Spinal Gabapentin and Antinociception: Mechanisms of Action

Spinal gabapentin has been known to show the antinoceptive effect. Although several assumptions have been suggested, mechanisms of action of gabapentin have not been clearly established. The present study was undertaken to examine the action mechanisms of gabapentin at the spinal level. Male Sprague-Dawley rats were prepared for intrathecal catheterization. The effect of gabapentin was assessed in the formalin test. After pretreatment with many classes of drugs, changes of effect of gabapentin were examined. General behaviors were also observed. Intrathecal gabapentin produced a suppression of the phase 2 flinching, but not phase 1 in the formalin test. The antinoceptive action of intrathecal gabapentin was reversed by intrathecal NMDA, AMPA, D-serine, CGS 15943, atropine, and naloxone. No antagonism was seen following administration of bicuculline, saclofen, prazosin, yohimbine, mecamylamine, L-leucine, dihydroergocristine, or thapsigargin. Taken together, intrathecal gabapentin attenuated only the facilitated state. At the spinal level, NMDA receptor, AMPA receptor, nonstrychnine site of NMDA receptor, adenosine receptor, muscarinic receptor, and opioid receptor may be involved in the antinociception of gabapentin, but GABA receptor, L-amino acid transporter, adrenergic receptor, nicotinic receptor, serotonin receptor, or calcium may not be involved.

Key Words: Analgesics, Non-Narcotic; Gabapentin; Injections, Spinal

INTRODUCTION

Gabapentin is an anticonvulsant that was synthesized as a structural analog to γ-aminobutyric acid (GABA) (1). Intrathecal or systemic delivery of gabapentin diminishes hyperalgesia in tissue injury pain models without affecting acute noxious stimuli threshold (2, 3). Furthermore, the antinociceptive effect of gabapentin is more powerful after intrathecal rather than systemic administration (4, 5). These findings suggest that gabapentin may alter the facilitated state and the major site of action of gabapentin may be the spinal cord. Although the mechanisms of action of gabapentin are not clear, the relations to specific receptors (1, 2) or substances (6), L-amino acid transporter (7), or voltage-dependent calcium channel (8) has been proposed as the sites of action of gabapentin.

Recently, understanding of neurotransmitters and systems such as serotoninergic, adrenergic, cholinergic, and purinergic receptors involved in nociceptive modulation in the spinal cord has been increased (9-12).

The formalin test is an experimental model which shows acute nociception followed by facilitated state which occurs secondary to the persistent afferent input generated by a local tissue injury.

Thus, the aim of the present study was to observe the effect of intrathecal gabapentin in the formalin test and to further evaluate the mechanisms of action of gabapentin at the spinal level.

MATERIALS AND METHODS

The studies were conducted under a protocol approved by the Institutional Animal Care Committee, Research Institute of Medical Science, Chonnam National University.

Male Sprague-Dawley rats (250-300 g) were used. Rats were housed in group cages on a 12-h night/day cycle with access to food and water at all times. For drug administration, an intrathecal catheter was implanted during enflurane anesthesia, as previously described (13). A polyethylene (PE-10) catheter was advanced caudally by 8.5 cm through an incision in the atlantooccipital membrane to the lumbar enlargement. The exterior part of the catheter was tunneled subcutaneously and exited at the top of head and plugged with a piece of steel wire. The skin was closed with 5-0 silk sutures. Rats showing neurologic deficits postoperatively were sacrificed immediately. After surgery, rats were kept in individual cages and allowed to recover for 4-5 days.

The following drugs were used in this study: gabapentin (1-aminomethyl cyclohexanecetic acid), D-serine (Sigma Chemical Co., St., Louis, MO, U.S.A.), NMDA (N-methyl-
D-aspartate, Research Biochemical Internationals [RBI], Natick, MA, U.S.A.), AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolopropanoate RBI), L-leucine (Sigma), bicuculline (Sigma), saclofen (RBI), prazosin hydrochloride (Sigma), yohimbine hydrochloride (Sigma), atropine sulfate (RBI), mecaminylamine hydrochloride (RBI), CGS 15943 (RBI), naloxone hydrochloride (Sigma), dihydroergocristine methanesulfonate (RBI), and thapsigargin (RBI). CGS 15943, AMPA, and thapsigargin were dissolved in DMSO. Yohimbine and dihydroergocristine were dissolved in distilled water. Saclofen and prazosin were dissolved in 0.1 N NaOH and methyl alcohol, respectively. Other drugs were prepared by dissolving them in normal saline. Intrathecal administration of drugs was performed using a hand-driven, gear-operated syringe pump. All the drugs were delivered in a volume of 10 μL solution, followed by an additional 10 μL of normal saline to flush the catheter.

Pinna reflex, corneal reflex, and motor function were examined at 5, 10, 20, 30, 40, 50, and 60 min after intrathecal administration of gabapentin (300 μg, n=5). Motor function was assessed by placing reflex, stepping reflex, and righting reflex.

For the nociceptive stimulus, 50 μL of 5% formalin solution was injected subcutaneously into the plantar surface of the hindpaw using a 30 gauge needle. The formalin injection produces a characteristic pain behavior, biphasic flinching/shaking of the injected paw. Such pain behavior was therefore quantified by periodically counting the incidences of spontaneous flinching/shaking of the injected paw. The numbers of flinching were counted for 1-min periods at 1 and 5 min and at 5-min intervals from 10 to 60 min. Two phases of spontaneous flinching were observed after the formalin injection. Phase 1 and phase 2 were defined as 0-9 and 10-60 min after formalin injection, respectively. After the observation period of 1 hr, animals were immediately sacrificed.

Four to five days after surgery, rats were placed in a restraint cylinder for the experiment. After a 15-20 min adaptation, rats were then assigned to one of the drug treatment groups. Control experiments were performed with saline. The rats were used only once.

Time course and dose-response of the antinociceptive action of intrathecal gabapentin (10, 30, 100, 300 μg) were determined. Gabapentin was administered 10 min before formalin injection.

To determine mechanisms of action of intrathecal gabapentin, many kinds of drugs were intrathecally given 10 min before the delivery of gabapentin (300 μg), and formalin was injected 10 min later. Doses of drugs for antagonism were chosen based on the preliminary experiments, which were the maximal doses without affecting the control formalin response. Drugs were as follows: 1) GABA receptor; GABA receptor antagonist, bicuculline 0.3 μg, GABA receptor antagonist, saclofen 30 μg, 2) NMDA receptor; NMDA 0.1 μg, 3) AMPA receptor; AMPA 0.003 μg, 4) nonstrychnine site agonist of NMDA receptor; D-serine 100 μg, 5) L-amino acid transporter competitor; L-leucine 100 μg, 6) opioid receptor; opioid antagonist, naloxone 0.3 μg, 7) adrenergic receptor; alpha-1 antagonist prazosin 3 μg, alpha-2 antagonist, yohimbine 10 μg, 8) cholinergic receptor; muscarinic antagonist, atropine 10 μg, nicotinic antagonist, mecaminylamine 10 μg, 9) serotonin receptor; serotonin antagonist, dihydroergocristine 3 μg, 10) adenosine receptor; adenosine antagonist CGS 15943 0.03 μg, and 11) calcium uptake inhibitor; thapsigargin 0.3 μg.

Data are expressed as mean ± SEM. The time response data are presented as the number of flinching. The dose-response data are presented as the percentage maximal possible inhibitory effect (%MPIE) in each phase.

The numbers of flinching were converted to %MPIE according to the following formula.

\[
%\text{MPIE} = \frac{\text{Sum of phase 1(2) count with drug}}{\text{Sum of phase 1(2) count in control group}} \times 100
\]

Dose-response data were analyzed by one-way analysis of variance (ANOVA) with Scheffe for post hoc. Comparison of antagonism for the effect of gabapentin was analyzed by unpaired t-test. \( p<0.05 \) was considered statistically significant.

RESULTS

Neither change of pinna reflex and corneal reflex nor motor

![Fig. 1. Time course effect of gabapentin in the formalin test. Gabapentin was intrathecally administered 10 min prior to injection of formalin into the hindpaw. Data are presented as the number of flinching. Each point on the graph represents mean ± SEM of 7-8 rats.](image-url)
Analgesic Mechanisms of Spinal Gabapentin

Impairment was noted after intrathecal administration of gabapentin. Gabapentin caused a decreased spontaneous activity and urination in some rats. Other abnormal behaviors were not observed. Subcutaneous injection of formalin into the hindpaw resulted in a biphasic flinching response of the injected paw. Fig. 1 shows the time course of the effect of intrathecal gabapentin, administered 10 min before formalin injection, in the formalin test. Intrathecal gabapentin did not alter the flinching response during phase 1. During phase 2, gabapentin produced a dose-dependent suppression of the flinching response (Fig. 2).

The antinociceptive effects of intrathecal gabapentin were antagonized by intrathecal NMDA, AMPA, D-serine (Fig. 3), CGS 15943, atropine, and naloxone (Fig. 4), whereas bicuculline, saclofen (Fig. 5), prazosin, yohimbine, mecamylamine (Fig. 6), L-leucine, dihydroergocristine, and thapsigargin
DISCUSSION

In these experiments, intrathecal gabapentin did not affect the flinching response of phase 1 in the formalin test, but it decreased the phase 2 response. The results of gabapentin observed in this study were consistent with the previous findings (2, 14). Therefore, these observations uphold that spinal gabapentin may alter the facilitated state evoked by persistent afferent input without effects on acute nociception.

(Fig. 7) did not reverse the antinociception of gabapentin.
Although the antinociceptive mechanisms of spinal gabapentin remain unclear, several hypotheses have been suggested. It has been reported that gabapentin decreases glutamate concentrations and inhibits the release of glutamate and glutamatergic synaptic transmission presynaptically (15-17). Glutamate acts on the NMDA receptor and non-NMDA receptor and shows the excitatory effect (18, 19). Further, AMPA-evoked neuronal response is inhibited by gabapentin (20, 21). These findings suggest that NMDA or AMPA receptor may be an action site of gabapentin. Our results that NMDA or

**Fig. 6.** The effect of prazosin, yohimbine, and mecamylamine on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. Prazosin, yohimbine, and mecamylamine were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min before formalin injection. Data are presented as the number of flinching or the percentage maximal possible inhibitory effect (%MPIE). Prazosin, yohimbine, and mecamylamine alone do not affect the control response (A), but none of them reverse the effect of GP (B). Each treatment group represents mean ± SEM of 5-6 rats.

**Fig. 7.** The effect of L-leucine, dihydroergocristine (HEC), and thapsigargin on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. L-leucine, HEC, and thapsigargin were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min before formalin injection. Data are presented as the number of flinching or the percentage maximal possible inhibitory effect (%MPIE). L-leucine, HEC, and thapsigargin alone do not affect the control response (A), but none of them reverse the effect of GP (B). Each treatment group represents mean ± SEM of 5-6 rats.
AMP A attenuated the antinociceptive effect of gabapentin in the present study, it could be supposed that gabapentin may act on the adenosine receptors in the spinal cord.

In addition, intrathecal gabapentin is more potent than systemic injection in terms of antinociception (5, 6), emphasizing that the spinal cord may be a major action site. The above-mentioned findings jointly suggest that gabapentin may act on a certain site or receptors at the spinal level and produce the antinociceptive effect.

In conclusion, spinal gabapentin exhibits antinociception in the facilitated state. These antinociceptive effects may be mediated through spinal NMDA receptor, AMPA receptor, nonstrychnine site of NMDA receptor, adenosine receptors, and muscarinic receptor.

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