Gabapentin Attenuates Nociceptive Behaviors in an Acute Arthritis Model in Rats

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ABSTRACT
In this study, we investigated the effectiveness of gabapentin (Neurontin), administered spinally with a microdialysis fiber, in reducing nociceptive behavioral responses induced by a knee joint inflammation model. This model is produced by injection of the knee joint with kaolin and carrageenan in rats. The resultant knee joint inflammation produces a secondary hyperalgesia to radiant heat applied to the hindpaw. Both pretreatment and post-treatment protocols were examined. Spinal administration of gabapentin (10 mg/ml) infused 1.5 h before induction of knee joint inflammation, although having no effect on the baseline, prevented the development of heat hyperalgesia. Gabapentin also prevented the development of other pain-related behaviors scored subjectively. Gabapentin had no effect, however, on the joint circumference increase typical in this model. In animals with fully developed knee joint inflammation, gabapentin produced a reversal of heat hyperalgesia. The paw withdrawal latency responses and subjective pain scores were no longer significantly different from baseline, but joint circumference increases remained. These data suggest that gabapentin is an effective antinociceptive agent when administered either before or after induction of knee joint inflammation acting through a central neurogenic mechanism.

Gabapentin [1-(aminomethyl)cyclohexane acetic acid; Neurontin] has been used extensively as a safe and effective oral anticonvulsant therapy for humans with partial or generalized epilepsy (Crawford et al., 1987; UK Gabapentin Study Group, 1990; Chadwick, 1992; US Gabapentin Study Group Number 5, 1993). Recent studies have also demonstrated its effectiveness in a number of painful conditions, including reduction of neuropathic pain (Rosner et al., 1996; Xiao and Bennett, 1996; Rosenberg et al., 1997; Gould, 1998; Merren, 1998) and postherpetic neuralgia (Segal and Rordorf, 1996). Gabapentin [1-(aminomethyl)cyclohexane acetic acid; Neurontin] has been used extensively as a safe and effective oral anticonvulsant therapy for humans with partial or generalized epilepsy (Crawford et al., 1987; UK Gabapentin Study Group, 1990; Chadwick, 1992; US Gabapentin Study Group Number 5, 1993). Recent studies have also demonstrated its effectiveness in a number of painful conditions, including reduction of neuropathic pain (Rosner et al., 1996; Xiao and Bennett, 1996; Rosenberg et al., 1997; Gould, 1998; Merren, 1998) and postherpetic neuralgia (Segal and Rordorf, 1996). In animal studies, either s.c. or spinal administration of gabapentin reduces formalin-induced nociceptive behaviors (Singh et al., 1996; Stanfa et al., 1997; Shimoyama et al., 1997a; Carlton and Zhou, 1998) and the spontaneous activity of spinal neurons of nerve injured rats (Chapman et al., 1998). The intrathecal injection of gabapentin produces a dose-dependent reversal of the thermal hyperalgesia evoked by mild thermal injury (Jun and Yaksh, 1998). Studies in this laboratory and others have previously shown that both the inflammation and hyperalgesia are reduced by spinal administration of the gabapentin analog isobutygaba in the knee joint inflammation model (Dirig et al., 1998; Houghton et al., 1998). Enhancement of the antinociceptive effects of mor-
inflammation model. In animals with inflamed knee joints, the effects on the PWL were highly correlated with the dorsal horn immunoreactive glutamate content (i.e., in animals where paw withdrawal response times were short, dorsal horn glutamate immunoreactivity was high) (Sluka and Westlund, 1993d). The PWL decreases and the measurable and stable amino acid increases were blocked by spinal administration of GABA_A, non-NMDA, or NMDA glutamate receptor antagonists (Sluka et al., 1993; Sluka and Westlund, 1993a,b,c). The resultant amplification and persistence of the nociceptive state attributable to events generated in the dorsal horn in this model are suggestive of an long-term potentiation-like or epileptiform event. These findings suggested that gabapentin might be effective in blocking the dorsal horn neurogenerative events induced after knee joint injection of kaolin and carrageenan that result in a persistent nociceptive and inflammatory state. Thus, in the following study, gabapentin was administered to the dorsal horn through a microdialysis fiber and the effects were noted on secondary hyperalgesic behavior in response to radiant heat.

**Materials and Methods**

All studies followed the guidelines of the Institutional Animal Care and Use Committee, in accordance with the guidelines of the National Institutes of Health. Forty-six animals in two experimental groups were treated 1) before and 2) after the induction of an experimental inflammation. Inflammation was induced with a knee joint injection of kaolin/carrageenan. Gabapentin or artificial cerebrospinal fluid (aCSF) was administered through a microdialysis fiber positioned in the dorsal horn for spinal treatment (surgically implanted the previous day) or s.c. in the nape of the neck for systemic release. All experiments were carried out by an observer who was blind to the drug treatment.

**Placement of Microdialysis Fibers.** Sprague-Dawley rats (220–270 g) were anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg i.p.). A microdialysis fiber (200 μm o.d., 45,000 molecular weight cutoff; Hospal AN69) was coated with epoxy resin, except for a 2-mm section. In 40 animals, the microdialysis fiber was placed in the dorsal horn. A small midline incision was made in the skin over the L1 vertebral level. The L1 vertebra was cleared of muscle, and a hole was drilled in both sides of the lamina. The microdialysis fiber was then passed through the holes in the vertebrae and transversely through the dorsal horn of the spinal cord. The microdialysis fiber lay between L1-L6 segments with the permeable 2-mm section of the fiber in the dorsal horn. The microdialysis fiber was connected to PE_20 tubing (Becton Dickinson) that was tunneled under the skin to the nape of the neck. The connecting joint between the microdialysis fiber and PE_20 tubing was stabilized with dental cement. The aCSF was pumped through the tubing at a rate of 5 μl/min for 1 h before the PE_20 tubing was sealed, and the animal was allowed to recover for 24 h. Once the rats were awake, they were examined for motor deficits; any rat that had motor deficits was excluded from the study.

As a systemic control for drug administration, the microdialysis fiber was implanted in the subcutaneous tissue at the nape of the neck in an additional six rats.

**Behavior Testing and Assessment of Arthritis.** The PWL responses to noxious radiant heat were tested as a standard measure of heat hyperalgesia (Hargreaves et al., 1988). A decrease in the PWL responses in animals with knee joint inflammation was interpreted as indicative of hyperalgesia (Lewis, 1942; Merskey and Bogduk, 1994). Because the radiant heat stimulus is applied to the plantar surface of the hindpaw at quite some distance from the inflamed knee joint, the measure reported represents secondary heat hyperalgesia.

On the day after fiber placement, animals were housed in small Lucite cubes on an elevated glass plate. Radiant heat was applied to the plantar surface of the hindpaw until the rat lifted the paw. The time which it took for this to occur was considered the PWL response time. Both paws were tested independently at 5-min intervals for a total of five readings. A mean of these five readings was used as the animal’s PWL response for each time point. In pretreatment rats (n = 12), PWL was measured 1) before the administration of any drugs (baseline), 2) after the drug had been infused for 1.5 h, and 3) 4 h after the induction of arthritis. In the post-treatment group (n = 18), the animals were tested 1) before the induction of arthritis in the knee joint (baseline), 2) 4 h after the induction of arthritis, and 3) 1.5 h after drug infusion (i.e., 5.5 h after the induction of arthritis).

The pain-related behavior, the extent of guarding of the hindpaw of the arthritis limb, was scored by two independent observers. To quantify these changes, the animals were graded by a subjective pain rating scale (0–5) modified from that described by Guilbaud and colleagues (Attal et al., 1990) where 0 is normal, 1 is curling of the toes, 2 is evasion of the paw, 3 is partial weight bearing, 4 is non-weight bearing and guarding, and 5 is avoidance of any contact with the hindpaw.

The circumference of the knee joint was also measured as in previous studies (Sluka et al., 1993a) using a flexible tape measure before the induction of arthritis (baseline), 4 h after the induction of arthritis (pretreatment group), and 1.5 h after drug infusion (5.5 h after the induction of arthritis in the post-treatment group).

**Induction of Arthritis.** Rats were anesthetized briefly with methohexital sodium (Brevital Sodium, 60 mg/kg i.p.) after baseline behavior tests (post-treatment group) or after infusion of the drug (pretreatment group). The knee joint was then injected with 0.1 ml of 3% kaolin and 3% carrageenan suspended in sterile saline and was flexed manually until the rat awoke (approximately 5–10 min.)

**Administration of Drug.** A dose-response curve was generated by examining the effects of gabapentin pretreatment in animals with knee joint inflammation (4 h) treated similarly to the experimental groups. In this pretreatment group, drugs were infused at concentrations of 0.1 (n = 4), 0.3 (n = 4), 3 (n = 4), 10 (n = 6), and 30 (n = 4) mg/ml. The gabapentin was dissolved in aCSF. The animals treated with the maximally effective dose, 10 mg/ml, were used in the pretreatment experimental group comparisons. The 10 mg/ml dose group was also used in the post-treatment experiments. A curve-fitting program (INPLOT Version 3.15; GraphPAD, San Diego, CA) was used to plot the log dose response for the effect of the varying concentrations of gabapentin in the microdialysis tube, as well as to generate the EC_50 value.

The experimental animals either received drug, gabapentin, or aCSF as a control. Both gabapentin and aCSF were infused through the microdialysis fiber at a rate of 5 μl/min. The drug concentration transferred across the microdialysis membrane is maximally 17% as determined in vitro by spectrophotometry (Beckman DU650). Therefore, with the diffusion barriers presented by the tissue, the neurons are likely to be exposed to a dose of gabapentin (<1.7 mg/ml) much lower than that inside the microdialysis fiber (10 mg/ml). The pH values of the gabapentin solution and aCSF were adjusted by bubbling with 95% CO_2/5% O_2 (approximately 7.4) before use. The drug was a gift from Parke-Davis and was synthesized at Parke-Davis Research Laboratories, a Division of Warner-Lambert (Ann Arbor, MI). Previous studies have characterized gabapentin analogs, R (+)-3- and R(-)-3-isobutyrglyba, in this model (Houghton et al., 1998).

**Statistical Analysis.** The results for each group were expressed as the average percent change from baseline ± S.E.M. Paired t tests were used to compare the test responses of each animal with its own baseline (p < .01) for PWL and circumference data. Nonparametric tests were used to analyze the spontaneous pain behavior scores because this is an ordinal scale. Pairwise comparisons for each treatment groups with their own baseline were made with the Wilcoxon sign test (p < .05).
Results

**Baseline Measures.** The baseline PWL, spontaneous behavior, and knee joint circumference of all 46 rats used in this study were measured before infusion of the drug or vehicle through the spinal cord or subcutaneously. The mean ± S.E.M. PWL responses and knee joint circumference for the total population were 10.31 ± 0.15 s and 5.33 ± 0.04 cm, respectively. No spontaneous pain-related behaviors were noted, and a score of zero was given.

Consequent Changes with Joint Inflammation. In Table 1, the baseline and outcome responses for the arthritic animals are presented for all measures. The data include the combined measures for the aCSF-treated arthritic control animals from both pretreatment and post-treatment groups. In these aCSF-treated arthritic control rats (n = 12), 4 h after the injection of kaolin and carrageenan, the PWL responses to noxious radiant heat decreased to 76% of baseline value. This decrease was significant (paired t test, p < .01) and indicated the presence of secondary heat hyperalgesia. There was also a significant change in the hindpaw posture of the rat indicative of spontaneous ongoing pain-related behavior. These postural changes representing spontaneous ongoing pain-related behavior were represented by a score of 1.25 ± 0.13 (p < .05). A significant 14% increase in knee joint circumference is noted compared with the baseline (paired t test, p < .01).

Dose Response of Gabapentin Pretreatment. The dose range of gabapentin infused through the microdialysis fiber was 0.1, 0.3, 3, 10, and 30 mg/ml in the pretreatment group (n = 4 for each dose except n = 6 for the dose of 10 mg/ml). The PWL responses for this dose range (expressed in mM) are illustrated on a logarithmic scale in Fig. 1. The EC50 value for gabapentin was 4.34 mM. The maximally effective dose, 10 mg/ml (58 mM), was chosen for subsequent experiments.

**Pretreatment: Effect of Gabapentin Infusion Directly into Spinal Cord before Induction of Knee Joint Inflammation.** Gabapentin or aCSF was infused through the microdialysis fiber into the spinal cord for 1.5 h before the knee joint was injected with kaolin and carrageenan. There was no significant effect on baseline of either the gabapentin or aCSF on any of the measures (Table 2 and Fig. 2A).

Four hours after injection of the knee joint with kaolin and carrageenan, the aCSF-treated animals had a significant reduction in their PWL responses and demonstrated significant spontaneous pain-related behaviors as expected. In contrast, gabapentin was effective in preventing the development of secondary hyperalgesia responses to the applied radiant heat. The PWL response to radiant heat and the posture of the hindpaw with arthritis were not significantly changed from baseline (Fig. 2, A and B) or that of the other hindlimb after gabapentin treatment. The circumference of the inflamed joint (Fig. 2C), however, was increased significantly 4 h after arthritis, similar to the aCSF arthritic control rats. Thus, although gabapentin was not effective in blocking the development of joint inflammation in the pretreated group of rats, gabapentin was highly effective in preventing the development of secondary heat hyperalgesia and measures of spontaneous pain-related behaviors.

Post-treatment: Effect of Gabapentin Infusion into Spinal Cord or s.c. after Induction of Knee Joint Inflammation. Three groups of animals received gabapentin or aCSF in post-treatment studies (Table 3 and Fig. 3). Two groups of rats were infused with the drug or vehicle through a microdialysis fiber implanted directly into the spinal cord. One group received the same dose of gabapentin systemically through a microdialysis fiber implanted subcutaneously at the nape of the neck. Secondary heat hyperalgesia and spontaneous pain-related behaviors were reversed only in arthritic animals receiving spinally administered after treatment with gabapentin.

Four hours after injection of kaolin and carrageenan, all animals infused with gabapentin spinally displayed reduced PWL responses and spontaneous pain-related behaviors. The PWL significantly decreased to about 81% of baseline measurements (paired t test, p < .01; Table 3 and Fig. 3A). The animals with arthritic hindpaws started curling their toes and decreasing their weight bearing. By 1.5 h after spinal gabapentin infusion, the PWL measurements returned back to the baseline and the toes flattened on the table (Fig. 3B); however, the drug did not reduce the amount of the joint swelling. After 4 h of arthritis and 1.5 h of drug infusion, the

![Dose Response Curve](image-url)

**Fig. 1.** Dose-response curve illustrating the antihyperalgesic effect of gabapentin administered spinally through a microdialysis fiber in the dorsal horn before induction of arthritis. Represented is the ability of gabapentin pre-treatment to inhibit the decrease in PWL responses observed after the induction of knee joint inflammation. The doses used (mM) are plotted on a logarithmic scale. Each point represents the average of percent of baseline for the five doses tested (mean ± S.E.M.). The EC50 value is indicated.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Effect of kaolin/carrageenan on pain-related measures</td>
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<tr>
<td>PWL s</td>
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<tr>
<td><strong>Baseline</strong></td>
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<td>Arthritis (4 h)</td>
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* p < .05.
** p < .01.
The circumference of the knee joint was significantly increased about 22% above baseline (Fig. 3C), similar to aCSF arthritic control and subcutaneously treated rats.

In the group that was infused with gabapentin s.c., the PWL responses to noxious radiant heat significantly decreased by 15% from baseline measurements 4 h after joint injection. After 1.5 h of drug infusion, the PWL responses continued to decrease to 82% of the baseline value, which was similar to aCSF control arthritic rats (Fig. 3A). Both the pain-related behavior score (Fig. 3B) and the circumference (Fig. 3C) of the inflamed joint increased significantly after 4 h arthritis and 1.5 h drug infusion (5.5 h post). Thus, gabapentin administered subcutaneously at the maximally effective spinal dose did not affect the PWL responses to radiant heat and spontaneous pain-related behavior, in contrast to the effectiveness of this dose when administered to the spinal cord.

### Table 2

<table>
<thead>
<tr>
<th>Pretreatment Group</th>
<th>Baseline PWL (% of baseline) ± SEM</th>
<th>PWL (1.5 h after drug infusion) ± SEM</th>
<th>PWL (4 h after joint injection) ± SEM</th>
<th>Behavior Score (4 h after joint injection) ± SEM</th>
<th>Circumference (4 h after joint injection) ± SEM</th>
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</thead>
<tbody>
<tr>
<td>Gabapentin (n = 6)</td>
<td>100 ± 0.67</td>
<td>105.18 ± 4.56</td>
<td>100.03 ± 4.37</td>
<td>0.67 ± 0.20</td>
<td>114.20 ± 1.53**</td>
</tr>
<tr>
<td>aCSF (n = 6)</td>
<td>100 ± 0.67</td>
<td>93.12 ± 6.31</td>
<td>74.47 ± 3.44**</td>
<td>1.33 ± 0.2*</td>
<td>114.87 ± 1.74**</td>
</tr>
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*p < .05.

**p < .01.

Fig. 2. The effect of gabapentin pretreatment on (A) PWL responses to radiant heat, (B) pain-related behavior, and (C) circumference. After baseline (base) PWL testing, animals received gabapentin (10 mg/ml) for 1.5 h through a spinal microdialysis fiber. The PWL scores were again determined (1.5 h drug), and then their knee joint was injected with kaolin/carrageenan and the PWL responses were tested again at 4 h postinjection (4 h post). Circumference was assessed and compared only at baseline and 4 h after knee joint injection. Comparisons were made with baseline. *significantly different from baseline, p < .05. **significantly different from baseline, p < .01. Open columns, aCSF (cord) (n = 6); solid columns, gabapentin (cord) (n = 6).

### Discussion

In the present study, we found that gabapentin was effective in both preventing and reversing the secondary heat hyperalgesia and spontaneous pain-related behaviors induced by kaolin/carrageenan knee joint inflammation. In both treatment groups, the significant finding is the ability of gabapentin to retain (or return) the PWL scores at baseline. Thus, gabapentin and isobutylgaba (Houghton et al., 1998) are more effective antihyperalgesic agents in this knee joint inflammation than other agents we tested previously, including non-NMDA and NMDA glutamate receptor antagonists, a GABA<sub>δ</sub> receptor antagonist, and NK1 and NK2 receptor antagonists (Sluka et al., 1993, 1997; Sluka and Westlund, 1993b). Nonsteroidal anti-inflammatory agents have also been shown to be effective in carrageenan-evoked thermal hyperalgesia, including with post-treatment administration (Dirig et al., 1998). The effectiveness of gabapentin and isobutylgaba in reducing the hyperalgesia and pain-related behavior after the arthritis is fully developed in this model suggests that they may have clinically relevant effects in inflammatory pain conditions.

Gabapentin had no effect in the normal state. After 1.5 h of gabapentin infusion in the animals before the induction of arthritis, there were no significant changes in the PWL responses to radiant heat compared with the baseline. This is in agreement with other reports of behavioral studies in the literature (Fields et al., 1997a,b). Interestingly, increases in individual dorsal horn neuronal response rate and duration are reported after treatment with gabapentin in normal animals despite decreases in activity in response to gabapentin in cells recorded after inflammation (Stanfa et al., 1997).

Subcutaneous systemic treatment through the microdialysis fiber in this dose range had no effect in the present study. This is in contrast to a study by Singh and colleagues (Fields et al., 1997b) in which gabapentin was effective in returning PWL responses to baseline in a postoperative pain model.
with a subcutaneously administered dose above 10 mg/kg. The differences in these results are easily explained by the methodological differences. Most notably, the dose administered to the animals (10 mg/kg) in the previous report would effectively be much higher than that given presently. The effective dose administered to the tissue after diffusion from the microdialysis fiber is maximally 17% of the concentration of the solution pumped through the fiber (10 mg/ml). Thus, the gabapentin is extremely potent when administered spinally and is effective in doses below effective systemic doses.

The effectiveness of a limited spinal administration in this dose range even in the absence of systemic effectiveness confirms that binding sites for gabapentin are present in the spinal cord dorsal horn, as noted by Jun and Yaksh (1998). Tritiated gabapentin binding sites in other brain regions (Hill et al., 1993) overlap regions containing binding sites for those of $^3$H$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and $^3$H$\alpha$-cyano-2,3-dihydroxy-7-nitroquinolinolinate (Nielsen et al., 1990) but not of $^3$H$\alpha$GABA (Taylor, 1995). Previous studies from this laboratory have indicated that NMDA, non-NMDA, and GABA$\alpha$ receptor mechanisms in the dorsal horn contribute to a potentiated long-term effect that amplifies and prolongs the altered nociceptive state in this inflammation model (Rees et al., 1994; Sluka et al., 1995). Likewise, the present study suggests that gabapentin is effective not only in blocking the development of the persistent nociception but also in reversing ongoing secondary hyperalgesia. The fact that gabapentin is effective in the secondary hyperalgesia testable in this model confirms the ability to act through central nervous system circuitry because secondary hyperalgesia is believed to require a central neuronal loop.

In contrast to our previous study demonstrating effectiveness of the gabapentin analog isobutyrgaba as an anti-inflammatory agent (Houghton et al., 1998), gabapentin was not effective in reducing the inflammation itself. The lack of an anti-inflammatory effect by gabapentin was also noted by Singh et al. (1996). We have also shown in previous studies a differential effectiveness of non-NMDA and GABA$\alpha$ receptor antagonists in attenuation of the neurogenic inflammatory response, whereas NMDA and non-NMDA and GABA$\alpha$ receptor antagonists are effective in attenuating the secondary hyperalgesia in this model.

In summary, gabapentin was effective in pretreatment and post-treatment protocols in a rat knee joint inflammation model against both the secondary heat hyperalgesia and the spontaneous pain-related behaviors. The extent of the inflammation measurable as a change in joint circumference was not affected. Thus, the present study indicates that gabapentin is effective in preventing and reducing the neurodegenerative events responsible for the persistent nociceptive state that develops in response to knee joint injection with

![Fig. 3. The effect of gabapentin after treatment on (A) PWL responses to radiant heat, (B) pain-related behavior, and (C) circumference. The three groups tested included animals in which aCSF or gabapentin (10 mg/ml) was infused into the spinal cord (cord), as well as animals in which gabapentin was infused subcutaneously from the microdialysis fiber placed at the nape of the neck. For measurement of PWL responses, tests were done at baseline (base), 4 h after knee joint injection (4 h post), and 1.5 h after dorsal horn drug infusion (5.5 h postarthritis). Pain-related behavior scores were compared with baseline (zero). Circumference was measured at baseline and at 5.5 h after knee joint injection. *Significantly different from baseline, $p < .05$. **Significantly different from baseline, $p < .01$. Open columns, aCSF (cord) ($n = 6$); cross-hatched columns, gabapentin (s.c.) ($n = 6$); solid columns, gabapentin (cord) ($n = 6$).]
kaolin and carrageenan in this rat model. These studies suggest that gabapentin would be a suitable add-on therapy effective in addressing the central neurogenic contribution to painful peripheral inflammatory conditions.

References


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