Enhanced Excitability of Sensory Neurons in Rats With Cutaneous Hyperalgesia Produced by Chronic Compression of the Dorsal Root Ganglion

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Zhang, Jun-Ming, Xue-Jun Song, and Robert H. LaMotte. Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. J. Neurophysiol. 82: 3359–3366, 1999. Pain and hyperalgesia can occur when the dorsal root ganglion (DRG) and its roots are deformed mechanically in association with injuries or diseases of the spine. To evaluate the electrophysiological changes that contribute to this sensory pathology, intracellular recordings were obtained in vitro from DRGs that had received a chronic mechanical compression [chronic compression of DRG (CCD)]. The compression was produced by inserting L-shaped rods ipsilaterally into the intervertebral foramina, one at L4 and the other at L5, in rats 1–14 days before the recording. Control rats received a sham operation. Postoperatively, the threshold force applied by punctate stimulation of the plantar surface of the hind paw decreased significantly on the foot ipsilateral to the CCD (mechanical hyperalgesia) but changed little on the contralateral foot or on either foot for control rats. DRG somata were viewed through a microscope during recording and classified as small, medium, and large according to their diameters. CCD cells in each size category were more excitable than those of comparable size from control rats as judged by the significantly lowered threshold currents and action potential voltage thresholds. Spontaneous activity was recorded in 11% of all the CCD cells tested. The spontaneous activity and/or changes in both the threshold currents and action potential thresholds were observed as early as one day after injury. The association of cutaneous hyperalgesia with changes in the electrophysiological properties of DRG cells suggests a possible role for intrinsic alterations in the membrane properties of compressed DRG cells in the production and persistence of chronic pain after certain spinal injuries or pathologies of the spine.

INTRODUCTION

Compression of the dorsal root ganglion (DRG) and its nerve roots is a possible consequence of such disorders as foraminal or spinal stenosis, disk herniations, degenerative disorders, tumors, or spinal injuries (e.g., Briggs and Chandraraj 1995; Poletti 1996). When an uninjured DRG is experimentally compressed by a mechanical stimulus, applied in vivo (Howe et al. 1977) or in vitro (Sugawara et al. 1996), action potentials may be evoked and firing may persist for several minutes after removal of the stimulus. The same stimulus applied to the adjacent spinal nerve or dorsal root elicits either a transient response or none at all. It is therefore possible that acute or chronic compression of the DRG may contribute, in some instances, to low back pain or sciatica and other painful sequelae of spinal injuries or disease.

Recently, it was discovered that a chronic implantation of a rod into the lumbar intervertebral foramen of the rat, presumably compressing the DRG, produced behavioral evidence of chronic cutaneous hyperalgesia on the ipsilateral hind paw (Hu and Xing 1998; Song et al. 1997, 1999). Extracellular electrophysiological recordings from teased dorsal root fibers from the chronically compressed DRG (CCD), revealed the presence of abnormal ectopic discharges in subpopulations of neurons with myelinated or unmyelinated axons. The discharge originated within the DRG and persisted in vivo (Hu and Xing 1998) and in vitro (Song et al. 1997) after removal of the implanted rod. The patterns of ectopic discharge were similar to those recorded from primary sensory neurons with transected peripheral axons (e.g., Burchiel 1984; DeSantis and Duckworth 1982; Devor 1994 for review; Kajander et al. 1992; Wall and Gutnick 1974; Zhang et al. 1997a,b). The presence of ectopic discharges originating from the compressed ganglion suggests that the DRG somata have become hyperexcitable despite having intact, functioning axonal processes.

In the present study, intracellular recordings from the DRG provide further evidence that an enhanced excitability of the somata may initiate the events leading to pain and hyperalgesia resulting from a chronic compression of the ganglion.

METHODS

Surgical procedure for rod implantation

Female Sprague-Dawley rats weighing 120–150g were anesthetized with pentobarbital sodium (40 mg/kg ip). After a midline incision from L4 to L6, the right paraspinal muscles were separated from the transverse process and the entry for the L5 and the L4 intervertebral foramina exposed. In each of 16 rats, an L-shaped rod of stainless steel (4 × 2 mm length and 0.6 mm diam) was inserted unilaterally into each foramen at an angle of 30° to the midline without exposing the ganglia. A slight twitch in the ipsilateral leg typically was observed during the insertion. The L-shape prevented any possibility of the rod moving into the cauda equina. The incision was closed in layers. An identical operation was performed in 13 additional rats without rod insertion (“control rats”).

Behavioral observations and tests

Foot withdrawal to mechanical stimulation was measured on each of 2 days before and on every other day after surgery until the day of electrophysiological recording. During each test, the rat was placed in a clear plastic cage with a floor of wire mesh with 1 × 1 cm openings.
The cage was elevated so that stimulation could be applied to each hind foot from beneath the rat. Von Frey filaments capable of exerting bending forces of 5, 10, 20, 40, 60, 80, and 120 mN, but each having the same tip diameter of 0.1 mm, were applied to 10 designated loci distributed over the plantar surface of the foot (LaMotte et al. 1998; Song et al. 1997, 1999). Each filament was applied alternately to each foot and to each locus. The filaments were applied in order of ascending force. The percentage of withdrawals was plotted as a function of force, and the threshold was defined as the force corresponding to a 50% withdrawal, as determined by linear interpolation. Mechanical hyperalgesia for a given rat was defined as a postoperative decrease of ≥20 mN from the mean preoperative threshold.

**Electrophysiological recording**

Recordings were obtained from CCD rats on postoperative days 1 (n = 2), 3 (n = 2), 6 (n = 4), 9 (n = 3), and 14 (n = 5). Recordings from control rats were obtained on postoperative days 1 (n = 2), 3 (n = 1), 6 (n = 2), 9 (n = 4), and 14 (n = 4). The rat was anesthetized with pentobarbital (50 mg/kg ip). The sciatic nerve was isolated from surrounding tissue, transected at the mid-thigh level, and its proximal portion traced to the ganglia. A laminectomy was then performed at the level of L3-L5. The L4 or L5 DRG and its dorsal roots were identified. The locations of the rods were checked immediately on exposure of the ganglia. Oxygenated artificial cerebrospinal fluid (ACSF), consisting of (in mM) 130 NaCl, 3.5 KCl, 1.25 NaH2PO4, 24 NaHCO3, 10 dextrose, 1.2 MgCl2, and 1.2 CaCl2 (pH = 7.2), was dripped periodically onto the surface of the ganglion during the surgical procedure to prevent drying and hypoxia. The ganglion and the attached peripheral nerve were removed from the rat and placed in a 35-mm petri dish filled with oxygenated ACSF. Under the dissecting microscope, the perineurium and epineurium were peeled away from the ganglion with fine forceps and the dorsal roots transected adjacent to the ganglion.

After 30 min in oxygenated ACSF, kept at room temperature (23–24°C), the ganglion and attached nerve were placed in the recording chamber and mounted on the stage of an upright microscope (BX50-W1, Olympus). A U-shaped stainless steel rod with two pieces of fine silver wire crossed from one side to the other was used to gently hold the ganglion in place within the recording chamber. The DRG was perfused continuously with oxygenated ACSF at a rate of 2 ml/min. The temperature was maintained at 35 ± 1 °C (mean ± SD) by a temperature controller (TC-344A, Warner Instruments).

DRG cells at different levels of the ganglion were visualized under differential interference contrast (DIC) (Fig. 1). To compare the structure of the ganglia in control and CCD rats, the images of the ganglia were captured by a frame grabber (Snappy, Japan) through a CCD camera (Hamamatsu, Japan). Intracellular, electrophysiological recordings were made from each cell with a microelectrode filled with 2 M potassium acetate (pH = 7.2). Satisfactory recordings were obtained with electrodes of 50–80 MΩ. Before electrode penetration, the DRG soma was classified visually by the diameter of its soma as small (≤30 μm), medium (31–49 μm), or large (≥50 μm). The electrophysiological data were collected with the use of single-electrode continuous current clamp (AxoClamp-2B, Axon Instruments) and analyzed with pClamp 6 software (Axon Instruments).

To compare the excitability of neurons from normal and CCD rats, we measured the threshold current, action potential (AP) threshold, resting membrane potential (Vm), input resistance (Rm), and afterhyperpolarization (AHP) of each DRG cell (e.g., Czeh et al. 1977; Villiere and McLachlan 1996). The Vm was taken 3 min after a stable recording first was obtained. Depolarizing currents of 0.05–4 nA (100-ms duration) were delivered in increments of 0.05 nA until an AP was evoked. The threshold current was defined as the minimum current required to evoke an action potential. The AP voltage threshold was defined as the first point on the rising phase of the spike at which the change in voltage exceeded 50 mV. The duration of the AP was measured at the threshold voltage. The AP amplitude was measured between the peak and the AP threshold. The Rm for each cell was obtained from the slope of a steady-state I-V plot in response to a series of hyperpolarizing currents, 100-ms duration delivered in steps of 0.05 nA from 0.2 to −2 nA. The AHP amplitude was measured from the valley peak to the baseline, and the AHP duration was measured at an amplitude half way between.

The axonal conduction velocity was measured by delivering a series of current pulses (3–5 mA, 0.2–0.6 ms) to the nerve at 1 Hz and recording the evoked action potential with the intracellular electrode. The conduction velocity was calculated by dividing the minimal latency of the evoked action potential into the distance between the stimulating electrode and the center of the ganglion.

**Statistical analysis**

Student’s t-tests were used to test the significance of differences in withdrawal thresholds and differences in electrophysiological measurements between control and CCD rats. A probability of 0.05 was chosen as the criterion for significance.
RESULTS

Mechanical threshold

Mechanical hyperalgesia was present in all 16 CCD rats including 2 rats tested only 1 day after surgery. The mean threshold on the foot ipsilateral to the CCD decreased from 57.8 ± 3.1 (SE) mN before surgery to 13.2 ± 3.7 mN after surgery, measured on the day of electrophysiological recording. The withdrawal thresholds for the two rats studied one day after surgery decreased from 50 and 60 mN to 5 and 15 mN, respectively. The postoperative withdrawal reflex was typically larger and of longer duration than the preoperative and often followed by paw licking. For the foot contralateral to the CCD, the difference between preoperative and postoperative mean thresholds, averaged for all rats tested, did not reach statistical significance (52.9 ± 3.9 before vs. 42 ± 6.0 mN after, t-test, P > 0.05). However, three rats each had a mean postoperative threshold on the contralateral foot that was >20 mN lower than the mean preoperative threshold. None of the control rats exhibited significant decreases in threshold on either foot over the same period of testing in which the CCD rats exhibited hyperalgesia. Their mean thresholds were 50.9 ± 1.9 before and 55.9 ± 2.9 mN after surgery.

Two CCD rats maintained a posture of standing with the ipsilateral foot lifted most of the time and occasionally held it lifted while walking. However, they did use the foot when prodded. Two other rats used the affected foot when walking but otherwise kept it lifted. These behavioral signs suggested the presence of mechanical hyperalgesia. Some of the rats occasionally licked the affected foot while holding it in a lifted position, possibly indicating the presence of spontaneous pain.

Neither autotomy nor deformation of the affected paws was observed in any rat suggesting that most peripheral axons remained intact despite the CCD.

Appearance of the ganglion

Under a dissection microscope, the inserted rod was found to be located on top of the ganglion in most cases and, in a few cases, alongside it as described by Song et al. (1999). The rod and the ganglion often were covered with connective tissue enriched with blood vessels. Removal of a centrally located rod often left a slightly depressed imprint on the ganglion. Under higher magnification, the appearances of each control and compressed ganglion was observed before electrophysiological recording. In most rats, increased vascularization could be seen on the surface of the ganglion, especially within the area that had been mechanically deformed by the inserted rod. On removal of the epineurium and perineurium, the ganglia from normal rats were clean and the neural somata well exposed. Scattered satellite cells could be seen between neurons (Fig. 1A). The compressed ganglia appeared to be covered by a layer of connective tissue that was somewhat difficult to remove. More nonneuronal cells could be seen around each neuronal cell body (Fig. 1B). These could represent an increase in satellite cells and/or macrophages (Lu and Richardson 1993).

Spontaneous discharge and discharge patterns

A cell was defined as spontaneously active if the spontaneous firing lasted more than three minutes after a stable record-

| TABLE 1. Electrophysiological characteristics of DRG cells from control and injured (CCD) ganglia |
|-----------------|-----------------|-----------------|-----------------|
|                 | Small           | Medium          | Large           |
|                 | Control         | CCD             | Control         | CCD             |
| Cell size, μm   |                 |                 |                 |
| n               | 26              | 36              | 27              | 26              |
| V_m, mV         | 28.96 ± 0.38    | 28.52 ± 0.38    | 40.95 ± 0.91    | 38.46 ± 0.85    |
| n               | 26              | 36              | 27              | 26              |
| AP threshold, mV|                 |                 |                 |
| n               | 26              | 36              | 27              | 16              |
| V_m, mV         | -61.94 ± 1.69   | -61.2 ± 1.67    | -63.91 ± 1.43   | -64.50 ± 1.30   |
| n               | 26              | 36              | 27              | 16              |
| AP duration, ms |                 |                 |                 |
| n               | 26              | 34              | 27              | 16              |
| V_m, mV         | 3.26 ± 0.24     | 3.38 ± 0.29     | 1.96 ± 0.13     | 2.27 ± 0.23     |
| n               | 26              | 34              | 27              | 16              |
| Threshold current, nA | 0.90 ± 0.08 | 0.58 ± 0.05** | 1.68 ± 0.14 | 0.72 ± 0.08** |
| n               | 26              | 34              | 27              | 26              |
| AHP amplitude, mV|                 |                 |                 |
| n               | 26              | 34              | 27              | 26              |
| V_m, mV         | 15.51 ± 1.03    | 15.2 ± 0.89     | 12.94 ± 1.17    | 13.9 ± 1.06     |
| n               | 26              | 34              | 27              | 26              |
| AHP duration, ms|                 |                 |                 |
| n               | 26              | 34              | 27              | 26              |
| V_m, mV         | 4.49 ± 0.35     | 4.18 ± 0.33     | 2.42 ± 0.16     | 4.47 ± 0.63**   |
| n               | 26              | 34              | 27              | 26              |
| V_max, mV/ms    | 188.45 ± 13.3   | 159.9 ± 12.0    | 191.9 ± 20.6    | 217.2 ± 22.9    |
| n               | 26              | 34              | 27              | 26              |
| AP amplitude, mV|                 |                 |                 |
| n               | 26              | 34              | 27              | 26              |
| V_m, mV         | 56.1 ± 2.0      | 61.2 ± 7.3      | 55.1 ± 2.2      | 62.8 ± 2.7*     |
| n               | 26              | 34              | 27              | 26              |
| CV, m/s         |                 |                 |                 |
| n               | 26              | 34              | 27              | 26              |
| V_m, mV         | 1.0 ± 0.13      | 1.13 ± 0.15     | 8.62 ± 2.13     | 8.42 ± 1.49     |
| n               | 8               | 9               | 6               | 8               |
| R_m, MΩ         |                 |                 |                 |
| n               | 20              | 33              | 17              | 26              |

Values are means ± SE and n = sample size. DRG, dorsal root ganglion; CCD, chronically compressed DRG; V_m, resting membrane potential; AP, action potential; AHP, after hyperpolarization; R_m, input resistance; CV, conduction velocity; V_max, maximum depolarization rate. * P < 0.05; ** P < 0.01.
significantly greater for CCD than for control cells. There were no such significant differences in these parameters for cells in the other two size categories (Table 1).

Measurements of the excitability of large-sized cells recorded the day after surgery were compared for CCD and control rats. The mean AP threshold was significantly lower for CCD than control cells ($255.96 \pm 2.01$ mV, $n = 6$ vs. $240.4 \pm 2.7$ mV, $n = 23$). Similarly, the mean threshold current was significantly lower for CCD than for control ($0.47 \pm 0.08$ nA, $n = 6$ vs. $1.89 \pm 0.21$ nA, $n = 24$). Comparable data were not obtained at this time for small- and medium-sized cells.

**Axonal conduction velocity**

The conduction velocity was measured in 32 of 109 (29%) CCD neurons, 5 of which were spontaneous active (4 Aβ and...
The afterdischarge evoked in one Aβ cell by a single stim-
ulus pulse lasted ~10 min.

**DISCUSSION**

**Intracellular mechanisms of enhanced excitability induced by DRG compression**

Because there was no significant change in the whole cell input resistance or resting membrane potential after the compression injury, the lowered threshold currents of CCD cells may be a consequence of the lowered AP thresholds. We do not know what ionic mechanisms are responsible for the alteration in the AP threshold. However, it has been hypothesized that the lowered AP threshold of DRG cells after peripheral axotomy may be a consequence of a higher concentration of sodium channels in the membrane of the cell body (Rizzo et al. 1995; Zhang et al. 1997a) and possibly a decreased K+ conductance as well (Everill and Kocsis 1997). The higher AP amplitude observed in medium sized CCD cells is consistent with the hypothesis that the lower AP action potential thresholds resulted from enhanced sodium currents.

Although an increase in sodium current might be expected to shorten the duration of AP duration, this effect might be negated by a decrease in K+ conductance, which has been shown recently for medium-sized DRG cells after sciatic axotomy (Everill and Kocsis 1997). Also, because the AP threshold was lower in CCD cells, the total sodium current may have been inactivated more in CCD than in control cells at threshold. Therefore the $V_{\text{max}}$ and the maximal amplitude of the AP in CCD cells might not change even though sodium current may have been enhanced.

Spontaneous activity was recorded from Aβ cells as early as the first postoperative day. The various patterns of spontaneous discharge were similar to those recorded from DRG cells in rats with peripheral nerve injury (Zhang et al. 1997b). The oscillation in membrane potential present in most spontaneously active A and C cells may be the trigger of the spontaneous activity.

The frequency distributions of firing thresholds indicate that the whole population of DRG neurons has shifted its firing thresholds leftward after CCD. Thus the significant decrease in firing threshold is not solely restricted to the spontaneously active neurons (11%). This result differs from that observed in dissociated neurons after a chronic constriction injury (CCI) where the altered excitability was found only in cells exhibiting spontaneous activity (Study and Kral 1996). It is possible that the difference in results may be partially explained by the different lesions in the two animal models. Because only 50% of the L4/L5 DRG neurons receive input from the sciatic nerve (Devor et al. 1985), the CCI injury will not affect all of the neurons (Munger et al. 1992), and some of the quiescent neurons from CCI rats may still have intact axons with no changes in the action potential characteristics. In the CCD rat, as the implanted rod applied a compressional force to the whole ganglion, each individual neuron is potentially affected.

**Extracellular factors modulating the enhanced excitability in vivo**

When a transient mechanical pressure was applied to the normal DRG in vivo, repetitive discharges persisted as long as 25 min after the stimulus was removed (Howe et al. 1977). Similar results were obtained in an in vitro study (Sugawara et
al. 1996). Rydevik et al. (1989) suggested that an increase in pressure within the DRG, measured after gentle compression of the ganglion with 2 mm wide forceps, may have resulted from intraneural edema and hemorrhage in the endoneurial space of the DRG. Qualitatively similar changes may have occurred in the present study after chronic compression of the DRG.

Hypoxia might enhance neuronal excitability during chronic compression of the ganglion. Spontaneous activity was generated in dorsal root fibers of normal DRG neurons, recorded in vitro, when the oxygen level in the perfusing solution was lowered (Sugawara et al. 1996). One consequence of the hypoxia was a lesser force required to elicit firing when acutely compressing the DRG. In other studies, substance P immunoreactivity was increased in the DRG after mechanical compression of the dorsal root (Cornefjord et al. 1995), suggesting a possible role of this neuropeptide in the production of lower back pain (Weinstein 1986; Weinstein et al. 1988). Bradykinin, serotonin, histamine, potassium ions, and prostaglandins, released from damaged tissue, can activate or sensitize nociceptors directly and might depolarize DRG somata if released in their proximity (Kress and Reeh 1996). A prolonged application of serotonin increased CAMP levels in sensory neurons in Aplysia and caused a detectable translocation of the catalytic subunit of PKA into the nucleus (Bacskai et al. 1993). PGE$_2$ is known to increase the excitability of dissociated rat DRG cells (e.g., Lopshire and Nicol 1997) and enhance the excitability of sensory neurons to inflammatory mediators such as bradykinin (Cui and Nicol 1995).

Inflammatory mediators may contribute to low back pain in patients with various spinal injuries or disorders of the spine such as intervertebral herniations (Devor 1996). Surgical exposure of the rat lumbar dorsal root and DRG subsequently reduced mechanical thresholds for ipsilateral foot withdrawal (Olmarker and Myers 1998). The additional trauma of mechanically deforming the root and exposing it and the DRG to material obtained from the nucleus pulposus resulted in a reduction in latencies of withdrawal to heating the ipsilateral foot. In the dog, an inflammatory response was produced in the spinal cord and dorsal roots after repetitive epidural injections of an extract from the nucleus pulposus (McCarron et al. 1987). An abnormally high concentration of phospholipase A$_2$, an inflammmogen, was found in lumbar disk tissue at the affected level in low back pain patients (Saal et al. 1990). Cytokines, such as interleukins, have been detected in lumbar herniated disk materials in humans (Kang et al. 1996; Yamagishi et al. 1996). Cavanaugh et al. (1997) found that the application of autologous nucleus pulposus to rabbit DRG in vitro evoked in nerve discharge lasting 1–3 min. Recently, cytokines (e.g., TNF-α) were found to induce thermal hyperalgesia and mechanical allodynia (Sommer et al. 1998) possibly via evoked activity in nociceptive afferent fibers (Sorkin et al. 1997), suggesting that inflammatory cytokines may contribute to hyperalgesia by increasing activity in nociceptive neurons. In CCD rats, it is possible that chronic compression may have caused the release of cytokines and other inflammatory mediators from the damaged tissue. It is also possible that a proliferation of satellite cells or an increase in resident or hematogenous macrophages, which occurs in the DRG after peripheral nerve injury (Lu and Richardson 1993) may occur within the compressed DRG. These cells might release inflammatory mediators and cytokines that alter the electrophysiological properties of sensory neurons.

Hyperexcitable DRG cells and neuropathic pain

Approximately half of the L$_4$/L$_5$ DRG neurons are reported to have axons in the sciatic nerve in normal rats (Devor et al. 1985). In the present study, conduction velocity was obtained in ~29% of L$_4$/L$_5$ DRG neurons in CCD rats but also in 37% of the cells from control rats. Although the obtained percentages were less than might be expected, they are approximately the same for cells from CCD and control ganglia. It therefore seems likely that the failure to obtain conduction velocity measurements in a higher percentage of recorded cells is due to inadequacies in the method of measurement rather than to any loss of axons due to the compression. In addition, the absence of autotomy and deformation of the ipsilateral foot, which occurs after peripheral axotomy (Devor and Raber 1983), is consistent with the supposition that the majority of the DRG cells retained intact axons during the chronic compression.

In a pilot study we recorded spontaneous activity in vivo from subpopulations of dorsal root fibers with cutaneous nociceptors with myelinated and unmyelinated axons 2 wk after a CCD (unpublished observations). Spontaneous activity of certain nociceptive primary afferents might induce central sensitization in the spinal cord, which in turn could induce secondary cutaneous hyperalgesia (e.g., Torebjörk et al. 1992).

In the present study, it was found that spontaneous activity and decreased threshold currents could be recorded as early as 1 day after surgery in CCD rats with mechanical hyperalgesia, suggesting a correlation between the increased neuronal excitability and the development of cutaneous hyperalgesia. We hypothesize that an increase in the excitability of the DRG cells, resulting from chronic compression and a consequent alteration of the local chemical environment of the DRG, could contribute to cutaneous pain and mechanical hyperalgesia during certain diseases and injuries of the spine.

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