

## Antiallodynic Effect of Intrathecal Gabapentin and Its Interaction with Clonidine in a Rat Model of Postoperative Pain

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**Background:** Systemic administration of gabapentin was shown previously to attenuate mechanical allodynia in a rat model of postoperative pain. Because intrathecal administration of gabapentin is effective in other hypersensitivity states, the authors tested its effect in the postoperative model, its interaction with another antiallodynic agent (clonidine), and a possible mechanism of gabapentin action (entry into sites of action *via* an L-amino acid transporter).

**Methods:** Male Sprague-Dawley rats were anesthetized with halothane, and an incision of the plantaris muscle of right hind paw induced punctate mechanical allodynia. Withdrawal threshold to von Frey filament application near the incision site was determined before and 2 h after surgery. Then, an intrathecal injection was performed and thresholds were determined every 30 min for 3 h thereafter.

**Results:** Paw incision induced a mechanical hypersensitivity (mechanical threshold > 25 g before incision and < 5 g after). Intrathecal gabapentin dose-dependently (10–100 µg) reduced mechanical allodynia. Intrathecal injection of an inhibitor of L-amino acid transporters or a competitor for this transporter, L-leucine, did not reverse the intrathecal effect of gabapentin. The ED<sub>50</sub> of intrathecal gabapentin, clonidine, and their combination were 51, 31, and 9 µg, respectively, and isobolographic analysis showed synergy between gabapentin and clonidine.

**Conclusions:** Intrathecal gabapentin is effective against tactile allodynia that occurs after paw incision, and interacts synergistically with clonidine. Unlike results *in vitro*, gabapentin does

not obligatorily need to enter cells *via* the L-amino acid transporter mechanism to achieve its effects *in vivo*. (Key words: α<sub>2</sub>-Adrenergic receptors; anticonvulsants; spinal cord.)

BRENNAN *et al.*<sup>1</sup> described a rat model of postoperative pain in which incision of the skin, fascia, and muscle of the plantar aspect of the hind paw results in reproducible, quantifiable mechanical allodynia that lasts at least 3 days.<sup>2</sup> The major advantage of this model is that it closely mimics the phenomena of primary and secondary hyperalgesia,<sup>3</sup> which are observed in patients after surgery and may contribute importantly to postoperative pain.<sup>4</sup>

The antiepileptic agent gabapentin produces antinociception or reduces hypersensitivity after inflammation or nerve injury in animals and shows effectiveness in the treatment of clinical chronic pain.<sup>5–11</sup> A single subcutaneous dose of gabapentin, 1 h before surgery, dose-dependently (3–30 mg/kg) blocks the development of mechanical allodynia after incision of the hind paw, with a minimum effective dose of 10 mg/kg.<sup>2</sup> Gabapentin is considerably more potent after intrathecal than systemic administration, but has not been evaluated after intrathecal administration in this model. One purpose of the current study was to determine the effectiveness, potency, and duration of action of intrathecally administered gabapentin in this model of postoperative allodynia.

The mechanism of action of gabapentin in reducing hypersensitivity in animals or producing analgesia in humans is unknown; however, several hypotheses have been suggested.<sup>12</sup> It has been suggested<sup>13</sup> that gabapentin must first enter synaptic terminals or cells to act, and studies of astrocytes, synaptosomes, and Chinese hamster ovary cells support an active entry of gabapentin *via* the L-amino acid transporter because it is blocked by increasing concentrations of the competing ligand L-leucine. We speculated that gabapentin might also necessitate active cellular uptake for effect *in vivo*; and a second aim of the current study was to test whether the

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actions of gabapentin could be inhibited by coadministered L-leucine or the specific L-amino acid transporter inhibitor, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH).

The  $\alpha_2$ -adrenergic agonist clonidine produces antinociception and reduces hypersensitivity states in animals after intrathecal injection, including in this postoperative model, and produces postoperative analgesia in humans.<sup>14-18</sup> Because gabapentin does not interact with  $\alpha_2$ -adrenergic receptors, it is conceivable that it could interact with clonidine in a synergistic manner to reduce postoperative allodynia. The third aim of the current study, was to evaluate, using standard isobolographic techniques, the nature of the interaction between intrathecal gabapentin and clonidine in this postoperative model.

## Materials and Methods

### *Surgical Preparation*

The studies were approved by the Animal Care and Use Committee of the Wake Forest University School of Medicine. Male Sprague-Dawley rats (250–300 g) obtained from Harlan (Indianapolis, IN) were used in all experiments. Animals were housed under a 12-h light-dark cycle (lights on at 7:00 AM), with food and water *ad libitum*. For intrathecal drug administration, a polyethylene catheter was inserted during halothane anesthesia, as previously described.<sup>19</sup> The catheter was passed caudally from the cisterna magnum to the level of lumbar enlargement (8.5 cm). Only animals without evidence of neurologic dysfunction after catheter insertion were studied. All studies were performed at least 5 days after insertion of the intrathecal catheter.

Paw incision was performed as described by Brennan *et al.*<sup>1</sup> Animals were anesthetized with halothane, the plantar surface of the right hind paw was prepared with 50% ethanol, and a 1-cm longitudinal incision was made through the skin and fascia, starting 0.5 cm from the edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. The wound was then closed with two silk sutures and covered with a polymixin B, neomycin, and bacitracin ointment.

### *Behavioral Testing*

For determining withdrawal threshold, rats were placed individually in plastic cages with a plastic mesh

floor. Animals were tested after accommodation to the environment, typically 20–30 min after being placed in the cage. Withdrawal threshold to punctate mechanical testing was determined using calibrated von Frey filaments (Stoelting Co., Wood Dale, IL), beginning with the 2.0-g filament. Filaments were applied vertically to an area adjacent to the wound at the heel for 4 s while the hair was bent. Brisk withdrawal or paw flinching was 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” method, as described by Chaplan *et al.*<sup>20</sup> Tests were performed in duplicate, with an approximate 3-min test-free period between withdrawal responses, and their average was used. Studies were performed on the first day after paw incision surgery. Only rats with marked allodynia (withdrawal threshold < 5 g) after paw incision were studied.

### *Experimental Treatments*

First, the effects of intrathecally administered gabapentin (10, 30, and 100  $\mu$ g randomly assigned) were evaluated. These doses correspond to those used in studies of other models of hypersensitivity.<sup>8,9</sup> The withdrawal threshold was determined before and 2 h after surgery, then every 30 min for 3 h after intrathecal injection. The test drug was injected intrathecally after testing 2 h after surgery.

To test whether BCH or L-leucine could attenuate the antiallodynic effect of gabapentin, 30  $\mu$ g gabapentin was injected intrathecally alone, or with 100  $\mu$ g BCH or 100  $\mu$ g L-leucine. Treatment assignment was random. In control experiments, rats received BCH or L-leucine alone, without gabapentin. Higher doses could not be studied because these were saturated solutions in saline.

Intrathecal clonidine was tested using cumulative dosing (5, 15, and 50  $\mu$ g) because preliminary studies showed that the antiallodynic effect of intrathecal clonidine in this model peaked around 30 min, with a stable effect of more than 60 min.

Based on similar ED<sub>50</sub> values of the two drugs, gabapentin and clonidine were combined in a 1:1 ratio to test their interaction. A dose-response was determined for this mixture; the ED<sub>50</sub> was determined; and the type of interaction was determined by isobolographic analysis according to the method described by Tallarida *et al.*<sup>21</sup>

### Side Effects

Placing and stepping reflexes were used to detect motor dysfunction in the gabapentin study groups. Sedation was determined by spontaneous activity and response to light stimulation.

### Drugs

Drugs used were clonidine hydrochloride (molecular weight, 267; Sigma Chemical, St. Louis, MO), gabapentin (molecular weight, 171; Parke-Davis, Ann Arbor, MI), L-leucine (molecular weight, 131.2; Sigma Chemical), and BCH (molecular weight, 155.2; Calbiochem-Novabiochem, La Jolla, CA). Drugs were dissolved in normal saline and delivered in a volume of 5  $\mu$ l, followed by a 10- $\mu$ l flush of normal saline.

### Data Analysis and Statistics

Withdrawal threshold data from von Frey filament testing were converted to percent of maximum possible effect (%MPE), according to the formula

$$\%MPE = (\text{post drug threshold} - \text{baseline postincision threshold}) / (\text{preincision threshold} - \text{baseline postincision threshold}) \times 100$$

The percent maximum possible effect data at time of peak effect after intrathecal gabapentin or gabapentin-clonidine combination injection (60 or 30 min, respectively) were used to calculate the respective ED<sub>50</sub> values and 95% confidence intervals using linear regression. Data are presented as the mean  $\pm$  SEM. The effect of BCH and L-leucine on postincisional allodynia or the antiallodynic effect of intrathecal gabapentin was determined by one-way or two-way analysis of variance.  $P < 0.05$  was considered to be significant.

## Results

### General Appearance

After intrathecal catheterization, all rats displayed normal grooming behavior, ambulation, and weight gain. Withdrawal threshold was less than 5 g on the day after paw incision for a majority of animals, and they were therefore included in the study.

### Gabapentin Alone

Intrathecal administration of gabapentin resulted in a dose-dependent increase in the withdrawal threshold evoked by application of von Frey filaments on the incised paw. The peak effect of intrathecal gabapentin

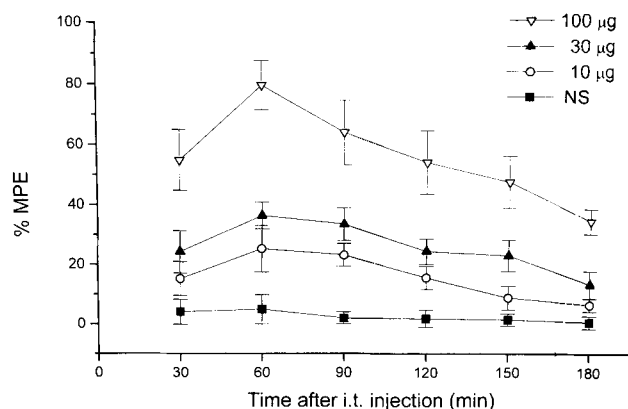


Fig. 1. Dose-dependent reduction in punctate mechanical allodynia (expressed as percent maximum possible effect [%MPE]) produced by intrathecal injection of gabapentin. Each point represents the mean  $\pm$  SEM of eight rats.

occurred 60 min after injection (fig. 1). The ED<sub>50</sub> value (95% confidence interval) of intrathecal gabapentin was 51  $\mu$ g (38–64  $\mu$ g).

### Effect of BCH and L-leucine

Intrathecal BCH 100  $\mu$ g or L-leucine 100  $\mu$ g alone produced no effect on withdrawal threshold after paw incision (fig. 2). Intrathecal coadministration of 100  $\mu$ g BCH or 100  $\mu$ g L-leucine with 30  $\mu$ g gabapentin did not significantly alter the antiallodynic effect of 30  $\mu$ g gabapentin (fig. 2).

### Interaction with Clonidine

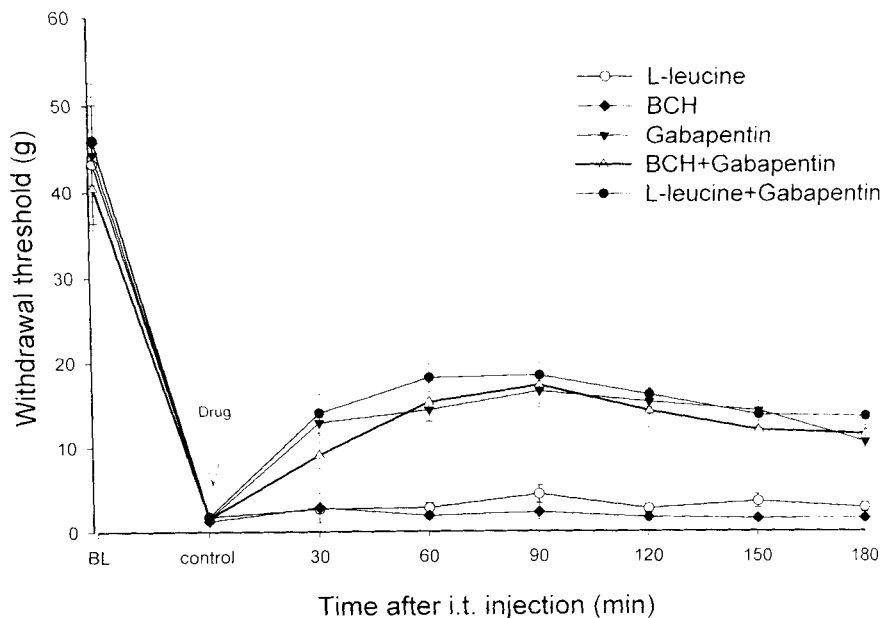
Intrathecal clonidine produced a dose-dependent increase in withdrawal threshold of the incised paw (fig. 3), with an ED<sub>50</sub> of 31  $\mu$ g (25–37  $\mu$ g). Combination of gabapentin with clonidine in a 1:1 ratio produced a dose-dependent increase in withdrawal threshold (fig. 4), with an ED<sub>50</sub> of 9  $\mu$ g (7–11  $\mu$ g). Isobolographic analysis indicated that there was no overlap between the confidence intervals of the experimentally determined combination ED<sub>50</sub> and the theoretical ED<sub>50</sub> of additivity (39  $\mu$ g; 36–41  $\mu$ g), indicating a synergistic interaction (fig. 5).

### Side Effects

No detectable motor dysfunction or sedation was observed after the studied doses of gabapentin. Clonidine alone produced sedation. In the gabapentin-clonidine combination 10 plus 10  $\mu$ g group, short-term (between 30 and 60 min) sedation was noted in five of eight rats.

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Fig. 2. Withdrawal threshold in rats treated with intrathecal injection of 100  $\mu$ g L-leucine, 100  $\mu$ g BCH, 30  $\mu$ g gabapentin alone, or 100  $\mu$ g gabapentin combined with L-leucine or 100  $\mu$ g BCH. L-leucine and BCH alone did not alter the postincisional withdrawal threshold (control) significantly (analyzed by one-way analysis of variance). L-leucine and BCH did not alter significantly the antiallodynic effect of intrathecal gabapentin (analyzed by two-way analysis of variance). Control data were obtained 2 h after surgery. Each group consisted of 6–12 rats. BL = baseline withdrawal threshold measured before paw incision surgery.



## Discussion

The postoperative paw incision model in rats developed by Brennan *et al.*<sup>1</sup> is considered to be useful, in that it results in primary and secondary hyperalgesia<sup>3</sup> and allodynia that parallels the postoperative course of patients.<sup>22</sup> This model may provide a tool to investigate agents that are promising for treating postoperative pain or understanding the adverse physiologic consequences of surgical trauma.<sup>4</sup> For these reasons, the acute effects of analgesics in this model may more accurately predict the human pharmacology of analgesia after surgery.

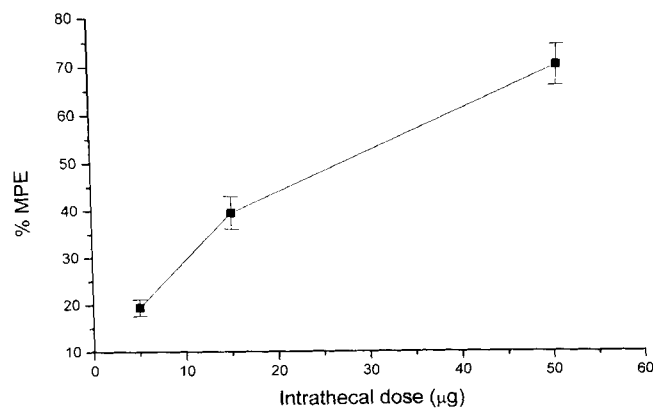


Fig. 3. Dose-dependent reduction in punctate mechanical allodynia (expressed as percent maximum possible effect [%MPE]) produced by intrathecal injection of clonidine. Each point represents the mean  $\pm$  SEM of eight rats.

Spinally administered  $\alpha_2$ -adrenergic agonists alter pain transmission by acting presynaptically on C fibers to reduce transmitter release, and postsynaptically to hyperpolarize dorsal horn nociceptors.<sup>23</sup> Intrathecal injection of clonidine produces pain relief in patients after surgery<sup>18</sup> and reduces allodynia in the rat paw incision model by an action on  $\alpha_2$ -adrenergic receptors.<sup>17</sup>  $\alpha_2$ -Adrenoceptor activation in the spinal cord also plays a role in analgesia from spinally administered norepinephrine<sup>24</sup> and from centrally administered opioids.<sup>25</sup>

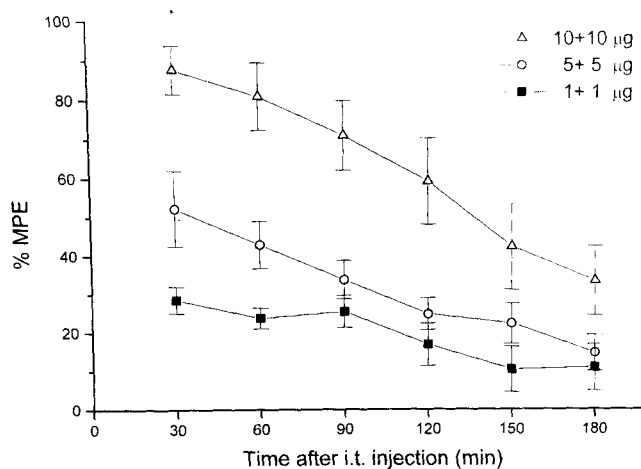
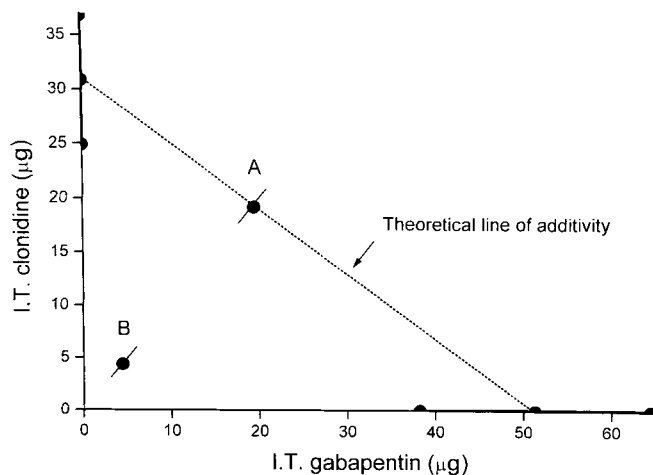


Fig. 4. Dose-dependent reduction in punctate mechanical allodynia (expressed as percent maximum possible effect [%MPE]) produced by intrathecal injection of gabapentin combined with clonidine. Each point represents the mean  $\pm$  SEM of eight rats.



**Fig. 5.** Isobologram of the interaction between intrathecal gabapentin and clonidine. The  $ED_{50}$  values for each agent are plotted on the  $x$ -axis and  $y$ -axis, respectively, and the thick lines present the 95% confidence intervals of each  $ED_{50}$ . The dotted line connecting these two points is the theoretical line of additivity. The theoretical additive  $ED_{50}$  point (A) and 95% confidence interval are calculated from the  $ED_{50}$  values and 95% confidence intervals of each agent. The experimentally observed  $ED_{50}$  point (B) with 95% confidence interval for the combinations lies outside the region defined by the theoretical line of additivity. The confidence intervals of A and B do not overlap, indicating a synergistic interaction.

Clonidine also reduces allodynia after nerve injury (spinal nerve ligation).<sup>14</sup> Although a portion of this effect may be caused by sympatholysis from clonidine,<sup>26-29</sup> it is unclear whether sympatholysis plays a role in the antiallodynic effect of clonidine after paw incision.

Gabapentin reduces pain in patients with chronic pain, especially with neuropathic symptoms,<sup>10,11</sup> and reduces hypersensitivity in a various animal pain models.<sup>5-9</sup> Yet the mechanisms by which gabapentin acts have not been elucidated. It has been suggested that gabapentin must first enter nerve terminals or cells to exert its effects, and that it does so *via* the L-amino acid transporter. This transporter is generally considered as one of the major  $Na^+$ -independent carriers for large neutral  $\alpha$ -amino acids, such as L-leucine, in mammalian cells. Uptake of gabapentin in rat brain cortex astrocytes, synaptosomes, and Chinese hamster ovary cells is 80–90% reduced by leucine, valine, isoleucine, phenylalanine, tryptophan, cysteine, histidine, or glutamine, and the L-amino acid transporter inhibitor BCH.<sup>13</sup> In contrast, we observed no effect of large doses of BCH and L-leucine on the behavioral effect of intrathecal gabapentin *in vivo*. These data are consistent with either an action of gabapentin on extracellular sites (such as access to the  $\alpha_2\delta$  subunit of voltage-sensitive  $Ca^{2+}$  chan-

nels<sup>12,30</sup>) or access to intracellular sites *via* other mechanisms.

Synergy between intrathecal gabapentin and clonidine observed in the current study could reflect pharmacokinetic or pharmacodynamic interactions. We did not measure drug concentrations and cannot, therefore, exclude a pharmacokinetic explanation. However, gabapentin and clonidine probably act through different mechanisms to reduce postoperative hypersensitivity, and activation of different mechanisms of action can result in synergy.<sup>15</sup>

As important as assessment of interaction for effectiveness with drug combinations is assessment of their interaction in producing side effects. Sedation is a major side effect of clonidine and may limit its usefulness in the treatment of pain. As expected, intrathecal clonidine produced sedation. Although short-term sedation was noted in the 10 plus 10  $\mu$ g gabapentin-clonidine combination group, this dosage produced a near-maximal effect in reducing postoperative allodynia, similar to more than 50  $\mu$ g clonidine alone (fig. 3). These data suggest that a clonidine-gabapentin combination could result in less sedation clinically than with clonidine alone. The other major side effect of clonidine is hypotension, and whether gabapentin alters this side effect was not assessed in the current study.

It could be argued that gabapentin exerts activity after systemic administration, and there is little rationale for study of this agent by intrathecal administration. There are at least two reasons intrathecal administration of gabapentin may be important. First, potency and effectiveness both may be increased with intrathecal administration. Indeed, the current study shows a dramatically increased potency of gabapentin in reducing postoperative allodynia compared with systemic administration.<sup>2</sup> In addition, the study of drug action and interaction after intrathecal administration may help to elucidate the mechanism of action of gabapentin, one of the few agents with proven effectiveness in hypersensitivity states in humans.

In summary, intrathecal gabapentin reduces punctate mechanical allodynia in an established rat model of postincisional pain. This effect does not necessitate entry of gabapentin into cells or nerve terminals *via* the L-amino acid transporter. Intrathecal gabapentin and clonidine interact in a synergistic manner in reducing postoperative allodynia, suggesting such a combination could have clinical usefulness after surgery. However, there is no injectable formulation of gabapentin available, and human trials must await appropriate preclinical

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screening for possible neurotoxicity from the intrathecal injection.

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