The Effect of Intrathecal Gabapentin on Mechanical and Thermal Hyperalgesia in Neuropathic Rats Induced by Spinal Nerve Ligation

Gabapentin decreases the level of glutamate and elevates that of γ-amino-butyric acid in the central nervous system. Gabapentin was shown to have antinociceptive effects in several facilitated pain models. Intrathecal gabapentin was also known to be effective in reducing mechanical allodynia in animals with neuropathic pain. In this study, we investigated to see whether intrathecal gabapentin produces antihyperalgesic effects on thermal and mechanical hyperalgesia in neuropathic rats and whether its effects are associated with motor impairment. To induce neuropathic pain in Sprague-Dawley rats, left L5 and L6 spinal nerves were ligated. After a week, lumbar catheterization into subarachnoid space was performed. Then, paw withdrawal times to thermal stimuli and vocalization thresholds to paw pressure were determined before and up to 2 hr after intrathecal injection of gabapentin. Also, motor functions including performance times on rota-rod were determined. Intrathecal gabapentin attenuated significantly thermal and mechanical hyperalgesia in neuropathic rats, but did not block thermal and mechanical nociception in sham-operated rats. Intrathecal gabapentin of antihyperalgesic doses inhibited motor coordination performance without evident ambulatory dysfunction. This study demonstrates that intrathecal gabapentin is effective against thermal and mechanical hyperalgesia, in spite of moderate impairment of motor coordination.

Key Words : *Gabapentin; Injections, Spinal; Hyperalgesia*

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INTRODUCTION

Peripheral nerve injury caused by trauma is associated with spontaneous pain, allodynia, and hyperalgesia. These neuropathic pain symptoms are often poorly relieved by conventional analgesics, such as opioids and non-steroidal anti-inflammatory drugs (1, 2). Although the mechanisms underlying neuropathic pain have not been fully understood, it is known that excitatory amino acids, including glutamate, play a key role in the alteration of the spinal sensory processing and the plasticity of dorsal horn neurons after nerve injury (3, 4). In the search for alternative treatment, anticonvulsants have been found as a pharmacological intervention for patients with neuropathic pain, because the mechanisms of convulsion may be similar to those of neuropathic pain. Gabapentin as an anticonvulsant drug has attracted recent attention because of its effectiveness against neuropathic pain in clinical trials and animal experiments. Although the mechanism of the antinociceptive action of gabapentin remains unclear, it has been demonstrated that gabapentin decreased glutamate concentration and elevated γ-amino-butyric acid (GABA) concentration in the central nervous system of rat (5-7).

Previous studies have shown that gabapentin produced an antinociceptive effect in the various facilitated pain models (8-14) and intrathecal gabapentin was effective against allodynia in neuropathic pain (15, 16). The antinociceptive effect of systemic administration of gabapentin was observed at doses below those producing its side effects, including behavior or motor dysfunction (17-19). However, it has been uncertain whether intrathecal gabapentin is effective in mechanical and thermal hyperalgeia in neuropathic pain induced by nerve injury and whether its effect is accompanied with any side effect on motor function.

Therefore, we examined whether intrathecal gabapentin produces the antinociceptive effect on thermal and mechanical hyperalgesia in neuropathic rats induced by spinal nerve ligation, and whether the antihyperalgesic effect of gabapentin is affected by motor dysfunction.

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley rats weighing 150-200 g were housed in separate cages and allowed to acclimate for 5-7 days by

using a 12/12 hr day/night cycle. The surgical preparation and the experimental protocol were approved by the Institutional Animal Care and Committee of the Samsung Biomedical Research Institute.

Surgical preparation

Ligation of the left L5 and L6 spinal nerves in rats was used in this study as an experimental model of neuropathic pain. Rats were anesthetized with 1% halothane in O_2 by a mask. The surgical procedure was performed, according to the method described by Kim and Chung (20). A dorsal midline incision was made from L3 to S2. The left L6 transverse process was resected in part to visualize L4, 5 spinal nerves. The left L5 spinal nerve was isolated and ligated tightly with 6-0 black silk just distal to the dorsal root ganglion. The left L6 spinal nerve was isolated below the iliac crest and ligated tightly with 6-0 black silk. After recovery from anesthesia, rats that were unable to withdraw the left hindpaw were excluded from the study. The rats in which the thresholds to thermal and mechanical stimuli after nerve ligation were decreased more than 20% than those before nerve ligation were included in the study. Sham control rats were prepared in the same way, except for nerve ligation. The animals were allowed to recover for 5-7 days before intrathecal cannulation. Intrathecal catheters (PE-10 tube) were inserted into lumbar subarachnoid space during halothane anesthesia, as previously described by Størkson et al. (21). Proper placement of the catheter was determined by the occurrence of hindpaws paralysis after an intrathecal injection of 10 μL of 2% lidocaine. All pharmacological experiments were conducted between 2 and 3 weeks after spinal nerve ligation. Each rat received only a single intrathecal injection of drugs.

Behavioral testing

Thermal response was determined by the left hindpaw withdrawal times using plantar tester (Stoelting Co, Wood Dale, U.S.A.) described by modified method of Hargreaves et al. (22). Rats were allowed to acclimate within plastic enclosures on a clear glass plate maintained at room temperature. A radiant heat source was controlled with a timer and focused onto the plantar surface of hindpaw encompassing the glabrous skin. Paw withdrawal stops both heat source and timer. A maximal cut-off of 30 sec was used to prevent tissue damage. Three trials, at least 10 min apart, were conducted and three withdrawal times were averaged to give a mean withdrawal time.

Mechanical response was measured by using Randall-Selitto algesiometer (Ugo Basile, Comerio, Italy), which generates a linearly increasing mechanical force. A mechanical stimulus was applied to the dorsal surface of the left hindpaw by a dome-shaped plastic tip. Mechanical thresholds were defined as the force in grams at which the rat vocalized. A maximal

cut-off of 400 g was used to prevent tissue damage. Two trials, at least 10 min apart, were conducted and two vocalization thresholds were averaged.

Motor function of hindpaws was evaluated by testing the animals' ability to stand and ambulate in a normal posture. We assessed the motor function by grading the ambulating behavior of rats (16) as the following: 2=normal; 1=limping; 0=paralyzed.

Motor coordination was tested using an accelerating rota-rod treadmill (Stoelting Co, Wood Dale, U.S.A.). The rota-rod was set in motion at a constant speed and the rats were placed into individual sections of the rota-rod. Once the rats were in position, the timers were set to zero and rota-rod was switched to accelerating mode. The rota-rod was operated at a rate of 4 rpm for 20 sec, and then at 8 rpm for 120 sec and at 16 rpm for 60 sec (19). The rats were trained in the test procedure for 5 days before collecting data. The performance times were recorded when the rats were unable to stay on the rota-rod and tripped on the plate. Two trials were performed at intervals of 10 min and performance times were averaged.

Experimental protocol

On experiment day, rats were acclimated for 30 min before testing. Then, baseline thresholds were determined. Rats were assigned randomly to four groups receiving intrathecal injection of normal saline (n=6) and three different doses of gabapentin (Parke-Davis, Ann Arbor, U.S.A.): 30 μg (n=6); 100 μg (n=6); 300 μg (n=6). These doses were based on the previous and our pilot studies. Drugs were dissolved in normal saline and delivered in a volume of 10 μL, followed by a 10 μL flush of normal saline, using a gear-driven microinjection syringe. The thermal and mechanical thresholds and the motor function in neuropathic rats and sham-operated rats were determined at 30, 60, and 120 min after treatment. The motor performance on the rota-rod was measured in sham operated rats. The response threshold data were calculated to a percentage of the maximum possible effect (%MPE) according to the following formula: %MPE=[(postdrug threshold-predrug threshold)/(cut-off threshold - predrug threshold)] × 100.

Statistical analysis

Data are presented as mean ± SD. Within each of the treatment groups, effects of drugs on thermal and mechanical hyperalgesia and motor coordination were compared with pre-treatment values by repeated-measures analysis of variance, followed by Dunnett analysis of least significance difference for multiple comparisons. The paw thresholds in response to thermal and mechanical stimuli before and after nerve ligation were compared using paired Student's t-test. A probability level <0.05 was considered to be statistically significant.

RESULTS

The tight ligation of spinal nerves produced a marked reduction in the thermal and the mechanical stimuli which were necessary to evoke paw withdrawal and vocalization. The paw withdrawal times were reduced significantly from 11.3 ± 1.5 to 7.7 ± 0.6 sec and the vocalization thresholds also from 213.2 ± 32.8 to 126.5 ± 21 g.

In spinal nerves-ligated rats, intrathecal gabapentin of 100 and 300 μg increased significantly the withdrawal times of the injured paw in response to thermal stimuli ($p<0.05$) in a dose-dependent manner. These effects of intrathecal gabapentin 300 μg were observed at 30 min and reached a maximum at 60 min (Fig. 1). The percentages of the maximum possible effect were 48% and 74% at doses of 100 and 300 μg, respectively. In sham-operated rats, however, intrathecal injection of three different doses of gabapentin did not increase the withdrawal times in response to thermal stimuli (Table 1).

In spinal nerves-ligated rats, intrathecal gabapentin also increased significantly the vocalization thresholds in response to mechanical stimuli ($p<0.05$), except for gabapentin 30 μg. This effect of intrathecal gabapentin at 300 μg was observed up to 120 min, whereas that of gabapentin 100 μg was not at 120 min (Fig. 2). The percentages of the maximum possible effect were 31% and 79% at doses of 100 and 300 μg, respectively. In sham-operated rats, however, intrathecal gabapentin did not increase the vocalization thresholds in response to mechanical stimuli, except for gabapentin 300 μg (Table 2).

In spinal nerves-ligated and sham-operated rats, intrathecal

Table 1. Changes of withdrawal time (sec) of left hindpaw to thermal stimuli after intrathecal injection in sham-operated rats

	Pre-injection	30 min	60 min	120 min
Vehicle	11.1 ± 0.9	11.5 ± 1.2	11.5 ± 1.4	11.3 ± 1.6
Gabapentin 30 μg	11.3 ± 2.0	10.5 ± 1.0	11.0 ± 1.5	11.1 ± 1.1
Gabapentin 100 μg	11.8 ± 1.6	11.2 ± 1.7	10.8 ± 1.5	11.0 ± 2.0
Gabapentin 300 μg	11.0 ± 1.7	12.0 ± 2.4	10.3 ± 1.4	10.3 ± 1.4

Data (sec) are mean $\pm$ SD (n=6/group).

Table 2. Changes of vocalization threshold (g) of left hindpaw to mechanical stimuli after intrathecal injection in sham-operated rats

	Pre-injection	30 min	60 min	120 min
Vehicle	229 ± 35	216 ± 51	220 ± 52	205 ± 32
Gabapentin 30 μg	208 ± 38	209 ± 19	214 ± 44	219 ± 26
Gabapentin 100 μg	203 ± 28	205 ± 33	217 ± 17	211 ± 26
Gabapentin 300 μg	211 ± 30	$337 \pm 51*$	$363 \pm 37*$	$363 \pm 43*$

Data (g) are mean $\pm$ SD (n=6/group). *: $p<0.05$ versus pre-injection.

Table 3. Changes of performance time (sec) on rota-rod after intrathecal injection in sham-operated rats

	Pre-injection	30 min	60 min	120 min
Vehicle	164 ± 15	170 ± 9	168 ± 8	172 ± 7
Gabapentin 30 μg	165 ± 14	169 ± 14	157 ± 21	155 ± 20
Gabapentin 100 μg	167 ± 10	$106 \pm 42*$	$89 \pm 44*$	$86 \pm 33*$
Gabapentin 300 μg	161 ± 14	$62 \pm 25*$	$83 \pm 27*$	$120 \pm 22*$

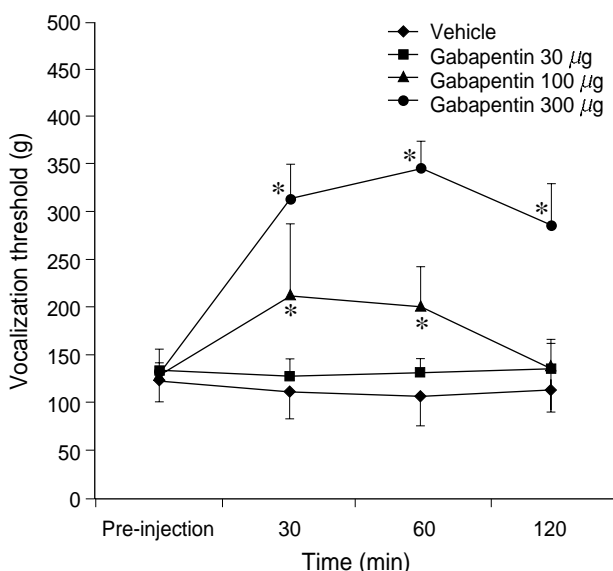Data (sec) are mean $\pm$ SD (n=6/group). *: $p<0.05$ versus pre-injection.

Fig. 1. Changes of withdrawal time of left hindpaw to thermal stimuli in spinal nerve-ligated rats. The withdrawal time is increased significantly ($p<0.05$) after intrathecal administration of gabapentin 100 and 300 μg. The symbols represent mean $\pm$ SD. *: $p<0.05$ versus pre-injection.
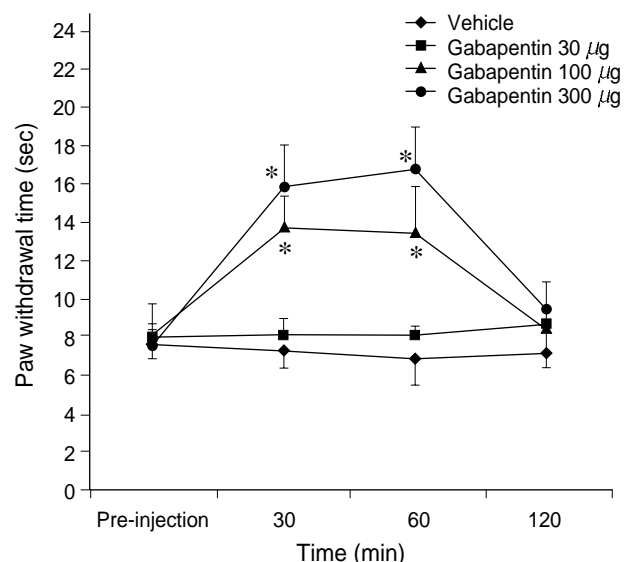

Fig. 2. Changes of vocalization threshold of left hindpaw to mechanical stimuli in spinal nerve-ligated rats. The vocalization threshold is increased significantly ($p<0.05$) after intrathecal administration of gabapentin 100 and 300 μg. The symbols represent mean $\pm$ SD. *: $p<0.05$ versus pre-injection.

gabapentin did not decrease the ambulating behavior scores. However, rats given intrathecal injection of gabapentin 300 μg showed the splayed hindpaws.

Intrathecal gabapentin decreased significantly the performance times on rota-rod ($p<0.05$) in sham-operated rats, except for gabapentin 30 μg (Table 3).

DISCUSSION

In the present study, intrathecal administration of gabapentin was effective against thermal and mechanical hyperalgesia in neuropathic pain induced by spinal nerve ligation and its effect was not limited by motor dysfunction. However, antihyperalgesic doses of intrathecal gabapentin inhibited the motor coordination performance without evident ambulatory dysfunction.

The antinociceptive effect of intrathecal gabapentin observed in the current study agrees with previous observations in which gabapentin was effective in the various facilitated pain models. It has been shown that gabapentin attenuated the various hypersensitive states induced by injection of formalin (8, 9), streptozocin (10), or substance P (14) and reduced paw incision-induced pain (11, 12) and burn-induced pain (13). In addition, intrathecal gabapentin decreased mechanical allodynia in neuropathic pain induced by nerve injury (15, 16). The efficacy of gabapentin in several pain models suggests the common mechanisms associated with the generation of a facilitated state of processing. Although the mechanism of antinociception of gabapentin has not been known, several studies suggested that the spinal cord is the primary site of drug action (8, 9, 11, 13, 15, 16). In our study, we found antihyperalgesic effect of intrathecal gabapentin at a higher dose than that producing antiallodynic effect in neuropathic rats. This dose was also higher than that producing antihyperalgesic effect in formalin-induced pain (8, 9), postoperative pain (11), and burn-induced pain (13). It may simply reflect a difference in the stimuli strength that intrathecal gabapentin has to overcome to produce an antinociceptive effect in various pain models. It also reflects that intrathecal gabapentin may produce different sensitivity to different kinds of abnormal pain.

Previous studies have shown that administration of gabapentin in normal rats did not alter the formalin-induced behaviors during phase 1 period (8, 9) and the response to physiologic pain (18). It suggested that antinociception of gabapentin is not analgesic but antihyperalgesic. We observed that intrathecal gabapentin of 300 μg in sham-operated rats did not increase the paw withdrawal times to thermal stimuli but increased the vocalization thresholds to mechanical stimuli. This finding is consistent partly with the recent observation that systemic gabapentin increased vocalization thresholds of the noninjured paw to mechanical stimuli in neuropathic rats (17). However, it does not support that rel-

atively high doses of intrathecal gabapentin may produce analgesic effect in normal physiologic pain. It is known that the vocalization response to paw pressure is a supraspinally integrated test and the paw withdrawal response to thermal stimuli is a spinally coordinated reflex. As gabapentin is poorly soluble in lipid (23), intrathecal gabapentin may spread cephalad easily and produce the supraspinal effect. Therefore, the vocalization response may be more susceptible than the paw withdrawal response to intrathecal gabapentin.

Although the mechanism of action of gabapentin is not clear, there are several evidences that *N*-methyl-D-aspartate (NMDA) and GABA-mediated events are involved in the pharmacological action of gabapentin. The glycine/NMDA agonist reversed the antihyperalgesic action of gabapentin (19). Furthermore, it blocked the thermal hyperalgesia induced by intrathecal NMDA (14). Gabapentin has been shown to increase GABA synthesis (5) and enhance the GABA release in brain regions (7). Although previous studies have failed to show any affinity for GABAA or GABAB sites or any known site associated with its receptor (15, 23, 24), gabapentin may play a physiologic role in the modulation of the glutaminergic and GABAergic functions that are involved in the central sensitization of the dorsal horn neurons induced by injury.

In our study, although intrathecal gabapentin at 300 μg caused the hindpaws to be splayed, it did not obtund the brisk withdrawal response of hindpaw to thermal stimuli in sham-operated rats, nor did it inhibit the ambulating ability. This finding suggests that antihyperalgesic effect of intrathecal gabapentin in neuropathic rats is not limited by motor dysfunction. Previous studies showed that intrathecal administration of gabapentin up to 100 μg caused no detectable motor weakness, as judged by placing-stepping reflexes and ambulating behavior or other visible behavioral changes, such as sedation and agitation (11, 14, 16). The doses of intrathecal gabapentin less than 300 μg did not alter motor responses, including paw withdrawal response to pinch (15). Furthermore, several observations pointed out a good separation between the antinociceptive and the side effects of gabapentin (16-18). Systemic gabapentin was effective in models of neuropathic pain after sciatic nerve constriction (16, 17) or formalin-induced pain (18) at doses below those producing behavior or locomotion dysfunction. However, we found that intrathecal gabapentin at the doses inducing antihyperalgesic effect produced motor impairment on rota-rod. It indicates that intrathecal administration of gabapentin has narrower ranges of margin of safety than systemic administration. As water-soluble gabapentin may move from the injected site to the brain through cerebrospinal fluid, the central nervous system that controls locomotion may be more vulnerable to intrathecal gabapentin than to systemic gabapentin.

In conclusion, the present study reveals that intrathecal injection of gabapentin is effective against the thermal and

mechanical hyperalgesia in neuropathic rats induced by spinal nerve ligation. This result suggests that gabapentin may exert potent effects on anomalous pain states with facilitated spinal processing by tissue or nerve injury. However, intrathecal gabapentin at antihyperalgesic doses also causes the impairment of the motor coordination, which may be considered as one of its side effects. Therefore, it remains to be determined whether intrathecal injection of gabapentin may be a safe intervention for neuropathic pain.

ACKNOWLEDGMENTS

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