Inflammatory Mediators Enhance the Excitability of Chronically Compressed Dorsal Root Ganglion Neurons

C. Ma, K. W. Greenquist, and R. H. LaMotte
Department of Anesthesiology, Yale University School of Medicine, New Haven, Connecticut

Submitted 15 July 2005; accepted in final form 22 December 2005

Ma, C., K. W. Greenquist, and R. H. LaMotte. Inflammatory mediators enhance the excitability of chronically compressed dorsal root ganglion neurons. J Neurophysiol 95: 2098–2107, 2006. First published December 28, 2005; doi:10.1152/jn.00748.2005. A laterally herniated disk, spinal stenosis, and various degenerative or traumatic diseases of the spine can sometimes lead to a chronic compression and inflammation of the dorsal root ganglion and chronic abnormal sensations including pain. After a chronic compression of the dorsal root ganglion (CCD) in rats, the somata in the dorsal root ganglion (DRG) become hyperexcitable, and some exhibit ectopic, spontaneous activity (SA). Inflammatory mediators have a potential role in modulating the excitability of DRG neurons and therefore may contribute to the neuronal hyperexcitability after CCD. In this study, an inflammatory soup (IS) consisting of bradykinin, serotonin, prostaglandin E2, and histamine (each $10^{-6}$ M) was applied topically to the DRG. The responses of DRG neurons were electrophysiologically recorded extracellularly from teased dorsal root fibers or intracellularly from the somata in the intact DRG or from dissociated neurons within 30 h of culture. In all three preparations, IS remarkably increased the discharge rates of SA CCD neurons and evoked discharges in more silent-CCD than control neurons. IS slightly depolarized the resting membrane potential and decreased the current and voltage thresholds of action potential in both intact and dissociated neurons, although the magnitude of depolarization or decrease in action potential threshold was not significantly different between CCD and control. IS-evoked responses were found in a proportion of neurons in each size category including those with and without nociceptive properties. Inflammatory mediators, by increasing the excitability of DRG somata, may contribute to CCD-induced neuronal hyperexcitability and to hyperalgesia and tactile allodynia.

INTRODUCTION

In humans, spinal stenosis or a laterally herniated disc can compress the dorsal root ganglion (DRG) causing swelling, paresthesias, and leg pain (Aota et al. 2001; Nordin et al. 1984). In animals, an experimental compression of the DRG increases endoneurial fluid pressure that in turn can decrease blood flow (Igarashi et al. 2005; Rydevik et al. 1989). The resulting ischemia along with any mechanical trauma caused by the compression could initiate an inflammatory reaction and the release of a variety of pro-inflammatory mediators derived from the circulation and from neurons as well as nonneuronal cells in the ganglion. Inflammatory mediators released in injured tissue include neurotrophins, cytokines, peptides, proteins, free radicals, histamine, bradykinin, serotonin, and prostanooids (Dray 1995).

After a chronic compression of the DRG (CCD), produced in rats by the implantation of a rod into the intervertebral foramen, the neuronal cell bodies (somata) in the ganglion become hyperexcitable and some exhibit ectopic, spontaneous activity (SA) (Hu and Xing 1998; Zhang et al. 1999). Hyperexcitable somata and SA originating in the DRG might contribute to CCD-induced cutaneous hyperalgesia for example by initiating and maintaining the sensitization of neurons in the dorsal horn or by amplifying activity generated from cutaneous receptors. The present study examines the effects of a soup of inflammatory mediators (IS) consisting of bradykinin, serotonin, prostaglandin E2, and histamine (each $10^{-6}$ M) in activating neuronal somata of different size after CCD. Multiple components of IS are derived from the circulatory system and from both neurons and nonneuronal cells in the ganglion such as satellite glia, fibroblasts, and macrophages (Lerner et al. 1992; McMahon et al. 2005). There is little information as to whether inflammatory mediators can enhance ongoing SA or generate SA in silent neurons in the DRG after CCD. However, the incidence of the SA, recorded electrophysiologically from the acutely decompressed DRG, is higher in vivo (20%) than it is in vitro in the absence of blood-borne cells and inflammatory mediators (10%) and still lower in acutely dissociated somata that are separated from their satellite glia and other nonneuronal cells (5%) (Hu and Xing 1998; Ma and LaMotte 2005; Song et al. 1999).

Although components of IS might be expected to activate certain nociceptive neurons with small somata, as can occur after their acute dissociation (Kress and Reeh 1996; Kress et al. 1997; Nicol et al. 1997), their effect on nonnociceptive versus nociceptive neurons with large somata and myelinated axons is largely unstudied. SA in larger neurons might contribute, if not to pain or hyperalgesia, to the nonpainful paresthesias that can occur during movement or certain postures in patients with DRG compression (Nordin et al. 1984). After CCD, the incidence of SA in large somata is approximately the same as it is for medium and small somata (Zhang et al. 1999).

The present study found that IS elicits action potentials in subpopulations of neurons of all sizes after CCD (but not in control neurons) including those with and without nociceptive properties. It is further shown that the increased excitability to IS after CCD is an endogenous property of the neuronal soma because it remains after acutely dissociating of somata from their axons and other cells in the ganglion. Preliminary data have been published in abstract form (Greenquist et al. 2002; Ma et al. 2003a).
METHODS

Surgical procedure for rod implantation

Fifty-four adult female Sprague-Dawley rats weighing 150–180 g were housed in groups of three or four in a climate-controlled room under a 12-h light/dark cycle. The use and handling of animals was approved by the Institutional Animal Care and Use Committee of the Yale University School of Medicine and was in accordance with guidelines provided by the National Institutes of Health and the International Association for the Study of Pain.

In CCD rats (n = 30), under pentobarbital sodium anesthesia (Nembutal, 50 mg/kg ip), the transverse process and intervertebral foramina of L₄ and L₅ were exposed unilaterally as previously described (Song et al. 1999). A stainless steel L-shaped rod (0.63 mm diam and 4 mm length) was inserted into each foramen, one at L₄ and the other at the L₅ ganglion. The incision was closed in layers. Five to 10 days after surgery, the correct placement of each implanted rod was confirmed when the ganglion was harvested for recording. Because all the rods were in the correct position (Song et al. 1999; Yao et al. 2003), all 60 DRGs from CCD animals were accepted for the experiment. Twenty-four unoperated rats were used as controls.

Chemical solutions

The chemical stimulus was an “inflammatory soup” (IS) consisting of bradykinin (BK), serotonin (5-HT), prostaglandin E₂ (PGE₂), and histamine (10⁻⁶ M) in artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM) 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 1.2 MgCl₂, 1.2 CaCl₂, and 180 dextrose. The solution was bubbled with 95% O₂-5% CO₂ and had a pH of 7.4 and an osmolality of 290–310 mosM. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. The decision to use 10⁻⁶ M IS was based on the results of published studies in which IS excited the endings of nociceptive afferents or the cell bodies of DRG neurons at the same dose or higher (Kessler et al. 1992; Lang et al. 1990). It was also based on the results of a pilot experiment with five SA CCD Aβ dorsal-root fibers in our laboratory. For each fiber, concentrations of 10⁻⁷, 10⁻⁸, 10⁻⁶, and 10⁻⁵ M were applied to the DRG for 3 min at interstimulus intervals of 40 min. None of the fibers responded to the lowest dose of IS (10⁻⁸ M). The peak discharge rate increased monotonically between doses of 10⁻⁷ and 10⁻⁵ M. Because the mean latency and duration of response saturated between the two highest doses, the next to highest dose of 10⁻⁶ M was chosen for use in the present study.

Rationale for the use of each of three physiological preparations

Our first preparation was intended to determine the extent to which IS, applied to the intact DRG, evoked action potentials in dorsal root fibers. The fiber-dissection and extracellular recording method allowed the sampling of SA and chemically evoked action potential activity in the entire DRG, i.e., the information that is actually sent to the DRG from the CNS. Our intracellular recordings in the intact DRG were confined to visualized somata on the surface of the DRG. Although we did not record from cells beneath the surface with this method, we were able to visualize and choose the size of each neuron from which information about passive and active membrane properties could be obtained. The advantage of the third preparation, i.e., recording from acutely dissociated neurons, is that the neuronal soma was separated from its axon and the effects of its neighbors, e.g., from chemical factors released by other cells that were adjacent to the soma in the intact ganglion. This allowed a test of whether the effects of IS were intrinsic to the soma. The use of sharp electrodes for both the intact DRG and dissociated neurons (and not patch electrodes for the latter) eliminated any differences in results due to the recording procedure, e.g., due to exchange of ions and chemicals between a patch pipette and the cell. The disadvantage of sharp electrode recording was not being able to isolate specific currents that could contribute to the excitatory effects of IS and its individual components.

Extracellular recording from dorsal root fibers of the intact ganglion

Extracellular recordings were obtained from 9 control (unoperated) and 11 CCD rats. As described previously (Zhang et al. 1997), the rats were first anesthetized with pentobarbital sodium (50 mg/kg ip). The L₄ and L₅ ganglia with attached dorsal roots and sciatic nerve were dissected surgically and placed in a recording chamber after the capsule was carefully removed. The DRG was perfused at a rate of 4 ml/min with ACSF. A two-way valve provided the means of switching between two reservoirs, one containing ACSF and the other, IS in ACSF. The perfusion solution (ACSF or IS in ACSF) was heated to maintain a bath temperature at 37°C. The dorsal root extended out from this chamber into an adjacent one that was filled with mineral oil. Action potentials from single dorsal root fibers were recorded extracellularly from dissected proximal endings of dorsal root microfilaments placed over a monopolar electrode. The discharges of single fibers were displayed on an oscilloscope and collected and analyzed with pClamp8 software from Axon Instruments (Union City, CA). Each neuron was initially classified by its conduction velocity (CV), defined as the distance between the simulation and recording site divided by the latency of an action potential (evoked by an electrical stimulation of the sciatic nerve). Neurons with unmyelinated axons (C-) had CVs of <2 m/s, whereas Aδ- and Aβ-neurons had CVs of 2–12 and >12 m/s, respectively (Waddell and Lawson 1990).

The basal discharge rate was defined as the mean discharge rate recorded for 3 min prior to the application of IS. IS was applied for 3 min and then washed out by the bath solution. To minimize the effects of tachyphylaxis, as investigated in a small number of experiments, the interval between two consecutive IS applications in the same DRG was >40 min.

Intracellular recordings from somata in the intact DRG

Fourteen CCD and 11 control rats were used for intracellular recording from visualized somata in intact DRG neurons, in vitro as previously described (Zhang et al. 1999). Briefly, under pentobarbital anesthesia (50 mg/kg ip), the L₄ and L₅ DRGs with their corresponding dorsal roots, spinal nerves, and sciatic nerve above the mid-thigh level were removed from the animal and transferred to a chamber perfused with oxygenated ACSF. The recording chamber was mounted on the stage of an upright microscope (BX50-WI, Olympus Optical, Tokyo). The bath solution was preheated to 36 ± 1°C (mean ± SD) by means of an in-line heater with a servo-controller (TC-344A, Warner Instrument, Hamden, CT). The intracellular recording electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL) and pulled on a Flaming/Brown micropipette puller (P-97, Sutter Instrument, Novato, CA). Electrodes were filled with 1.0 M KCl (impedance: 40–80 MΩ) and positioned by a micromanipulator (MIS-5000, Burleigh Instruments, Fisher, NY).

Electrophysiological recordings were collected with continuous current-clamp mode using an AxoClamp-2B (Axon Instruments, CA), stored digitally via a Digidata 1322A interface, and analyzed off-line with pClamp8 software (Axon Instruments). The electrode resistance was balanced by a bridge circuit in the amplifier. A neuron was accepted for study only when it exhibited a resting membrane potential (RMP) more negative than −45 mV. Current steps, each 100-ms duration were delivered in increments of 0.05 nA from −0.5 to 4 nA through the intracellular recording electrode. The input resistance (Rᵢ, MΩ) was obtained from the slope of a steady-state I-V plot in response to a series of hyperpolarizing currents steps from −0.5 to

J Neurophysiol • VOL 95 • APRIL 2006 • www.jn.org
−0.05 nA. The current threshold (CT, nA) of action potential was defined as the minimal depolarization current required to evoke an action potential. The voltage threshold (VT, mV) of action potential was defined as the minimal depolarization current required to evoke an action potential. The voltage threshold (VT, mV) of action potential was defined as the first point on the rising phase of the spike at which the change in voltage exceeded 50 mV/ms. The action potential amplitude (\( A_{P_{\text{amp}}} \), mV) was measured between the resting potential to the peak of the action potential. The conduction velocity of each neuron was measured via electrical stimulation delivered with a suction electrode to the dorsal root (CVdr). The action potential trace was differentiated (dV/dt) off-line using pClamp 8 software.

The presence of an inflection in the falling phase of the action potential, evoked by electrical stimulation of the dorsal root, is characteristic of nociceptive DRG neurons in vivo (Koerber et al. 1988; Ma et al. 2003b; Bitter and Mendell 1992; Stebbing et al. 1999). The inflection is detected as a slight plateau on the differentiated trace of action potential (Fig. 5, D and G). Because the CCD surgery did not change the shape of action potential of DRG (Zhang et al. 1999), we used the inflection criterion for putative nociceptor in this study. When multiple action potentials were encountered, only the first action potential was used.

Although it is common practice in some studies to deliver repetitive hyperpolarizing steps during chemical application to monitor the \( R_{\text{m}} \), we did not do so because in hyperexcitable CCD neurons such pulses sometimes evoke rebounding action potentials that could interfere with our observation of an IS-induced response. Instead we delivered the aforementioned series of current steps to test both \( R_{\text{m}} \) and action potential threshold once before, during, and after IS application.

IS \( (10^{-4} \text{M in ACSF}) \) was applied locally to the cell body of DRG neurons through a micropipette with a 100 μm diameter tip and a pressure-controlled drug application system (Automate). The tip of the micropipette was located ~100 μm from the neuron under study. Pilot data indicated that neurons responded (by depolarization) within 5 s when switching from normal ACSF to ACSF containing 20 mM KCl, 1 MgCl₂, 3 CaCl₂, 25 sorbitol, and 10 HEPES, adjusted to pH 7.2 with NaOH. The DRGs were digested for 20 min with collagenase A (1 mg/ml) and then further digested for another 20 min with collagenase D (1 mg/ml; Boehringer Mannheim) and papain (30 fibers/ml, Worthington Biochemical, Lakewood, NJ) in ACSF containing 0.5 mM EDTA and 2 μg cysteine at 37°C. The cells were dissociated by triturating 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 0.1 mg/ml polyornithine and 1 mg/ml laminin (Boehringer Mannheim). The culture medium contained equal amounts of Dulbecco’s modified Eagle medium and F12 (Gibco, Grand Island, NY) with 10% FCS (HyClone Laboratories, Logan, UT) and 1% Penicillin (100 U/ml)/Streptomycin (0.1 mg/ml); Life Technologies, Rockville, MD). The cells were incubated at 37°C (5% CO₂ balanced air) for 1 h after which culture medium without the trypsin inhibitor was added.

Intracellular recordings were obtained within 24–30 h after the dissociation. Within this time period, dissociated CCD neurons exhibited electrophysiological properties, such as the incidence of spontaneous activity, that were similar to those of somata in the intact DRG after CCD (Ma and LaMotte 2005). Coverslips were transferred to a recording chamber mounted on the stage of the upright microscope (as described). The chamber was perfused with a preheated (36 ± 1°C) bath solution containing (in mM) 130 NaCl, 3 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose, adjusted to pH 7.4 and osmolarity 300 mosM. The bottom of the recording chamber was made of aluminum and was electrically warmed to assist in maintaining the bath temperature in the recording chamber at 36 ± 1°C. IS \( (10^{-6} \text{M in bath solution}) \) was applied topically through bath perfusion. To avoid possible tachyphylaxis, the effect of IS was studied on only one neuron per coverslip. The intracellular recording methods were the same as described for the intact ganglion except for the absence of conduction velocity.

**Statistical analyses**

Student’s t-test (SigmaStat Version 2.03, SPSS, San Rafael, CA) was used to determine the statistical significance of differences between the electrophysiological parameters obtained from DRG neurons under different experimental conditions (CCD vs. control). Paired t-tests were used to determine the significance of differences in the number of action potentials evoked by current injection in the same neuron before and during IS application. \( \chi^2 \) tests were used to assess differences between experimental groups in the incidence of SA and the incidence of IS-evoked responses. A probability of 0.05 was chosen as the criterion for significance.

**RESULTS**

IS applied to the intact DRG evoked action potentials in dorsal root fibers of CCD but not in control neurons

Extracellular recordings were obtained from 70 single fibers in the L₄/L₅ dorsal roots of CCD rats and from 43 single fibers in control animals. None of the 14 C, 11 Aδ, and 18 Aβ fibers (“control” fibers) from control rats exhibited SA. Among the fibers in CCD rats, 47 exhibited spontaneous discharges prior to the application of IS and were classified as SA CCD. The remaining fibers were categorized as silent CCD. Our sampling bias of purposively searching for fibers that were SA accounted for the higher proportion of CCD neurons that were SA as opposed to silent.

The spontaneous discharge patterns in CCD neurons were classified as regular/continuous (11%), bursting (32%), or irregular (57%) as illustrated in Fig. 1. Aside from significantly...
greater discharge rates in A than in C fibers, the response profiles evoked by IS were remarkably similar for fibers of each type. IS application evoked an increase in the discharge rates in 38 of 45 (84%) SA CCD fibers (Fig. 1, A–D). IS also evoked discharges in 7 of the 23 (30%) silent CCD fibers tested (Fig. 1E). In contrast, only one control (silent) fiber responded to IS applied to the intact DRG.

FIG. 1. Typical patterns of spontaneous activity (SA) and typical responses to inflammatory soup (IS) in chronic compression of the dorsal root ganglion (CCD) neurons recorded extracellularly from dorsal root fibers. Spontaneous discharges in dorsal root ganglion (DRG) neurons were classified as continuous (A), bursting (B) and irregular (C, D) before IS application. All SA CCD neurons responded to IS with an increase in discharge rates. A: continuous SA in an Aβ neuron; B: bursting SA, in an Aδ neuron, that changed to a continuous pattern during IS; C: irregular SA in a C neuron; D: irregular SA, in an Aδ neuron, that became continuous during IS. E: irregular discharge evoked by IS in a silent Aδ neuron. Time scale: 1 s for A, B, C, and E, 200 ms for D.

FIG. 2. The percentage of extracellularly recorded dorsal root fibers that responded to IS applied to the intact DRG. The percentage is defined for each fiber type and experimental condition as the number of responsive neurons divided by the total number of neurons tested (top of each bar) × 100. ■, □, △, and □, percentages for SA CCD, silent CCD and control neurons, respectively.

Intracellular recordings were obtained from 89 somata of the intact DRG neurons and from 60 dissociated DRG somata. SA was present in 15% of the 48 intact CCD neurons and 13% of the 31 dissociated CCD neurons, but none of the 41 intact, control neurons and only 1 of the 29 dissociated, control neurons. Neurons exhibiting SA were found in each size category both in the intact DRG and after dissociation (Fig. 3). In the intact DRG, for each category of somal size, the mean current and voltage thresholds of action potential (CT and VT) were significantly lower for CCD than for control neurons (Table 1). There were no significant differences between CCD and control neurons in RMP, Rm, or APamp. The electrophysiological properties recorded from intact DRG neurons in Table 1 are similar to those previously published for CCD and control animals (Song et al. 2003a; Zhang et al. 1999).

The electrophysiological properties of dissociated CCD and control neurons (Table 2) were similar to those of intact DRG neurons with two exceptions: first, for dissociated neurons, CCD resulted in lower mean CTs and VTs for M and L neurons but not for S neurons. Second, for dissociated L neurons, the mean RMP was more depolarized after CCD than under control conditions.

Thus CCD increased the excitability of neurons in each size category as evidenced by the increased incidence of SA and the lower than normal action potential thresholds. For the most part, the same characteristics of CCD-induced hyperexcitability remained after the DRG somata were dissociated and placed in culture in agreement with previous results from our laboratory (Ma and LaMotte 2005).
TABLE 1.  Effects of IS on electrophysiological properties of intact DRG neurons from control and CCD rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>CCD</td>
<td>Control</td>
<td>CCD</td>
</tr>
<tr>
<td>Size, μm</td>
<td>9</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>28.2 ± 0.8</td>
<td>28.3 ± 0.5</td>
<td>39.1 ± 0.6</td>
</tr>
<tr>
<td>dRMP, mV</td>
<td>−60.8 ± 2.7</td>
<td>−62.6 ± 3.0</td>
<td>−63.3 ± 1.5</td>
</tr>
<tr>
<td>Vr, MΩ</td>
<td>6.6 ± 1.9†</td>
<td>4.1 ± 1.0†</td>
<td>2.7 ± 0.7†</td>
</tr>
<tr>
<td>dVr, MΩ</td>
<td>66.1 ± 14.0</td>
<td>59.6 ± 12.0</td>
<td>35.1 ± 4.8</td>
</tr>
<tr>
<td>Amp, mV</td>
<td>11.4 ± 7.9</td>
<td>−3.0 ± 7.9</td>
<td>0.1 ± 2.3</td>
</tr>
<tr>
<td>VT, mV</td>
<td>69.8 ± 3.5</td>
<td>70.7 ± 2.4</td>
<td>63.9 ± 3.2</td>
</tr>
<tr>
<td>dVT, mV</td>
<td>−5.5 ± 2.3‡</td>
<td>−2.9 ± 1.0‡</td>
<td>−3.1 ± 1.1‡</td>
</tr>
<tr>
<td>CT, nA</td>
<td>0.60 ± 0.12*</td>
<td>1.08 ± 0.15</td>
<td>0.79 ± 0.20*</td>
</tr>
<tr>
<td>dCT, nA</td>
<td>−0.22 ± 0.08‖</td>
<td>−0.31 ± 0.13‖</td>
<td>−0.29 ± 0.09‖</td>
</tr>
<tr>
<td>dCT, %</td>
<td>−34.3 ± 10.4</td>
<td>−26.3 ± 9.2</td>
<td>−33.5 ± 12.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = sample size. Results were obtained from intact dorsal rat ganglion (‡) DRG by intracellular recording. Neurons were classified into 3 categories based on the size of the soma. Size, mean diameter of the soma; RMP, resting membrane potential; Rmp, input resistance; Amp, amplitude of action potential; VT, voltage threshold of action potential; CT, current threshold of action potential. ΔM(ACT, resp.) (IS)-induced changes in electrophysiological properties (during IS — before IS application); dCT%, Percentage of IS-induced change in CT [100 × (during IS — before IS) / before IS]. *, P < 0.05, chronic compression of the DRG (CCD) vs. control. †, P < 0.05, during IS vs. before IS, i.e. IS-induced changes (d) were significant.

CCD somata were more responsive to IS than control somata

Responses to topical IS application were recorded intracellularly from somata of 48 CCD neurons and 41 control neurons in the intact DRG and from the dissociated somata of 31 CCD and 29 control neurons. Of the 31 CCD neurons, IS increased the discharge rate of six of seven intact and three of four dissociated neurons (Fig. 3, A–C). Of the silent CCD neurons, IS induced action potential discharges in 7 of 41 (15%) intact neurons and 4 of the 31 dissociated neurons (15%; Fig. 3, D and E). Of the control neurons, only one silent neuron in the intact DRG responded with action potentials. IS did not increase the discharge rate of the single, dissociated, control neuron with SA.

The percentages of neurons exhibiting action potentials in response to IS were similar for each of the three size categories for both intact and dissociated neurons (Fig. 4, A and B, respectively). The percentages were combined for SA and silent neurons. The total percentage of neurons responsive to IS was significantly higher in CCD- than in control neurons both for the intact DRG (27 vs. 2.4%, respectively) and for the dissociated condition (23 vs. 0%; P < 0.05, χ2 test).

The patterns of SA recorded from intact and dissociated CCD neurons (Fig. 3, A–C) were similar to those recorded from dorsal root fibers (Fig. 1, A–D). Most SA CCD neuron responded to IS with an increase in discharge rate that was accompanied for some neurons by a change in pattern (Fig. 3, B and C). During IS application, the mean discharge frequency increased from 20.6 ± 3.5 to 75.0 ± 10.8 Hz in the intact DRG and from 36.7 ± 8.8 to 56.7 ± 8.8 Hz in the dissociated neurons. The increase in discharge rate during IS application was usually accompanied by a slight depolarization of the RMP.

The presence of an inflection on the falling phase of the action potential, evoked by electrical stimulation of the dorsal root (Fig. 5, D and G), is characteristic of nociceptive DRG neurons in vivo (Koerber et al. 1988; Ma et al. 2003b; Ritter and Mendell 1992; Stebbing et al. 1999). All but one of the S neurons recorded from the intact DRG were infected (most with C-fiber CVs) and therefore could be categorized as the C-nociceptor group. The L and M neurons (most with A-fiber unclassified).
included both nociceptive and nonnociceptive neurons in both the control and CCD groups.

**Neurons in the intact DRG.** In the intact DRG, IS typically induced a slight depolarization in RMP and/or a reduction in the action potential threshold (CT and VT) (Fig. 5, for typical responses in L, M and S neurons). For both CCD and control neurons, in most of the size categories, IS evoked a significant change in RMP, CT, and VT ($P < 0.05$, paired $t$-test, Table 1). The $R_{in}$ increased during IS application in ~45% of neurons, decreased in 30%, and remained unchanged in the rest 25% neurons. These diverse responses resulted in a relative large variation of $d$-$R_{in}$ (IS induced change in $R_{in}$) and thus accounted for the absence of statistical significance ($P > 0.05$, paired $t$-test, Table 1). The changes in $R_{in}$ were not significantly correlated either with cell types or the changes in RMP and action potential threshold. The $AP_{amp}$ decreased slightly but significantly during IS application, along with the depolarized RMP in both CCD and control neurons ($P < 0.05$, paired $t$-test, Table 1).

We defined IS-induced excitation as a depolarization in RMP by $\geq 2$ mV and/or a decrease in CT for $\geq 0.2$ nA. By this criterion, most control (78% S, 69% M, and 75% L) and CCD (78% C, 70% M, and 79% L) neurons were excited by IS. The magnitude of RMP depolarization, change in $R_{in}$, decrease in $AP_{amp}$, CT, or VT, or the percentage decrease in CT was not significantly different for CCD and control neurons within each size category (Table 1). However, because of the CCD induced increase in excitability, CCD neurons were more likely than control to exhibit action potential discharges in response to IS.

**Dissociated Neurons.** Most dissociated neurons, like those in the intact DRG, were excited during the application of IS. The excited, dissociated neurons included both control (75% S, 80% M, and 82% L) and CCD (88% S, 75% M, and 82% L; Fig. 6). With one exception, CCD and control neurons did not differ significantly in the IS-induced magnitude of RMP depolarization, change in $R_{in}$, decrease in $AP_{amp}$, CT, and VT, and

**FIG. 4.** The percentage of intracellularly recorded somata that responded to IS with action potential discharges. The recordings were obtained from somata of small (S), medium (M), and large (L) diameter in the intact DRG (A) or in culture after dissociation (B). Percentage is defined for each neuronal type and experimental condition as the number of responsive neurons divided by the total number of neurons tested (top of each bar) $\times$ 100. ■ and □, SA neurons and silent neurons that responded to IS with action potential discharges, respectively. *, 1 SA neuron in this group did not respond to IS and was not included in the graph.

CVs) could be categorized as either inflected (putative nociceptive) or uninflected (nonnociceptive). We found that IS excited noninflected as well as inflected L and M neurons in both CCD and control groups. The changes in RMP, CT and VT produced by IS were similar for both inflected and noninflected neurons in each group. Thus the neurons excited by IS

**FIG. 5.** Effects of IS in depolarizing the membrane potential and lowering the current threshold of CCD somata in the intact DRG. Intracellular recordings were obtained from a large (A–C), a medium (D–F), and a small-sized (G–I) CCD neuron. Conduction velocities, obtained by electrically stimulating the dorsal root, were used to classify the neurons as Aβ (A), Aδ (D), and C (G). Insets in A, D, and G display differentiated action potential traces ($dV/dt$) of these indicate an inflection on the falling phase of the action potential (arrow) for the C and Aδ but not the Aβ neuron. Each neuron responded to a 60-s application of IS with a 4–10- mV depolarization (B, E, and H). The current threshold of each neuron decreased remarkably during IS application (C, F, and J). J: current protocol to measure current threshold in C, F, and J. Vertical scale: A–F: 10 mV; J: 1 nA. Time scale: A and D: 2 ms; G: 4 ms; B, E, and H: 10 s; C, F, I, and J: 20 ms.
the percentages of decrease in CT within each size category. The exception was the significantly higher percentage of decrease in the CT for CCD- than for control neurons of small size (Table 2).

For both CCD and control neurons, the effects of IS in evoking action potentials or decreasing the action potential threshold were not consistently related to the magnitude of the depolarization of RMP. Without an obvious change in RMP (<1 mV), IS evoked action potentials in one silent CCD neuron, increased discharge rates in two SA CCD neurons, and reduced action potential thresholds in four control and four CCD neurons. On the other hand, six control and seven CCD neurons were depolarized by IS (≥3 mV) without a corresponding change in action potential thresholds. In a silent CCD neuron, action potential discharges, accompanied by ~5-mV depolarization, was induced by IS application but not by current injections of ±2 nA that elicited a depolarization of ~10 mV in RMP (Fig. 7). These observations suggested that excitatory effects of IS were only partially or not at all due to the depolarization of the RMP.

Current injection during IS evoked more action potentials in CCD neurons but not in control neurons

The mean numbers of action potentials evoked by a 100-ms current injection of 1× and 1.5× current threshold level were compared for CCD and control neurons, before and during IS application. The results are presented separately for the intact and dissociated groups of neurons (Fig. 8, A and B, respectively). Because we found no significant differences in the numbers of action potentials evoked in small, medium, and large neurons, the data for each size category were combined within each of the two groups. Neurons in the intact and dissociated groups responded in similar fashion. For CCD neurons, but not for control neurons, more action potentials were evoked during IS than before IS (P < 0.01, paired t-test) for both 1× and 1.5× CT levels. Before IS application, there were no significant differences for either group between control and CCD neurons in the number of action potentials evoked by current injections of either 1× or 1.5× CT (P > 0.05, t-test). However, during IS application, CCD neurons (both intact and dissociated) exhibited significantly more action potentials than controls at 1× (P < 0.05, t-test) although not at 1.5× CT (probably due to the larger variation in response to the latter). Therefore IS decreased accommodation in CCD neurons but not in control neurons.

Discussion

DRG neurons are more excitable and more responsive to IS after CCD

Using both extra- and intracellular recordings from intact DRG neurons, we found that IS remarkably increased the discharge rates of SA CCD neurons. IS-induced changes in RMP or action potential threshold were similar in CCD and control neurons. However, silent (non-SA) CCD neurons were more likely to respond to IS than silent control neurons. The excitatory effects of IS in evoking action potential discharges and decreasing the action potential threshold were not invariably related to the depolarization of RMP in both CCD and control neurons. Another finding was that IS decreased accommodation in CCD neurons but not in control neurons. These results were found not only in the intact DRG but also in dissociated neurons. Because the dissociated somata were immediately surrounded by few or no satellite cells and were not in the proximity of other neurons, the similarity of results obtained from intact and dissociated neurons suggest that the IS-induced increases in excitability were intrinsic to the neuron.

In the absence of tissue injury or inflammation, IS or one or more of its components can evoke action potentials in subpopulations of primary afferent neurons, in vivo (Lang et al. 1990; Steen et al. 1996) as well as depolarize membrane potential and reduce the action potential threshold in vitro (Cardenas et al. 2001; Gold and Traub 2004; Song et al. 2003b). In the present study, IS produced a small depolarization of the resting potential of most neurons in both control and CCD ganglia including those with nociceptive and nonnociceptive
ceptive properties. Although the effects of individual inflammatory mediators may differ for large versus small DRG neurons, the combination of multiple mediators was found to have similar effects on all three size categories of neuron. In agreement with results obtained by Song et al. (2003a), we found that IS produced a small depolarization of the membrane potential and a decrease in the action potential thresholds of most (~80%) control A and C neurons in the intact DRG, recorded in vitro. Also in agreement with the effects of a chronic constriction injury (CCI) of the peripheral nerve (Song et al. 2003a), IS enhanced, after CCD, the rate of ongoing ectopic discharges and evoked action potentials in silent DRG neurons. However, in relation to the effects of IS on control neurons, CCI but not CCD significantly enhanced the magnitude of depolarization possibly because of a higher concentration of IS for the CCI study (10^-5 vs. 10^-6 M). Thus, despite differences in the type of injury (CCI but not CCD eliciting a partial anatomy and neuritis of the peripheral nerve), the similarity in the response to IS suggests common underlying ionic channel mechanisms.

Possible mechanisms underlying the IS-induced excitation of DRG neurons

Multiple ion channels may be involved in the mechanisms underlying the excitatory effects of IS. One possible candidate is the tetrodotoxin-resistant (TTX-R) Na^+ current, which is increased by both 5-HT and PGE_2 in a subpopulation of capsaicin-sensitive DRG neurons (Cardenas et al. 1997; Gold et al. 1996). BK depolarizes and evokes action potentials in a subpopulation of TTX-R primary afferent neurons via B2 receptor (Jeftinija 1994). One subtype of TTX-R Na^+ channel, Nav1.8 (SNS/PN3), may contribute to the action potential threshold without affecting the RMP and R_in, whereas another subtype, Nav1.9 (NaN), modulates the RMP (for review, Gold 2000). PGE_2 also suppresses an outward potassium current (Nicol et al. 1997). These effects are mediated by the camp/protein kinase A and C (PKA and PKC) pathways (Cui and Nicol 1995; Evans et al. 1999; Gold et al. 1998). Serotonin activates one nociceptive-like subpopulation of DRG neurons, but not another, through the activation of a 5-HT_4 receptor-coupled cAMP-dependent signaling pathway that increases a TTX-R Na^+ current via PKA-mediated phosphorylation (Cardenas CG et al. 1997; Cardenas LM et al. 2001). Calcium and Ca^{2+}-activated K^+ channels, which are modulated by 5-HT (Cardenas et al. 1995), may also contribute to the action potential discharges by affecting the accommodative properties of DRG neurons. As for nonnociceptive neurons, 5-HT increases hyperpolarization-activated current (_{i_H}) in medium- and large-diameter DRG neurons via activation of 5-HT_7 receptors; this shifts the voltage dependence of _{i_H} to more depolarized potentials and increases neuronal excitability (Cardenas et al. 1999). PGE_2, but not 5-HT, increases a tetrodotoxin-sensitive (TTX-S) Na^+ current in subpopulations of small- and medium-diameter neurons (Cardenas et al. 1997).

Dissociated DRG neurons are by no means uniform in their functional properties and have been classified not only by size but according to other factors such as their differences in electrophysiological characteristics, chemosensitivity and immunoreactivity (Cardenas et al. 1995; Petruska et al. 2002). Such differences, for example in the expression of receptors and various ionic currents between one cell type and another, are likely to contribute to individual differences we have observed in responses to IS, such as changes in the magnitude of depolarization, accommodation and R_in.

The wide range of changes in R_in suggests the possibility of different underlying mechanisms. For example, R_in could be decreased by a depolarization of the RMP and an increase in _{i_H} currents (Cardenas et al. 1999) or increased by the inhibition of K^+ currents (Nicol et al. 1997) or unaffected by an enhancement of Na^+ currents (Cardenas et al. 1997; Gold et al. 1996) or reduction of Ca^{2+} currents (Cardenas et al. 1995).

Although the absolute amount of depolarization induced by IS appeared to be small (~5 mV) in both CCD and control neurons, it was sufficient to elicit action potentials in hyperexcitable CCD neurons exhibiting lower than normal action potentials and, in some cases, SA. The candidate mechanisms we have discussed, while contributing to increased excitability, would not necessarily lead to the excitation of action potentials, particularly in those silent CCD the action potential thresholds of which would not be reached by a slight depolarization of the membrane potential. The mechanisms of excitation by IS deserve further study particularly with the use of patch-clamp recording and the isolation of individual currents.

The decrease of AP amplitude during IS application was probably due to the depolarization in resting potential, which reduced the driving force of voltage-gated sodium currents. The possible effect of TTX-R and/or TTX-S Na^+ current upregulation on increasing the AP amplitude could be compensated by the depolarization of resting potential. Nevertheless, we did observe increased AP amplitude in a few neurons (n = 6, from both CCD and control animals) excited by IS but without obvious change in resting potential.
Cardenas et al. (1999) found that 5-HT increased $I_H$ current and facilitated the anode-break excitation in subpopulations of large and medium neurons. We did not find that IS decreased accommodation in control neurons. However, it was possible that accommodation decreased only in a subpopulations of control neurons exhibiting an increase in $I_H$ current during IS application. The lower dose of 5-HT used in our study (1 vs. 2106 C. MA, K. W. GREENQUIST, AND R. H. LA MotTE

Functional significance of the excitatory effects of inflammatory mediators on DRG neurons after injury

We found that the neurons responsive to IS were in each size or CV category and included those with and without nociceptive properties. Although the intracellular recording from intact DRG only sampled neurons from the surface of ganglion, this method has been used in vivo and included most types of peripheral receptive fields (Ma et al. 2003b). We found more SA CCD neurons from extracellular recording than from intracellular recording probably because the searching method in the prior was biased toward the SA units. Michaelis et al. (2000) discovered that after peripheral nerve axotomy, muscle spindle units were more likely to develop spontaneous discharge than cutaneous units. However, because the CCD model directly injured the DRG but not the peripheral nerve, the SA neurons found in CCD animal were not limited to muscle spindles but included neurons in each size and CV category (Song et al. 2003a; Zhang et al. 1999). These included neurons with myelinated axons and an inflection on the falling phase of their action potentials—a characteristic of nociceptive but not muscle-spindle neurons (Koerber et al. 1988; Ma et al. 2003b; Ritter and Mendell 1992; Stebbing et al. 1999).

Among the neuropathic or inflammatory pain models, the CCD model is unique in that the injury and inflammation are located in the ganglion as opposed to the spinal nerve [spinal nerve ligation or Chung model (Kim and Chung 1992), the peripheral nerve [chronic nerve constriction injury or CCI model (Bennett and Xie 1988)] or in the innervated tissue [injection of complete Freund’s adjuvant, CFA (Stein et al. 1988)]. After CCD surgery, the somata of DRG neurons are no longer mechanically compressed but also very likely surrounded by a mixture of inflammatory mediators released by inflamed tissues, macrophages, and lymphocytes. However, during the in vitro experiments, in the intact or dissociated neurons, the DRG somata are no longer compressed and are deprived of their in vivo, chemical environment. Our finding that IS enhanced SA and evoked discharges in CCD neurons under these deprived conditions suggest that an even higher level of excitability may occur in vivo during CCD, thereby contributing to the higher incidence of SA observed in vivo as opposed to in vitro (Hu and Xing 1998; Ma and LaMotte 2005; Song et al. 1999). It is possible that such abnormal ectopic discharge in a subpopulation of nociceptive neurons could induce a chronic sensitization of dorsal horn neurons that receive a convergent input from both nociceptive and nonnociceptive primary afferent neurons. Thus inflammatory mediators, by increasing the excitability of DRG somata, might contribute to CCD-induced hyperalgesia and tactile allodynia (Ma et al. 2003b).

ACKNOWLEDGMENTS

We thank D. Donnelly and H. Yao for technical assistance.

GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-14624 and NS-38317.

REFERENCES


Kessler W, Kirchhoff C, Reeh PW, and Handwerker HO. Excitation of cutaneous afferent nerve endings in vitro by a combination of inflammatory.