Increased Sensitivity of Sensory Neurons to Tumor Necrosis Factor α in Rats With Chronic Compression of the Lumbar Ganglia

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Liu, Baogang, Huiqing Li, Sorin J. Brull, and Jun-Ming Zhang. Increased sensitivity of sensory neurons to tumor necrosis factor α in rats with chronic compression of the lumbar ganglia. J Neurophysiol 88: 1393–1399, 2002; 10.1152/jn.00021.2002. Proinflammatory cytokines may sensitize primary sensory neurons and facilitate development of neuropathic pain processes after peripheral nerve injury. The goal of this study was to determine whether responses of dorsal root ganglion (DRG) neurons to exogenous tumor necrosis factor α (TNF-α) are altered in a chronically compressed DRG (CCD) injury model. Extracellular recordings from teased dorsal root microfilaments demonstrated that acute topical application of TNF-α to the DRG for 15 min evoked C- and Aβ-fiber responses in both normal and CCD rats. However, the response latency was significantly shorter, and the peak discharge rate was higher, in CCD fibers than in normal fibers. Intracellular recordings from small- and large-sized neurons showed that TNF-α induced greater depolarization and greater decrease in rheobase in CCD neurons than in normal neurons. The proportion of both small- and large-sized neurons that were responsive to TNF-α increased significantly after CCD injury. Furthermore, TNF-α altered the discharge patterns of large, spontaneously active neurons in addition to enhancing their discharge rates. However, the depolarization caused by TNF-α in such neurons was minor (<2 mV). Inflammatory cytokines such as TNF-α increased the sensitivity of sensory neurons in normal and CCD rats. The CCD injury itself, on the other hand, increased neuronal responses to inflammatory cytokines.

INTRODUCTION

Lumbar disk herniation in humans is often associated with severe low back pain and sciatica resulting from chemical irritation with or without mechanical compression of the dorsal root ganglia (DRG) and/or nerve roots. Endogenous inflammatory agents are believed to play a critical role in the generation of pain and hyperalgesia, because antiinflammatory drugs have been shown to be very effective in relieving pain in patients with radiculopathy.

Among several inflammatory agents that have been detected in the herniated disk, tumor necrosis factor α (TNF-α), a potent proinflammatory cytokine, is believed to be important in the initiation of local inflammation (Igarashi et al. 2000). Recent behavioral studies in our laboratory demonstrated that chronic local application of TNF-α to the normal, intact ganglion significantly increased cutaneous sensitivity to mechanical stimulation (Homma et al. 2002). In vitro electrophysiological recordings from unjured primary sensory neurons indicate that acute topical application of TNF-α to the ganglion induces protein kinase A (PKA)-mediated activities in DRG neurons with myelinated or unmyelinated axons (see companion paper). Similar effects were reported previously when TNF-α was applied directly to nerve trunks (Sorkin et al. 1997) or injected into peripheral receptive fields in vivo (Junger and Sorkin 2000).

There is evidence that TNF-α and other inflammatory cytokines such as interleukin-1β may directly modulate the activity in various classes of neurons. TNF-α was found to reduce K+ conductance in Aplysia (Sawada et al. 1990), and retinal ganglion neurons (Diem et al. 2001). On a slower time scale (24 h instead of 20–60 min), TNF-α affects calcium currents in cultured sympathetic (Soliven and Albert 1992) and hippocampal neurons (Furukawa and Mattson 1998).

TNF-α can be synthesized and released by a variety of cell types during inflammatory as well as neuropathic processes (Creange et al. 1997; Tchelingerian et al. 1993; Wagner et al. 1998) and contributes to the development of pain and hyperalgesia in animal models of local inflammation or peripheral neuropathy (Cunha et al. 1992; DeLeo and Colburn 1995; Sommer et al. 1998a; Wagner et al. 1998; Woolf et al. 1997). It has been demonstrated that antisera, or soluble receptors that reduce endogenous TNF-α in neuropathic animal models, reduce thermal as well as mechanical hyperalgesia (Lindenlaub et al. 2000; Sweitzer et al. 2001).

Recently it was reported that rats develop cutaneous hyperalgesia to radiant heat and tactile stimuli on the plantar surface of the foot after chronic compression injury of the ipsilateral DRG produced by implantation of a metal rod in each of the L4 and L5 intervertebral foramina (Song et al. 1999). Ectopic discharges originating in the compressed ganglia were electrophysiologically recorded in vitro from 9% of the myelinated dorsal root fibers (Song et al. 1999). The patterns of ectopic discharge were similar to those recorded from primary sensory neurons with transected peripheral axons (Burchiel 1984; De-Santis and Duckworth 1982; Devor 1994; Wall and Gutnick 1974; Zhang et al. 1997). Enhanced excitability was demonstrated in all three types of sensory neurons with or without spontaneous activity (Zhang et al. 1999). This DRG chronic compression preparation provides a human model of DRG...
compression from an acutely herniated lumbar disk, spinal stenosis, tumors, or other injuries or diseases of the spinal cord.

In the present study, using extracellular and intracellular techniques, we compared the sensitivity of DRG neurons to exogenously applied TNF-α between normal rats and rats that had been subjected to chronic compression injury of the DRG.

METHODS

CCD injury animal model

Young female Sprague-Dawley rats weighing 100 g at the time of surgery were anesthetized with intraperitoneal injection of pentobarbital sodium (40 mg/kg ip). After a midline incision was made from L1 to L6, the right paraspinal muscles were separated from the transverse processes and the L4 and L5 intervertebral foramina were exposed. In each of 47 rats, an L-shaped stainless steel rod (4 × 2 mm in length and 0.6 mm diam) was inserted unilaterally into each ganglion. The incision was then closed in layers and prophylactic

Extracellular electrophysiological recording

Extracellular recordings were obtained from 17 normal and 27 CCD rats on postoperative days 7 to 14. As described previously (Zhang et al. 1997), the rats were first anesthetized with pentobarbital sodium (40 mg/kg ip). The right L4 or L5 ganglia with attached dorsal roots (length: ~2 cm) and sciatic nerve (length: ~3 cm) were dissected surgically and placed in a recording chamber after the capsule was carefully peeled off. The DRG was perfused at a rate of 5 ml/min with oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 3.5 KCl, 1.25 NaH2PO4, 24 NaHCO3, 10 glucose, 1.2 MgCl2, and 1.2 CaCl2 (pH = 7.3). The perfusion solution was heated to maintain the bath temperature at 37°C. The dorsal root was placed to extend out of this chamber into an adjacent mineral oil-filled chamber where microfilament recordings were performed. The spinal/sciatic nerve (length: ~40 mm) was placed in an adjacent chamber containing mineral oil and was positioned in contact with a bipolar stimulating electrode. Each chamber contents were separated by petroleum jelly (Vaseline) to prevent the solutions from mixing.

Dorsal root microfilaments were teased apart under a dissecting microscope. The proximal end of a dissected microfilament was placed on a fine silver electrode for single-fiber recording. The discharges of single fibers were displayed on a digital oscilloscope and collected via an interfaced Spike 2 data-acquisition system (Cambridge Electronic Design, Cambridge, U.K.) on an interfaced Pentium III PC. The conduction velocity (CV) of each fiber was measured via electrical stimulation delivered in decreasing steps of 0.05 nA from 0.2 to ~50 nA.

After surgical dissection, the right L4 or L5 DRG from each of 22 normal and 20 CCD rats was placed in the recording chamber and mounted on the stage of an upright microscope (BX50-WI, Olympus, Japan). A U-shaped stainless steel wire on which three to four fine nylon fibers spanned the two sides was used to gently hold the ganglion immersed at the bottom of the chamber. The DRG was continuously perfused with oxygenated ACSF at a rate of 2 ml/min, and the temperature was maintained at 37 °C as described previously (Zhang et al. 1999).

DRG cells were visualized under differential interference contrast (DIC) through a CCD camera (Hamamatsu, Japan). Intracellular electrophysiological recordings were made from each cell with a micro-electrode filled with 2.5 M potassium acetate (pH = 7.2). Satisfactory recordings were obtained with electrodes of 50–80 MΩ. Before electrode penetration, the DRG soma was visually classified according to its diameter as small (≤30 µm) or large (≥50 µm). The electrophysiological data were collected with the use of single-electrode continuous current clamp (AxoClamp-2B, Axon Instruments, Foster City, CA) and analyzed with Clampex 8 software (Axon Instruments).

After a stabilization-recording period of 3 min, TNF-α at 0.001 ng/ml was applied to the DRG for 5 min, followed by washout with ACSF for ≥30 min. A TNF-α concentration of 0.001 ng/ml was chosen as it was the lowest dose tested in the extracellular studies (see the companion paper). To compare the sensitivity of normal and CCD rat neurons to TNF-α, we measured the changes in the threshold current, action potential (AP) threshold, resting membrane potential (Vm), input resistance (Rm) and afterhyperpolarization (AHP) of each DRG cell (Czech et al. 1977; Pellegrino et al. 1984) after topical application of TNF-α for 5 min (Fig. 1). Vm was first measured 3 min after a stable recording was obtained and was measured again after 5 min of TNF-α application. Depolarizing currents of 0.05–4.0 nA (100-ms pulse duration) were delivered in increments of 0.05 nA until an action potential (AP) was evoked. The threshold current (rheobase) was defined as the minimum current required to evoke an AP. 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/ms. The duration of the AP was measured at the AP threshold level. 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 of 100-ms duration, delivered in decreasing 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 amplitude half way between.
Data acquisition and statistical analyses

A mean basal ("control") discharge rate was computed as the mean number of spikes/s (± SE) for 15 min before delivery of TNF-α. For each fiber, the maximal or peak effect of TNF-α on the fiber’s activity was defined as the discharge rate in the 5-min interval (spikes·s⁻¹·5 min⁻¹) following drug administration that exhibited the greatest increase from the peak basal rate. Student’s t-test was used to compare the different peak discharges and the response latencies between normal and CCD fibers. Paired t-test or Wilcoxon signed-rank test was used to compare the changes in AP parameters before and after topical TNF-α application. χ² test or Mann-Whitney rank-sum test was used to compare the incidence of neuronal response to TNF-α between normal and CCD rats. A P value of <0.05 was considered statistically significant.

RESULTS

Extracellular microfilament recording

C FIBERS FROM NORMAL AND CCD RATS. A total of 40 C fibers with CV <2 m/s were selected for this series of experiments, 18 from normal rats and 22 from CCD rats. Eight of the 18 normal and 9 of the 22 CCD fibers were spontaneously active prior to drug application, while the remaining fibers were initially quiescent.

As shown in Fig. 2A, TNF-α evoked responses in 11 of 13 quiescent fibers from CCD rats and in 4 of 10 quiescent fibers from normal rats (P < 0.05, χ² test). The peak discharge rates of the 11 CCD fibers that responded to TNF-α averaged 0.68 ± 0.32 spikes/s, which is significantly higher than the average discharge rate in normal rats (0.09 ± 0.03 spikes/s, n = 4; Fig. 2B). Furthermore, the response latency for TNF-α to evoke discharges was shorter in CCD rats (13.4 ± 4.2 min, n = 11) than in normal rats (22.9 ± 7.9 min, n = 4; P < 0.05, Student’s t-test; Fig. 2C). Seven of 11 CCD fibers and 3 of 4 normal fibers recovered completely after washout with ACSF.

TNF-α also enhanced the discharge rate in eight of nine spontaneously active CCD fibers and in all eight spontaneously active normal fibers (Fig. 2D). The mean basal discharge rates prior to TNF-α application were 0.03 ± 0.01 and 0.04 ± 0.02 spikes/s for CCD and normal neurons, respectively. After TNF-α application, the mean peak discharge rate of the nine CCD fibers increased to 0.20 ± 0.21 spikes/s, which was higher than that of normal fibers (0.13 ± 0.16 spikes/s; P = NS, Student’s t-test). Figure 2D shows that a similar response was induced by TNF-α applications in spontaneously active C fibers from normal and CCD rats. Similar to quiescent fibers, the response latency of spontaneously active fibers was shorter in CCD neurons than normal neurons (Fig. 2C).

Aβ FIBERS FROM NORMAL AND CCD RATS. A total of 36 Aβ fibers (CV >15 m/s) were tested in this series of experiments, 18 CCD fibers (8 quiescent and 10 spontaneously active) and 18 normal fibers (15 quiescent and 3 spontaneously active). The mean basal discharge rate for the 10 spontaneously active CCD fibers prior to TNF-α application was 6.0 ± 3.6 spikes/s. Following TNF-α application, six of eight (75%) quiescent CCD fibers responded after a mean latency of 16.6 ± 4.4 min. Only two of these six fibers returned to electrical silence after 30–40 min washout with ACSF. In contrast, only 4 of 15 (27%) of the normal quiescent fibers responded to TNF-α application and did so after a much longer latency (23.3 ± 7.4 min) than CCD fibers (P < 0.05, Student’s t-test). Seven of 10 (70%) spontaneously active CCD fibers responded to TNF-α application by increasing discharge rate from an average of 5.4 ± 3.1 to 12.0 ± 5.6 spikes/s (P < 0.01, Wilcoxon signed-rank test, n = 10). Discharge of one fiber was inhibited; two fibers did not show significant changes in discharge rate. One of three (33%) normal spontaneously active fibers responded with a slight increase in discharge rate following TNF-α application.

Microelectrode intracellular recording

A total of 68 small neurons (39 normal and 29 CCD) and 68 large neurons (22 normal, 35 quiescent CCD, and 11 spontaneously active CCD) were studied.

SMALL SIZE DRG NEURONS FROM NORMAL AND CCD RATS.

Normal neurons. Acute topical application of TNF-α significantly increased (depolarized) membrane potential of normal neurons from −61.2 ± 1.7 mV prior to TNF-α application to −59.1 ± 1.9 mV 5 min after TNF-α application (P = 0.006, Wilcoxon signed-rank test, n = 39; Fig. 3A). Of 39 normal
neurons, 23 were depolarized, 9 were hyperpolarized, and the remaining 7 neurons did not respond to TNF-α. For the depolarized neurons, the mean increase was 4.6 ± 0.7 mV (n = 23; 1–13 mV range). Acute topical application of TNF-α decreased the rheobase in 19 of 39 neurons, increased it in 9 neurons, and induced no changes in the remaining 11 neurons. The mean rheobase for all the neurons tested decreased significantly, from 0.69 ± 0.06 to 0.59 ± 0.06 nA after TNF-α application (P = 0.004, Wilcoxon signed-rank tests; Fig. 3B). In addition, the mean maximal depolarizing rate was decreased significantly from 161 ± 11.3 to 146 ± 8.1 mV/s (P = 0.017, Wilcoxon signed-rank test), and the AP amplitude decreased from 50.2 ± 1.9 to 47.6 ± 1.8 ms (P = 0.034, paired t-test). There was no significant change in the action potential threshold, the maximal repolarizing rate, or the afterhyperpolarization after 5 min of TNF-α application.

**CCD neurons.** Similar to normal neurons, CCD neurons responded to topical TNF-α application by increasing the membrane potential and decreasing rheobase. The mean membrane potential increased from −62.6 ± 1.6 to −58.4 ± 1.6 mV (P < 0.001, paired t-test, n = 29; Fig. 3A). Of the 29 small CCD neurons tested, 27 neurons were depolarized by TNF-α, and 2 neurons were minimally hyperpolarized (<2 mV). The depolarization for the 27 neurons averaged 5 ± 0.8 mV, ranging between 1 and 15 mV. TNF-α decreased the rheobase in 20 of 29 neurons, increased it in 4 neurons, and produced no change in 5 neurons. For all 29 neurons tested, the rheobase decreased from an average of 0.64 ± 0.06 to 0.53 ± 0.06 nA (P < 0.001, paired t-test; Fig. 3B). There were no significant changes in any other action potential parameters.

**CCD vs. normal neurons.** Compared to normal, uninjured neurons, small CCD neurons were more sensitive to TNF-α. TNF-α-induced changes in membrane potential (5.0 vs. 4.6 mV, P > 0.05, Mann-Whitney rank-sum test) and rheobase (0.11 vs. 0.08 nA, P > 0.05, Mann-Whitney rank-sum test) were both slightly greater in CCD than normal neurons; the proportion of neurons that were depolarized by TNF-α was significantly higher in CCD (93%) than in normal rats (59%; P < 0.05, χ² test). Similarly, the rheobase was decreased by TNF-α in 49% of normal neurons and 69% of CCD neurons (P < 0.05, χ² test; Fig. 3C).

**LARGE-SIZED DRG NEURONS FROM NORMAL AND CCD RATS.**

**Normal neurons.** Acute topical application of TNF-α produced minor depolarization in 11 of 22 (50%) normal large neurons. The depolarization in the 11 responsive neurons averaged 1.55 ± 0.45 mV (1- to 6-mV range). The mean membrane potential immediately before and 5 min after TNF-α application was −64.1 ± 1.6 and −63.7 ± 1.4 mV, respectively (P > 0.05, paired t-test). Only 3 of 22 (14%) tested neurons showed a decreased rheobase (<0.2 nA) after a 5-min of TNF-α application; the rest of the neurons were either slightly hyperpolarized or no change. No statistical difference was observed in any other action potential parameters (Fig. 4, A and C–E).

**Quiescent CCD neurons.** In CCD neurons, TNF-α induced a greater effect on the membrane properties than in normal neurons (Fig. 4, A and B). Thirty of 35 (86%) CCD neurons were depolarized after topical application of TNF-α. As a result, the mean membrane potential for the 35 neurons tested increased (depolarized) from −64.6 ± 1.3 to −60.8 ± 1.4 mV (P < 0.001, paired t-test, n = 35; Fig. 4C). The depolarization averaged 5.1 ± 0.6 mV, ranging between 1 and 17 mV (depolarized cells only). Of the rest of the neurons that were not depolarized by TNF-α application, two neurons were slightly hyperpolarized, and three neurons did not respond to TNF-α. In 21 of 35 (60%) neurons, the rheobase decreased; for the group of 35 neurons, the mean rheobase decreased from a control value of 0.92 ± 0.07 to 0.81 ± 0.06 nA (P = 0.007, paired t-test) following TNF-α application (Fig. 4, B and D). In addition, the action potential duration was increased by TNF-α application from an average of 0.80 ± 0.05 to 0.85 ± 0.06 ms (P = 0.016, paired t-test, n = 35; Fig. 4E). There were no significant changes in other action potential parameters of CCD neurons.

**Spontaneously active CCD neurons.** Eleven large spontaneously active neurons with different discharge patterns were studied. Of the 11 neurons, 3 exhibited low-frequency irregular discharges; 3 neurons had regular bursting discharges; and the remaining 5 neurons had irregular high-frequency discharges. Topical application of TNF-α to the DRG for 4–10 min significantly increased the discharge rate of all three neurons that exhibited low-frequency discharges. After washout with ACSF, the responses in two of these three neurons returned to baseline level within 13 and 20 min, respectively (Fig. 5A). One neuron failed to recover after a 30-min ACSF washout. TNF-α also enhanced the discharge rate of the three neurons with regular bursting discharges, with an average of 2.5-mV depolarization. In addition, the discharge pattern of two neurons changed from a regular bursting discharge to an irregular high-frequency discharge following TNF-α application and recovered after washout with ACSF for 10 and 30 min, respectively (Fig. 5B). Among the remaining five neurons, three
depolarized (average of 1.2 mV), one neuron did not change, and another one hyperpolarized. However, there was no significant change in the discharge rate or the discharge patterns after TNF-α application. For all the spontaneously active neurons tested (n = 11), a slight but significant depolarization was induced by TNF-α application. The mean membrane potential before and after TNF-α application was −66.2 ± 2.8 and −64.4 ± 2.7 mV, respectively (P = 0.02, paired t-test).

**CCD vs. normal neurons.** Compared to normal neurons, large CCD neurons were more sensitive to TNF-α as demonstrated by a higher percentage of neurons that were depolarized by TNF-α in CCD (86%) than in normal neurons (50%; P < 0.05, χ² test). Similarly, 60% of CCD neurons responded to TNF-α application with decreased rheobase, while in normal neurons, the responsive proportion was 14%. In addition, the action potential duration was increased in 60% of CCD neurons, which is greater than in normal neurons (30%; P < 0.05, χ² test; Fig. 4F).

**DISCUSSION**

This study demonstrates that acute topical application of TNF-α evokes action potentials and enhances neuronal excitability of DRG neurons, whether or not previously exposed to compression injury. However, CCD neurons are more sensitive to TNF-α application than normal neurons.

**Extracellular microfilament recording**

Both Aβ and C fibers from control and CCD rats responded to topical application of TNF-α. The response of silent Aβ and C fibers to TNF-α application was similar in CCD rats. The latency of TNF-2-evoked responses was shorter in CCD than in normal neurons, and the mean peak discharge rate was higher in CCD than in normal neurons. However, the response magnitude was lower in spontaneously active fibers than in quiescent ones. The reasons are unknown; however, as discussed in the companion paper, it is possible that in spontaneously active neurons with or without compression injury, certain ion channels (e.g., K⁺ channels) responsible for TNF-α-evoked responses might have been partially inactivated prior to chemical
application, leading to a reduced response to TNF-α application.

Dose-response comparisons would provide further information about the relative differences in TNF sensitivity between normal and CCD fibers and help determine if CCD neurons have a lower response threshold. However, the long excitatory effect of TNF-α after a single application, and the difficulties in maintaining a reliable recording of C-fibers for over 2–3 h limited our ability to conduct such an experiment. The concentration of TNF-α (1 ng/ml) may be higher than the physiological level in the rat sciatic nerve (George 1999). However, under certain pathologic conditions, DRG neurons can be exposed to relatively high concentrations of TNF-α released from herniated nucleus pulposus (Igarashi et al. 2000). In addition, in our extracellular recording experiments, the concentration of TNF-α that actually reached the somata in the DRG could be considerably lower than that in the perfusion solution. Responses of DRG neurons to TNF-α at lower concentrations (as shown in the companion paper) suggest a physiological effect of TNF-α.

**Intracellular recording**

Low concentration TNF-α was used during the intracellular recording experiments to detect possible changes in the membrane properties of DRG neurons, which are not measurable with extracellular recording techniques. Results are in agreement with current extracellular studies: chronic compression injury enhanced TNF-α sensitivity of DRG neurons, demonstrating that CCD neurons are hyperexcitable not only to electrical (Zhang et al. 1999) but also to chemical stimulation.

TNF-α enhanced excitability of normal DRG neurons with either myelinated or unmyelinated axons. The decreased rheobase is probably caused by depolarization, as no changes in action potential threshold or \( R_{\text{m}} \) were observed in response to TNF-α application. The decreased maximal depolarization rate of small DRG neurons can be explained by lowered action potential amplitude without alteration in action potential duration. In small CCD neurons, no significant changes in action potential duration, action potential amplitude, or maximal depolarization rate were observed on TNF-α application. This lack of response could be due to changes in action potential configurations by compression injury prior to TNF-α application (Zhang et al. 1999). TNF-α increased the discharge rate of spontaneously active large DRG neurons with only minor depolarization (1–2 mV). This suggests that low degrees of depolarization induced by lower concentrations of TNF-α may not be enough to generate spontaneous activity in normal DRG neurons but may be sufficient to evoke action potentials or enhance spontaneous activity in cells that are hyperexcitable following peripheral nerve or lumbar ganglia injury.

The latency for TNF-α to evoke action potentials as observed in extracellular fiber recordings is longer than the latency to induce depolarization as obtained in our intracellular recording experiments. The reasons are twofold: first, in the intracellular study, most cells that were tested were on the surface of the ganglion, whereas in extracellular fiber recording study, it is very likely that a large number of cells were located beneath the superficial layer. Second, it is possible that during the extracellular recording study, a certain level of depolarization may have occurred before the generation of spikes.

The ionic mechanisms underlying TNF-α-induced depolarization are not clear. However, evidence suggests strongly that \( K^+ \) conductance is involved in changes in neuronal excitability following peripheral nerve injury (Everill and Kocsis 1999). Increased neuronal excitability caused by a low \( K^+ \) conductance is normally accompanied by an increase in the input resistance and a decrease in the rheobase (Bal and McCormick 1993). In the current study, TNF-α did not induce significant changes in the input resistance but significantly decreased the rheobase, which likely resulted from depolarization of the membrane potential. This hypothesis is supported by previous studies in which TNF-α decreased \( K^+ \) conductance in *Aplysia* abdominal neurons (Sawada et al. 1999a and retinal ganglion neurons (Diem et al. 2001). In the companion paper, we report that TNF-α-induced sensory responses are blocked by specific antagonists of PKA pathway. Thus it is possible that when deposited at the ganglia, TNF-α activates the PKA pathway and induces phosphorylation of certain types of \( K^+ \) channel and results in their closure. The prolonged action potential duration found in large DRG neurons supports this hypothesis.

The mechanisms underlying the enhanced sensitivity of CCD neurons to TNF-α are not clear. There is evidence that the levels of TNF-α and TNF-α receptors are increased in peripheral nerves that underwent previous loose ligation injury (Shubayev and Myers 2000). Other studies showed that injection of antibody to TNF-α receptor1 or TAPI (which is believed to downregulate TNF-α receptor1 protein level) can reduce thermal and mechanical allodynia (Sommer et al. 1997, 1998b). In a recent study from our own laboratory, we reported that local administration of mixed, soluble TNF-α receptors partially reversed the CCD-induced mechanical hyperalgesia.

An alternative explanation for the enhanced neuronal TNF-α sensitivity after CCD injury could be the lower pH of the intracellular environment induced by the inflammatory reaction. This local inflammation could have facilitated the self-embedding of TNF-α into the cell membrane to form minipores, a hypothesis suggested by Kagan et al. (1992). Finally, changes in potassium activity due to compression injury, if any, could directly alter the sensitivity of DRG neurons to TNF-α.

In summary, results from our present study indicate that TNF-α may further enhance excitability of CCD neurons and that CCD sensory neurons become more sensitive to exogenous TNF-α. Our results suggest that clinically, inflammatory cytokines (such as those released from the herniated disks) may cause pain and hyperalgesia by activating sensory neurons of the lumbar ganglia.

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**REFERENCES**


