Anesthesiology
2000; 92:500-6

Synergistic Effect between Intrathecal Non-NMDA Antagonist and Gabapentin on Allodynia Induced by Spinal Nerve Ligation in Rats

Shao-Rui Chen, M.D., James C. Eisenach, M.D.,† Patrick P. McCaslin, M.D.,‡ Hui-Lin Pan, M.D., Ph.D.§*

Background: **Glutamate and non–*N*-methyl-D-aspartate (NMDA) receptors have been implicated in the development of neuroplasticity in the spinal cord in neuropathic pain. The spinal cord has been identified as one of the sites of the analgesic action of gabapentin. In the current study, the authors determined the antiallodynic effect of intrathecal 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in a rat model of neuropathic pain. Also tested was a hypothesis that intrathecal injection of CNQX and gabapentin produces a synergistic effect on allodynia in neuropathic rats.**

Methods: **Allodynia was produced in rats by ligation of the left L5 and L6 spinal nerves. Allodynia was determined by application of von Frey filaments to the left hind paw. Through an implanted intrathecal catheter, 10–100 μg gabapentin or 0.5–8 μg CNQX disodium (a water-soluble formulation of CNQX) was injected in conscious rats. Isobolographic analysis was performed comparing the interaction of intrathecal gabapentin and CNQX using the ED_{50} dose ratio of 15:1.**

Results: **Intrathecal treatment with gabapentin or CNQX produced a dose-dependent increase in the withdrawal threshold to mechanical stimulation. The ED_{50} for gabapentin and CNQX was 45.9 ± 4.65 and 3.4 ± 0.22 μg, respectively. Intrathecal injection of a combination of CNQX and gabapentin produced a strong synergistic antiallodynic effect in neuropathic rats.**

Conclusions: **This study shows that intrathecal administration of CNQX exhibits an antiallodynic effect in this rat model of neuropathic pain. Furthermore, CNQX and gabapentin, when combined intrathecally, produce a potent synergistic antiallodynic effect on neuropathic pain in spinal nerve–ligated rats. (Key words: AMPA; glutamate; kainate; neuropathic pain; NMDA receptors; spinal cord.)**

PERIPHERAL nerve injury caused by surgery or trauma is associated with spontaneous pain, hyperalgesia (increased pain intensity in response to noxious stimuli), and allodynia (normally innocuous stimuli become painful).[1,2] These neuropathic pain symptoms often are poorly relieved by conventional analgesics,[1,2] which has prompted the search for alternative interventions. The mechanisms underlying neuropathic pain are not understood completely. Previous studies have shown that excitatory amino acids, such as glutamate, play a role in the alteration of the spinal sensory processing and the plasticity of dorsal horn neurons after nerve injury.[3,4] Although spinal *N*-methyl-D-aspartate (NMDA) receptors mediate a nociceptive action and intrathecal injection of NMDA antagonists are analgesic,[5-7] the clinical usefulness of these antagonists is hampered by the side effects on motor function, cognition, and behavior.[8-11] In a previous study, Chaplan *et al.*[10] observed that intrathecal administration of a non-NMDA antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX), has a short-term effect on tactile allodynia induced by spinal nerve ligation in rats. Because the analgesic action of DNQX is largely limited by its side effect on the motor function, it is uncertain whether its antiallodynic effect is caused by the motor dysfunction. In rats subjected to the sciatic nerve injury, Mao *et al.*[12] reported that intrathecal injection of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) has no effect on thermal hyperalgesia, but its effect on allodynia was not tested. Therefore, the analgesic effect of intrathecal administration of non-NMDA receptor antagonists on neuropathic pain warrants further study. Therefore, one of the aims of this study was to further define the role of non-NMDA receptors in the maintenance of allodynia induced by spinal nerve ligation in rats.

Anticonvulsants have been used as an alternative treat-

* Research Staff, Department of Anesthesiology.

† Francis M. James III Professor of Anesthesiology.

‡ Assistant Professor of Anesthesiology.

§ Assistant Professor of Anesthesiology, Physiology, and Pharmacology.

Received from the Departments of Anesthesiology, Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina. Submitted for publication May 4, 1999. Accepted for publication September 21, 1999. Supported in part by National Institutes of Health grants HL60026 (to Dr. Pan) and GM35523 (to Dr. Eisenach), Bethesda, Maryland.

Address reprint requests to Dr. Pan: Department of Anesthesiology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. Address electronic mail to: hpan@wfubmc.edu

ment for patients with neuropathic pain.[1] Gabapentin (Neurontin; Parke-Davis Pharmaceutical Research, Ann Arbor, MI) is a new anticonvulsant agent that can effectively relieve chronic pain in patients.[13,14] However, its pharmacologic actions are different from other substances that interact at γ-aminobutyric acid (GABA) synapses because it does not bind to GABA receptors or any known neurotransmitter receptors.[15,16] Intrathecal administration of gabapentin is capable of attenuating allodynia in a rat model of neuropathic pain,[17] but the mechanisms of its antinociceptive action remain to be established. Previous studies have suggested that one of the sites of the analgesic action of gabapentin is the spinal cord, and its effect may be mediated through voltage-gated calcium channels.[17,18] Gabapentin has been used clinically in conjunction with other anticonvulsants. However, its interaction with other agents, such as non-NMDA receptor antagonists, has not been evaluated. In the current study, we tested a hypothesis that intrathecal coadministration of a non-NMDA antagonist and gabapentin produces a synergistic effect on allodynia induced by spinal nerve ligation in rats.

Materials and Methods

Surgical Procedure

Male rats (Harlan Sprague-Dawley) weighing 150–180 g at the time of acquisition were used in this study. Ligation of the L5 and L6 spinal nerves in rats was used in this study as an experimental model of neuropathic pain because it produces profound and sustained tactile allodynia, which resembles the condition observed in patients with neuropathic pain.[19,20] During halothane anesthesia, the left L5 and L6 spinal nerves were isolated under a surgical microscope and ligated tightly with 5-0 silk suture, according to the method described by Kim and Chung.[19] The animals were allowed to recover for 5–7 days before intrathecal cannulation. Intrathecal catheters (PE-10 tubing) were inserted in rats during halothane anesthesia, as previously described by Yaksh and Rudy.[21] Intrathecal catheters were advanced 8 cm caudal through an incision in the cisternal membrane and secured to the musculature at the incision site. Only animals with no evidence of neurologic deficits after catheter insertion were studied. All pharmacologic experiments were conducted between 3 and 4 weeks after spinal nerve ligation because tactile allodynia develops within 1 week after surgery and lasts for at least 6–8 weeks. Each rat received only a single intrathecal injection of drugs. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee at the Wake Forest University School of Medicine.

Behavioral Testing

The mechanical threshold was determined before and after spinal nerve ligation in all animals. To quantify mechanical sensitivity of the paw, rats were placed in individual plastic boxes on a mesh floor, which allowed access to their hind paw. A series of calibrated von Frey filaments (Stoelting Co., Wood Dale, IL) were applied perpendicularly to the plantar surface of the left paw with sufficient force to bend the filaments for 6 s. Brisk withdrawal or paw flinching were considered as positive responses. In the absence of a response, the filament of next greater force was applied. In the presence of a response, the filament of next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the "up–down" calculating method, as described in detail by Chaplan *et al.*[20,22] Each trial was repeated 2 or 3 times at approximately 2-min intervals, and the mean value was used as the force to produce withdrawal responses. Motor dysfunction was evaluated by testing the animals' ability to stand and ambulate in a normal posture and to place and step with the hind paws. We assessed the motor function in a simple manner by grading the ambulation behavior of rats as the following: 2 = normal; 1 = limping; 0 = paralyzed. The rats were tested for 2 min once every test period.

Experimental Protocols

Effect of Intrathecal CNQX. This protocol was used to determine the dose–response and time course of the analgesic action of intrathecal injection of a non-NMDA receptor antagonist, CNQX. Other non-NMDA receptor antagonists, such as 2,3-dihydro-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) and DNQX, were not chosen for this study because of their effect of impairment on motor function after intrathecal injection or their poor solubility in saline.[10,12] Rats were acclimated for 30 min before testing. Then, baseline withdrawal thresholds to von Frey filament stimulation were determined. Rats were assigned randomly to three groups receiving intrathecal injection of three different doses of CNQX: 0.5 μg (n = 6); 2 μg (n = 7); and 8 μg (n = 7). These doses were selected based on our pilot study in which we found that 0.2 μg CNQX had no effect, whereas 10 μg CNQX produced a maximal effect on the paw with-

drawal responses to mechanical stimulation. The mechanical thresholds were determined 15, 30, 45, 60, 90, and 120 min after treatment.

Effect of Intrathecal Gabapentin. This protocol was used to determine the dose-dependency and time course of the analgesic action of intrathecal injection of gabapentin. After the baseline withdrawal threshold was measured, separate animals were randomly organized in the following three groups for intrathecal administration of three doses of gabapentin: 10 μg (n = 7); 30 μg (n = 7); and 100 μg (n = 7). These doses were chosen based on preliminary experiments and a previous study.[17] A previous study by Hwang and Yaksh[17] showed that the antiallodynic effect of intrathecal gabapentin in this animal model lasts about 4 h.

Effect of CNQX–Gabapentin Combination. To study the interaction between CNQX and gabapentin, an isobolographic analysis was performed.[23] The respective ED_{50} values were calculated from the dose-response curve of CNQX or gabapentin alone. Then, a dose-response curve was obtained by administration of both agents in a constant dose ratio (gabapentin:CNQX, 15:1, see Results) based on the ED_{50} values of the single drug. Twenty additional rats randomly received one of the following combinations of gabapentin and CNQX: 4.5:0.3 μg (n = 7); 18:1.2 μg (n = 7); or 72:4.8 μg (n = 6). The mechanical thresholds were determined 15, 30, 45, 60, 90, and 120 min after intrathecal treatment. Subsequently, from the dose-response curve of the combined drugs, the ED_{50} value of the total dose of the mixture was determined using linear regression. In addition, we performed experiments using separate rats (n = 6) to show that the effect of CNQX plus gabapentin was reversible. We evaluated the effect of a combination of CNQX and gabapentin (4.8:72 μg) for the first 5 h and 20 h after intrathecal injection.

Both drugs for intrathecal injection were dissolved in normal saline and administered in a volume of 5 μl, followed by a 10-μl flush with normal saline. Previous studies have shown that intrathecal injection of saline has no effect on the allodynic condition in spinal nerve-ligated rats.[10,22] Gabapentin was supplied by Parke-Davis Pharmaceutical Research (Ann Arbor, MI) and CNQX disodium, a water-soluble formulation of CNQX, was purchased from Research Biochemical International (Natick, MA).

To calculate the ED_{50} value of each drug, the response threshold data were converted to a percentage of the maximum possible effect (%MPE) according to the following formula:

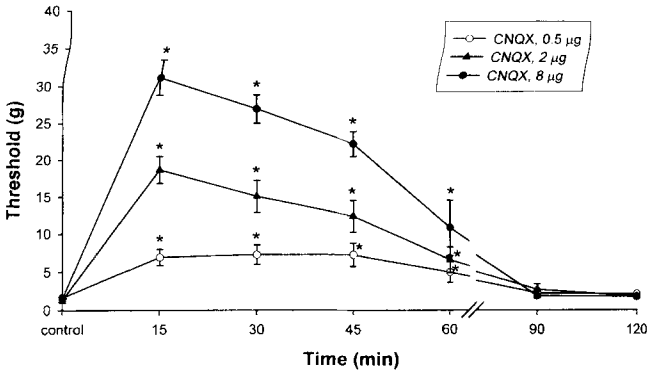

Fig. 1. Time course and dose–response effect of intrathecal injection of CNQX on tactile mechanical allodynia caused by ligation of spinal nerves in rats. Data are presented as the mean ± SD. *$P < 0.05$ compared with pretreatment control.

$$\%MPE = [(\text{postdrug threshold} - \text{baseline})/(\text{preligation threshold} - \text{baseline threshold})] \times 100$$

The baseline threshold was the withdrawal threshold after nerve ligation and recovery. ED_{50} was determined using linear regression for each drug. Isobolographic analysis using the ED_{50} for maximal effect was performed as described previously.[23] Data are presented as the mean ± SD. The paw withdrawal thresholds in response to mechanical stimulation before and after nerve ligation were compared using a paired Student *t* test. Effects of drugs on allodynia were determined by repeated-measures analysis of variance, followed by the Tukey *post hoc* test. $P < 0.05$ was considered to be statistically significant.

Results

The tight ligation of spinal nerves reliably produced allodynia, as defined by a marked reduction in the tactile stimulus necessary to evoke withdrawal of the paw ipsilateral to the nerve lesion. The paw withdrawal threshold before spinal nerve ligation was 28.7 ± 2.4 g. The mechanical threshold decreased significantly to 2.4 ± 0.5 g ($P < 0.05$) within 7 days after nerve ligation and remained stable for at least 6 weeks in all animals studied.

Intrathecal injection of CNQX increased significantly the withdrawal threshold in a dose-dependent manner (fig. 1). Blockade of non-NMDA receptors with CNQX produced a profound but less-sustained reduction in allodynia. The antiallodynic effect of intrathecal injec-

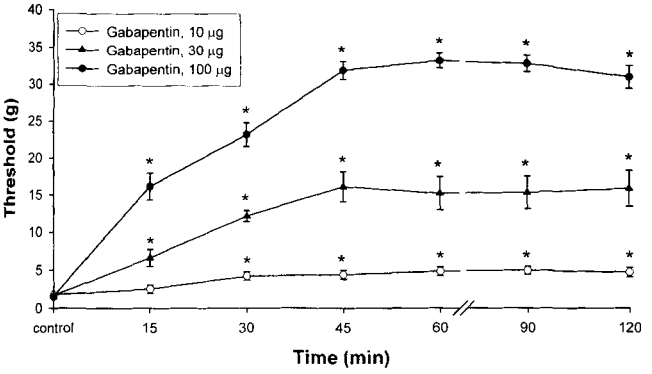

Fig. 2. Time course and dose–response effect of intrathecal injection of gabapentin on tactile mechanical allodynia induced by ligation of spinal nerves in rats. Data are presented as the mean ± SD. *P < 0.05 compared with pretreatment control.

tion of three different doses of CNQX quickly reached a maximum within 30 min, then gradually decreased and lasted approximately 60 min (fig. 1). The ED_{50} of CNQX was 3.4 ± 0.22 μg.

Intrathecal administration of gabapentin also produced a dose-dependent antiallodynic effect (fig. 2). The ED_{50} of gabapentin was 45.9 ± 4.65 μg. In contrast to the time course of the effect of CNQX, intrathecal injection of gabapentin gradually increased the baseline withdrawal thresholds, with a peak effect at 45 min (fig. 2). The antiallodynic effect of gabapentin lasted for at least 2 h (fig. 2).

Intrathecal coadministration of gabapentin and CNQX in a constant ratio of the ED_{50}s showed a significant increase in the withdrawal threshold to mechanical stimulation in a dose-dependent fashion (fig. 3). Isobolographic analysis showed that this interaction was synergistic because the dose of CNQX plus gabapentin

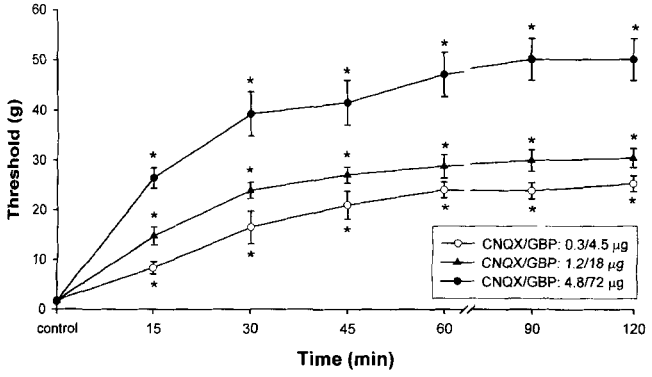

Fig. 3. Dose–response of intrathecal coadministration of CNQX and gabapentin (GBP) on withdrawal responses to application of von Frey filaments in spinal nerve–ligated rats. Data are presented as the mean ± SD. *P < 0.05 compared with control.

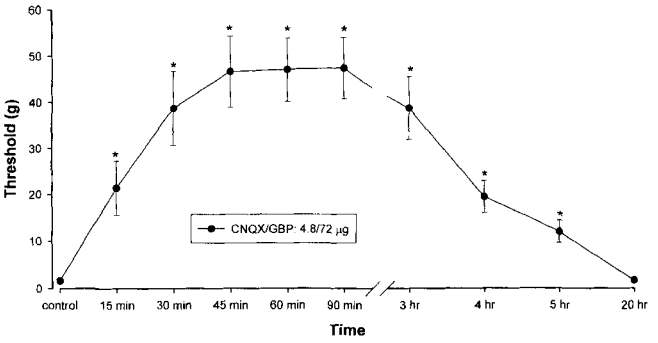

Fig. 4. Time course of intrathecal administration of CNQX plus gabapentin (GBP) on withdrawal responses to application of von Frey filaments in spinal nerve–ligated rats. Data are presented as the mean ± SD. *P < 0.05 compared with control.

necessary to produce a 50% return to presurgery withdrawal threshold was significantly less than that calculated as necessary for an additive interaction (fig. 4). The antiallodynic effect of CNQX plus gabapentin decreased gradually toward the control level over a 5-h period, and the tactile threshold returned completely to the baseline level within 20 h (fig. 5).

Animals receiving intrathecal injection of CNQX, gabapentin, or coadministration of gabapentin plus CNQX did not exhibit motor dysfunction, as judged by the placing-stepping reflex and ambulation behavior or other visible behavioral changes, such as sedation or agitation. The ambulation score was not affected by CNQX, gabapentin, and their combination in the doses used. All rats tested reached the maximum score.

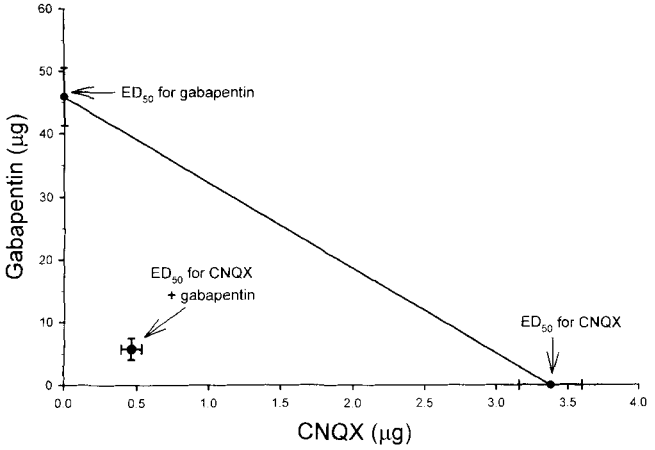

Fig. 5. Isobolograms for the interaction of intrathecal CNQX and gabapentin. The ED_{50} values for the single agent is plotted on the x- and y-axes. The ED_{50} for the drug combination lies below the additive line, indicating a strong synergy. Data are presented as the mean ± SD.

Discussion

There are two new observations from the current study. First, intrathecal administration of a non-NMDA receptor antagonist, CNQX, attenuated significantly the allodynic condition in a rat model of neuropathic pain without evident effect on motor function. These results support the notion that spinal non-NMDA receptors are important for the maintenance of pain behaviors in this rat model of neuropathic pain. Second, intrathecal CNQX and gabapentin produced a potent synergistic analgesic effect in spinal nerve-injured rats. Therefore, data from the current study suggest that intrathecal coadministration of non-NMDA receptor antagonists and gabapentin may be more effective for treatment of chronic neuropathic pain.

Excitatory amino acids, such as glutamate and aspartate, are important in the transmission of nociceptive inputs in the dorsal horn of the spinal cord.[3,5,6] These excitatory amino acids are known to facilitate spinal sensory transmission and contribute to the enhanced excitability of dorsal horn neurons in the chronic pain state through activation of NMDA and non-NMDA receptors.[3,5,24] Non-NMDA receptors are important for the processing of noxious and innocuous mechanical stimuli in the dorsal horn of the spinal cord.[5,6] The non-NMDA receptors are ligand-gated ion channels and are permeable to sodium and calcium ions. These receptors can be activated by kainate or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), from which these pharmacologic names are derived. Non-NMDA receptors mediate fast excitatory neurotransmission in the central nervous system, including the spinal cord dorsal horn neurons.[5,25] Receptors for AMPA and kainate both are expressed in the dorsal horn of the spinal cord.[4,26] Non-NMDA receptors are present in the superficial lamina of the dorsal horn of the spinal cord and are activated by stimulation of nociceptors and mechanoreceptors.[26,27] It has been reported that spinal non-NMDA receptors are upregulated after peripheral nerve injury,[24] and non-NMDA receptor antagonists suppress dorsal horn neuronal responses to mechanical stimuli in rats subjected to spinal nerve ligation.[3] The antiallodynic effects of non-NMDA antagonists on neuropathic pain remain controversial because one previous study failed to show such an analgesic effect in a neuropathic pain model induced by sciatic nerve ligation.[12] Although DNQX has been used intrathecally in the same neuropathic pain model,[10] the focus of that study was on NMDA, not on non-NMDA receptors. Furthermore, the analgesic effect of DNQX is largely limited by its effect (or the vehicle used; *i.e.*, NaOH) on the motor function.[12] In the current study, to avoid the undesirable effects of some non-NMDA antagonists (*i.e.*, NBQX and DNQX) and their vehicles on the motor function, we evaluated the antiallodynic action of a preformed water-soluble formulation of CNQX in a well-established rat model of neuropathic pain. We observed that intrathecal injection of CNQX up to 8 μg produced no detectable motor dysfunction, which is in agreement with the findings of others using this compound.[7,28] Our results indicate that intrathecal CNQX possesses an antiallodynic effect on this neuropathic pain model without affecting the motor function. This information is important in establishing the role of spinal non-NMDA receptors in the maintenance of allodynia in this rat model of neuropathic pain. Leem *et al.*[3] demonstrated that NMDA and non-NMDA receptor antagonists differentially suppress the responses of spinal dorsal horn neurons to noxious and non-noxious mechanical stimuli, respectively. Thus, the preferential effect of CNQX on innocuous peripheral input may be the basis of its antiallodynic effect on this rat model of neuropathic pain. Recently, the effect of another non-NMDA antagonist, LY29358, on capsaicin-induced allodynia has been shown in humans.[29] We found that intrathecal administration of CNQX produced a significant antiallodynic effect, which lasted approximately 60 min. Therefore, blockade of non-NMDA receptors alone may be insufficient to produce a sustained antiallodynic action in this model. For this reason, it would be logical to combine this class of drugs with other agents for a prolonged effect.

The antiallodynic effect of intrathecal gabapentin observed in the current study is similar to what has been reported by others.[17] Intrathecal injection of gabapentin had a slow onset of action but was long-lasting ($>$ 2 h). Systemically administered gabapentin reduces the responses of spinal dorsal horn neurons to mechanical stimuli, indicating that the spinal cord is one of the sites of action of gabapentin.[18] Although the mechanisms of the antinociceptive action of gabapentin in the spinal cord remain unclear, previous studies have suggested that gabapentin treatment elevates GABA concentration and decreases glutamate concentration in rat brain tissues.[30,31] However, gabapentin may have effects through other receptors or ion channels. For example, recent studies have shown that gabapentin has a high affinity for the $\alpha_2\delta$-subunit of voltage-sensitive calcium channels in the brain tissue,[32] an action that may reduce intracellular calcium accumulation and attenuate neuronal excitability after

nerve injury. Calcium channel activation is know to play a role in allodynia development because intrathecal injection of N-type calcium channel blockers alleviates allodynia induced by spinal nerve ligation in rats.[33] Conversely, *in vitro* electrophysiologic experiments have failed to show any effect of gabapentin on voltage-sensitive calcium channels.[15] Further study is necessary to elucidate the pharmacologic action of gabapentin in the spinal cord.

The interaction between intrathecal gabapentin and non-NMDA receptor antagonists on chronic neuropathic pain has not been studied. The current study indicates that coadministration of gabapentin and CNQX resulted in a greater reduction of allodynia induced by ligation of spinal nerves in rats. This functional synergy may result from a distinct drug interaction that acts independently to inhibit spinal nociceptive processing. Because activation of non-NMDA receptors, N-type calcium channels, and a decrease in GABA availability in the spinal cord have been considered to play a role in the development of neuropathic pain,[3,5,33] the synergistic action between intrathecal gabapentin and CNQX may be achieved by blockade of non-NMDA receptors, inhibition of voltage-gated calcium channels, and an increase in GABA content in the spinal cord. As shown in this study, the benefits of these two agents, when combined, are not only reflected by the increased potency (synergistic interaction), but also characterized by a rapid and prolonged duration of analgesia in this rodent model of neuropathic pain. It should be acknowledged that the threshold after intrathecal gabapentin plus CNQX (72: 4.8 μg; fig. 3) was significantly higher than the preligation threshold. It appears that the combination of these two drugs produces an antinociceptive effect in addition to a reversal of the allodynic condition in this neuropathic pain model. However, their interaction in inhibiting noxious sensory inputs in the spinal cord was not studied in the current study. Alternatively, because non-NMDA antagonists attenuate painful responses to the paw formalin injection in rats,[34,35] it is possible that a large dose of CNQX alone could produce an antinociceptive effect. We avoided using a high dose of CNQX in this study because it may cause unspecific effects, as reported by Ren *et al.*[7]

In summary, this study shows that spinal non-NMDA receptors play an important role in the maintenance of neuropathic pain. Furthermore, intrathecal administration of a non-NMDA receptor antagonist plus gabapentin has a synergistic antiallodynic effect in this model of neuropathic pain. The synergy may arise from a decrease in excitatory neurotransmission (CNQX) accompanied by inhibiting calcium channels (gabapentin) in the spinal cord. Therefore, intrathecal administration of non-NMDA receptor antagonists and gabapentin may represent an alternative therapy for patients with neuropathic pain conditions.

References

1. MacFarlane BV, Wright A, O'Callaghan J, Benson HA: Chronic neuropathic pain and its control by drugs. Pharmacol Ther 1997; 75:1-19

2. Arner S, Meyerson BA: Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988; 33:11-23

3. 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

4. Tolle TR, Berthele A, Schadrack J, Zieglgansberger W: Involvement of glutamatergic neurotransmission and protein kinase C in spinal plasticity and the development of chronic pain. Prog Brain Res 1996; 110:193-206

5. 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

6. Dougherty PM, Willis WD: Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-D-aspartic acid and substance P. Pain 1991; 47:85-93

7. Ren K, Williams GM, Hylden JL, Ruda MA, Dubner R: The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. Eur J Pharmacol 1992; 219:235-43

8. Max MB, Byas-Smith MG, Gracely RH, Bennett GJ: Intravenous infusion of the NMDA antagonist, ketamine, in chronic posttraumatic pain with allodynia: A double-blind comparison to alfentanil and placebo. Clin Neuropharmacol 1995; 18:360-8

9. McQuay HJ, Carroll D, Jadad AR, Glynn CJ, Jack T, Moore RA, Wiffeh PJ: Dextromethorphan for the treatment of neuropathic pain: A double-blind randomised controlled crossover trial with integral n-of-1 design. Pain 1994; 59:127-33

10. Chaplan SR, Malmberg AB, Yaksh TL: Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J Pharmacol Exp Ther 1997; 280:829-38

11. Wei HB, Jakeman LB, Hunter JC, Bonhaus DW: Pharmacological characterization of N-methyl-D-aspartate receptors in spinal cord of rats with a chronic peripheral mononeuropathy. Neuropharmacology 1997; 36:1561-9

12. Mao J, Price DD, Hayes RL, Lu J, Mayer DJ: Differential roles of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy. Brain Res 1992; 598:271-8

13. Mellick GA, Mellicy LB, Mellick LB: Gabapentin in the management of reflex sympathetic dystrophy. J Pain Symptom Manage 1995; 10:265-6

14. Rosenberg JM, Harrell C, Ristic H, Werner RA, de Rosayro AM: The effect of gabapentin on neuropathic pain. Clin J Pain 1997; 13:251-5

15. Rock DM, Kelly KM, Macdonald RL: Gabapentin actions on

ligand- and voltage-gated responses in cultured rodent neurons. Epilepsy Res 1993; 16:89–98

16. 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

17. 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

18. 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

19. Kim SH, Chung JM: An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1993; 50:355–63

20. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53:55–63

21. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 7:1032–6

22. Pan H-L, Chen SR, Eisenach JC: Role of spinal nitric oxide in antiallodynic effect of intrathecal clonidine in neuropathic rats. ANESTHESIOLOGY 1998; 89:1518–23

23. Tallarida RJ, Porreca F, Cowan A: Statistical analysis of drug-drug and site-site interactions with isobolograms. Life Sci 1989; 45:947–61

24. Harris JA, Corsi M, Quartaroli M, Arban R, Bentivoglio M: Up-regulation of spinal glutamate receptors in chronic pain. Neuroscience 1996; 74:7–12

25. Neugebauer V, Lucke T, Schaible HG: N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the hyperexcitability of dorsal horn neurons during development of acute arthritis in rat's knee joint. J Neurophysiol 1993; 70:1365–77

26. Furuyama T, Kiyama H, Sato K, Park HT, Maeno H, Takagi H, Tohyama M: Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. Brain Res Mol Brain Res 1993; 18:141–51

27. Popratiloff A, Kharazia VN, Weinberg RJ, Laonipon B, Rustioni A: Glutamate receptors in spinal motoneurons after sciatic nerve transection. Neuroscience 1996; 74:953–8

28. Coderre TJ, Van Empel I: The utility of excitatory amino acid (EAA) antagonists as analgesic agents: I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. Pain 1994; 59:345–52

29. Sang CN, Hostetter MP, Gracely RH, Chappell AS, Schoepp DD, Lee G, Whitcup S, Caruso R, Max MB: AMPA/kainate antagonist LY293558 reduces capsaicin-evoked hyperalgesia but not pain in normal skin in humans. ANESTHESIOLOGY 1998; 89:1060–7

30. Loscher W, Honack D, Taylor CP: Gabapentin increases aminooxyacetic acid-induced GABA accumulation in several regions of rat brain. Neurosci Lett 1991; 128:150–4

31. 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

32. Brown JP, Dissanayake VU, Briggs AR, Milic MR, Gee NS: Isolation of the [^{3}H]gabapentin-binding protein/$\alpha_2\delta$ Ca^{2+} channel subunit from porcine brain: Development of a radioligand binding assay for $\alpha_2\delta$ subunits using [^{3}H]leucine. Anal Biochem 1998; 255:236–43

33. Chaplan SR, Pogrel JW, Yaksh TL: Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia. J Pharmacol Exp Ther 1994; 269:1117–23

34. Hunter JC, Singh L: Role of excitatory amino acid receptors in the mediation of the nociceptive response to formalin in the rat. Neurosci Lett 1994; 174:217–21

35. Simmons RM, Li DL, Hoo KH, Deverill M, Ornstein PL, Iyengar S: Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. Neuropharmacology 1998; 37:25–36