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## Antagonism of the Antinocifensive Action of Halothane by Intrathecal Administration of GABA<sub>A</sub> Receptor Antagonists

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**Background:** The hind brain and the spinal cord, regions that contain high concentrations of  $\gamma$ -aminobutyric acid (GABA) and GABA receptors, have been implicated as sites of action of inhalational anesthetics. Previous studies have established that general anesthetics potentiate the effects of  $\gamma$ -aminobutyric acid at the GABA<sub>A</sub> receptor. It was therefore hypothesized that the suppression of nocifensive movements during anesthesia is due to an enhancement of GABA<sub>A</sub> receptor-mediated transmission within the spinal cord.

**Methods:** Rats in which an intrathecal catheter had been implanted 1 week earlier were anesthetized with halothane. Core temperature was maintained at a steady level. After MAC determination, the concentration of halothane was adjusted to that at which the rats last moved in response to tail clamping. Saline, a GABA<sub>A</sub>, a GABA<sub>B</sub>, or a glycine receptor antagonist was then injected intrathecally. The latency to move in response to application of the tail clamp was redetermined 5 min later, after which the halothane concentration was increased by 0.2%. Response latencies to application of the noxious stimulus were measured at 7-min intervals during the subsequent 35 min. To determine whether these antagonists altered baseline response latencies by themselves, another experiment was conducted in which the concentration of halothane was not increased after intrathecal administration of GABA<sub>A</sub> receptor antagonists.

**Results:** Intrathecal administration of the GABA<sub>A</sub> receptor antagonists bicuculline (0.3  $\mu$ g) or picrotoxin (0.3, 1.0  $\mu$ g) antagonized the suppression of nocifensive movement produced

by the small increase in halothane concentration. In contrast, the antinocifensive effect of the increase in halothane concentration was not attenuated by the GABA<sub>B</sub> receptor antagonist CGP 35348 or the glycine receptor antagonist strychnine. By themselves, the GABA<sub>A</sub> receptor antagonists did not alter response latency in rats anesthetized with sub-MAC concentrations of halothane.

**Conclusions:** Intrathecal administration of bicuculline or picrotoxin, at doses that do not change the latency to pinch-evoked movement when administered alone, antagonized the suppression of noxious-evoked movement produced by halothane concentrations equal to or greater than MAC. These results suggest that enhancement of GABA<sub>A</sub> receptor-mediated transmission within the spinal cord contributes to halothane's ability to suppress nocifensive movements. (Key words: Anesthetics, volatile: halothane. Receptors: GABA<sub>A</sub>. Spinal cord: antinociception; pain.)

GENERAL anesthetics block the motor response to noxious stimulation at concentrations greater than those that suppress learning, consciousness, or thermoregulation and less than those that suppress autonomic responsiveness.<sup>1-4</sup> This observation suggests that the antinocifensive component is mediated independently of the other components of general anesthesia. Recent studies of the site(s) within the central nervous system at which isoflurane or halothane act to suppress nocifensive movement suggest that this effect is independent of an action on forebrain structures because the antinocifensive potency of these inhalational anesthetics is unchanged in decerebrate rats<sup>5</sup> or rats with focal cryogenic lesions of the parietal cortex.<sup>6</sup> In addition, more than twice as much isoflurane is required to suppress nocifensive movement in goats in which the forebrain is preferentially anesthetized.<sup>7</sup> The observation that acute spinal transection does not alter the antinocifensive potency of isoflurane suggests that inhalational anesthetics act at the level of the spinal cord to suppress nocifensive movement.<sup>8</sup> This idea is supported by the recent finding that the isoflurane concentration required to suppress nocifensive movement is lower when the goat brain stem and spinal cord to-

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gether, without the forebrain, are perfused than it is with whole-body perfusion.<sup>9</sup> These studies suggest that the spinal cord is at least as sensitive to isoflurane as is the entire central nervous system.

Although these studies provide preliminary insight into the sites at which inhalational anesthetics act to suppress nocifensive movement, they offer little information about the pharmacologic mechanism(s) of action. A common property of a wide spectrum of general anesthetics, including inhalational anesthetics, barbiturates, alcohols, and 2,6-diisopropyl phenol (propofol) is their ability to enhance the actions of  $\gamma$ -aminobutyric acid (GABA) at the GABA<sub>A</sub> receptor.<sup>10</sup> GABA plays an important role in the modulation of nociceptive transmission in the spinal cord. The spinal cord contains high concentrations of GABA<sup>11,12</sup> and high densities of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors.<sup>13,14</sup> Intrathecal administration of isoguvacine, a GABA<sub>A</sub> receptor agonist, or L-baclofen, a GABA<sub>B</sub> receptor agonist, produces antinociception.<sup>15-18</sup> In contrast, GABA<sub>A</sub> receptor antagonists, and to some extent GABA<sub>B</sub> receptor antagonists, produce hyperalgesia and allodynia when intrathecally administered.<sup>15,19-22</sup> The current study therefore examined whether the suppression of nocifensive movement by an inhalational general anesthetic is mediated by an action at GABA<sub>A</sub> or GABA<sub>B</sub> receptors in the spinal cord.

## Methods and Materials

Male Sprague-Dawley rats weighing 250–375 g were instrumented with intrathecal catheters that terminated at the thoracolumbar junction of the spinal cord as described by Yaksh and Rudy.<sup>23</sup> Briefly, rats were anesthetized with 2–3% halothane and, under aseptic conditions, one end of a PE-10 catheter was introduced through the atlantooccipital membrane and threaded 8.6 cm caudally in the spinal cord subarachnoid space. The other end was externalized at the dorsum of the head. Rats were allowed to recover for 1 week after surgery, during which neurologic function and weight were monitored daily. Rats exhibiting motor dysfunction as a consequence of catheter implantation were excluded from the study.

### Experimental Design

The acute experiments were performed 1 week after intrathecal catheter implantation. Rats were initially anesthetized with halothane in an airtight con-

tainer. After induction, 1.1–1.5% halothane was administered by a nose cone. The halothane concentration was monitored throughout the experiment by a Datex Capnomac Anesthetic Monitor (Helsinki, Finland) and measured as the average of concentrations sampled at 3 Hz over a 1-min period.

Core body temperature was monitored *via* a rectal probe and maintained at 37.0–39.0°C for the duration of the experiment using a warm water blanket, a plastic cover, and a heat lamp. Although core body temperature varied among rats, each individual rat was maintained within 0.5°C of its core body temperature determined 40–70 min after anesthetic induction. After a 40-min equilibration period at 1.0–1.4% halothane, the rat was tested for a motor response to the application of a 1-cm serrated alligator clip to the proximal third of the tail. Application of the clip evoked a vigorous escape response in awake rats and was judged to be very painful when applied to a fold of the investigator's skin. The clip was oscillated for 1 min or until the rat responded by purposeful movement of one or more of its limbs. In addition to recording the occurrence of nocifensive movement, the latency to movement was measured to the nearest second. Rats in which application of the clip did not elicit nocifensive movement were assigned the cutoff latency of 60 s. If application of the clip evoked a movement, then the halothane concentration was increased by 0.2%. If no response occurred, the concentration of halothane was decreased by 0.2%. Forty minutes were allowed for equilibration at each new concentration of halothane, after which the response to application of the clip was redetermined. During MAC determination, the clip was not applied at intervals less than 40 min. The above process was repeated until two independent move-no move crossovers were obtained. The average of the midpoints obtained in each crossover was defined as the minimum anesthetic concentration (MAC).<sup>4</sup>

After determination of MAC, the halothane concentration was then either maintained at or adjusted to and then equilibrated at the level at which the rat had most recently responded to stimulation. The rat was then tested to confirm its responsiveness to application of the clip at this concentration of halothane. Within 15 min of the confirmatory response, either saline, 0.3  $\mu$ g bicuculline methiodide, 0.3  $\mu$ g or 1.0  $\mu$ g picrotoxin, 1.0  $\mu$ g strychnine hemisulfate, or 30  $\mu$ g CGP 35348 was injected intrathecally. Five minutes later, the rat's

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INTRATHECAL GABA<sub>A</sub> ANTAGONISTS AND HALOTHANE

response to application of the alligator clip was redetermined. The concentration of halothane was then increased by 0.2% and responsiveness was redetermined at 7-min intervals for the subsequent 35-min period. In 14 rats, the concentration of halothane was not increased, but remained at the sub-MAC concentration, and responsiveness was redetermined as described earlier to evaluate the effects of saline or antagonist on baseline responsiveness. Each rat received only one dose of a drug and the identity of the drug was unknown to the person testing the rat. At the conclusion of the experiment, rats were killed with an overdose of chloral hydrate or pentobarbital, exsanguinated, and the location and patency of the catheter was verified by visual inspection and injection of india ink.

Although the initial determination of MAC was made by conventional methods, pharmacodynamic factors precluded a conventional redetermination of MAC after intrathecal drug administration. MAC determination requires that two independent move-no move crossovers be obtained, a process that can take 3–4 h in the case of halothane with its 40-min equilibration time. This requirement was at odds with the comparatively short duration of action of intrathecally administered drugs. In the case of the agents used in this study, onset to effect occurs within 5 min with peak effect observed by 15 min after administration.<sup>15,21</sup> The effect of these drugs is substantially diminished 40 min after administration. Although continuous infusion of the antagonists would enable a conventional determination of MAC, this approach was not attempted. It was considered unlikely that the doses (both molar amount and volume) of the antagonists in the spinal cord subarachnoid space could be titrated to levels that (1) were consistently just sufficient for receptor antagonism for a period of 3–4 h, (2) did not eventually redistribute beyond the lumbar segments of the spinal cord, and (3) did not eventually accumulate to concentrations that produce allodynia or seizures, or alter baseline response latency. Thus, it was necessary for the purposes of this study to forgo a determination of the change in MAC and rather examine the drug effects on the decrease in motor responses produced by a small increase in halothane concentration from just below MAC to just above MAC.

#### Drugs

All drugs were obtained from Sigma Chemical (St. Louis, MO), with the exception of CGP 35348, which was obtained from Ciba-Geigy (Basel, Switzerland). The

solutions were freshly prepared, passed through a 0.2- $\mu$ m filter and their pH was adjusted to a range of 6.9–7.2. Drugs were administered in a volume of 10  $\mu$ l followed by 10  $\mu$ l saline to clear the catheter. The doses of antagonists were based on their ability to antagonize the effects of their respective receptor agonists in the spinal cord of the rat without altering responsiveness to noxious or non-noxious stimuli. For example, 0.3  $\mu$ g bicuculline produces a 3.4-fold rightward shift in the dose-response curve of intrathecally administered isoguvacine but does not alter response latency to noxious thermal stimuli or produce touch-evoked allodynia in awake rats<sup>15</sup> (unpublished observations, DL Hammond). Intrathecal administration of 30  $\mu$ g CGP 35348 shifts the dose-response curve of intrathecally administered baclofen tenfold to the right but does not alter response latency to noxious thermal stimuli or produce touch-evoked allodynia<sup>21,22</sup> (unpublished observations, DL Hammond). Analogous information was not available for either strychnine or picrotoxin. Therefore, the doses of these antagonists were based on literature reports, and confirmed in preliminary dose-ranging experiments, of doses that were submaximal for the production of touch-evoked allodynia and overt "pain" or motor behaviors such as scratching, vocalization, or myoclonic twitches after intrathecal administration in the rat. In the case of picrotoxin, this dose corresponded to 1.0  $\mu$ g<sup>24</sup> (unpublished observations, DL Hammond). In the case of strychnine, intrathecal administration of 2.8  $\mu$ g was reported not to produce significant biting, scratching, twitching, or vocalization in the rat.<sup>24</sup> However, in our preliminary studies, 1.5 or 3.0  $\mu$ g strychnine produced myoclonic seizures, biting of the flanks, and distress vocalization; the intensity was dose-dependent in nature. The dose of 1.0  $\mu$ g was thus chosen because it was just subthreshold for these effects and for touch-evoked allodynia (unpublished observations, DL Hammond).

#### Statistical Analysis

The effects of the GABA or glycine receptor antagonists on the latency to response were compared to that of saline by two-way analysis of variance for repeated measures. *Post hoc* comparisons of individual mean values were made by Newman-Keuls test. Fisher's exact test was used to compare the percentage of rats in the saline- and drug-treated groups that moved in response to application of the alligator clip. A *P* value of less than or equal to 0.05 was considered significant.

## Results

### Core Body Temperature and MAC

During early experiments, it became evident that core temperature influenced the probability of movement in response to application of the alligator clip. Examination of the relationship between core temperature and response to application of the alligator clip during MAC determination indicated that rats were more likely to move at warmer temperatures than at colder temperatures.<sup>25</sup> As shown in figure 1, core temperature had the greatest effect on the probability of nocifensive movement at intermediate concentrations of halothane. At 1.20–1.39% halothane, rats with temperatures of 37.0–37.5°C moved in only 23% of trials whereas rats with a core temperature of 38.5–39.0°C moved in 75% of trials. Because of this trend, core body temperature was recorded at the time of each stimulus trial and was maintained at a steady level throughout the experiment. The mean temperature deviation over time within each treatment group was less than 0.1°C. Two-way analysis of variance for repeated measures showed no significant difference in core body temperature among the different treatment groups and no significant difference within each treatment group over time. Under conditions in which core body temperature was controlled within a range of 37.0–39.0°C, the mean MAC of halothane was  $1.10 \pm 0.02$  ( $n = 49$ ) and did not differ among drug treatment groups ( $P > 0.2$ ). This value is in good agreement with previous reports.<sup>6,26,27</sup>

### Effect of GABA and Glycine Receptor Antagonists on Responses at Sub-MAC Concentrations of Halothane

Responsiveness to application of the alligator clip was redetermined 5 min after the intrathecal administration of saline or antagonist and before the concentration of halothane was increased, *i.e.*, while the rats were still at a sub-MAC concentration of anesthetic. Within treatment group comparisons revealed that intrathecal administration of saline did not alter either the latency to respond or the percentage of rats that moved in response to the alligator clip as compared to its baseline value (figs. 2A and 2B). Similarly, intrathecal injection of 0.3 µg picrotoxin (figs. 2A and 2B) or 1.0 µg strychnine (figs. 3A and 3B) did not alter the latency to response or the percentage of rats that moved in response to application of the alligator clip as compared to their respective baseline values. In rats that received 30 µg CGP 35348, a modest increase ( $P <$

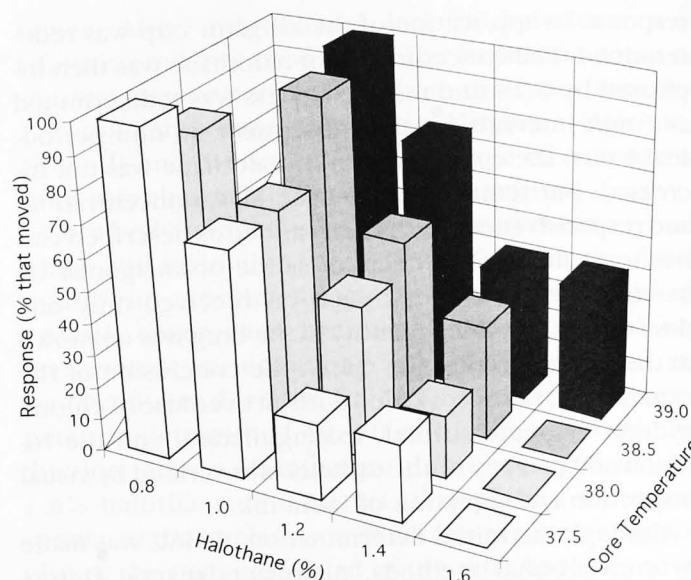


Fig. 1. Histogram of the relationship between core body temperature, inspired halothane concentration, and the percentage of rats that responded to application of an alligator clip to the proximal third of the tail. No rats with a core temperature of greater than 38.0°C were tested at an anesthetic concentration of 0.80–0.99%.

0.01) in response latency, but no decrease in the percentage of rats that moved occurred (fig. 3A and 3B). This increase could be attributed to one rat whose latency increased to 55 s at this one time. However, in rats receiving intrathecal injections of either 0.3 µg bicuculline or 1.0 µg picrotoxin a significant decrease in response latency occurred 5 min later as compared to their respective baseline values (figs. 2A and 2B). Importantly, between-group comparisons indicated that none of the antagonist treatment groups differed from the saline control group with respect to the latency to movement or the percentage of rats that moved at either the baseline timepoint or 5 min after intrathecal injection.

Nonetheless, these results prompted an ancillary study to further examine whether bicuculline or picrotoxin decreased baseline response latency when administered by themselves to a rat maintained at a sub-MAC concentration of halothane and whether repetitive application of the alligator clip induced sensitization in saline-treated rats. For this experiment, either saline, 0.3 µg bicuculline, or 1.0 µg picrotoxin was administered intrathecally and responsiveness to application of the alligator clip was redetermined for the subsequent 40 min in the absence of an increase in halothane concentration, *i.e.*, while the rats remained at a sub-

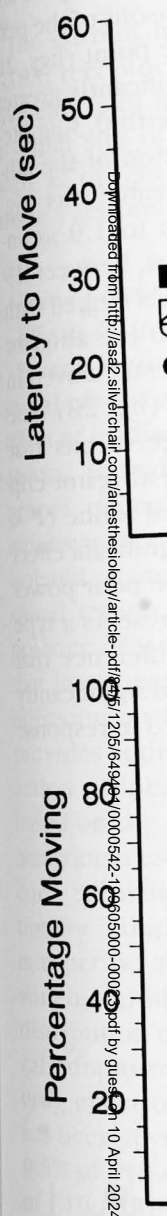


Fig. 2. Effect of 0.3 µg picrotoxin on the antinociceptive response of these drugs to the application of an alligator clip. "B" is 5 min before the clip (arrowhead), which the control group. Symbols represent individual data points. Response latency was redetermined from those of point (\* $P < 0.05$ ).



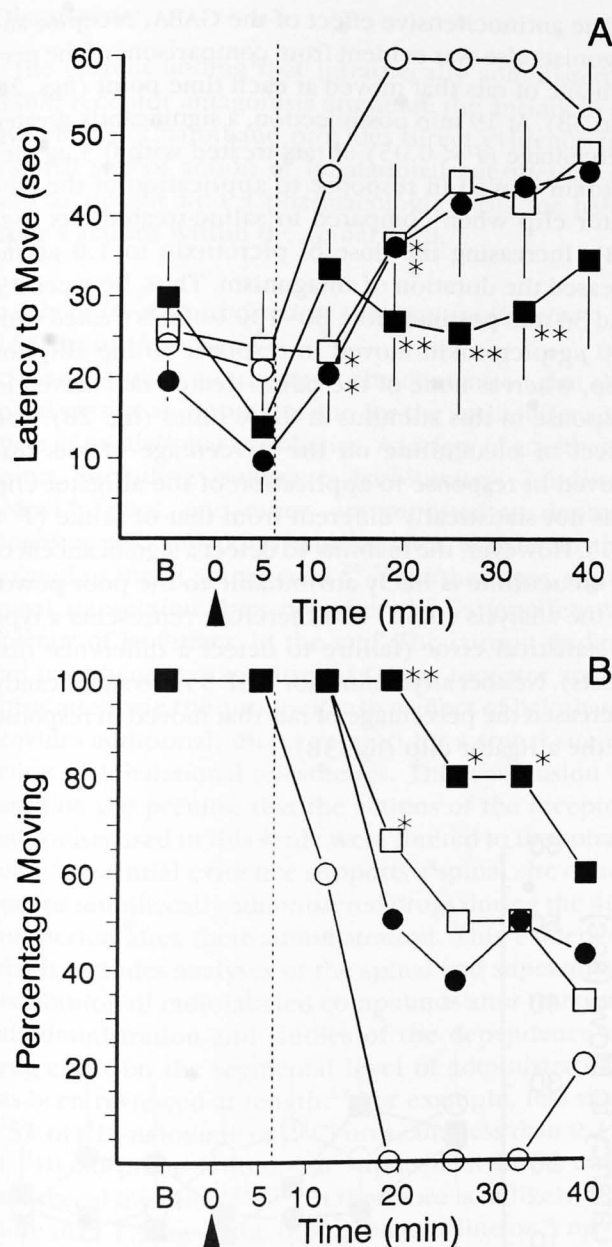
INTRATHECAL GABA<sub>A</sub> ANTAGONISTS AND HALOTHANE

Fig. 2. Effect of saline (○;  $n = 5$ ), 0.3  $\mu$ g bicuculline (●;  $n = 8$ ), 0.3  $\mu$ g picrotoxin (□;  $n = 6$ ), or 1.0  $\mu$ g picrotoxin (■;  $n = 5$ ) on the antinocifensive effect of halothane in the rat. (A) Effects of these drugs on latency to movement in response to application of an alligator clip to the proximal third of the tail. (B) Percentage of rats that moved in response to application of the clip. "B" indicates the baseline measure determined 5–15 min before the intrathecal administration of drug at time zero (arrowhead). The dashed vertical line indicates the time at which the concentration of halothane was increased by 0.2%. Symbols represent the mean  $\pm$  SE. Error bars that are not visible were encompassed by the symbol. Asterisks indicate response latencies or percentages that are significantly different from those of saline-treated rats at the corresponding time point (\* $P < 0.05$ ; \*\* $P < 0.01$ ).

MAC concentration of halothane. Although within-treatment group comparisons to baseline latency indicated that latency to movement decreased 19–33 min after intrathecal administration of picrotoxin ( $P < 0.05$ ), latency to movement was similarly decreased 26–40 min after the intrathecal administration of saline ( $P < 0.05$ ). No significant change in latency to movement occurred after intrathecal administration of picrotoxin. Importantly, between-group comparisons indicated that response latencies in picrotoxin- or bicuculline-treated rats did not differ from those of saline-treated rats at any time (fig. 4).

#### Effect of GABA and Glycine Receptor Antagonists on the Antinocifensive Action of Halothane

After redetermination of responsiveness 5 min after intrathecal drug administration, the concentration of halothane was then increased by approximately 0.2%. The mean increase in halothane concentration for all animals was  $0.22 \pm 0.01\%$  and did not differ among the treatment groups ( $P > 0.5$ ). In saline-treated rats, the latency to movement increased and the percentage of rats responding to the alligator clip decreased at 12 min after the increase in halothane concentration (figs. 2A and 2B). These effects stabilized by 19 min at which time none of the saline-treated rats moved in response to oscillation of the alligator clip for 1 min. The 12- and 19-min postinjection time points corresponded to 7 and 14 min, respectively, after the increase in halothane concentration.

The antinocifensive effects of the small increase in the concentration of halothane were attenuated in rats pretreated with either bicuculline or picrotoxin (fig. 2). In rats pretreated with 0.3  $\mu$ g of bicuculline, the response latency determined at 12 and 19 min postinjection (corresponding to 7 and 14 min after the increase in halothane) was significantly less than in saline-treated rats (fig. 2A). In rats pretreated with 0.3  $\mu$ g picrotoxin, the response latency determined after the increase in halothane concentration was significantly less than in saline-treated rats at 12 and 19 min (fig. 2A). Increasing the dose of picrotoxin to 1.0  $\mu$ g resulted in a more prolonged attenuation of the effects of halothane. Response latency was significantly less than that in saline-treated rats at 19, 26, and 33 min (fig. 2A). By comparison, intrathecal pretreatment with either 1  $\mu$ g strychnine, a glycine receptor antagonist, or 30  $\mu$ g CGP 35348, a GABA<sub>B</sub> receptor antagonist, did not attenuate the antinocifensive effects of halothane (figs. 3A and 3B).

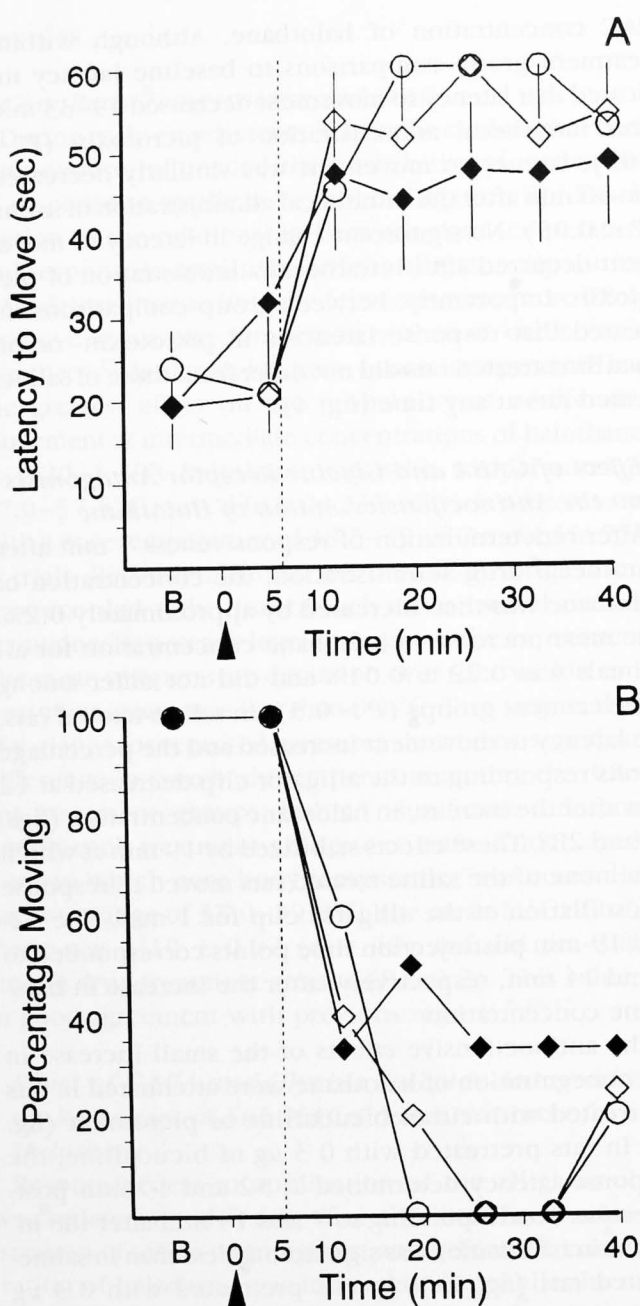


Fig. 3. Effect of saline ( $\circ$ ;  $n = 5$ ), 30  $\mu$ g CGP 35348 ( $\blacklozenge$ ;  $n = 6$ ), or 1.0  $\mu$ g strychnine ( $\diamond$ ;  $n = 5$ ) on the antinocifensive effect of halothane in the rat. (A) Effects of these drugs on latency to movement in response to application of an alligator clip to the proximal third of the tail. (B) Percentage of rats that moved in response to application of the clip. "B" indicates the baseline measure determined 5–15 min before the intrathecal administration of drug at time zero (arrowhead). The dashed vertical line indicates the time at which the concentration of halothane was increased by 0.2%. Symbols represent the mean  $\pm$  SE. Error bars that are not visible were encompassed by the symbol. Asterisks indicate response latencies or percentages that are significantly different from those of saline-treated rats at the corresponding time point (\* $P < 0.05$ ; \*\* $P < 0.01$ ).

The antinocifensive effect of the GABA<sub>A</sub> receptor antagonists also was evident from comparisons of the percentage of rats that moved at each time point (figs. 2B and 3B). At 19 min postinjection, a significantly greater percentage ( $P < 0.05$ ) of rats treated with 0.3  $\mu$ g picrotoxin moved in response to application of the alligator clip when compared to saline-treated rats (fig. 2B). Increasing the dose of picrotoxin to 1.0  $\mu$ g increased the duration of antagonism. Thus, between 19 and 33 min postinjection, 80–100% of rats treated with 1.0  $\mu$ g picrotoxin moved in response to the alligator clip, whereas none of the saline-treated rats moved in response to this stimulus at these times (fig. 2B). The effect of bicuculline on the percentage of rats that moved in response to application of the alligator clip was not statistically different from that of saline ( $P < 0.1$ ). However, the inability to detect a significant effect of bicuculline is likely attributable to the poor power of the analysis (0.46) and therefore represents a type II statistical error (failure to detect a difference that exists). Neither strychnine nor CGP 35348 significantly increased the percentage of rats that moved in response to the alligator clip (fig. 3B).

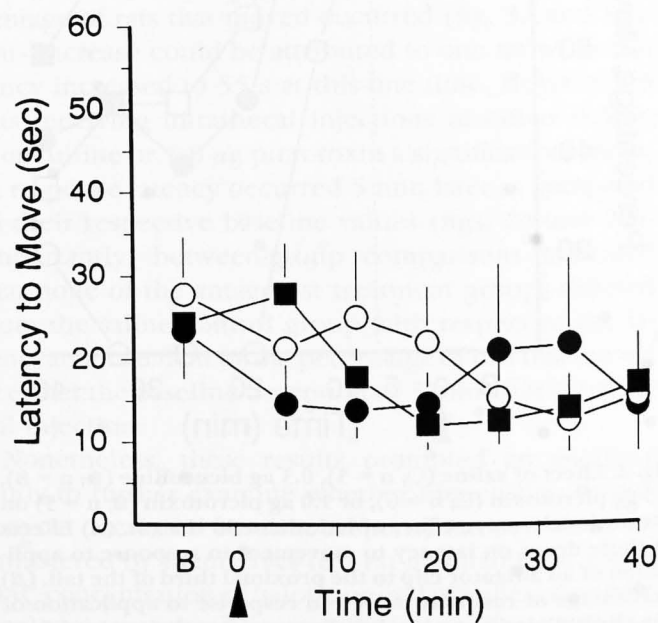


Fig. 4. Effect of saline ( $\circ$ ;  $n = 5$ ), 0.3  $\mu$ g bicuculline ( $\bullet$ ;  $n = 5$ ), or 1.0  $\mu$ g picrotoxin ( $\blacksquare$ ;  $n = 4$ ) on the latency to movement in response to application of an alligator clip to the proximal third of the tail at sub-MAC concentrations of halothane. "B" indicates the baseline measure determined 5–15 min before the intrathecal administration of drug at time zero (arrowhead). Symbols represent the mean  $\pm$  SE. Error bars that are not visible were encompassed by the symbol.

## Discussion

The current findings support the role of GABA<sub>A</sub> receptors in the antinocifensive effects of halothane at a spinal site of action, as well as insight into the actions of

Role of the Spinal GABA<sub>A</sub> Receptor

Several studies have shown that the spinal cord is a site of action for the antinocifensive effects of halothane. Inhaled agents, including propyl picrotoxin, have been shown to be effective in isolated spinal cord preparations. The antinocifensive effect of halothane is likely mediated by its action on the spinal GABA<sub>A</sub> receptor. The current findings support this hypothesis by showing that the antinocifensive effect of halothane is blocked by the GABA<sub>A</sub> receptor antagonist picrotoxin. The effect of bicuculline on the antinocifensive effect of halothane was not statistically significant, which may be due to the poor power of the analysis. The inability to detect a significant effect of bicuculline is likely attributable to the poor power of the analysis (0.46) and therefore represents a type II statistical error (failure to detect a difference that exists). Neither strychnine nor CGP 35348 significantly increased the percentage of rats that moved in response to the alligator clip (fig. 3B).



INTRATHECAL GABA<sub>A</sub> ANTAGONISTS AND HALOTHANE

## Discussion

The current finding that intrathecally administered GABA<sub>A</sub> receptor antagonists attenuate the antinocifensive effects of halothane provides direct evidence for a spinal site of action of inhalational anesthetics, as well as insight into a pharmacologic basis for halothane's actions within the spinal cord.

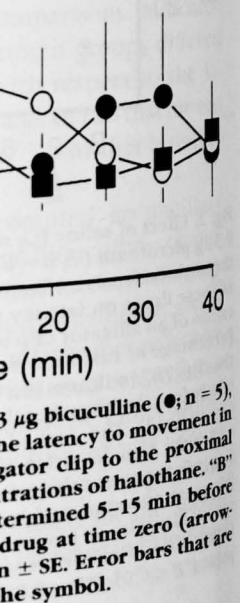
*Role of the Spinal Cord in the Antinocifensive Effects of Halothane*

Several studies now support the contention that the spinal cord is an important site for the antinocifensive effect of inhalational anesthetics. A variety of anesthetic agents, including isoflurane, barbiturates, 2,6-diisopropyl phenol, and ether, are reported to depress monosynaptic and polysynaptic reflex activity in the isolated or intact spinal cord.<sup>28-31</sup> Furthermore, acute spinal transection does not alter the antinocifensive potency of isoflurane in the rat.<sup>8</sup> The current finding that intrathecally administered GABA<sub>A</sub> receptor antagonists attenuate the antinocifensive effect of halothane provides additional, direct support for a spinal site of action of inhalational anesthetics. This conclusion is based on the premise that the actions of the receptor antagonists used in this study were limited to the spinal cord. Substantial evidence supports a spinal site of action for intrathecally administered drugs during the 40-min period after their administration. This evidence, which includes analyses of the spinal and supraspinal distribution of radiolabeled compounds after intrathecal administration and studies of the dependence of drug effect on the segmental level of administration, has been reviewed at length.<sup>32</sup> For example, less than 0.5% of [<sup>3</sup>H]-naloxone or [<sup>14</sup>C]-urea, and less than 0.1% of [<sup>3</sup>H]-morphine is found at supraspinal levels after intrathecal injection.<sup>23,33,34</sup> It therefore is unlikely that more than 1.5 ng of the 0.3  $\mu$ g bicuculline or 5 ng of the 1.0  $\mu$ g picrotoxin administered in the current study would be expected to reach supraspinal sites. These amounts are insufficient to alter nociceptive responsiveness when administered directly to supraspinal sites.<sup>35-37</sup> Additionally, local application of GABA<sub>A</sub> receptor antagonists at several supraspinal sites produces antinociception, rather than nociception.<sup>35-39</sup> Thus, in the unlikely event that pharmacologically relevant amounts (40-100 ng) of bicuculline or picrotoxin were to reach supraspinal sites, these drugs would be expected to enhance, rather than attenuate the antinocifensive effects of halothane.

Although anesthetic actions within the spinal cord appear to be sufficient to block nociceptive movement,<sup>40</sup> an additional action of inhalational anesthetics at supraspinal structures cannot be excluded. For example, activation of GABA<sub>A</sub> receptors in the periaqueductal gray or raphe magnus results in an enhanced responsiveness to noxious stimuli,<sup>35-37,41</sup> presumably by inhibiting the activity of neurons in these nuclei that project to the spinal cord and inhibit nociceptive transmission.<sup>42-44</sup> As halothane and other inhalational anesthetics potentiate the actions of GABA at the GABA<sub>A</sub> receptor,<sup>10</sup> it is likely that halothane can act supraspinally at sites in the periaqueductal gray or raphe magnus to inhibit the activity of these neurons and so enhance spinal nociceptive transmission by decreasing tonic descending inhibition. Indeed, nociceptive inhibitory neurons in the raphe magnus are inhibited by supramac concentrations of isoflurane.<sup>45</sup> By comparison, in the spinal cord, enhancement of GABAergic transmission results in antinociception. Selective antagonism of the actions of halothane at spinal GABA<sub>A</sub> receptors may concomitantly permit the expression of or unmask the pronociceptive actions of halothane at supraspinal sites. Thus, the ability of intrathecally administered GABA<sub>A</sub> receptor antagonists to attenuate the antinocifensive effects of inhalational anesthetics may reflect both a direct antagonism of the actions of halothane at GABA<sub>A</sub> receptors in the spinal cord and an indirect unmasking of a pronociceptive action of halothane at supraspinal GABA<sub>A</sub> receptors. Clarification of the contribution of supraspinal sites of action must await complementary studies of the effects of supraspinally administered GABA<sub>A</sub> and GABA<sub>B</sub> receptor ligands on the antinocifensive potency of halothane.

*GABA<sub>A</sub> Receptors Mediate the Antinocifensive Effects of Halothane in the Spinal Cord*

This study used two GABA<sub>A</sub> receptor antagonists of different physicochemical structure and different mechanisms of action to assess the contribution of GABA<sub>A</sub> receptors to the antinocifensive effects of halothane. Bicuculline is a competitive antagonist of GABA at the GABA<sub>A</sub> receptor, whereas picrotoxin is a non-competitive antagonist of the chloride channel associated with the GABA<sub>A</sub> receptor.<sup>46</sup> Both bicuculline and picrotoxin effectively antagonized the antinocifensive effect of a small increase in halothane concentration. In contrast, antagonists for either the closely related glycine receptor or the GABA<sub>B</sub> receptor were ineffective. These findings suggest that the antinocifensive ef-



fects of threshold concentrations of inhalational anesthetics result from an enhancement of the actions of GABA at GABA<sub>A</sub> receptors within the spinal cord.

Intrathecal administration of GABA<sub>A</sub> receptor antagonists similarly antagonized the suppression of nociceptive reflexes by systemic barbiturates.<sup>47</sup> However, the large doses of bicuculline (25 µg) or picrotoxin (12 µg) used in that study induce seizures when administered alone.<sup>47</sup> Thus, the antagonism of the antinocifensive effects of the barbiturate may simply have been due to gross alterations of excitability in the spinal cord. This confounder was not an issue in the current study because the doses of GABA<sub>A</sub> receptor antagonists were carefully chosen to be just sufficient for antagonism of their receptor, yet too low to enhance sensitivity to innocuous mechanical stimuli, augment baseline nociceptive motor responses, or produce myoclonic twitches in awake rats. Although intrathecal administration of GABA<sub>A</sub> receptor antagonists can produce allodynia and hyperalgesia, the minimum doses that produce such effects are threefold- to tenfold higher<sup>15</sup> (unpublished observations, DL Hammond) than those used to antagonize halothane in the current study. The allodynia, hyperalgesia, and spontaneous myoclonic twitches that are commonly reported after intrathecal administration of GABA<sub>A</sub> receptor antagonists in unanesthetized rats,<sup>19,20</sup> occur after administration of doses of 30–60 µg, 100- to 200-fold higher than the doses used in the current study. Administration of these high doses also causes an increase in the spontaneous activity of dorsal horn cells.<sup>48</sup>

The lack of effect of the low doses of GABA<sub>A</sub> receptor antagonists used in this study was further verified in rats anesthetized with sub-MAC concentrations of halothane, a condition in which the actions of GABA at the GABA<sub>A</sub> receptor may be enhanced<sup>49</sup> and so might be more sensitive to antagonism by bicuculline or picrotoxin. Although initial experiments indicated that the latency to nocifensive movement in rats anesthetized with sub-MAC concentrations of halothane was significantly decreased 5 min after intrathecal administration of 0.3 µg bicuculline or 1.0 µg picrotoxin, this observation was not replicated in an ancillary study. In this study, rats treated with 0.3 µg bicuculline or 1.0 µg picrotoxin did not differ from saline-treated rats at any time before or after drug administration. The most parsimonious explanation for this finding is that there is little or no potentiation of GABA<sub>A</sub> receptor-mediated transmission in the lightly anesthetized condition and that an increase in the tonic activation of GABA<sub>A</sub> recep-

tor-mediated transmission occurs only at anesthetic levels equal to or greater than MAC. Taken together, these findings indicate that the antagonism of the antinocifensive effects of halothane by intrathecal administration of low doses of bicuculline or picrotoxin cannot be ascribed to a nonspecific excitation or a general reduction in inhibition in the spinal cord, but rather to selective antagonism of the GABA<sub>A</sub> receptor.

#### *Possible Sites of Action Within the Spinal Cord*

Although Bárány<sup>50</sup> suggested that general anesthetics suppress central nervous system activity in proportion to the number of synapses in the pathway, general anesthetics preferentially suppress monosynaptic reflexes.<sup>51</sup> Thus, the effect of general anesthetics on somatomotor transmission is more likely due to the differential vulnerability of specific synapses (*i.e.* the Ia afferent to  $\alpha$ -motoneuron synapse *vs.* the polymodal nociceptor to dorsal horn cell synapse) to modulation by general anesthetics than to the cumulative number of synapses used.<sup>51,52</sup> The current results suggest that synapses with GABA<sub>A</sub> receptors, located either on the presynaptic terminal or on the postsynaptic membrane, may be selectively targeted for enhancement by halothane at concentrations near the threshold for blocking nocifensive movements. The depression of somatomotor activity by general anesthetics in the spinal cord may be mediated by inhibition of excitatory neurotransmitter release *via* GABA<sub>A</sub> receptors situated presynaptically on the terminals of Ia afferents,<sup>53–56</sup> low threshold primary afferents,<sup>57</sup> and myelinated nociceptors.<sup>58,59</sup> In addition, GABA<sub>A</sub> receptors located postsynaptically may mediate inhibition of dorsal horn cells,<sup>60,61</sup> Ia interneurons,<sup>62</sup> and motoneurons.<sup>63–65</sup> Thus, activation or potentiation of GABA<sub>A</sub> synaptic transmission, at numerous sites within the spinal cord, is likely to contribute to the suppression of somatomotor transmission by inhalational general anesthetics.

In conclusion, the current results provide direct evidence that the spinal cord is an important site of action for the antinocifensive action of inhalational anesthetics. These results further indicate that enhancement of the action of GABA at spinal GABA<sub>A</sub> receptors is one mechanism by which halothane exerts its antinocifensive actions.

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INTRATHECAL GABA<sub>A</sub> ANTAGONISTS AND HALOTHANE

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## Involvement and Bicultural Consciousness

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**Background:** Glutamate receptor-mediated excitotoxicity is an important mechanism in the pathogenesis of neurodegenerative diseases. Inhibitory neurotransmission is essential for the regulation of glutamate release and receptor activation. The present study was designed to investigate the role of inhibitory neurotransmission in the development of chronic pain in a rat model of neuropathic pain. **Methods:** Male Sprague-Dawley rats were subjected to a unilateral sciatic nerve ligation. The effects of intrathecal administration of the GABA<sub>A</sub> receptor antagonist bicuculline (100 μg) on the development of mechanical allodynia were studied. The effects of bicuculline on the expression of Fos and Fos-related antigen (FRA) in the spinal cord were also examined. **Results:** Intrathecal administration of bicuculline (100 μg) significantly reduced the development of mechanical allodynia in the ligated rats. The expression of Fos and FRA in the spinal cord was also significantly reduced by the administration of bicuculline. **Conclusions:** The present study suggests that inhibitory neurotransmission plays a role in the development of chronic pain in a rat model of neuropathic pain.

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