalian cell line and in spinal sensory neurons. J Neurosci 2001;21:5952–61.

Dib-Hajj SD, Tyrrell L, Black JA, Waxman SG. NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. Proc Natl Acad Sci USA 1998a;95: 8963–9.

Dib-Hajj SD, Black JA, Cummins TR, Kenney AM, Kocsis JD, Waxman SG. Rescue of α-SNS sodium channel expression in small dorsal root ganglion neurons after axotomy by nerve growth factor in vivo. J Neurophysiol 1998b;79:2668–78.

Djouhri L, Fang X, Okuse K, Wood JN, Berry CM, Lawson SN. The TTx-resistant sodium channel $Na_v1.8$ (SNS/PN3): expression and correlation with membrane properties in rat nociceptive primary afferent neurons. J Physiol 2003;550:739–52.

England S, Bevan S, Docherty RJ. PGE_2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic-AMP-protein kinase A cascade. J Physiol 1996;495: 429–49.

Fang X, Djouhri L, Black JA, Dib-Hajj SD, Waxman SG, Lawson SN. The presence and role of the tetrodotoxin-resistant sodium channel $Na_v1.9$ (NaN) in nociceptive primary afferent neurons. J Neurosci 2002;22: 7425–33.

Fanger GR, Erhardt P, Cooper GM, Mandel G. ras-Independent induction of rat brain type II sodium channel expression in nerve growth factor treated cells. J Neurochem 1993;61:1977–80.

Fjell J, Cummins TR, Davis BM, Alber KM, Fried K, Waxman SG, Black JA. Sodium channel expression in NGF-overexpressing transgenic mice. J Neurosci Res 1999a;57:39–47.

Fjell J, Cummins TR, Fried K, Black JA, Waxman SG. In vivo NGF deprivation reduces SNS/PN3 expression and TTX-R sodium currents in IB4-negative DRG neurons. J Neurophysiol 1999b;81:803–10.

Fjell J, Hjelstrom P, Hormuzdiar W, Milenkovic M, Aglieco F, Tyrrell L, Dib-Hajj S, Waxman SG, Black JA. Localization of the tetrodotoxin-resistant sodium channel NaN in nociceptors. NeuroReport 2000;11: 199–202.

Gold MS. Tetrodotoxin-resistant Na^+ currents and inflammatory hyperalgesia. Proc Natl Acad Sci USA 1999;96:7645–9.

Gold MS, Reichling DB, Shuster MJ, Levine JD. Hyperalgesic agents increase a tetrodotoxin-resistant Na^+ current in nociceptors. Proc Natl Acad Sci USA 1996;93:1108–12.

Gold MS, Levine JD, Correa AM. Modulation of TTX-R I_{Na} by PKC and PKA and their role in PGE_2-induced sensitization of rat sensory neurons in vitro. J Neurosci 1998;18:10345–55.

Gold MS, Zhang L, Wrigley DL, Traub RJ. Prostaglandin E_2 modulates TTX-T I_{Na} in rat colonic sensory neurons. J Neurophysiol 2001;88: 1512–22.

Gould III HJ, England JD, Liu ZP, Levinson SR. Rapid sodium channel augmentation in response to inflammation induced by complete Freund's adjuvant. Brain Res 1998;802:69–74.

Gould III HJ, Gould TN, Paul D, England JD, Liu ZP, Reeb SC, Levinson SR. Development of inflammatory hypersensitivity and augmentation of sodium channels in rat dorsal root ganglia. Brain Res 1999;824: 296–9.

Gould III HJ, Gould TN, England JD, Paul D, Liu AP, Levinson SR. A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain. Brain Res 2000;854:19–29.

Hains BC, Black JA, Waxman SG. Primary motor neurons fail to up-regulate voltage-gated sodium channel $Na_v1.3$/brain type III following axotomy resulting from spinal cord injury. J Neurosci Res 2002;70: 546–52.

Iadarola MJ, Brady LS, Draisci G, Dubner R. Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: stimulus specificity, behavioral parameters and opioid receptor binding. Pain 1988;35:313–26.

Khasar SG, Gold MS, Levine JD. A tetrodotoxin-resistant sodium current mediates inflammatory pain the rat. Neurosci Lett 1998;256:17–20.

Kim CH, Oh Y, Chung JM, Chung K. The changes in expression of three subtypes of TTX-sensitve sodium channels in sensory neruons after spinal nerve ligation. Mol Brain Res 2001;95:153–61.

Kim CH, Oh Y, Chung JM, Chung K. Changes in expression of three subtypes of tetrodotoxin sensitive sodium channel expression in the axotomized dorsal root ganglion in the rat. Neurosci Lett 2002;323: 125–8.

Leffler A, Cummins TR, Dib-Hajj SD, Hormuzdiar WN, Black JA, Waxman SG, GDNF GF. reverse changes in repriming of

TTX-sensitive Na^+ currents following axotomy of dorsal root ganglion neurons. J Neurophysiol 2001;88:650–8.

Mandel G, Cooperman SS, Maue RA, Goodman RH, Brehm P. Selective induction of brain type II Na channels by nerve growth factor. Proc Natl Acad Sci USA 1988;85:924–8.

Oaklander AL, Belzberg AJ. Unilateral nerve injury down-regulates mRNA for Na^+ channel SCN10A bilaterally in rat dorsal root ganglia. Mol Brain Res 1997;52:162–5.

Rush AM, Brau ME, Elliott AA, Elliott JR. Electrophysiological properties of sodium current subtypes in small cells from adult rat dorsal root gangia. J Physiol 1998;511:771–89.

Sangameswaran L, Delgado SG, Fish LM, Koch BD, Jakeman LB, Stewart GR, Sze P, Hunter JC, Eglen RM, Herman RC. Structure and function of a novel voltage-gated tetrotodoxin-resistant sodium channel specific to sensory neurons. J Biol-Chem 1996;271:5953–6.

Sleeper AA, Cummins TR, Dib-Hajj SD, Hormuzdiar W, Tyrrell L, Waxman SG, Black JA. Chanes in expression of two tetrodotoxin-resistant sodium channels and their currents in dorsal root ganglion neurons after sciatic nerve injury by not rhizotomy. J Neurosci 2000;20:7279–89.

Tanaka M, Cummins TR, Ishikawa K, Dib-Hajj SD, Black JA, Waxman SG. SNS $Na^($ channel expression increases in dorsal root ganglion neurons in the carrageenan inflammatory pain model. NeuroReport 1998;9:967–72.

Toledo-Aral JJ, Moss BL, He Z-J, Koszowski AG, Whisenand T, Levinson SR, Wolff JJ, Silos-Santiago I, Halegoua S, Mandel G. Identification of PN1, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. Proc Natl Acad Sci USA 1997;94:1527–32.

Urbano FJ, Buno W. Neurotrophin regulation of sodium and calcium channels in human neuroblastoma cells. Neuroscience 2000;96:439–43.

Vidaltamayo R, Sanche-Soto MC, Hiriart M. Nerve growth factor increases sodium channel expression in pancreatic beta cells: implications for insulin secretion. Fed Am Soc Exp Biol J 2002;16:891–2.

Waxman SG, Kocsis JK, Black JA. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. J Neurophysiol 1994;72:466–71.

Woolf CJ, Safieh-Garabedian B, Ma Q-P, Crilly P, Winters J. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience 1994;62:327–31.

Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. Proc Natl Acad Sci USA 1999;96:7723–30.