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Spinal Gabapentin and Antinociception: Mechanisms of Action

Spinal gabapentin has been known to show the antinociceptive 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 SD 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 antinociceptive 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*

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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 serotonergic, 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 3-0 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] cyclohexanacetic 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-methtyl-4-isoxazolepropionate RBI), L-leucine (Sigma), bicuculline (Sigma), saclofen (RBI), prazosin hydrochloride (Sigma), yohimbine hydrochloride (Sigma), atropine sulfate (RBI), mecamylamine 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; $GABA_A$ antagonist, bicuculline 0.3 μg, $GABA_B$ 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, mecamylamine 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

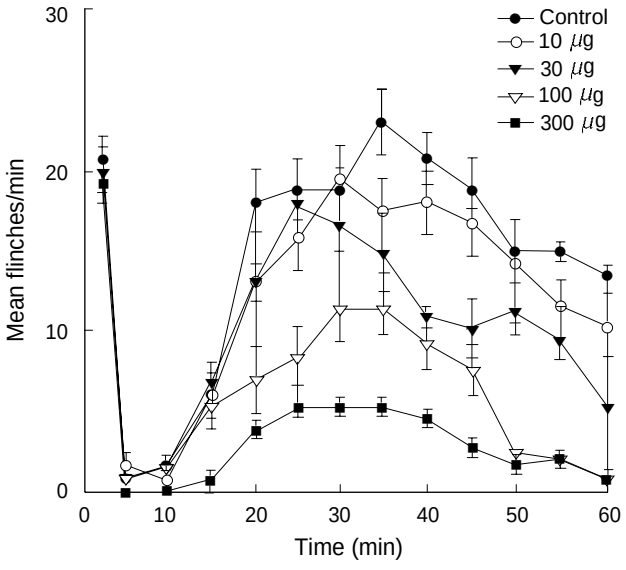

Fig. 1. Time course effect of gabapentin in the formalin test. Gabapentin was intrathecally administared 10 min prior to injection of formalin into the hindpaw. Data are presented as the number of flinching. Each point on the graph represents mean ±SEM of 7-8 rats.

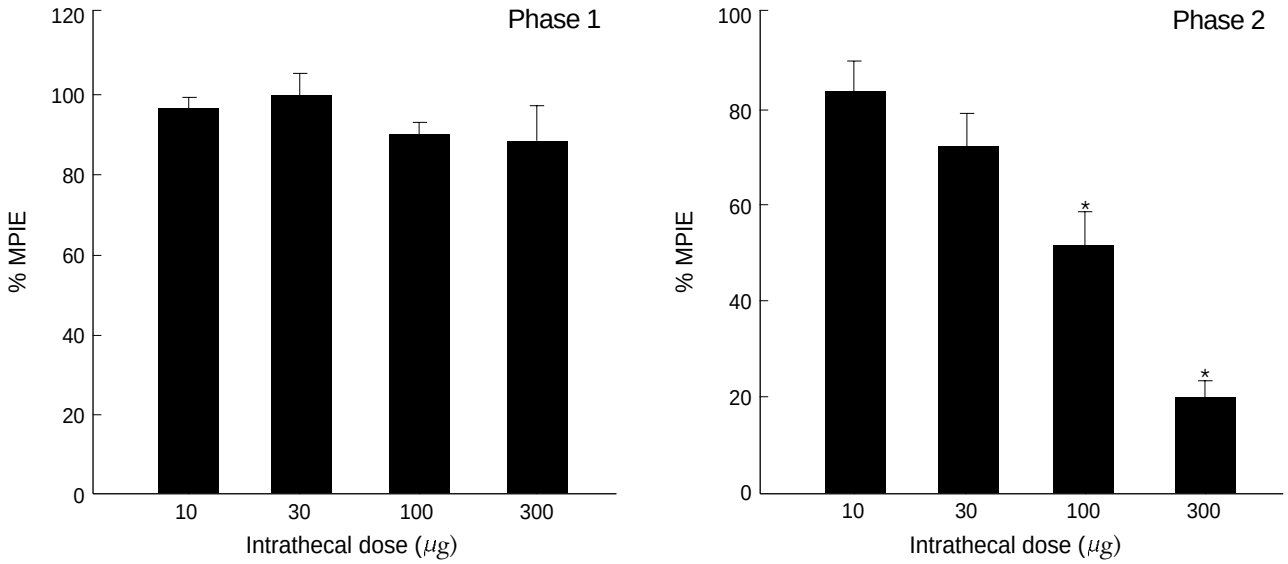

Fig. 2. Dose response effect of gabapentin in the formalin test. Data are presented as the percentage maximal possible inhibitory effect (%MPIE). Intrathecal gabapentin dose-dependently suppresses only phase 2 flinching response. Each point on the graph represents mean±SEM of 7-8 rats. C: control, *p<0.001 compared with control.

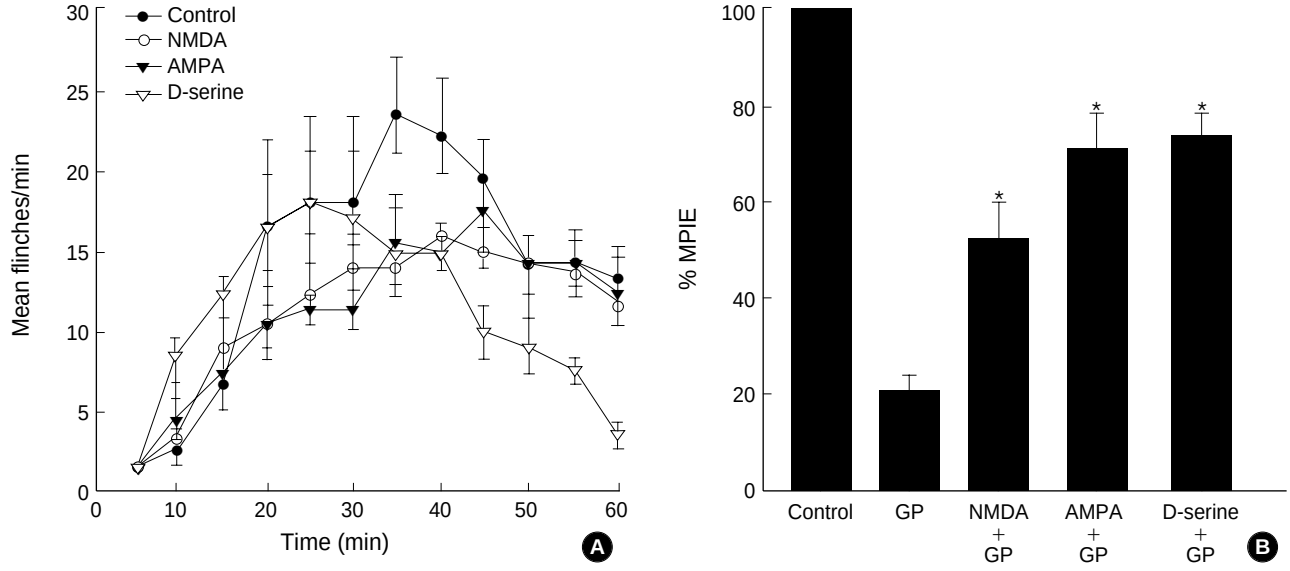

Fig. 3. The effect of NMDA, AMPA, and D-serine on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. NMDA, AMPA, and D-serine were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min before formalin injection. Data are presented as the number of flinching or the percentage maximal possible inhibitory effect (%MPIE). NMDA, AMPA, and D-serine alone do not affect the control response (A), but all of them reversed the effect of GP (B). Each treatment group represents mean±SEM of 5-6 rats. *p<0.05, compared with GP.

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

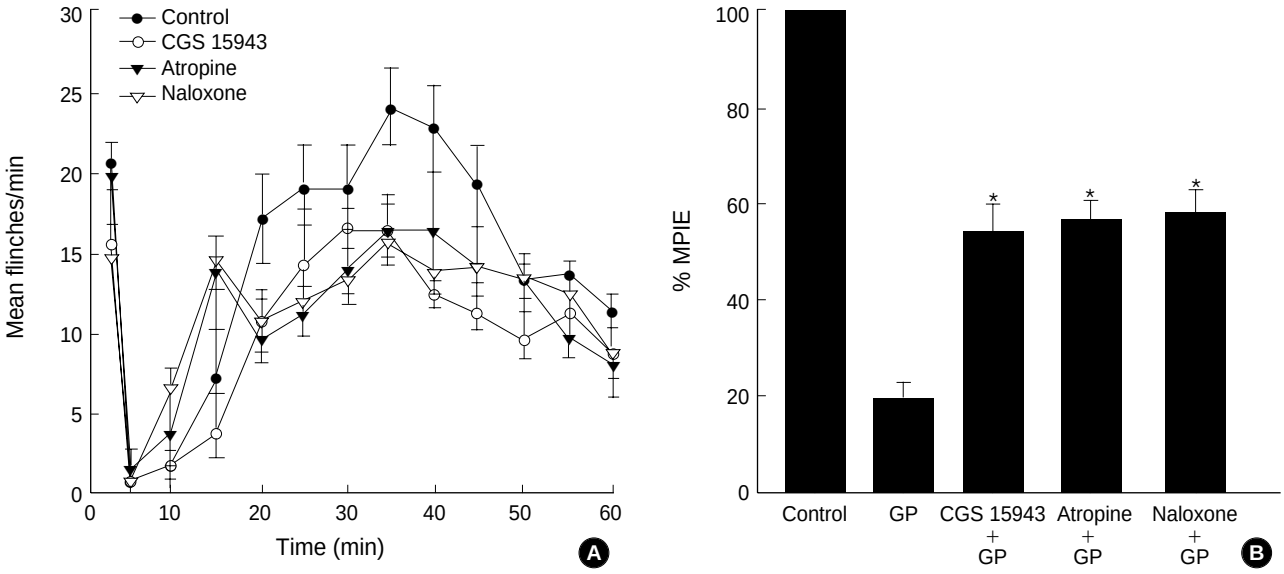

Fig. 4. The effect of CGS 15943, atropine, and naloxone on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. CGS 15943, atropine, and naloxone were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min before formalin injection. Data are presented as the number of flinching or the percentage maximal possible inhibitory effect (%MPIE). CGS 15943, atropine, and naloxone alone do not affect the control response (A), but all of them reverse the effect of GP (B). Each treatment group represents mean±SEM of 5-6 rats. *p<0.05, compared with GP.

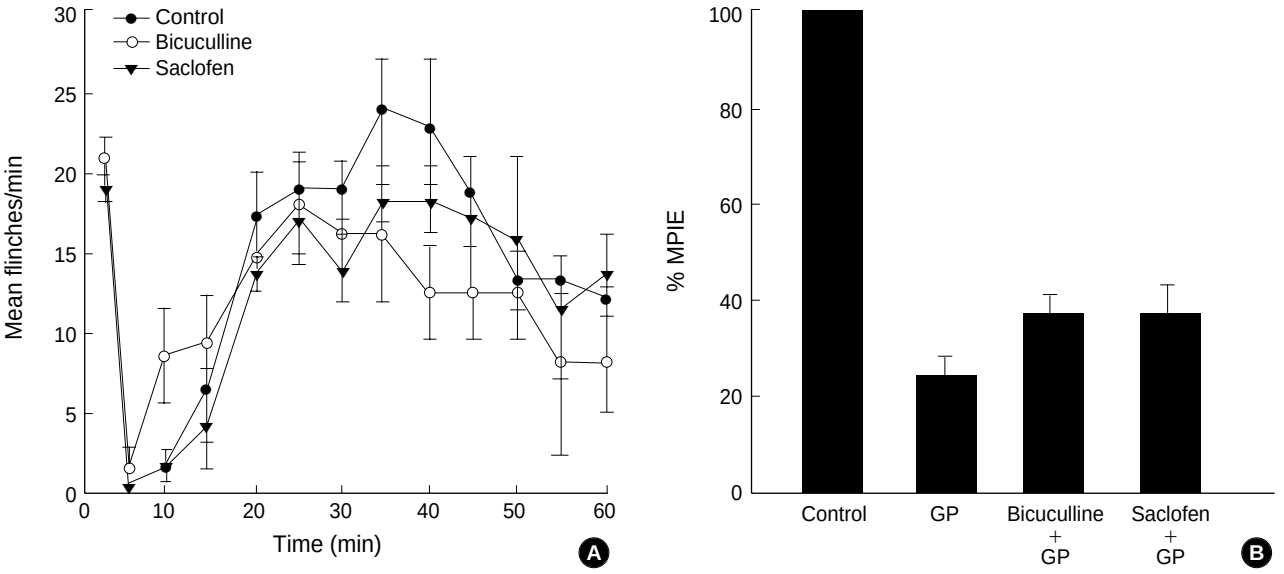

Fig. 5. The effect of bicuculline and saclofen on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. Bicuculline and saclofen were intrathecally administered 20 min prior to injection of formalin. Intrathecal GP was given 10 min before formalin injection. Data are presented as the number of flinching or the percentage maximal possible inhibitory effect (%MPIE). Bicuculline and saclofen alone alone do not affect the control response (A), but neither bicuculline nor saclofen reverse the effect of GP (B). Each treatment group represents mean±SEM of 5-6 rats.

(Fig. 7) did not reverse the antinociception of gabapentin.

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.

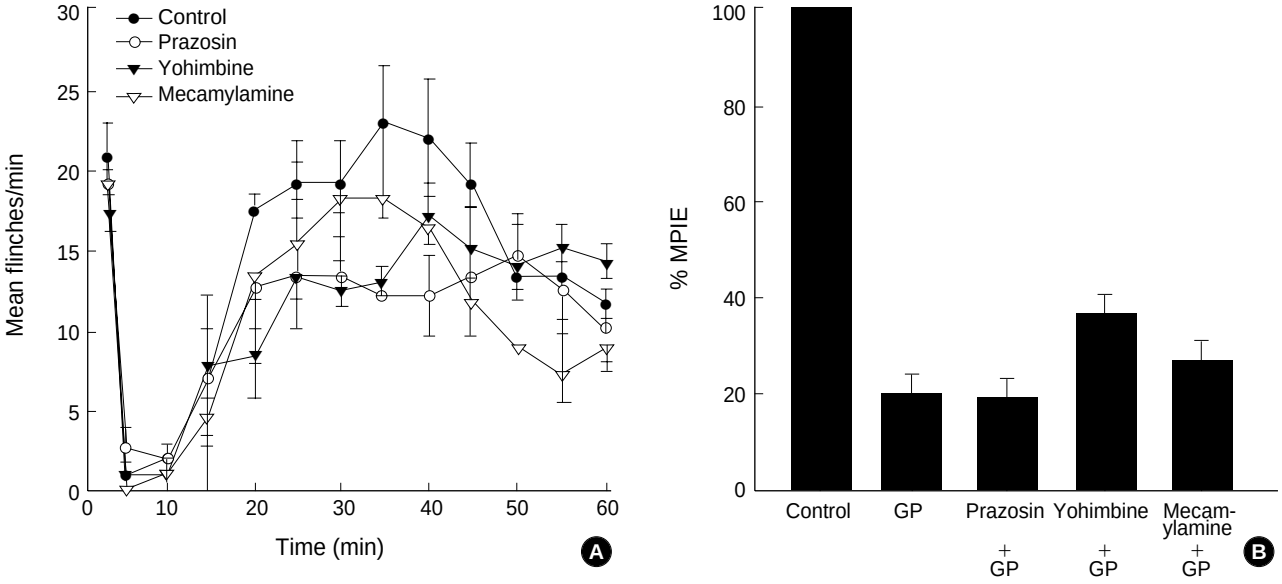

Fig. 6. The effect of prazosin, yohimbine, and mecamylamine on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. Prazosin, yohimbine, and mecamylamine were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min before formalin 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.

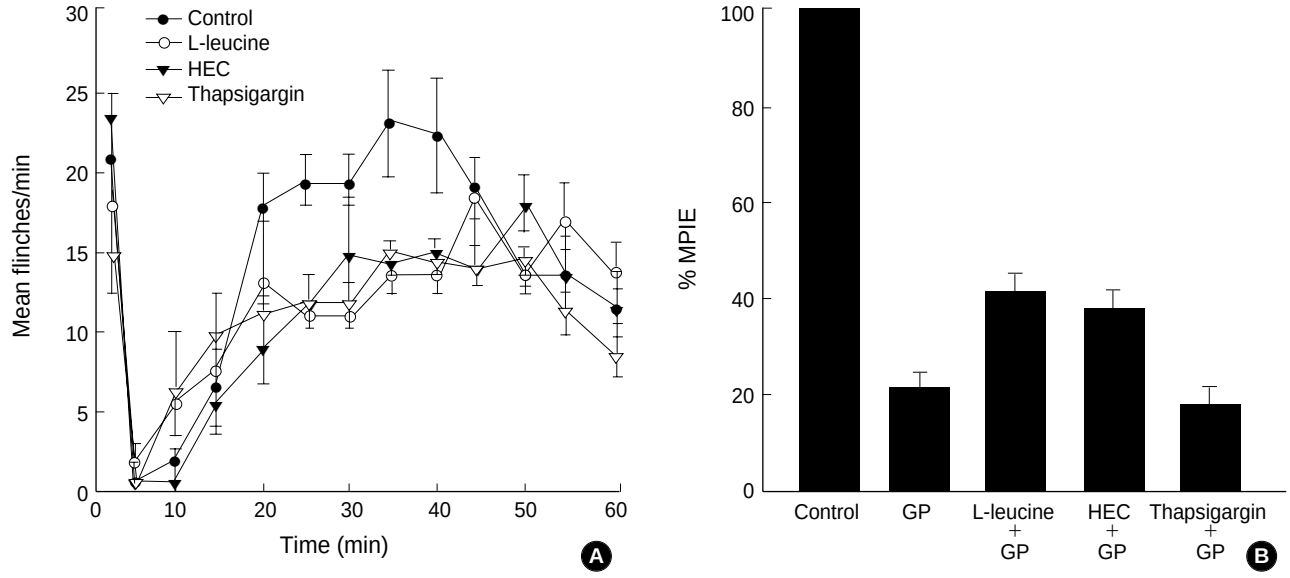

Fig. 7. The effect of L-leucine, dihydroergocristine (HEC), and thapsigargin on the antinociception of gabapentin (GP, 300 μg) during phase 2 of the formalin test. L-leucine, HEC, and thapsigargin were intrathecally administered 20 min prior to the injection of formalin. Intrathecal GP was given 10 min 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.

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 presynatically (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

AMPA attenuated the antinociceptive effect of gabapentin support these observations. Additionally, an agonist at the nonstrychnine site of NMDA receptor complex reversed the antinociception of gabapentin. This observation is also consistent with others (2, 22). Therefore, NMDA receptor, AMPA receptor, and the nonstrychnine site of NMDA receptor may be action sites of gabapentin. Previous studies have shown that gabapentin increases the concentration, the rate of synthesis, and the release of GABA (1, 6). However, intrathecal administration of $GABA_A$ or $GABA_B$ receptor antagonists did not reverse the antiallodynic effects produced by gabapentin (23). In the current study, the antinociceptive action of gabapentin was not affected by either $GABA_A$ or $GABA_B$ receptor antagonists. Thus, it could be supposed that spinal GABA receptors may not be directly involved in the antinociceptive effect of intrathecal gabapentin. It has been proposed that gabapentin crosses several membrane barriers in the body via a specific amino acid transporter and competes with leucine, isoleucine, valine, and phenylalanine for transport (1, 7). However, intrathecal L-leucine and BCH did not antagonize the antiallodynic effect of intrathecal gabapentin (24). Hence, gabapentin may not have to enter nerve terminal or cells via L-amino acid transporter, which was supported by our results.

The $\alpha_2\delta$ subunit of voltage-sensitive calcium channels has been suggested as the binding site of gabapentin (8). However, electrophysiological studies did not exhibit an action of gabapentin on voltage-sensitive calcium channels (25). Although we did not investigate the role of calcium channels for the antinociception of gabapentin, it may not inhibit the calcium uptake. Gabapentin augments the level of serotonin (26) and intrathecal serotonin produces an antinociceptive effect being mediated through serotonin receptors (9). Our study showed that the antinociceptive effect of gabapentin was not reduced by serotonin antagonist. Hence, the serotinin receptor does not seem to be involved in the effect of gabapentin. Spinal adrenergic receptors and cholinergic receptors are active to modulate the nociceptive information (10, 11). In this study, the effect of gabapentin against nociception was not diminished by neither adrenergic antagonists nor nicotinic antagonist, while it was reduced by muscarinic antagonist. Accordingly, it may be conceivable that the antinociception of gabapentin is mediated by muscarinic receptor. Interestingly, naloxone reversed the effect of gabapentin, which implicates that there are certain connections between the effect of gabapentin and opioid receptors.

Adenosine is an endogenous purine compound with various effects on the modulation of nociceptive information at the spinal level (27). The antinociception of intrathecal adenosine is mediated through adenosine receptors in dorsal horn of the spinal cord (12). Both A_1 and A_2 subtypes of adenosine receptors have been identified in the substantia gelatinosa of the dorsal horn (28). Considering that the antinociceptive effect of gabapentin was reversed by adenosine antagonist 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.

REFERENCES

1. Taylor CP, Gee NS, Su TZ, Kocsis JD, Welty DF, Brown JP, Dooley DJ, Boden P, Singh L. *A summary of mechanistic hypotheses of gabapentin pharmacology. Epilepsy Res 1998; 29: 233-49.*
2. Yoon MH, Yaksh TL. *The effect of intrathecal gabapentin on pain behavior and hemodynamics on the formalin test in the rat. Anesth Analg 1999; 89: 434-9.*
3. Yoon MH, Yaksh TL. *Evaluation of interaction between gabapentin and ibuprofen on the formalin test in rats. Anesthesiology 1999; 91: 1006-13.*
4. Field MJ, Holloman EF, McCleary S, Hughes J, Singh L. *Evaluation of gabapentin and S-(+)-isobutylgaba in a rat model of postoperative pain. J Pharmacol Exp Ther 1997; 282: 1242-6.*
5. Chapman V, Suzuki R, Chamarette HL, Rygh LJ, Dickenson AH. *Effects of systemic carbamazepine and gabapentin on spinal neuronal responses in spinal nerve ligated rats. Pain 1998; 75: 261-72.*
6. Petroff OA, Hyder F, Rothman DL, Mattson RH. *Effects of gabapentin on brain GABA, homocarnosine, and pyrrolidinone in epilepsy patients. Epilespia 2000; 41: 675-80.*
7. Su TZ, Lunney E, Campbell G, Oxender DL. *Transport of gabapentin, a γ-amino acid drug by system L α-amino acid transporters: a comparative study in astrocyte, synaptosomes, and CHO cells. J Nerurochem 1995; 64: 2125-31.*
8. Bryans JS, Davies N, Gee NS, Dissanayake VU, Ratcliffe GS, Horwell DC, Kneen CO, Morrell AI, Oles RJ, O'-Toole JC, Perkins GM, Singh L, Suman-hauhan N, O'-Neill JA. *Identification of novel ligands for the gabapentin binding site on the alpha2delta subunit of a calcium channel and their evaluation as anticonvulsant* agents. *J Med Chem 1998; 41: 1838-45.*
9. Bardin L, Lavarenne J, Eschalier A. *Serotonin receptor subtype involved in the spinal antinociceptive effect of 5-HT in rats. Pain 2000; 86: 11-8.*
10. Hao S, Takahata O, Iwasaki H. *Antinociceptive interaction between spinal clonidine and lidocaine in the rat formalin test: an isobolographic analysis. Anesth Analg 2001; 92: 733-8.*
11. Grant GJ, Piskoun B, Bansinath M. *Intrathecal administration of liposomal neostigmine prolongs analgesia in mice. Acta Anaesthesiol Scand 2002; 46: 90-4.*

12. Bantel C, Childers SR, Eisenach JC. *Role of adenosine receptors in spinal G-protein activation after peripheral nerve injury. Anesthesiology 2002; 96: 1443-9.*
13. Yaksh TL, Rudy TA. *Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 17: 1031-6.*
14. Shimoyama N, Shimoyama M, Davis AM, Inturrisi CE, Elliott KJ. *Spinal gabapentin is antinociceptive in the rat formalin test. Neurosci Lett 1997; 222: 65-7.*
15. Goldlust A, Su TZ, Welty DF, Taylor CP, Oxender DL. *Effects of anticonvulsant drug gabapentin on the enzymes in metabolic pathways of glutamate and GABA. Epilepsy Res 1995; 22: 1-11.*
16. Shimoyama M, Shimoyama N, Hori Y. *Gabapentin affects glutamatergic excitatory neurotransmission in the rat dorsal horn. Pain 2000; 85: 405-14.*
17. Maneuf YP, McKnight AT. *Block by gabapentin of the facilitation of glutamate release from rat trigeminal nucleus following activation of protein kinase C or adenylyl cyclase. Br J Pharmacol 2001; 134: 237-40.*
18. Leem JW, Choi EJ, Park ES, Paik KS. *N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptor antagonists differentially suppress dorsal horn neuron responses to mechanical stimuli in rats with peripheral nerve injury. Neurosci Lett 1996; 211: 37-40.*
19. Dougherty PM, Palecek J, Paleckova V, Sorkin LS, Willis WD. *The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. J Neurosci 1992; 12: 3025-41.*
20. Chizh BA, Scheede M, Schlutz H. *Antinociception and (R,S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid antagonism by gabapentin in the rat spinal cord in vivo. Naunyn Schmiedebergs Arch Pharmacol 2000; 362: 197-200.*
21. Fink K, Meder W, Dooley DJ, Gothert M. *Inhibition of neuronal Ca(2+) influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. Br J Pharmacol 2000; 130: 900-6.*
22. Partridge BJ, Chaplan SR, Sakamoto E, Yaksh TL. *Characterization of the effects of gabapentin and 3-isobutyl-gamma-aminobutyric acid on substance P-induced thermal hyperalgesia. Anesthesiology 1998; 88: 196-205.*
23. Hwang JH, Yaksh TL. *Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. Reg Anesth 1997; 22: 249-56.*
24. Cheng J, Pan H, Eisenach JC. *Antiallodynic effect of intrathecal gabapentin and its interaction with clonidine in a rat model of postoperative pain. Anesthesiology 2000; 92: 1126-31.*
25. Rock DM, Kelly KM, Macdonald RL. *Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons. Epilepsy Res 1993; 16: 89-98.*
26. Rao ML, Clarenbach P, Vahlensieck M, Kraetzschmar S. *Gabapentin augments whole blood serotonin in healthy young men. J Neural Transm 1988; 73: 129-34.*
27. Sawynok J. *Purines in pain management. Curr Opin CPNS Invest Drugs 1999; 1: 27-38.*
28. Choca JI, Green RD, Proudfit HK. *Adenosine A1 and A2 receptors of the substantia gelatinosa are located predominantly on intrinsic neurons: an autoradiography study. J Pharmacol Exp Ther 1988; 247: 757-64.*