Gabapentin Antinociception in Mice with Acute Herpetic Pain Induced by Herpes Simplex Virus Infection

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Received August 16, 2000; accepted October 17, 2000 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

The effects of systemic and local injections of gabapentin, a novel anticonvulsant agent, were tested on nociceptive behaviors in mice with acute herpetic pain. Transdermal injection with herpes simplex virus type-1 (HSV-1) produced nociceptive hypersensitivity of the infected hind paw to innocuous (allodynia) and noxious mechanical stimulation (hyperalgesia) with von Frey filaments. Systemic administration of gabapentin (10–100 mg/kg, peroral) produced a dose-dependent inhibition of both allodynia and hyperalgesia; gabapentin (30–300 mg/kg) did not affect locomotor activity. Intrathecal injection of gabapentin (10–100 μg/animal) also attenuated dose dependently both nociceptive hypersensitivities. In contrast, intraplantar, intracerebroventricular, and intracerebroventricular administration of gabapentin (10–100 μg/animal) had no effect on the HSV-1-induced nociceptive hypersensitivities. Pretreatment with naltrexone (1 mg/kg) inhibited antinociceptive effect of morphine (5 mg/kg), but not gabapentin (100 mg/kg). Repeated administration of morphine (5 mg/kg, four times) led to tolerance of antinociceptive action, whereas gabapentin (100 mg/kg, four times) had antinociceptive effect even after the forth administration. The present results suggest that gabapentin is effective in the treatment of acute herpetic pain without apparent adverse effects, and antinociceptive action of gabapentin is mainly mediated by actions on the spinal cord.

Gabapentin [1-(aminomethyl)cyclohexaneacetic acid], a novel anticonvulsant agent, is currently in clinical use as an “add-on” therapy in patients with partial seizures resistant to conventional therapies (Goa and Sorkin, 1993). Although gabapentin is a synthetic analog of γ-aminobutyric acid (GABA) and easily penetrates into the central nervous system, it does not interact with either GABA_A or GABA_B receptors (Taylor et al., 1998). It has been recently reported that gabapentin alleviates neuropathic pain, including postherpetic neuralgia, reflex sympathetic dystrophy, and diabetic neuropathy (Mellick et al., 1995; Rosner et al., 1996; Rosenberg et al., 1997). The clinical efficacy has been supported by studies using various animal models of pain. For example, systemic injection of gabapentin reverses allodynia of rats with neuropathy induced by partial ligation of the sciatic nerve (Pan et al., 1999) and prevents the development of thermal hyperalgesia in a rat model of postoperative pain (Field et al., 1997). Moreover, local (spinal or peripheral) injection of gabapentin reduces formalin-induced nociceptive behaviors (Carlton and Zhou, 1998; Yoon and Yaksh, 1999). Thus, there are several lines of evidence for effectiveness of gabapentin in the management of various pain in animals and human subjects; however, the analgesic mechanisms and site of action of gabapentin are still unknown.

Recently, we have developed a mouse model of acute herpetic pain (Takasaki et al., 2000a). When inoculated with herpes simplex virus type-1 (HSV-1) on the skin of the hind paw of the mouse, zosteriform skin lesions developed unilaterally in a corresponding dermatome after a 4-day latent period. Such mice showed aversive responses to innocuous tactile stimulation (designated as allodynia) and noxious mechanical stimulation (designated as hyperalgesia). These nociceptive responses became apparent when HSV-1 replicated in the dorsal root ganglion and eruption developed on the skin. We showed that HSV-1-induced allodynia and hyperalgesia were attenuated dose dependently by systemic (peroral) administration of gabapentin (Takasaki et al., 2000b). Thus, this study was conducted to determine the site and characteristics of antinociceptive action of gabapentin in mice with acute herpetic pain.

Materials and Methods

Animals. Female BALB/c mice weighing about 20 g (6 weeks old at the start of experiments; Japan SLC, Shizuoka, Japan) were used. They were housed six per cage under controlled temperature (22 ± 1°C) and humidity (55 ± 10%). The room was lighted from 7:00 AM to 7:00 PM and during the behavioral test. Food and water were freely available. HSV-1 inoculation and behavioral experiments were done in the infection room of Molecular Genetics Research Center, Toyama Medical and Pharmaceutical University, Toyama, Japan.

ABBR EVIATION S: GABA, γ-aminobutyric acid; HSV-1, herpes simplex virus type-1; NMDA, N-methyl-D-aspartate.
Virus Infection. The mice were inoculated with HSV-1 as described (Takasaki et al., 2000a). Briefly, HSV-1 (7401H strain, 1 × 10^6 plaque-forming units in 10 μl) was inoculated on the depilated shin skin of the right hind paw (5 × 5 mm) after scarification with 27-gauge needles. The contralateral hind paw was without inoculation.

Behavioral Test. Allodynia and hyperalgesia of the hind paw were assessed as described (Takasaki et al., 2000a). The mice were placed individually in a plastic cage (11 × 18 × 15 cm) with a wire mesh bottom. After acclimation period of at least 15 min, von Frey filaments with bending forces of 0.03 g (innocuous stimulation) and 1.20 g (noxious stimulation) were pressed perpendicularly against the plantar skin and held for 3 to 5 s with it slightly buckled. The stimulation of the same intensity was applied six times to each hind paw at intervals of several seconds. The responses to these stimuli were ranked as follows: 0, no response; 1, move away from von Frey filament; and 2, immediate flinching or licking of the hind paw.

Nociceptive score was calculated as follows:

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\text{Nociceptive score (%)} = \frac{\sum (\text{average score of each animal}) \times 100}{2 \times (\text{no. of animals tested})}
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Nociceptive tests were performed according to the guidelines published in a Guest Editorial in Pain on ethical standards for investigations of experimental pain in animals (Zimmermann, 1983).

Drug Administration. Gabapentin was synthesized at Department of Organochrome Design and Synthesis, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University (Takasaki et al., 2000b). It was dissolved in tap water for peroral injection or in physiological saline for intrathecal (Hylden and Wilcox, 1980), intraplantar, intracisternal (Ueda et al., 1979), and intracerebroventricular (Haley and McCormick, 1957) injections. Intrathecal, intracisternal, and intracerebroventricular injections were given in a volume of 5 μl and intraplantar injection was given in a volume of 20 μl. Morphine hydrochloride (Sankyo, Tokyo, Japan) was dissolved in physiological saline and administered s.c. Effects of gabapentin and morphine were tested on day 6 and in some case on day 7 postinoculation. The μ-opioid receptor antagonist naltrexone (Sigma, St. Louis, MO) was dissolved in physiological saline and administered s.c. at a dose of 1 mg/kg 15 min before morphine and gabapentin injection.

Locomotor Activity Test. Mice were placed individually in a wheel cage (25 cm in diameter and 6 cm in width), and locomotor activity was measured as the number of wheel revolutions. Locomotor activity was assessed from 1 to 2 h after gabapentin administration.

Data Analysis. Unless otherwise mentioned, the means of data are presented together with S.E.M. Results of behavioral experiments were analyzed with one-way ANOVA or repeated measures two-way analysis of variance and post hoc Dunnett’s multiple comparisons; \( p < 0.05 \) was considered significant.

Results

HSV-1 inoculation on the unilateral hind paw of mouse produced allodynia (nociceptive response to 0.03-g von Frey filament) and hyperalgesia (nociceptive response to 1.20-g von Frey filament). Allodynia and hyperalgesia of the HSV-1-inoculated hind paw became apparent since day 5 postinoculation and persisted at least until day 8 (Fig. 1). HSV-1 inoculation did not affect the responses of the contralateral (uninoculated) hind paw to von Frey filaments until at least day 8 postinoculation (Fig. 1).

Effects of Systemic Gabapentin on HSV-1-Induced Alloodynia and Hyperalgesia. Systemic administration of gabapentin (10–100 mg/kg p.o.) produced a significant, dose-dependent inhibition of allodynia and hyperalgesia induced by HSV-1 infection; the 100-mg/kg dose of gabapentin produced complete and partial inhibition in allodynia and hyperalgesia, respectively (Fig. 2, A and B). In contrast, gabapentin did not affect the behavioral responses of the contralateral (uninfected) hind paw to von Frey filaments (Fig. 2, C and D). Locomotor activity was not affected by antinociceptive and higher doses of gabapentin (30–300 mg/ kg) (Fig. 3).

Site of Antinociceptive Action of Gabapentin. Intrathecal injection of gabapentin (10–100 μg/animal) produced a significant, dose-dependent inhibition of both allodynia and hyperalgesia (Fig. 4, A and B). The inhibitory effects of the highest dose (100 μg/animal) peaked 60 min after injection and subsided by 120 min. Intrathecal gabapentin had no effect on the response of the contralateral hind paw at doses of 10 to 100 μg/animal (data not shown). Intraplantar, intracisternal, and intracerebroventricular injections of gabapentin did not affect allodynia and hyperalgesia induced by HSV-1 infection (Fig. 5, C–H). Any abnormal behaviors, including decrease or increase of locomotor activity, were not observed after local injections of gabapentin.

Effect of Naltrexone on Antinociceptive Action of Gabapentin and Morphine. The inhibitory effects of gabapentin (100 mg/kg p.o.) on allodynia and hyperalgesia induced by HSV-1 infection were not affected by pretreatment with naltrexone (1 mg/kg s.c., −15 min) (Fig. 6, A and B).
Morphine (5 mg/kg s.c.) attenuated both allodynia and hyperalgesia induced by HSV-1 infection. The effects of morphine were almost completely antagonized by pretreatment with naltrexone (1 mg/kg s.c.) (Fig. 6, C and D).

**Tolerance to Antinociceptive Effects.** Repeated administration of gabapentin (100 mg/kg p.o., twice a day) produced constant inhibition on allodynia and hyperalgesia for at least 2 days; the antinociception was apparent even after the fourth administration (Fig. 7, A and B). On the other hand, the antinociceptive effects of morphine (5 mg/kg s.c.) were rapidly decreased after repeated administration (twice a day); the effects of the third and fourth administration were significantly smaller than that of the first administration (Fig. 7, C and D).

**Discussion**

The mice infected with HSV-1 exhibited allodynia and hyperalgesia since day 5 postinoculation and such nociceptive hypersensitivities persisted until at least day 8, confirming our previous observation (Takasaki et al., 2000a). The occurrence of the nociceptive hypersensitivity may be due to the infection and especially proliferation of HSV-1 in the dorsal root ganglia, because the amount of HSV-1 DNA (Takasaki et al., 2000a) and the number of HSV-1 antigen-positive cells (Takasaki et al., 2000b) in the dorsal root ganglia peaked on day 5 postinoculation.

In the present study, systemic injection of gabapentin markedly attenuated both allodynia and hyperalgesia induced by HSV-1 infection. Peroral administration of antinociceptive doses (30 and 100 mg/kg) and even the higher dose (300 mg/kg) of gabapentin did not affect locomotor activity, suggesting that gabapentin does not have sedative and motor deficit effect at doses tested. Thus, antiallodynic and antihyperalgesic actions of gabapentin may not be mediated by sedation or motor deficits. Importantly, in patients with postherpetic neuralgia (Segal and Rordorf, 1996), idiopathic trigeminal neuralgia (Sist et al., 1997) or painful diabetic neuropathy (Gorson et al., 1999), gabapentin eliminates pain without serious side effects such as somnolence or dizziness that cause either discontinuation or reduction of the dose.

One important finding in this study is that only intrathecal, but not intraplantar, intracisternal, and intracerebroventricular injection of gabapentin was effective against tactile-evoked allodynia and mechanical hyperalgesia in mice infected with HSV-1, suggesting that the antiallodynic and antihyperalgesic actions of gabapentin are mediated primarily by the action on the spinal cord, but not on peripheral and supraspinal regions. Coincident with our results, intrathecal gabapentin is effective on tactile-evoked allodynia in rats with surgically induced neuropathic pain (Hwang and Yaksh, 1997) and on formalin-induced pain behavior in the rat (Yoon and Yaksh, 1999).

Because high levels of specific binding of [3H]gabapentin were detected in the outer layers of the cerebral cortex (Hill et al., 1993), the brain is considered to be a site of antinoci-
ceptive action. In the present study, however, intracisternal and intracerebroventricular injections of gabapentin failed to attenuate both allodynia and hyperalgesia, suggesting that the brain is not a primary site of antinociceptive action of gabapentin at least in acute herpetic pain.

It has been reported that intraplantar injection of gabapentin (6–600 μg/animal) attenuates nociceptive behavior induced by formalin injection (Carlton and Zhou, 1998), suggesting the peripheral action of gabapentin. In this study, however, local injection of gabapentin into the region of stimulation had no effect on acute herpetic pain. In our mouse model of herpetic pain, nociceptive response may be mainly due to the propagation of HSV-1 in the dorsal root ganglia, but not to the peripheral inflammation (Takasaki et al., 2000a). Peripheral injection of gabapentin may be effective against the pain of peripheral inflammation, but not to neuropathic pain.

In contrast to morphine, antinociceptive action of gabapentin was not blocked by pretreatment with naltrexone, a μ-opioid receptor antagonist, suggesting that antiallodynic and antihyperalgesic effects of gabapentin are not mediated by μ-opioid receptors. Furthermore, although repeated administration of morphine produced apparent tolerance to its antinociceptive effects, tolerance did not develop to the antinociceptive action of gabapentin. These results are consistent with previous report that repeated administration of gabapentin for 6 days did not lead to development of tolerance to the antinociceptive effects in formalin test (Field et al., 1997). It is noteworthy that the duration of antinociceptive effects of gabapentin is longer than that of morphine (Takasaki et al., 2000b).
to von Frey filaments. It was reported that gabapentin (30–300 mg/kg s.c.) failed to show an antinociceptive effect in transient pain models in rats (Field et al., 1997). Intrathecal gabapentin (30–300 µg/animal) also has no effects on the thermal escape latency of the normal hind paw in rats (Jun and Yaksh, 1998). Similarly, in clinical use, gabapentin does not inhibit pain induced by strong stimuli, although it was claimed to be particularly effective against tactile- and cold-allodynia (Attal et al., 1998). Therefore, gabapentin seems to have antiallodynic and antihyperalgesic effects but not antinociceptive effect on nociceptor-specific pain; gabapentin may be effective against nociceptive hypersensitivity induced by tissue damage or neuropathy.

There are many pharmacological investigations about gabapentin; however, the mechanisms of antiallodynic and antihyperalgesic action of gabapentin are still unknown. It has been shown that gabapentin binds with a high affinity to the α2δ-subunit of voltage-dependent calcium channel (Gee et al., 1996). Although the physiological role of the α2δ-subunit is not well understood, it appears to be common to all types of voltage-dependent calcium channels. It has been reported that intrathecal application of N-type voltage-dependent calcium channel antagonist attenuates mechanical allodynia induced by tight ligation of L5 and L6 spinal nerves in rats (Chaplan et al., 1994), and that N- and P-type channel antagonists produced antinociception in formalin test in rats (Malmberg and Yaksh, 1994; Diaz and Dickenson, 1997). These findings raise the possibility that the subunit of the voltage-dependent calcium channel may play an important role in mechanism of action of gabapentin.

Another potential of analgesic mechanisms of gabapentin is the blockade of glutamatergic systems and NMDA receptor in spinal dorsal horn. Recently, it has been reported that gabapentin decreases the amplitudes of both non-NMDA and NMDA receptor-mediated excitatory postsynaptic currents in dorsal horn neurons of the rat spinal cord slice (Shimoyama et al., 2000). Furthermore, it has been reported that D-serine, an antagonist at the strychnine-insensitive glycine site on the NMDA receptor, inhibits antihyperalgesic effects of gabapentin in thermal injury rats (Jun and Yaksh, 1998). These findings suggest that gabapentin may also have an inhibitory effect on glutamatergic excitatory neurotransmission in the dorsal horn. Voltage-dependent calcium channels (N- and P-types) localized at synaptic sites are involved in the release of transmitters, such as glutamate (Turner et al., 1999). Thus, blockade of voltage-dependent calcium channels and inhibition of glutamate release in the spinal dorsal horn may be involved in the antinociceptive action of gabapentin. Whether glutamatergic release is increased in the spinal dorsal horn of HSV-1-infected mice and whether gabapentin attenuates glutamate release are interesting questions to be elucidated in future studies.

In summary, we demonstrated that gabapentin markedly attenuated nociceptive hypersensitivity induced by HSV-1 infection in mice; the effect may be mediated primarily by the spinal action. Tolerance did not develop to the antinociceptive action of gabapentin and behavioral abnormalities such as sedation and motor dysfunction were not apparent after antinociceptive doses of gabapentin. Thus, gabapentin may be useful in the treatment of the acute herpetic pain.

Acknowledgments
We thank Drs. Hideo Nemoto and Hiroki Takahata for the synthesis of gabapentin.

References


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