

# Stereospecific Effect of Pregabalin on Ectopic Afferent Discharges and Neuropathic Pain Induced by Sciatic Nerve Ligation in Rats

Shao-Rui Chen, M.D.,\* Zemin Xu, M.S.,\* Hui-Lin Pan, M.D., Ph.D.†

**Background:** The new anticonvulsants, gabapentin and pregabalin, are effective in the treatment of neuropathic pain. The sites and mechanisms of their analgesic action are not fully known. The authors have previously demonstrated that systemic gabapentin suppresses ectopic afferent discharges recorded from injured sciatic nerves in rats. In the current study, they further examined the stereospecific effect of pregabalin on neuropathic pain and afferent ectopic discharges in a rodent model of neuropathic pain.

**Methods:** Tactile allodynia and thermal hyperalgesia were induced by partial ligation of the left sciatic nerve in rats. Single-unit activity of afferent ectopic discharges was recorded from the sciatic nerve proximal to the site of ligation.

**Results:** Intravenous injection of 10–30 mg/kg pregabalin dose-dependently attenuated tactile allodynia ( $n = 10$ ) and thermal hyperalgesia ( $n = 8$ ). The stereoisomer of pregabalin, *R*-3-isobutylgaba, had no analgesic effect in this dose range. Furthermore, intravenous injection of pregabalin, but not *R*-3-isobutylgaba, significantly inhibited the ectopic discharges from injured afferents in a dose-dependent manner (from  $20.8 \pm 2.4$  impulses/s during control to  $2.3 \pm 0.7$  impulses/s after treatment with 30 mg/kg pregabalin,  $n = 15$ ). Pregabalin did not affect the conduction velocity of afferent fibers and the response of normal afferent nerves to mechanical stimulation.

**Conclusions:** These data strongly suggest that the analgesic effect of pregabalin on neuropathic pain is likely mediated, at least in part, by its peripheral inhibitory action on the impulse generation of ectopic discharges caused by nerve injury.

CHRONIC neuropathic pain remains a significant clinical problem. Conventional analgesics, such as opioids and nonsteroidal antiinflammatory drugs, possess limited efficacy and serious side effects in the treatment of this condition.<sup>1</sup> Considerable efforts have been made in the discovery of new drugs with an improved side effect profile that would be more effective in the treatment of chronic pain syndromes.<sup>2–5</sup> Controlled clinical trials have shown that gabapentin can reduce chronic pain associated with diabetic neuropathy and postherpetic neuralgia.<sup>5,6</sup> Pregabalin, formerly known as *S*(+)-3-isobutylgaba or CI-1008, is another novel anticonvulsant agent under clinical development for its analgesic proper-

ty.<sup>2,7–9</sup> Similar to gabapentin, pregabalin produces analgesic actions on various forms of pain caused by surgical incision, inflammation, and diabetic neuropathy.<sup>2,7–9</sup> Pregabalin consistently manifests a greater potency than gabapentin in preclinical studies.<sup>2,7–9</sup> Gabapentin and pregabalin are both 3-alkylated  $\gamma$ -aminobutyric acid analogs, and neither compound interacts with  $\gamma$ -aminobutyric acid type A ( $GABA_A$ ) or type B ( $GABA_B$ ) receptors.<sup>10,11</sup> Although a recent study suggests that gabapentin activates postsynaptic ( $GABA_B$ ) receptors in the rat hippocampus,<sup>12</sup> its relevance to the antinociceptive effect of gabapentinoids is unclear. Currently, the precise site and mechanisms of the analgesic action of pregabalin remain to be determined.

Although the pathogenesis of allodynia and hyperalgesia after peripheral nerve injury is still unclear, continuous discharges from ectopic foci are known to contribute to the development and maintenance of a hyperexcitable state of spinal dorsal horn neurons.<sup>13,14</sup> Clinical studies also suggest that the ectopic discharges from the afferent nerves likely are a source of ongoing spontaneous pain, and it dynamically maintains a state of central hypersensitivity that underlies evoked-pain syndromes, such as allodynia and hyperalgesia, in patients with painful neuropathies.<sup>15,16</sup> Our recent study has shown that the ectopic discharge activity from injured peripheral afferent nerves is suppressed by the therapeutic doses of gabapentin.<sup>17</sup> However, it remains to be determined whether pregabalin has a similar effect on ectopic discharges originating from injured peripheral afferents. Therefore, the major objective of the current study was to examine the stereospecific effect of pregabalin on the ectopic discharge activity from the injured sciatic nerve and neuropathic pain induced by nerve injury in rats.

## Materials and Methods

Male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 225–250 g were used in this study. During halothane anesthesia, the left sciatic nerve was exposed and isolated at the mid thigh, and one third to one half of the nerve was ligated tightly with a 5.0 silk suture, according to the method described previously.<sup>18</sup> We chose this rat model of neuropathic pain because of the stable and reproducible allodynia after nerve ligation. This model was used also because of the ease of electrophysiologic recordings from the sciatic nerve proximal

\* Research Staff, † Associate Professor and Director of Basic Research in Anesthesiology.

Received from the Department of Anesthesiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, and the Department of Anesthesiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina. Submitted for publication April 6, 2001. Accepted for publication August 6, 2001. Supported by grant Nos. GM64830 and NS41178 from the National Institutes of Health, Bethesda, Maryland.

Address correspondence to Dr. Pan: Department of Anesthesiology, H187, Penn State University College of Medicine, 500 University Drive, Hershey, Pennsylvania 17033-0850. Reprints will not be available from the authors. Address electronic mail to: hpan@psu.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

to the ligation site. The animals were allowed to recover for 10–14 days after nerve ligation. Then, the right jugular vein was cannulated with a polyethylene 50 tubing, and the catheter was externalized to the back of the neck during halothane anesthesia. After recovery for 3 days after cannulation, the rats were used for mechanical and thermal testings. We and others have shown that stable tactile allodynia and thermal hyperalgesia develop within 1 week after nerve ligation and last for at least 4 weeks.<sup>17,18</sup> Therefore, all the final studies were conducted between 2 and 3 weeks after sciatic nerve ligation. The behavioral testing was conducted between 8:00 and 11:30 AM. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State College of Medicine (Hershey, PA) and conformed to National Institutes of Health guidelines for the ethical use of animals. All efforts were made to minimize pain and the number of animals used.

#### *Behavioral Assessment of Tactile Allodynia*

To evaluate the mechanical sensitivity of the injured hind paw, rats were placed in individual plastic boxes on a mesh floor and were allowed to acclimate for 30 min. A series of calibrated von Frey filaments (Stoelting Co., Wood Dale, IL) were applied perpendicularly to the plantar surface of the left hind paw with sufficient forces to bend the filaments for 6 s. Brisk withdrawal or paw flinching was considered to be a positive response. In the absence of a response, the filament of next greater force was applied. In the presence of a response, the filament of next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal response was calculated by using the “up-down” method, as described in detail previously.<sup>17,19</sup> Each trial was repeated two to three times at approximately 2-min intervals. Unlike the noxious heat and pressure tests in which cutoff values are often used to avoid causing tissue damages, we did not use a cutoff value for the von Frey filament testing. After obtaining a consistent baseline, pregabalin or *R*-3-isobutylgaba was injected intravenously. The actual doses of pregabalin or *R*-3-isobutylgaba injected were 10, 10, and 10 mg/kg, separated by 15 min, to yield cumulative doses of 10, 20, and 30 mg/kg, respectively. The plasma half-life of pregabalin is similar to that of gabapentin, which is about 4 h in rats.<sup>11,20</sup> In the preliminary study, we found that intravenous pregabalin had no effect on allodynia in doses less than 10 mg/kg. Pregabalin and *R*-3-isobutylgaba (Parke-Davis Pharmaceutical Research, Ann Arbor, MI) were dissolved in normal saline and injected in a volume of 0.2 ml followed by a 0.1-ml flush with saline. The mechanical thresholds were determined every 15–30 min after injection.

#### *Behavioral Assessment of Thermal Hyperalgesia*

To assess quantitatively the thermal sensitivity of the injured hind paw, rats were placed on the glass surface of a thermal testing apparatus (model 336; IITC Inc./Life Science Instruments, Woodland Hills, CA). The rats were allowed to acclimate for 30 min before testing. The temperature of the glass surface was maintained constant at 30°C. A mobile radiant heat source located under the glass was focused onto the left hind paw of each rat. The paw withdrawal latency was recorded by a timer. The apparatus was adjusted at the beginning of the study so that the baseline paw withdrawal latency in normal rats was approximately 12 s. This setting (the light beam intensity) then was kept unchanged throughout the study. The cutoff of 30 s was used to prevent potential tissue damage.<sup>21</sup> After the baseline was measured, the paw withdrawal latency was determined every 15–30 min after injection of pregabalin or its stereoisomer, *R*-3-isobutylgaba, as described.

#### *Recording of Single-unit Activity of Afferent Nerves*

Additional animals were used for electrophysiologic studies. Allodynic conditions were first verified in all rats before afferent nerve recording experiments. Rats were anesthetized with an intraperitoneal injection of sodium phenobarbital (45 mg/kg). The right jugular vein and left carotid artery were cannulated for administering drugs and monitoring blood pressure, respectively. The trachea was cannulated, and the rat was ventilated artificially with a respirator (SAR-830; IITC Inc./Life Science Instruments). Arterial blood gases were analyzed with a blood gas analyzer and maintained within physiologic limits. Body temperature was maintained in the range of 37–38°C with a circulating water-heating pad and heat lamps throughout the experiment. The fascia and sheath overlying the left sciatic nerve were removed carefully. The nerve then was draped on a platform and covered with warm mineral oil. Small nerve filaments were teased gently from the nerve segment approximately 1 cm proximal to the ligated site under an operating microscope (model M900; D.F. Vasconcellos S.A., São Paulo, Brazil). Single-unit afferent nerve activity was recorded with a bipolar stainless electrode. The nerve filaments were dissected gradually until the single-unit activity of afferents was isolated. The action potential of the nerve was amplified, filtered with a bandpass filter of 100–1,000 Hz, and monitored through an audioamplifier (model AM8; Grass Instruments, West Warwick, RI) and a storage oscilloscope (TDS 210; Tektronix, Wilsonville, OR). The neurogram was recorded on a thermal-sensitive recorder (model K2G; Astro-Med, West Warwick, RI). The single-unit afferent was identified initially by examining the waveform and the spike amplitude on the oscilloscope at a rapid sweep speed as well as by checking the recorded sound frequency related to each spike

activity. Furthermore, the signals were digitized at a sampling rate of 20 kHz and recorded into a Pentium computer (Dell, Austin, TX) through an analog-to-digital interface card for subsequent off-line analysis. An amplitude threshold was set for the recorded action potential of nerve fibers. When an event was detected, the associated waveform (6 ms) was extracted and displayed continuously in a separate software oscilloscope window (Experimental Workbench; DataWave Technology, Inc., Longmont, CO). Single-unit recording was ensured by checking the constancy of the shape and polarity of the displayed spike wave form. Discharge frequency was quantified by using data acquisition and analysis software (DataWave Technology, Inc.), and a histogram was created for each afferent nerve.

After the spontaneous discharge activity of a single-unit afferent fiber was identified, the baseline discharge was recorded for 10–15 min. Then, pregabalin or *R*-3-isobutylgaba was injected intravenously at cumulative doses of 10, 20, and 30 mg/kg, each separated by 15 min. The animals were given doses at intervals identical to those used for the behavioral studies. We used the following two criteria to ensure that the recorded activity was ectopic discharge originating from the neuromas<sup>17</sup>: (1) recorded nerve fibers had no receptive field in the peripheral tissue, and (2) at the end of recording, the ectopic discharge activity was increased by direct stimulation of the neuroma but was not altered by transecting the nerve distal to the neuroma site. The saline control group was not included in this study because we have shown that intravenous saline has no effect on the baseline ectopic discharge and allodynia caused by nerve ligation.<sup>17</sup>

In addition, after observing the inhibitory effect of pregabalin on the ectopic discharge from injured afferents, we determined whether pregabalin had any effect on responses of normal A $\delta$  and C fibers to mechanical stimulation (these normal afferent fibers usually have no spontaneous discharges). Single-unit activity of afferent fibers was recorded from the left sciatic nerve in separate normal rats. After the receptive fields of afferents were precisely located on the skin, afferent responses to stimulation of the receptive field with calibrated von Frey filaments were examined before and after intravenous injection of 30 mg/kg pregabalin.

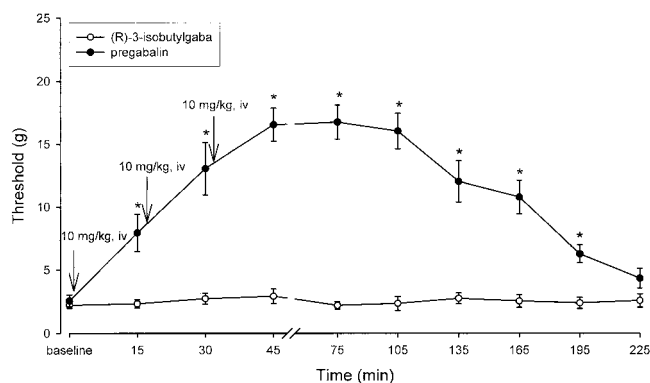
The conduction velocity of normal afferents was measured by electrical stimulation of the sural nerve, and the conduction velocity of injured afferent fibers was determined through electrical stimulation of the sciatic nerve proximal to the ligation site. Conduction time was determined by measuring the time interval from the signal of electrical stimulation to recording of the evoked afferent's action potential, displayed on the oscilloscope. C- and A $\delta$ -fiber afferents were classified as those with a conduction velocity of less than 2.5 and 2.5–15 m/s, respectively.<sup>17</sup>

All the behavioral data collected were normally distributed, as determined by the Komogorov-Smirnov test. Thus, the data are presented as mean  $\pm$  SEM, and parametric tests were chosen for statistical analysis. The ectopic discharge activity of afferents was averaged over 10–15 min before and after each treatment. Paw withdrawal threshold or latency in response to mechanical or thermal stimulation before and after nerve ligation, and the conduction velocity and the evoked response of normal afferents by mechanical stimulation before and after pregabalin treatment were compared by a paired Student *t* test. The effects of pregabalin and *R*-3-isobutylgaba on allodynia-hyperalgesia and afferent ectopic activity were determined by analysis of variance followed by the Tukey *post hoc* test.  $P < 0.05$  was considered to be statistically significant.

## Results

### *Effect of Pregabalin and the Stereoisomer on Mechanical Allodynia*

Paw withdrawal threshold in response to application of von Frey filaments before sciatic nerve ligation was  $22.8 \pm 2.5$  g. The mechanical threshold decreased significantly ( $2.2 \pm 0.4$  g,  $P < 0.05$ ) within 7 days after nerve ligation and remained stable for at least 4 weeks. Two rats were excluded from the study because the withdrawal threshold was more than 8 g one week after nerve ligation. Intravenous injection of 10–30 mg/kg pregabalin significantly increased the paw withdrawal threshold in 10 rats in a dose-dependent manner (fig. 1). Pregabalin administration was not associated with overt behavioral or motor function changes, assessed by testing the animals' ability to stand and ambulate in a normal posture and to place and step with the hind paws.<sup>22</sup> Intravenous injection of 10–30 mg/kg *R*-3-isobutylgaba did not significantly affect the allodynia in eight other rats (fig. 1).



**Fig. 1.** Effect of intravenous (iv) injection of 10–30 mg/kg pregabalin ( $n = 10$ ) or *R*-3-isobutylgaba ( $n = 8$ ) on mechanical thresholds determined by paw withdrawal response to von Frey filaments. The timing of three injections is indicated by arrows. Data are presented as mean  $\pm$  SEM. \* $P < 0.05$  versus pretreatment control.

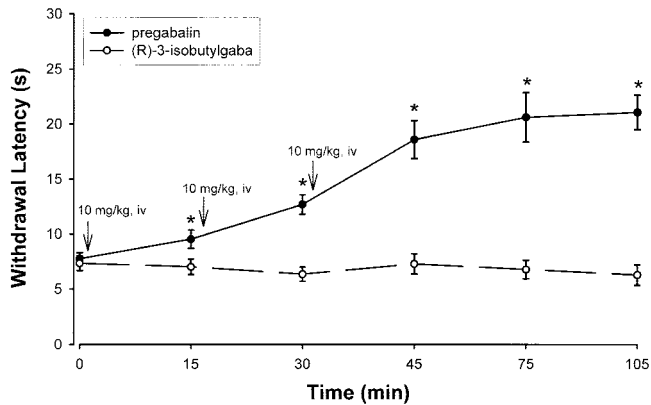


Fig. 2. Effect of intravenous (iv) injection of 10–30 mg/kg pregabalin ( $n = 8$ ) or *R*-3-isobutylgaba ( $n = 6$ ) on the withdrawal latency of the left hind paw in response to radiant heat stimulation. The timing of three injections is indicated by arrows. Data are presented as mean  $\pm$  SEM. \* $P < 0.05$  versus pretreatment control.

#### Effect of Pregabalin and the Stereoisomer on Thermal Hyperalgesia

The withdrawal latency of the left hind paw in response to the radiant heat stimulation before sciatic nerve ligation was  $12.5 \pm 0.8$  s. The thermal threshold of the left injured paw decreased significantly ( $7.6 \pm 0.9$  s,  $P < 0.05$ ) within 7 days after ligation of the left sciatic nerve. Intravenous injection of 10–30 mg/kg pregabalin significantly increased the paw withdrawal latency in eight rats in a dose-dependent manner (fig. 2). However, intravenous injection of 10–30 mg/kg *R*-3-isobutylgaba did not significantly affect the thermal hyperalgesia in the left hind paw ( $n = 6$ , fig. 2).

#### Electrophysiologic Recording Studies

A total of 27 afferent fibers with ectopic discharges (15 tested for pregabalin and 12 tested for the isomer) were recorded from the injured left sciatic nerve in 27 rats. These afferents exhibited typical spontaneous bursting discharge activity (fig. 3), as characterized in detail previously.<sup>14</sup> Intravenous injection of 10–30 mg/kg pregabalin significantly decreased the ectopic discharge activity of 15 afferent fibers in a dose-dependent fashion (figs. 3 and 4). The conduction velocity was measured in 10 of 15 afferents in the pregabalin group. There were eight A $\delta$  fibers with a conduction velocity ranging from 3.0 to 13.8 m/s. The conduction velocities of those two C fibers were 0.6 and 1.5 m/s, respectively. We paid attention to changes in the interspike interval of afferent firing and to the on-off bursting cycle. As the dose of pregabalin increased, the average duration of off periods lengthened progressively as that of on periods shortened gradually (fig. 3), but the interspike intervals did not change. By contrast, intravenous injection of 10–30 mg/kg *R*-3-isobutylgaba did not significantly attenuate the ectopic discharge frequency of 12 separate afferent fibers (11 A $\delta$  fibers and 1 C fiber) during the

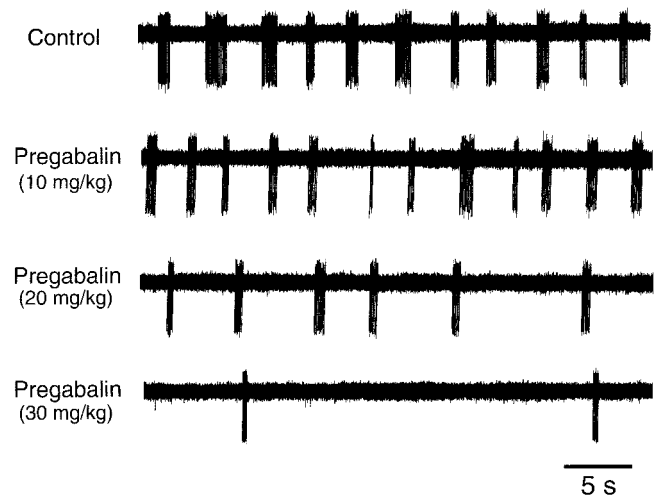


Fig. 3. Original representative neurograms showing dose-dependent inhibitory effect of intravenous pregabalin on ectopic discharge activity of an injured sciatic afferent fiber (conduction velocity = 4.6 m/s). The neurograms were sampled between 12–14 min after each injection. The cumulative doses of pregabalin are shown on the left.

entire recording period of 45–60 min (fig. 4). Additionally, we observed that intravenous injection of 30 mg/kg pregabalin had no effect on the spontaneous discharges of five afferent fibers recorded from uninjured axons running in the sciatic nerve (from  $2.2 \pm 0.6$  impulses/s to  $2.1 \pm 0.5$  impulses/s,  $P > 0.05$ ).

In 12 normal rats, intravenous injection of 30 mg/kg pregabalin did not alter the response of 12 normal afferent fibers to mechanical stimulation, evoked by application of calibrated von Frey filaments with bending forces of 2, 5, and 25 g applied to the afferents' receptive fields (fig. 5). Among 12 normal afferents, 9 were A $\delta$  fibers (conduction velocity,  $6.8 \pm 1.3$  m/s), and 3 were C fibers (conduction velocity,  $1.1 \pm 0.4$  m/s). Intravenous injection of 30 mg/kg pregabalin had no effect on the conduction velocity of these 12 afferents.

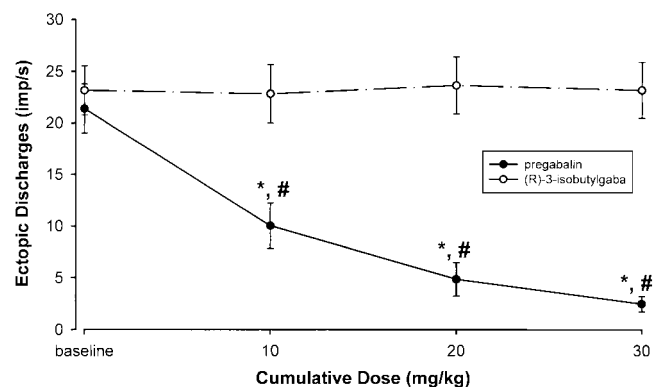


Fig. 4. Effect of intravenous injection of 10–30 mg/kg pregabalin ( $n = 15$ ) or *R*-3-isobutylgaba ( $n = 12$ ) on spontaneous discharge activity recorded from injured afferent fibers. The points represent averaged discharge activity during the entire recording period between doses. Data are presented as mean  $\pm$  SEM. \* $P < 0.05$  versus pretreatment control. # $P < 0.05$  versus corresponding values in *R*-3-isobutylgaba group.

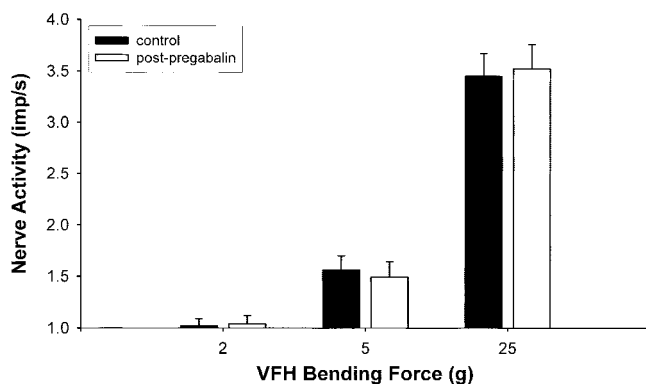


Fig. 5. Lack of effect of intravenous injection of 30 mg/kg pregabalin on the responses of normal afferents ( $n = 12$ ) elicited by topical application of von Frey filaments (VFH). Data are presented as mean  $\pm$  SEM. Receptive fields of all afferents were located in the left hind paw.

## Discussion

In the current study, we examined the effect of pregabalin at therapeutically relevant doses on ectopic discharges of injured afferent nerves in a rat model of neuropathic pain. The major finding of the current study is that pregabalin stereospecifically inhibited the ectopic discharge activity from the injured peripheral afferent nerve. We observed that intravenous injection of pregabalin dose-dependently reversed tactile allodynia and thermal hyperalgesia caused by partial sciatic nerve ligation. Furthermore, the observed analgesic effect of pregabalin paralleled with its inhibitory effect on the discharge frequency of ectopic afferent activity. Conversely, the stereoisomer, *R*-3-isobutylgaba, neither produced analgesic effect nor altered the frequency of ectopic discharges in this rat model of neuropathic pain. Therefore, these data provide new evidence that the peripheral action of pregabalin on the generation of ectopic discharge activity from injured afferent fibers likely constitutes an additional mechanism by which pregabalin produces an analgesic effect on neuropathic pain.

Previous studies indicate that high-frequency discharges from ectopic sites in primary afferents after nerve injury cause hypersensitivity of spinal cord dorsal horn neurons, which contributes to the sustained neuropathic pain states.<sup>13,14,23</sup> Although pregabalin and gabapentin are effective in various pain models, the inhibitory action of pregabalin on ectopic discharge activity from injured peripheral afferents has not been studied. We recently have demonstrated that therapeutic doses (30–90 mg/kg, intravenous) of gabapentin are capable of suppressing ectopic discharge activity generated from injured afferent nerves in rats.<sup>17</sup> Furthermore, gabapentin inhibits the excitatory synaptic input to the spinal dorsal horn neurons through a presynaptic site in rats with diabetic neuropathic pain.<sup>24</sup> These data suggest that the inhibitory action of gabapentinoids on injured afferent nerves is relevant to their analgesic effect on

neuropathic pain. The peripheral action of gabapentinoids is also supported by the finding that local injection of gabapentin or pregabalin attenuates nociception elicited by formalin in rats.<sup>25</sup> However, one study has reported that intraperitoneal injection of a single dose (50 mg/kg) of gabapentin has no effect on spontaneous ectopic discharges recorded from the spinal dorsal root in rats subjected to L<sub>5</sub>–L<sub>6</sub> spinal nerve ligation.<sup>26</sup> It is unclear whether the discrepancy is due to different animal models (sciatic nerve *vs.* spinal nerve ligation), different recording procedures (proximal sciatic nerve *vs.* dorsal root recordings), or different routes of drug administration (intravenous *vs.* intraperitoneal). It is also possible that the ectopic discharges may originate from the dorsal root ganglia rather than the injured nerve axons in that study.<sup>26</sup> It is uncertain whether different mechanisms exist for generating ectopic discharges from the neuroma and dorsal root ganglia. Therefore, the possibility that gabapentin may affect ectopic activity only from neuromas, not from dorsal root ganglia, requires further study.

The site of analgesic action of gabapentinoids has been considered to be centrally mediated.<sup>2,9</sup> As demonstrated in the current study, intravenous injection of 10–30 mg/kg pregabalin suppressed ectopic discharge activity generated from injured afferent nerves. Therefore, in addition to the possible effect of pregabalin on sensitized spinal neurons caused by nerve injury,<sup>27</sup> the effect of pregabalin on ectopic afferent activity may contribute to its analgesic action by directly eliminating the nociceptive afferent input to the spinal cord. Our data indicate that pregabalin has a rapid effect on ectopic discharges, which is consistent with its effect on tactile allodynia and thermal hyperalgesia. Therefore, the hypersensitivity of spinal dorsal horn neurons developed in this model may be highly dependent on the ectopic afferent barrage. As such, elimination of abnormal input by pregabalin could rapidly reverse the neuropathic pain condition. Alternatively, the effect of intravenous injection of pregabalin on ectopic discharges may not account entirely for its analgesic effect, which may be a result of its combined central and peripheral actions. In this regard, pregabalin can minimize the peripheral nociceptive input to the spinal cord, which could reduce spinal neurotransmitter release and normalize the hypersensitivity state of spinal dorsal horn neurons. Data from this study provide a new rationale for the use of systemic pregabalin as a potent analgesic agent in neuropathic pain.

In the thermal testing experiments, we observed that pregabalin not only reversed thermal hyperalgesia but also decreased the thermal sensitivity that was significantly different from the value measured before nerve ligation in the same rats. It is generally considered that the thermal nociceptive response is mediated largely by unmyelinated C fibers.<sup>28</sup> In rats subjected to nerve ligation, most C-fiber afferents distal to the site of ligation

degenerate.<sup>29</sup> Therefore, the paw thermal sensitivity of the injured side is expected to decrease after nerve ligation. However, in the presence of ectopic afferent discharge activity, such abnormal sensory input could sensitize the spinal dorsal horn neurons, resulting in not only tactile allodynia but also thermal hyperalgesia mediated by the remainder of C-fiber afferents. Therefore, it is likely that after pregabalin treatment, the ectopic afferent activity is minimized, which could normalize the sensitivity of spinal cord dorsal horn neurons. This may explain the reduced thermal sensitivity of the injured hind paw after pregabalin treatment in this rat model of neuropathic pain. It should be acknowledged that phenotypic changes in noninjured nociceptive-nonnociceptive fibers may also contribute to the development of heat hyperalgesia.

Our study provides further support that gabapentin and pregabalin act at a common site to reduce ectopic discharges in a stereospecific fashion. Both voltage-decendent  $\text{Na}^+$  channels and voltage-dependent  $\text{Ca}^{++}$  channels are closely related to the generation of ectopic discharge activity of injured afferents.<sup>14,30</sup> Recent studies have shown that gabapentin has a high affinity to the  $\alpha_2\delta$  subunit of  $\text{Ca}^{++}$  channels in the brain tissue, and pregabalin interacts with this site in a stereoselective manner.<sup>31,32</sup> No definite evidence is yet available to link the analgesic action of gabapentinoids with the  $\alpha_2\delta$  subunit of  $\text{Ca}^{++}$  channels. Although one study failed to show an effect of gabapentin on voltage-sensitive  $\text{Ca}^{++}$  channels,<sup>10</sup> another study has shown that gabapentin can block  $\text{Ca}^{++}$  current in cortical neurons.<sup>33</sup> The  $\alpha_2\delta$  subunit is common to all voltage-dependent  $\text{Ca}^{++}$  channels,<sup>34,35</sup> which are widely distributed throughout the peripheral and central nervous systems. The physiologic role of the  $\alpha_2\delta$  subunit is not clear and is considered to increase the functional expression of calcium channel complexes.<sup>34,36</sup> There are three subtypes of  $\alpha_2\delta$  subunits,<sup>37</sup> and gabapentin may target specifically the  $\alpha_2\delta$ -1 subunit.<sup>37</sup> It remains unclear which  $\alpha_2\delta$  subtype is associated with the neuropathic pain and afferent ectopic discharges. Primary afferent neurons are known to express  $\text{Ca}^{++}$  channels.<sup>38</sup> A recent study has shown a pronounced upregulation of  $\alpha_2\delta$ -1 subunit of  $\text{Ca}^{++}$  channels in the dorsal root ganglion after nerve ligation.<sup>39</sup> It has been reported that administration of N-type  $\text{Ca}^{++}$  channel blockers, SNX-111 and SNX-124, onto the site of nerve injury attenuates thermal hyperalgesia and tactile allodynia induced by sciatic nerve ligation in rats.<sup>40</sup> Further studies are required to show the binding site of gabapentin and pregabalin at the neuroma site and the role of the  $\alpha_2\delta$  subunit of voltage-dependent  $\text{Ca}^{++}$  channels in the maintenance of neuropathic pain and generation of ectopic discharges. Our *in vivo* data indicate that pregabalin suppresses the ectopic discharge activity from neuromas but did not affect the conduction velocity and the response of normal afferent fibers to tactile

mechanical stimulation. Because we did not test the effect of pregabalin in the response of normal afferent nerves to thermal stimulation, the observed lack of pregabalin effect on stimulus-evoked parameters in normal fibers applies only to mechanical stimuli.

In summary, we found that intravenous injection of 10–30 mg/kg pregabalin, but not *R*-3-isobutylgaba, significantly attenuated the tactile allodynia and thermal hyperalgesia induced by partial sciatic nerve ligation in rats in a dose-dependent manner. Furthermore, the same doses of pregabalin, but not its stereoisomer, dose-dependently inhibited the ectopic discharge activity from injured afferent nerves. However, the evoked response of normal afferent fibers and the conduction velocity of afferent nerves were not affected by pregabalin. Therefore, this study provides new information that systemic pregabalin produces analgesic effect on neuropathic pain, a stereospecific action that may be mediated, at least in part, by inhibition of the impulse generation of ectopic afferent discharges at the site of nerve injury.

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