Effects of a Chronic Compression of the Dorsal Root Ganglion on Voltage-Gated Na\(^+\) and K\(^+\) Currents in Cutaneous Afferent Neurons

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Tan, Z. Y., D. F. Donnelly, and R. H. LaMotte. Effects of a chronic compression of the dorsal root ganglion on voltage-gated Na\(^+\) and K\(^+\) currents in cutaneous afferent neurons. J Neurophysiol 95: 1115–1123, 2006; doi:10.1152/jn.00830.2005. A chronic compression of the dorsal root ganglion (CCD) produces ipsilateral cutaneous hyperalgesia that is associated with an increased excitability of neuronal somata in the compressed ganglion, as evidenced by spontaneous activity and a lower rheobase. We searched for differences in the properties of voltage-gated Na\(^+\) and K\(^+\) currents between somata of CCD- and control (unoperated) rats. CCD was produced in adult rats by inserting two rods through the intervertebral foramina, one compressing the L\(_4\) and the other, the ipsilateral, L\(_5\) dorsal root ganglion (DRG). After 5–9 days, DRG somata were dissociated and placed in culture for 16–26 h. Cutaneous neurons of medium size (35–45 μm), Fluorogold-labeled from the hindpaw, were selected for whole cell patch-clamp recording of action potentials and ion currents. In comparison with control neurons, CCD neurons had steady-state activation curves for TTX-sensitive (TTX-S) Na\(^+\) currents that were shifted in the hyperpolarizing direction, and CCD neurons had enhanced TTX-resistant (TTX-R) Na\(^+\) current. CCD neurons also had smaller, fast-inactivating K\(^+\) currents (K\(_{\text{f}}\)) at voltages from −30 to 50 mV. The reduction in K\(_{\text{f}}\), the hyperpolarizing shift in TTX-S Na\(^+\) current activation, and the enhanced TTX-R Na\(^+\) current may all contribute to the enhanced neuronal excitability and thus to the pain and hyperalgesia associated with CCD.

INTRODUCTION

In animal models of neuropathic pain that produce hyperalgesia and allodynia, dorsal root ganglion (DRG) neurons often exhibit signs of hyperexcitability, such as ectopic spontaneous discharges, a reduction in rheobase, and a greater number of action potentials (APs) evoked by sustained depolarizing currents (Abdulla and Smith 2001a; Kim et al. 1998; Moore et al. 2002; Song et al. 2003a,b; Zhang et al. 1999).

The ionic mechanisms contributing to the hyperexcitability of DRG neurons after peripheral inflammation or neuronal injury have been widely studied, but results have differed between laboratories and models. For instance, after transection of the sciatic nerve, there is an up-regulation of rapidly repriming TTX-sensitive (TTX-S) Na\(^+\) channels and a down-regulation of two isoforms of TTX-resistant (TTX-R) Na\(^+\) channels (Abdulla and Smith 2002; Black et al. 1999; Dib-Hajj et al. 1999; Sleeper et al. 2000; Waxman et al. 1994). In addition, voltage-gated K\(^+\) currents and Ca\(^{2+}\) current are down-regulated as well (Abdulla and Smith 2001b; Everill and Kocsis 1999; Ishikawa et al. 1999; Yang et al. 2004). After inflammation of peripheral tissues by carrageenan injection, there is an up-regulation of mRNA and currents for both TTX-S and TTX-R Na\(^+\) channels (Black et al. 2004; Tanaka et al. 1998). In addition, gastric ulcers in rats and ileitis in guinea pigs are associated with a reduction of K\(^+\) currents in DRG neurons (Dang et al. 2004; Stewart et al. 2003).

In our laboratory, chronic compression of the DRG (CCD) is used in the rat as a model of the ganglion compression that can occur in humans after, for instance, foraminal or spinal stenosis, disk herniations, degenerative disorders, tumors, or spinal injuries. In this model, a rod is implanted into the lumbar intervertebral foramen, causing a chronic compression of the DRG (Hu and Xing 1998). These rats develop a cutaneous hyperalgesia and tactile allodynia on the ipsilateral foot. Electrophysiologic recordings of DRG neurons from CCD rats evidence an enhanced excitability including a decrease in the current and voltage thresholds required to elicit APs and an increase in the incidence of spontaneous activity (Hu and Xing 1998; Song et al. 2003a,b; Zhang et al. 1999).

The purpose of this study was to study the effects of CCD on both AP properties and voltage-gated Na\(^+\) and K\(^+\) currents in cutaneous, medium-sized, dissociated DRG neurons. Our findings suggest that a negative shift of activation for voltage-gated Na\(^+\) channels, an increase in TTX-R Na\(^+\) current, and a decrease in fast-inactivating K\(^+\) currents could contribute to neuronal hyperexcitability in these neurons after CCD, including the decreased current and voltage threshold for APs and an increased capability to produce multiple APs during a sustained depolarization.

METHODS

Fluorogold labeling of cutaneous neurons

Female Sprague-Dawley rats (140–160 g) were anesthetized with pentobarbital sodium (40 mg/kg, ip). A fluorogold solution in distilled water (1%, 0.05 ml; Molecular Probes, Eugene, OR) was injected into the right lateral plantar region at several points (Oyelese and Kocsis 1996; Yao et al. 2003) to retrogradely label the cell bodies of cutaneous afferent neurons.

CCD surgery

Immediately after fluorogold labeling, one group of rats received no surgery and served as unoperated controls. In the other group of rats, the ipsilateral, right transverse process, and intervertebral foramina of L\(_4\) and L\(_5\) were exposed as described previously (Hu and Xing 1998; Song et al. 1999). A stainless steel L-shaped rod (0.63 mm diam and 4 mm in length) was inserted into each foramen, one at L\(_4\) and the...
other at the L₅ ganglion. Another group of rats received no additional surgery and were used as controls. Sham operations (surgery before and without implantation) were not considered necessary because of the absence of behavioral effects of such surgery or electrophysiological differences between neurons from previously unoperated versus sham-operated controls (Zhang et al. 1999).

Cell preparation

Five to 9 days after Flurogold injection, the rats were deeply anesthetized with pentobarbital sodium (40 mg/kg, ip), and the L₃ and L₅ lumbar DRGs were exposed. In CCD rats, the correct placement of each implanted rod was confirmed. DRGs were removed from control or CCD rats and placed in complete saline solution (CSS) for cleaning and mincing. The CSS contained the following (in mM): 137 NaCl, 5.3 KCl, 1 MgCl₂, 3 CaCl₂, 25 sorbitol, and 10 HEPES. The connective tissue of the DRGs was digested for 25 min with collagenase A (1 mg/ml; Boehringer Mannheim, Mannheim, Germany) and for another 25 min with collagenase D (1 mg/ml; Boehringer Mannheim) and papain (30 U/ml; Worthington, Lakewood, NJ) in CSS containing 0.5 mM EDTA and 2 μg of cysteine at 37°C as described previously (Rizzo et al. 1995; Yao et al. 2003). The cells were dissociated by trituration in culture medium containing 1 mg/ml bovine serum albumin and 1 mg/ml trypsin inhibitor (Boehringer Mannheim) and plated on glass coverslips coated with polyornithine and laminin. The culture medium contained equal amounts of DMEM (Invitrogen, San Diego, CA) and F-12 (Invitrogen) with 10% fetal calf serum (HyClone, Logan, UT) and 1% penicillin and streptomycin (Invitrogen). The cells were incubated at 37°C (5% CO₂ balance air) and laminin. The culture medium contained equal amounts of DMEM and balanced salt solution (BSS). The culture medium contained 0.5 mM EDTA and 2 μg of cysteine at 37°C as described previously (Rizzo et al. 1995; Yao et al. 2003). The cells were dissociated by trituration in culture medium containing 1 mg/ml bovine serum albumin and 1 mg/ml trypsin inhibitor (Boehringer Mannheim) and plated on glass coverslips coated with polyornithine and laminin. The culture medium contained equal amounts of DMEM (Invitrogen, San Diego, CA) and F-12 (Invitrogen) with 10% fetal calf serum (HyClone, Logan, UT) and 1% penicillin and streptomycin (Invitrogen). The cells were incubated at 37°C (5% CO₂ balance air) for 1 h, after which culture medium without the inhibitor was added. Cells were cultured for 14–24 h before recording.

Electrophysiological recording

Coverslips were transferred to a recording chamber that was mounted on the stage of an upright microscope (BX50-WI, Olympus Optical, Tokyo, Japan). Neurons selected for recording were 35–45 μm in diameter and were positive for Flurogold fluorescence. Patch pipettes (0.8–2 MΩ) were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL). Neurons were recorded at room temperature in whole cell mode (Multiclamp 700A, Axon Instruments, Union City, CA) using pClamp 8.01 software (Axon Instruments). Data were acquired with a 12-bit A/D converter at 10–50 kHz. The voltage drop across the access resistance was compensated at 50–100 mV. The chamber was filled with normal bath solution. Chemicals and other bath solutions were applied locally through a perfusion system (ALA Scientific Instruments, Union City, CA) using a drug application system (ALA Scientific Instruments, Union City, CA) using a drug application system (ALA Scientific Instruments, Union City, CA) using a drug application system (ALA Scientific Instruments, Union City, CA) using a drug application system (ALA Scientific Instruments, Union City, CA). The bath solution contained (in mM) 140 NaCl, 3 KCl, 1 MgCl₂, 2 CaCl₂, 0.1 Na₂SO₄, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with HEPES, and the osmolality was adjusted to 300–310 mosm. The pipette solution contained (in mM) 140 CsF, 10 NaCl, 10 EGTA, and 10 HEPES. The pH was adjusted to 7.2, and the osmolality was 290–300 mosm.

Solutions for recording under current clamp

The bath solution contained (in mM) 145 NaCl, 3 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with NaOH, and the osmolality adjusted to 300–310 mosm. The pipette solution contained (in mM) 138 KCl, 1 CaCl₂, 2 MgCl₂, 10 EGTA, and 10 Na-HEPES. The pH was adjusted to 7.2, and the osmolality was 290–300 mosm.

Solutions for recording sodium currents

Sodium currents were measured under a reduced sodium gradient to reduce space clamp problems. The bath solution contained (in mM) 35 NaCl, 105 TEA Cl, 4 4-aminopyridine (4-AP), 2 BaCl₂, 0.1 CaCl₂, 0.1 CdCl₂, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with HEPES, and the osmolality was adjusted to 300–310 mosm. The pipette solution contained (in mM) 140 CsF, 10 NaCl, 10 EGTA, and 10 HEPES. The pH was adjusted to 7.2 with CsOH, and the osmolality to 290–300 mosm. To isolate TTX-R Na⁺ currents, TTX (300 nM) was added to the bath solution.

Solutions for recording potassium current

The K⁺ bath solution contained (in mM) 140 choline Cl, 3 KCl, 1 MgCl₂, 1 CaCl₂, 0.1 CdCl₂, and 10 HEPES. The pH was adjusted to 7.4 with Tris-base, and the osmolality was adjusted to 300–310 mosm. The pipette solution contained (in mM) 100 choline Cl, 40 KCl, 1 MgCl₂, 10 EGTA, and 10 HEPES. The pH was adjusted to 7.2 with Tris-base, and osmolality to 290–300 mosm.

Data analysis

Data were analyzed using pClampfit 8.1 (Axon Instruments) and SigmaPlot 8.0 (SPSS, Chicago, IL). Data are expressed as means ± SE. DRG rat and control neurons were compared by Student’s t-test and χ² tests. In the case of multiple comparisons, for instance, over a voltage range, data were initially analyzed using an ANOVA with repeated measures. If this revealed a significant difference, Student’s t-tests were applied for post hoc testing with a Bonferroni correction applied to the probability criterion for significance that was initially set at P < 0.05.

For the analysis of steady-state activation, the peak sodium conductance (gₙa) at different potentials was calculated, as a chord conductance, from the corresponding peak current, Iₚa

\[ g_{na} = \frac{I_{na}}{V_{na} - V_{s}} \]

where \( V_{s} \) is the reversal potential for sodium current. The conductance values were fitted to a Boltzman function using a least-squares fit to characterize the activation characteristics of gₙa

\[ g_{na} = I_{na} / (1 + \exp(V_{na} - V_p / k_g)) \]

where \( V_p \) is the voltage for half-maximum activation and \( k_g \) is the slope factor.

The steady-state inactivation parameter (hₜ) was calculated at each prepulse potential by dividing the current evoked after a given prepulse potential by the current evoked after the maximal hyperpolarization potential. The inactivation parameter was fitted to a Boltzman function for determination of the half inactivation potential, \( V_h \), and the slope factor

\[ k_h h_{t}(V) = 1 / (1 + \exp[(V - V_h) / k_h]) \]

For the analysis of kinetics, the time constants for activation (\( \tau_{na} \)) and inactivation (\( \tau_{h} \)) for TTX-S and TTX-R sodium currents were measured at each potential over the voltage range of −40 to 10 mV.
FIG. 1. Action potential (AP) properties under current-clamp recording. A: series of current steps, each 2-ms duration, were injected in increments of 100 pA to a control (left) and a chronic compression of the dorsal root ganglion (CCD; right) neuron. Current threshold (rheobase) was the minimal amplitude of current that elicited an AP. Voltage threshold was taken as the 1st point on the upstroke of an AP where the rising rate exceeded 50 mV/ms (arrows). Both current and voltage thresholds were lower for the CCD than for the control neuron. B: accommodation in response to an injected current of 2.5 times rheobase and 1-s duration. Control neuron discharged 1 AP, whereas the CCD neuron discharged 9 APs.

A two-term exponential function was used to fit the falling phase of 4-AP–sensitive K\textsubscript{S} currents. The current amplitude (I\textsubscript{rat} and I\textsubscript{slow}) and time constant (\(\tau\text{r,at}\) and \(\tau\text{r,slow}\)) for each cell were determined.

RESULTS

Passive membrane properties and AP characteristics

Fifty-five cutaneous DRG neurons of medium size were recorded by whole cell patch clamp. Thirty-one were from control rats and 24 from CCD. In addition, 26 control and 23 CCD cells were used to compare the capability of repetitive firing induced by the injection of current at 2.5 times rheobase.

The mean rheobase current was significantly smaller for CCD than for control neurons (4.1 ± 0.7 vs. 7.9 ± 1.4 pA/pF; \(P < 0.05\), Student’s \(t\)-test). In addition, the voltage threshold was significantly more negative for CCD than for control neurons (−33.8 ± 1.9 vs. −26.0 ± 1.9 mV; \(P < 0.01\), Student’s \(t\)-test; Fig. 1). No significant difference was found between control and CCD group in cell capacitance, resting membrane potential, input resistance, AP amplitude, AP duration, AHP amplitude, or AHP duration (Table 1; Student’s \(t\)-test). The percentage of cells discharging multiple (>2) APs was significantly higher in CCD than in control neurons (13/23 vs. 5/26; \(P < 0.01\), \(\chi^2\) test; Fig. 1).

Voltage-gated Na\textsuperscript{+} currents

Voltage-gated Na\textsuperscript{+} current was measured (Fig. 2) first in the absence of TTX to obtain the total Na\textsuperscript{+} current and then in the presence of TTX to obtain the TTX-resistant (TTX-R) current. By subtracting the latter from the former, the TTX-sensitive (TTX-S) current was obtained. The activating and inactivating kinetics were faster for TTX-S currents than for TTX-R currents (Fig. 2).

TTX-S currents

TTX-S currents were activated at approximately −50 mV for CCD and control neurons and reached peak current values near −20 mV (Fig. 3A). Student’s \(t\)-tests were used to test for differences in current between CCD and control groups. Peak current values were not significantly different between control and CCD neurons (CCD: −136.4 ± 27.1 pA/pF; \(n = 16\); control: −143.3 ± 18.0 pA/pF; \(n = 18\)). However, at −40 mV the mean current amplitude was significantly higher for the CCD group (−111.8 ± 24.4 pA/pF) than for the control group (−156.2 ± 30.1 pA/pF).

TABLE 1. Membrane properties of control and CCD neurons

<table>
<thead>
<tr>
<th>Diameter, (\mu\text{m})</th>
<th>(C_{m}), pF</th>
<th>(V_{m}), mV</th>
<th>(R_{m}), M(\Omega)</th>
<th>AP Rheobase, (\mu\text{A}/\text{pF})</th>
<th>AP VTH, mV</th>
<th>AP Duration, ms</th>
<th>AHP Amplitude, mV</th>
<th>AHP Duration, ms</th>
<th>MS/non-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 31</td>
<td>38.5 ± 0.5</td>
<td>87.2 ± 5.8</td>
<td>−56.2 ± 1.5</td>
<td>122.5 ± 15.6</td>
<td>7.9 ± 1.4</td>
<td>−26.0 ± 1.9</td>
<td>86.5 ± 3.9</td>
<td>1.30 ± 0.15</td>
<td>17.1 ± 1.1</td>
</tr>
<tr>
<td>CCD 24</td>
<td>38.7 ± 0.5</td>
<td>83.9 ± 5.8</td>
<td>−55.0 ± 1.6</td>
<td>94.3 ± 13.3</td>
<td>4.1 ± 0.7*</td>
<td>−33.8 ± 1.9*</td>
<td>85.7 ± 4.0</td>
<td>1.18 ± 0.11</td>
<td>15.9 ± 1.4</td>
</tr>
</tbody>
</table>

Values are mean ± SE; \(n\) is number of cells. \(C_{m}\), cell capacitance; \(V_{m}\), membrane potential; \(R_{m}\), input resistance; AP rheobase, current threshold of action potential; VTH, voltage threshold; AHP, afterhyperpolarization; MS, number of cells discharging multiple (>2) sparks to current step at 2.5× rheobase; non-MS, number of cells discharging ≤2 spikes. *\(P < 0.05\), †\(P < 0.01\) (Student’s \(t\)-test, significant differences between control and CCD); +\(P < 0.01\) (\(\chi^2\) test, significant difference between control and CCD).
(–44.6 ± 15.0 pA/pF). No significant difference in current amplitude was found between CCD and control groups at higher activation voltages.

The TTX-S current for each neuron was converted to a conductance and fitted to a Boltzman function (Fig. 4A). The mid-point of activation was significantly more negative in CCD than in control neurons (–40.9 ± 2.2 mV, n = 16 vs. –32.9 ± 1.4 mV, n = 18, respectively; Student’s t-test). However, the slope factor was not significantly different between CCD and control neurons (4.1 ± 0.7 vs. 4.0 ± 0.6 mV, respectively). The steady-state inactivation parameter, h, was also fitted to a Boltzman function for each neuron. Unlike the activation function, the two groups did not differ in their steady-state inactivation characteristics. The half-inactivation potentials were –59.7 ± 3.4 mV (n = 14) for CCD and –57.4 ± 1.8 mV (n = 16) for control neurons. Slope values were 4.0 ± 0.6 mV for the CCD group (n = 14) and 5.1 ± 0.4 mV for control cells (n = 16).

The time constants of activation and inactivation of the Na current were measured at voltages within the range of –40 to 10 mV for CCD (n = 12) and control (n = 17) neurons (Fig. 5). Repeated-measures ANOVAs were used to compare time constants for control and CCD neurons across the entire range of voltages (without post hoc analyses). The mean inactivation time constant was significantly lower in the CCD group than that in control group (df = 1.27; F = 11.153; P < 0.01; Fig. 5A). However, there was no significant difference in the mean activation time constant between CCD (n = 12) and control (n = 17) neurons (df = 1.27; F = 0.791).

**TTX-R currents**

Because some neurons expressed little or no TTX-R current, analyses of TTX-R current magnitude/kinetics were only undertaken on cells whose ratio of peak TTX-R to TTX-S currents was >10% at –20 mV. This criterion reduced the population to nine cells from the control group and nine cells from the CCD group. The relative proportion of cells that met the above criteria was not different between control (39.1%) and CCD (42.9%; P > 0.05, χ² test). The mean diameters of these cells were 39.7 ± 1.2 μm for control and 40.4 ± 1.0 μm for the CCD group.

TTX-R currents activated at around –40 mV for both CCD and control groups (Fig. 3B) and reached maximal levels at –20 mV (Fig. 3B). The current amplitudes at –30 and –20 mV were significantly higher for the CCD group (–89.1 ± 23.1 and –109.7 ± 17.5 pA/pF, n = 9) than for the control group (–37.5 ± 12.8 and –61.9 ± 16.0 pA/pF, n = 9, Student’s t-test, respectively).

There was no significant difference between CCD and control groups in activation and steady-state inactivation (Fig. 4B, Student’s t-test). The mean mid-points and mean slope factors of activation curves were –25.9 ± 2.9 and 7.5 ± 1.8 mV for CCD (n = 9) and –28.6 ± 2.1 and 5.8 ± 1.1 mV for control (n = 9). The mid-points and slope factors of inactivation curves were –42.0 ± 3.3 and 2.5 ± 0.5 mV for CCD (n = 7) and –43.2 ± 4.0 and 4.5 ± 1.2 mV for control (n = 9).

The time constants of activation and inactivation were measured for voltages within the range of –40 to 10 mV (Fig. 5B).

**FIG. 2.** Representative families of voltage-gated sodium currents. Currents are shown for a control neuron (A, C, and E) and a CCD neuron (B, D, and F). For each neuron, membrane potential was held at –70 mV. Whole cell sodium currents were elicited by a series of 50-ms test pulses ranging from –70 to +50 mV in 10-mV steps. A and B: total sodium currents. C and D: TTX-resistant (TTX-R) currents obtained during application of 500 nM TTX. E and F: TTX-sensitive (TTX-S) currents obtained by subtracting TTX-R currents from total.
There were no significant differences in the mean activation or mean inactivation time constants between CCD (control) and CCD (repeated-measures ANOVA).

Voltage-gated K$^+$ currents

Voltage-gated K$^+$ currents were recorded as shown in Fig. 6. The command potential protocol was initially undertaken from a holding potential of $-100$ mV and repeated from a holding potential of $-30$ mV. Because the inactivating components inactivated at $-30$ mV and the sustained current, K$_{dr}$, was evoked from $-30$ mV, a subtraction of the current waveforms elicited at the two holding potentials yielded the inactivating current, K$_a$ (Fig. 6).

The amplitudes of K$_a$ currents for CCD neurons ($n = 15$) were significantly lower than those of controls ($n = 16$; df = 1,29; $F = 13.89$; $P < 0.001$, repeated-measures ANOVA; Fig. 7A). For example, at $+20$ mV, the mean K$_a$ currents were $34.0 \pm 5.1$ pA/pF for CCD neurons and $60.9 \pm 7.5$ pA/pF for controls. Post hoc comparisons of differences between individual means were performed using a sequence of Student's t-test starting at test pulses of $-40$ mV until significant differences were obtained for $-30$ and $-20$ V ($P < 0.01$, using Bonferroni corrections). The differences between individual means for all voltages positive to $-20$ mV were clearly significant as these were even greater than those tested. In contrast, there was no significant difference between CCD and control neurons in the amplitudes of K$_{dr}$ currents from $-60$ to $+50$ mV (repeated-measures ANOVA; df = 1,29; $F = 0.13$; $P = 0.73$; Fig. 7B).
In other experiments, $K_a$ was separated from $K_{dr}$ using the channel blocker 4-AP (6 mM) to preferentially block the $K_a$ current (Fig. 8, A and B). The current evoked in the presence of 4-AP was subtracted from the total current (without 4-AP) to obtain the 4-AP–sensitive current. The mean amplitude of the 4-AP–sensitive current for CCD neurons (43.3 ± 6.1 pA/pF, $n = 17$) was significantly lower than that of controls (85.2 ± 9.9 pA/pF, $n = 18$) at a test pulse depolarized from −100 to +50 mV ($P < 0.01$, Student’s $t$-test; Fig. 8C). At the same test pulse, no significant difference was found in the amplitude of the 4-AP–insensitive currents between CCD (116.4 ± 10.2 pA/pF, $n = 17$) and control neurons (138.8 ± 11.2 pA/pF, $n = 18$, Student’s $t$-test; Fig. 8C).

The fast and slow inactivating components of the 4-AP–sensitive $K_a$ currents were compared after fitting the inactivating phase to a two-term exponential function (Table 2). The fast inactivating component of $K_a$ currents was significantly smaller for CCD than that for control neurons (Student’s $t$-test; Table 2). However, there was no significant difference in the slower inactivating component between CCD and control neurons. There was also no significant difference in the fast and slow time constants between CCD and control cells (Table 2).

**DISCUSSION**

Previous studies showed that CCD causes an increase in the excitability of DRG somata recorded in the intact ganglion (Zhang et al. 1999) and in ganglion neurons after dissociation (Ma and LaMotte 2005). Both of these studies used sharp electrode recording and did not label neurons from their peripheral receptive fields. In this study, we confirmed the enhanced excitability produced by CCD using whole cell patch-clamp recordings and focused on one particular neuronal population—cutaneous neurons of medium size that are labeled after Fluorogold injection to the hindpaw.

Intact neurons with medium-sized cell bodies and myelinated axons include those that have peripheral nociceptors and others that have low-threshold mechanoreceptors (e.g., Fang et al. 2005; Ma et al. 2003). Nonnociceptive neurons with myelinated axons are thought to contribute to pain or altered pain states after peripheral inflammation or nerve injury by contributing, for example, to the production or maintenance of central sensitization or to the allodynia believed to be caused by the sensitization (Ma and Woolf 1996; Neumann et al. 1996; Noguchi et al. 1995). After CCD, medium-sized neurons can develop abnormal spontaneous activity and/or exhibit AP activity in response to inflammatory mediators (as can subpopulations of neurons...
A decrease in $K_\text{a}$ and an increase in TTX-R current would be expected to prolong the AP duration by reducing the repolarization current and enhancing the duration of Na influx. However, AP duration was not significantly altered in CCD. This was likely caused by the enhanced speed of inactivation of TTX-S current observed after CCD, which may have balanced the prolonging actions of TTX-R and $K_\text{a}$ on AP duration.

Changes in Na channel expression or activity have been observed in other models of neuropathic pain, but the direction of change has been variable. A partial or total transection of the sciatic nerve caused an up-regulation of Nav 1.3, a TTX-S isoform that is usually expressed only in the neonatal period (Black et al. 1999; Dib-Hajj et al. 1999; Waxman et al. 1994). Nav 1.3 is similarly up-regulated after carrageenan inflammation of the ipsilateral paw (Black et al. 2004; Tanaka et al. 1998). Although we did not identify changes in specific isoforms after CCD, we observed no change in the magnitude or steady-state properties associated with the inactivation of TTX-S current in our neurons. This suggests that there was no major change in TTX-S isoforms expressed in our cells. However, there was a leftward shift in the voltage of activation for the TTX-S current by 8 mV. Although the reason for this shift is not identified, a similar shift in activation is observed in hippocampal cells during exposure to pertussis toxin, suggesting that activation of a G protein–coupled pathway may occur that shifts the voltage of activation of a G protein–coupled inflammatory pathway that may occur with CCD (Ma et al. 1994). Results on TTX-R currents have been variable. We observed an up-regulation of TTX-R current after CCD—a result also observed in small neurons after carrageenan inflammation (Black et al. 2004; Tanaka et al. 1998). In contrast, the message for TTX-R current isoforms decreased after sciatic nerve transection, but it is unclear whether the membrane current was similarly decreased (Black et al. 1999; Dib-Hajj et al. 1999; Waxman et al. 1994). The variability of expression changes in different models of neuropathic pain suggest that multiple mechanisms may give rise to enhanced excitability of DRG neurons, resulting in a similar behavioral outcome.

Unlike changes in Na channel expression, changes in $K^+$ current have been relatively consistent among models of neuropathic pain. After transection of the sciatic nerve, $K^+$ currents ($K_\text{a}$, $K_{\text{dr}}$, and calcium-activated $K^+$ currents) decreased in DRG neurons (Abdulla and Smith 2001b; Everill and Kocsis 2000) and an increased density of hyperpolarization-activated cation current ($I_H$) that may contribute to increased repetitive firing (Yao et al. 2003). The hyperexcitability of medium-sized neurons may therefore contribute to the cutaneous hyperalgesia that develops ipsilateral to a CCD injury (Song et al. 1999; Zhang et al. 1999).

The main signs of hyperexcitability in cutaneous, medium-sized neurons were a decrease in the threshold current/voltage required to generate an AP and an increased capability to repetitively fire. Several factors may contribute to the enhanced excitability observed in CCD neurons—a hyperpolarizing shift in the TTX-S activation curve, an enhanced magnitude of TTX-R current, and a reduction in $K_\text{a}$ current in CCD compared with control neurons. The shift in TTX-S activation would cause a net increase in inward current at a more hyperpolarized potential and lead to a more negative voltage threshold for a regenerative depolarization to occur. An increase in TTX-R current would also support a more negative threshold voltage, because the threshold for a regenerative depolarization (Table 1) is above the threshold for TTX-R activation (Fig. 3). A decrease in $K_\text{a}$ currents could also lead to a decrease in the accommodative properties of neurons after CCD, thereby increasing their capacities to fire repetitively (Yoshimura and de Groat 1999).

Changes in Na channel expression or activity have been observed in other models of neuropathic pain, but the direction of change has been variable. A partial or total transection of the sciatic nerve caused an up-regulation of Nav 1.3, a TTX-S isoform that is usually expressed only in the neonatal period (Black et al. 1999; Dib-Hajj et al. 1999; Waxman et al. 1994). Nav 1.3 is similarly up-regulated after carrageenan inflammation of the ipsilateral paw (Black et al. 2004; Tanaka et al. 1998). Although we did not identify changes in specific isoforms after CCD, we observed no change in the magnitude or steady-state properties associated with the inactivation of TTX-S current in our neurons. This suggests that there was no major change in TTX-S isoforms expressed in our cells. However, there was a leftward shift in the voltage of activation for the TTX-S current by 8 mV. Although the reason for this shift is not identified, a similar shift in activation is observed in hippocampal cells during exposure to pertussis toxin, suggesting that activation of a G protein–coupled inflammatory pathway may occur with CCD (Ma et al. 1994). Results on TTX-R currents have been variable. We observed an up-regulation of TTX-R current after CCD—a result also observed in small neurons after carrageenan inflammation (Black et al. 2004; Tanaka et al. 1998). In contrast, the message for TTX-R current isoforms decreased after sciatic nerve transection, but it is unclear whether the membrane current was similarly decreased (Black et al. 1999; Dib-Hajj et al. 1999; Waxman et al. 1994). The variability of expression changes in different models of neuropathic pain suggest that multiple mechanisms may give rise to enhanced excitability of DRG neurons, resulting in a similar behavioral outcome.
The increased expression of TTX-R and reduced expression of $K_a$ may both contribute to the tendency of CCD neurons to generate multiple APs during a sustained depolarization. TTX-R currents in DRG (primarily Nav.1.8) inactivate at more depolarized potentials than TTX-S currents and thus would be available to mediate a regenerative depolarization during a prolonged stimulus. Previous work from our laboratory also showed an increased expression of hyperpolarization-activated current ($I_h$) in CCD neurons. This current is activated during the AHP that follows an AP and leads to a sustained depolarizing current, resulting in repetitive firing (Yao et al. 2003). Thus multiple changes in Na channel expression, $K_a$ channels, and $I_h$ currents may contribute to repetitive AP generation in CCD neurons.

In summary, this study identified several changes in Na$^+$ and K$^+$ current characteristics that may explain the enhanced excitability of DRG neurons after CCD. These are 1) a negative shift in the voltage of activation of TTX-S currents but no change in overall level of expression, 2) an increase in TTX-R current expression, and 3) a decreased expression of a fast inactivating K$^+$ current.

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