

## Inhibition of Nociception-induced Spinal Sensitization by Anesthetic Agents

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**Background:** Subcutaneous injection of dilute formalin in the hind paw of the rat produces a biphasic nociceptive response. Initial C-fiber activity is accompanied by flinching of the paw for about 5 min (phase 1), followed by cessation of activity and resumption of flinching beginning 15 min after injection and lasting about 40 min or more (phase 2). The second phase depends on changes in dorsal horn cell function that occur shortly after the initial C-fiber discharge. It was previously shown that isoflurane, administered during phase 1, reduced phase 2 activity, but a combination of isoflurane and nitrous oxide given throughout phase 1 did not suppress spinal sensitization. The same model was used to determine the effects of several inhalation and intravenous anesthetic agents on phase 2 of the formalin test.

**Methods:** The formalin test was carried out on male Sprague-Dawley rats. Animals anesthetized briefly with halothane to facilitate formalin injection, were compared to animals that received 1 MAC anesthesia from 5 min before to 6 min after formalin injection using halothane, enflurane, isoflurane, desflurane, or 70% N<sub>2</sub>O, or a combination of nitrous oxide plus 1 MAC halothane. Animals that were given intravenous saline immediately before injection of formalin were compared to animals given either 20 mg/kg intravenous thiopental just before formalin injection or 10 mg/kg intravenous propofol just before and 3 mg/kg immediately after formalin injection. Flinches/minute were counted at 1 and 5 min after formalin injection and thereafter at 5-min intervals for 1 h. The total of 1- and 5-min flinches were considered phase 1 activity and the total of 10-60-min flinches were considered phase 2. Total phase 2 activity was compared between groups using one-way analysis of variance.

**Results:** Animals that received halothane, enflurane, isoflurane, desflurane, or nitrous oxide during phase 1 demonstrated a significant decrease in phase 2 activity when compared to controls, while those that received a combination of nitrous oxide and halothane exhibited no difference. Animals that received intravenous thiopental anesthesia during phase 1 demonstrated no difference in phase 2 activity when compared to controls, whereas those that received propofol during phase 1 demonstrated a significant decrease of phase 2 activity.

**Conclusions:** Volatile anesthetics or nitrous oxide significantly suppress spinal sensitization, whereas the combination of nitrous oxide plus halothane causes no suppression. Thiopental does not affect spinal sensitization, whereas propofol causes significant suppression. These results may have important implications regarding the development of postoperative pain. (Key words: Anesthetics, inhalation. Anesthetics, intravenous. Hyperalgesia. Opioids. Spinal cord.)

IN recent years, there has been increasing interest in the possible effects of anesthetic agents on the processing of nociceptive activity in the spinal cord. The formalin test, which causes local tissue injury of the paw, has been used as a model for tonic pain. A reliable biphasic response is produced, characterized by several minutes of flinching immediately after injection (phase 1), followed by cessation of activity, and then resumption of flinching (phase 2), which can last for 40 min or more. Phase 2 activity depends on facilitation of spinal transmission that is evoked by C-fiber activity, generated immediately after the noxious stimulus.<sup>1,2</sup> Nociceptive sensitization, an increase in dorsal horn cell response to noxious stimulation, is thought to be responsible for the second phase of the formalin test.<sup>3</sup> Similarly, many of the clinical features of postoperative pain in humans are considered a consequence of injury-induced sensitization of dorsal horn neurons.<sup>4</sup>

It is well accepted that excitatory amino acids, including glutamate and aspartate, are the principle nociceptive neurotransmitters involved in the activation of dorsal horn cells by primary afferent terminals. N-methyl-D-aspartate (NMDA) receptors in the dorsal horn are ordinarily unresponsive to excitatory amino acids released from primary afferent terminals. However, after repetitive noxious stimulation, these receptors are enabled, and their activation leads to a series of intracellular events that magnify and prolong the neural responses to subsequent sensory stimuli.<sup>5-7</sup> Recent studies have suggested that both inhalation and intravenous anesthetic agents may affect spinal cord processing and modulate the response to nociceptive stimuli.<sup>8-12</sup> However, the mechanism by which this might occur is a matter for debate.

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The effect of anesthetic agents on spinal sensitization may be an important determinant of the severity of postoperative pain. Both inhalation and intravenous anesthetic agents have been shown to depress excitatory neurotransmission in the spinal cord.<sup>8,11-15</sup> However, such depressant effects do not necessarily indicate that these drugs are capable of blocking the sensitizing effects of a noxious stimulus. The effects of several inhalation anesthetics on the rat paw formalin test have been documented in several recent studies. In all of these studies, the anesthetics were administered during and for a short period after formalin injection and were discontinued before the onset of phase 2. Abram and Yaksh<sup>15</sup> showed that isoflurane produced a 34% reduction in phase 2 activity, whereas the combination of isoflurane and nitrous oxide failed to suppress phase 2. O'Connor and Abram<sup>13</sup> showed a similar reduction in phase 2 activity in animals exposed to halothane. Goto *et al.*<sup>16</sup> found that nitrous oxide suppressed phase 2, whereas halothane alone or a combination of halothane and nitrous oxide had no effect.

In light of the conflicting data regarding the effect of inhalation anesthetics on spinal sensitization, we sought to determine (1) whether volatile anesthetics reliably block spinal sensitization, (2) whether nitrous oxide alone blocks sensitization, (3) whether nitrous oxide indeed interferes with the ability of volatile anesthetics to block sensitization, and (4) whether equipotent concentrations of commonly used volatile anesthetics have differing abilities to block sensitization. We also sought to determine whether commonly used intravenous anesthetics were capable of suppressing spinal sensitization.

## Methods

The following studies were carried out under a protocol approved by the Animal Research Facility of the Zablocki Veterans's Administration Center. Male Sprague-Dawley rats weighing between 250 to 350 g were used for these studies.

### *Animal Preparation: Intravenous Catheter Insertion*

Animals that received intravenous agents were implanted with intravenous catheters, introduced under halothane anesthesia, into the right internal jugular vein *via* an incision on the right side of the neck, and tunnelled subcutaneously to the occipital area. These an-

imals were allowed to recover for 4-6 h, after which formalin testing was performed.

### *Formalin Test*

The formalin test was carried out as previously described.<sup>17</sup> To investigate the effect of halothane, enflurane, isoflurane, desflurane, nitrous oxide, and nitrous oxide plus halothane anesthesia on spinal sensitization, the paradigms listed below were used. Animals that received volatile anesthetics were placed in a Plexiglas induction box until immobile and then transferred to a nonrebreathing mask anesthesia system. Animals that received nitrous oxide alone were transferred to an enclosed Plexiglas chamber filled with 70% N<sub>2</sub>O and oxygen. Anesthetic concentrations were confirmed using a Criticare POET infrared gas analyzer in the inspiratory limb of the breathing system.

To provide a rough assessment of analgesia at the time of formalin injection, animals were tested by firmly pinching the metatarsals of the hind paw just before formalin injection, and the presence of a withdrawal response was determined. Doses of thiopental and propofol were determined on the basis of their ability to suppress response to paw-pinch for at least 5 min in a separate group of five animals. Before formalin injection, paw-pinch was tested to confirm lack of response in each animal. Animals that displayed withdrawal to paw-pinch were not used in the study.

Once awake, animals routinely displayed a flinching, withdrawal movement of the injected hind paw. Flinches per minute were recorded at 1 and 5 min after injection (including the periods of anesthesia) and at 5-min intervals thereafter for 1 h. The animals were killed with an overdose of barbiturate.

To investigate the effect of inhalation anesthetic agents on the second phase of the formalin test, the following paradigms were used. The concentrations of volatile anesthetics used represent 1 MAC for male rats.<sup>18,19</sup>

The inhalation anesthetic control group ( $n = 10$ ) underwent standard formalin testing. The rats were individually allowed to breathe 3% halothane until immobile. They were removed from the anesthetic and immediately given 50  $\mu$ l 5% formalin into the dorsum of the right hind paw using a 30-G needle.

The halothane group ( $n = 8$ ) was anesthetized with 3% halothane 10 min before formalin injection. When immobile, the concentration of halothane was reduced to 1.0% and maintained at that concentration for 5 min. Subcutaneous formalin was injected, and anesthesia was



maintained with 1.0% halothane. Anesthesia was discontinued 6 min after formalin injection.

The enflurane group ( $n = 7$ ) was anesthetized with 4% enflurane 10 min before formalin injection. When immobile, the concentration of enflurane was reduced to 2.2% and maintained at that concentration for 5 min. Subcutaneous formalin was injected, and anesthesia was maintained with 2.2% enflurane. Anesthesia was discontinued 6 min after formalin injection.

The isoflurane group ( $n = 5$ ) was anesthetized with 3% isoflurane 10 min before formalin injection. When immobile, the concentration of isoflurane was reduced to 1.4% and maintained at that concentration for 5 min. Subcutaneous formalin was injected, and anesthesia was maintained with 1.4% isoflurane. Anesthesia was discontinued 6 min after formalin injection.

The desflurane group ( $n = 7$ ) was anesthetized with 9% desflurane 10 min before formalin injection. When immobile, the concentration of desflurane was reduced to 7.7% and maintained at that concentration for 5 min. Subcutaneous formalin was injected, and anesthesia was maintained with 7.7% desflurane. Anesthesia was discontinued 6 min after formalin injection.

The nitrous oxide group ( $n = 8$ ) received 70%  $N_2O$  for 10 min before formalin injection. One-percent halothane was added 1 min before formalin injection to ensure immobilization for injection. Subcutaneous formalin was injected, and halothane anesthesia was immediately discontinued; then animals were transferred to another chamber, where 70%  $N_2O$  was maintained for 6 min after formalin injection.

The halothane plus nitrous oxide group ( $n = 6$ ) was anesthetized with 3% halothane and 70%  $N_2O$  until immobile, at which time the halothane was reduced to 1%. They were maintained at those concentrations for 5 min before formalin injection. Subcutaneous formalin was injected, and anesthesia was maintained with 1% halothane and 70%  $N_2O$  for 6 min after formalin injection. Anesthesia was then discontinued.

To demonstrate that the findings of our study were not the result of investigator bias, we elected to study four control animals and four animals anesthetized with isoflurane (as in the control and isoflurane groups above) in a blinded fashion. One investigator anesthetized the animals, administered the formalin, and performed the 1-, 5-, 10-, and 15-min counts. A second investigator, who did not know the treatment that each animal had received, performed the 20-60-min counts.

To investigate the effect of intravenous thiopental and propofol on the second phase of the formalin test, the following paradigms were used.

The intravenous anesthetic control group ( $n = 7$ ) underwent standard formalin testing. The rats were individually allowed to breathe 3% halothane until immobile. They were removed from the anesthetic and immediately given 1 ml intravenous saline followed by formalin injection.

The thiopental group ( $n = 6$ ) was anesthetized with 20 mg/kg intravenous thiopental immediately before formalin injection.

The propofol group ( $n = 10$ ) was anesthetized with 10 mg/kg intravenous propofol immediately before formalin injection. A second dose of 3 mg/kg intravenous propofol was given 1 min after formalin injection to maintain anesthesia throughout phase 1.

After injection, all animals were placed in clear Plexiglas chambers for observation. Coordinated spontaneous movement typically was noted within 1 min after cessation of brief halothane, within 2 min after desflurane, and within 5 min after cessation of prolonged halothane, halothane plus nitrous oxide, enflurane, or isoflurane injection. Animals that received nitrous oxide alone were somewhat uncoordinated but remained active and upright. The time to return of spontaneous coordinated movement after administration of intravenous anesthetic agents was noted.

#### Data Analysis

The total number of flinches was determined for all of the phase 1 (1-5 min) and phase 2 (10-60 min) observations for each animal. The total number of flinches during phase 2 was recorded for each animal and compared by one-way analysis of variance (StatView II). *Post hoc* comparisons were done using Scheffé's *F* test.

## Results

#### Inhalation Anesthetic Data

Paw-pinch was suppressed in all animals during 1 MAC volatile agent administration and during nitrous oxide and nitrous oxide plus halothane exposure. Flinching was noted during phase 1 for animals that received brief halothane anesthesia for formalin injection only (inhalation anesthetic control group). Little or no flinching was noted during phase 1 for animals that received volatile anesthetic agents from 5 min be-



**Table 1. Effects of Inhalation Anesthetics on Phase 1 and Phase 2 Response**

Inhalation Group	N	Mean No. of Flinches		Phase 2 (% of control)
		Phase 1	Phase 2	
Controls	10	8 ± 1	134 ± 14	100
Halothane	8	0	81 ± 10*	60
Enflurane	7	0	90 ± 8*	67
Isoflurane	5	1 ± 1	65 ± 21*	49
Desflurane	7	0	57 ± 17*	42
N <sub>2</sub> O	8	0	84 ± 14*	63
N <sub>2</sub> O/halothane	6	0	125 ± 9	92

\* Significantly different from controls ( $P < 0.05$ ).

fore to 6 min after formalin injection (halothane, enflurane, isoflurane, and desflurane groups), and none was seen in the groups that received nitrous oxide or nitrous oxide plus halothane anesthesia during that interval. Mean total numbers of flinches in phase 1 and 2 for all inhalation anesthesia groups are shown in table 1. Mean numbers of flinches/minute are shown in figures 1 and 2.

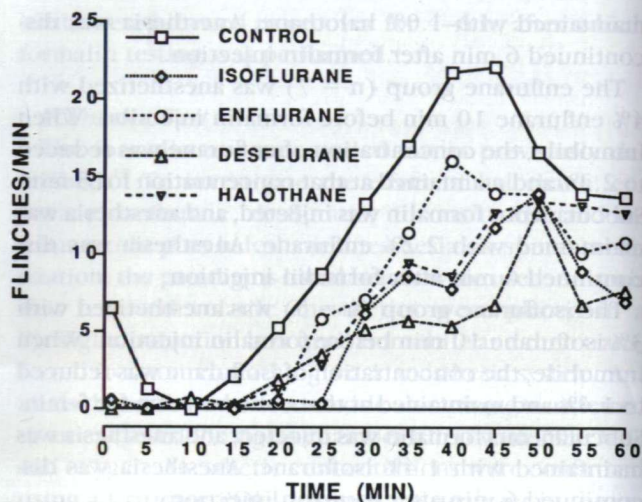
Animals in the halothane, enflurane, isoflurane, desflurane, and nitrous oxide groups demonstrated a significant decrease (33–58%) in phase 2 activity when compared to the inhalation anesthetic control group (fig. 1 and table 1). Phase 2 activity of animals in the nitrous oxide plus halothane group was nearly identical to that of the control group (fig. 2 and table 1).

The mean total number of flinches during phase 2 for the blinded control group was 140 and for the blinded isoflurane group was 78 ( $P < 0.05$ ), representing 55% of the control value. This result is essentially identical to that of the corresponding unblinded groups.

#### Intravenous Anesthetic Data

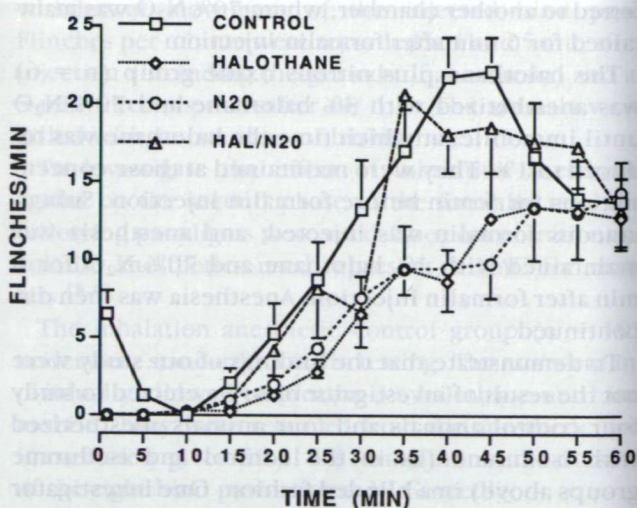
The mean time to return of spontaneous coordinated movement was 27 min for thiopental and 16 min for propofol after the intravenous doses used in this study. Animals that received thiopental anesthesia for formalin injection demonstrated no decrease in phase 1 activity but continued to flinch despite being otherwise unresponsive. Animals in the propofol group demonstrated a reduction in phase 1 activity when compared to controls, although this did not reach statistical significance ( $P = 0.06$ ; fig. 3 and table 2).

Phase 2 activity for animals in the thiopental group was essentially identical to activity for animals in the



**Fig. 1.** Mean number of flinches per minute plotted as a function of time after injection of formalin for the inhalation control group and the groups that received halothane, enflurane, isoflurane, or desflurane from 5 min before to 6 min after formalin injection.

intravenous anesthesia control group (intravenous saline). In several animals, phase 2 flinching began before recovery from anesthesia. Animals in the propofol group demonstrated a significant reduction in phase 2 activity ( $P < 0.05$ ) when compared to controls (fig. 3 and table 2).



**Fig. 2.** Mean number of flinches per minute plotted as a function of time after injection of formalin for the inhalation control group and the groups that received halothane, nitrous oxide, or the combination of halothane and nitrous oxide from 5 min before to 6 min after formalin injection. Error bars represent SEM.



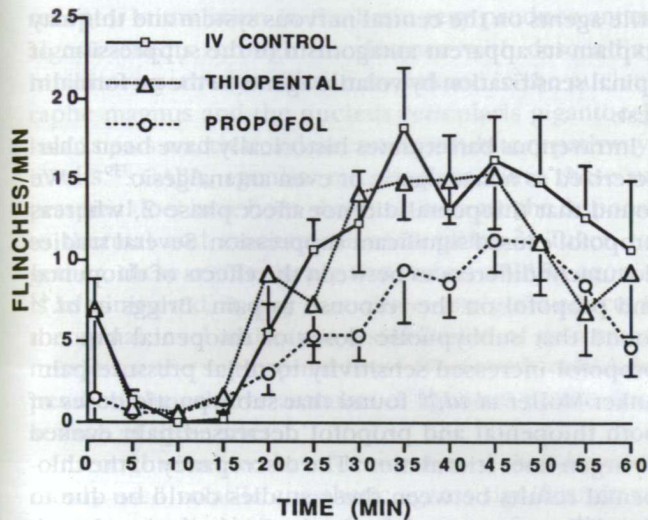


Fig. 3. Mean number of flinches per minute plotted as a function of time after injection of formalin for the intravenous control group and the groups that received thiopental or propofol before formalin injection. Error bars represent SEM.

## Discussion

This study provides further evidence that inhalation anesthetics can influence spinal cord processing of noxious stimuli. Abram and Yaksh<sup>15</sup> showed that isoflurane produced significant attenuation of spinal sensitization using the rat paw formalin test model and demonstrated that this effect was unlikely to be the result of ongoing anesthesia during the second phase. However, Goto *et al.*<sup>16</sup> found no attenuation of sensitization by halothane in this model. It is unclear why their data disagrees with that of the current study. It is conceivable that their failure to demonstrate an effect was related to the small number of animals tested.

In the current study, all inhalation anesthetics caused suppression of phase 2 activity. The decrease in activity was in the range of 33–58%, with desflurane producing the most suppression. This is a moderate effect compared to the combined effect of isoflurane plus intrathecal morphine (80% suppression)<sup>15</sup> or halothane plus intrathecal morphine (82% suppression).<sup>14</sup> In the study by Abram and Yaksh,<sup>15</sup> sensitization was not reduced further by raising the isoflurane concentration from 1% to 2.5% (MAC-BAR). This implies that a plateau can be reached, above which an increase in anesthetic concentration will not result in a further increase in suppression of spinal sensitization. They proposed the concept of MAC-FAC, the level of anesthesia required to prevent the postinjury state of facilitated processing.

It appears unlikely that MAC-FAC can be achieved with inhalation agents alone. However, the addition of other agents, such as intrathecal opioid,<sup>14,15</sup> or perhaps other classes of analgesics may produce profound blockade of spinal facilitation when combined with inhalation anesthetics.

General anesthesia causes a loss of consciousness and abolishes reactions to painful stimuli. Although the exact mechanisms underlying anesthesia are not adequately understood, several recent studies suggest the spinal cord as an important site of anesthetic effect. Rampil<sup>12</sup> assessed the relative roles of the brainstem and spinal cord as sites of anesthetic action and found that the site of anesthetic inhibition of motor response to pain may be in the spinal cord. In another study, Rampil *et al.*<sup>10</sup> showed that the minimum alveolar concentration of isoflurane that inhibits nociceptive responses is not affected by precollicular decerebration, an indication that the mechanism by which isoflurane produces unresponsiveness is independent of forebrain structures. Antognini and Schwartz<sup>11</sup> demonstrated that subcortical structures are important in the behavioral response to painful stimuli by showing a 240% increase in the minimum alveolar concentration of isoflurane when the goat brain was selectively anesthetized.

Many studies suggest that inhalation anesthetic agents may specifically suppress transmission of nociception in the spinal cord. One way this may be achieved is by modifying the activity of NMDA receptors in dorsal horn neurons.<sup>6,7</sup> These receptors have been shown to mediate nociceptive sensitization of the spinal cord,<sup>1,3</sup> and inhalation anesthetic agents may inhibit the development of this facilitated state. Savola *et al.*<sup>20</sup> demonstrated a marked inhibitory effect by isoflurane on spinal neurotransmission, depressing the response to both substance P and NMDA. Namiki *et al.*<sup>8</sup> demonstrated a direct action by halothane on the response of wide dynamic range neurons (neurons of the dorsal horn of the spinal cord that influence transmission and integration of nociceptive information) to noxious stimuli.

Table 2. Effects of Intravenous Agents on Phase 1 and Phase 2 Response

Intravenous Group	N	Mean No. of Flinches		Phase 2 (% of control)
		Phase 1	Phase 2	
Controls	7	8 ± 2	114 ± 20	100
Thiopental	6	8 ± 5	105 ± 26	92
Propofol	10	2 ± 1	68 ± 11*	59

\* Significantly different from intravenous controls ( $P < 0.05$ ).



Although the mechanism by which inhibition of transmission of noxious stimulation by inhalational anesthetic agents is achieved is speculative, it has been well documented that inhalation anesthetics enhance GABA<sub>A</sub> mediated sensory inhibition,<sup>21-24</sup> and it has been suggested that inhalation anesthetics may produce suppression of spinal cord sensitization through GABA<sub>A</sub>-mediated agonism.<sup>25</sup>

The effects of nitrous oxide on spinal sensitization are difficult to explain. Analgesia produced by nitrous oxide has been shown to be mediated by endogenous opioids in several studies,<sup>26-29</sup> probably *via* the  $\kappa$  receptor.<sup>30</sup> Analgesic action by nitrous oxide also has been attributed to depressant action on dorsal horn neurons in the spinal cord,<sup>31-33</sup> perhaps *via* activation of a supraspinal descending inhibition system.<sup>34</sup> In this study, we found an apparent mutual antagonism of suppression of spinal cord sensitization by the combination of halothane and nitrous oxide. Lack of suppression by a combination of a volatile agent and nitrous oxide was first demonstrated by Abram and Yaksh,<sup>14</sup> using isoflurane and nitrous oxide. Goto *et al.*<sup>16</sup> also demonstrated a lack of effect of a volatile anesthetic agent plus nitrous oxide, although, in contrast to our findings, they found no evidence of inhibition of spinal sensitization by halothane alone.

Interestingly, nitrous oxide also has been shown to reverse the electroencephalographic burst suppression produced by isoflurane, suggesting that it may oppose the effect of isoflurane on the central nervous system. Yli-Hankala *et al.*<sup>35</sup> studied the effects of nitrous oxide on burst suppression patterns during stable isoflurane anesthesia in patients and found that there was a significant decrease in the proportion of electroencephalographic suppression time when air was replaced by nitrous oxide. They concluded that the electroencephalographic effects of nitrous oxide and isoflurane are not additive and that nitrous oxide opposes depression by isoflurane of the central nervous system. A similar antagonistic effect of nitrous oxide on the electroencephalogram has been reported for desflurane<sup>36</sup> and isoflurane.<sup>37</sup> Cole *et al.*<sup>38</sup> have demonstrated a less than additive effect of nitrous oxide on the minimum alveolar concentrations of halothane, enflurane, and isoflurane. Taken together, these studies indicate that nitrous oxide may interfere with the depressant effects of vol-

atile agents on the central nervous system and this may explain its apparent antagonism of the suppression of spinal sensitization by volatile agents in the rat formalin test.

Intravenous barbiturates historically have been characterized as nonanalgesic or even antanalgesic.<sup>39-41</sup> We found that thiopental did not affect phase 2, whereas propofol caused significant suppression. Several studies document differences between the effects of thiopental and propofol on the response to pain. Briggs *et al.*<sup>42</sup> found that subhypnotic doses of thiopental but not propofol increased sensitivity to tibial pressure pain. Anker-Moller *et al.*<sup>43</sup> found that subhypnotic doses of both thiopental and propofol decreased pain evoked by argon laser stimulation. The discrepancy of the thiopental results between these studies could be due to the different types of noxious stimulation produced. For instance, we found that the dose of thiopental administered suppressed the paw-pinch but failed to abolish flinching during phase 1, even though animals remained immobile. Similarly, the formalin test examines yet another aspect of pain perception, *i.e.*, sensitization to subsequent afferent stimuli, and we have demonstrated suppression of sensitization with propofol but not thiopental.

It is possible that the lack of phase 2 effect of thiopental may relate to persistent antanalgesia<sup>41</sup> as opposed to lack of suppression of spinal sensitization. Nevertheless, the clinical implications are the same. These data suggest that patients who receive thiopental as an induction agent may have more severe postoperative pain than those who receive propofol.

Several studies show a direct effect of intravenous anesthetic agents on transmission of nociceptive stimuli, perhaps through actions at the GABA<sub>A</sub> receptor/chloride channel complex. Results of a study by Jewett *et al.*<sup>13</sup> suggest that some of the analgesic actions of the barbiturates and propofol are mediated in the dorsal horn and that these actions could be accounted for, at least in part, by enhancement of transmission through channels linked to GABA<sub>A</sub> receptors. Several other studies also demonstrate that propofol<sup>44,†</sup> and barbiturates<sup>45-49</sup> act partially through GABA<sub>A</sub>-receptor agonist effects. The question may be asked why each of these drugs with such similar receptor agonism profiles should exhibit different results in the formalin test? GABA receptors are located in the spinal cord and at the supraspinal level. Collins *et al.*<sup>50</sup> showed that pentobarbital reduced tonic inhibition of some spinal dorsal horn neurons. It is conceivable, therefore, that GABA

† Yamamura T, Ohsuka H, Furmido H, Tsutahara S, Kemmotsu O: Does propofol enhance GABA-mediated synaptic transmission? (abstract). *ANESTHESIOLOGY* 75:A588, 1991.



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receptor stimulation in the brain may produce antanalgesic effects through interference with descending inhibitory pathways. Glutamate release in the nucleus raphe magnus and the nucleus reticularis gigantocellularis pars  $\alpha$  activates descending inhibitory pathways in rats.<sup>51</sup> GABA<sub>A</sub> agonists microinjected into these supraspinal loci have been shown to increase the response to thermal and mechanical nociception.<sup>52</sup> Taken together, these results imply that, when a GABA agonist is administered, it may have direct analgesic effects at the spinal level and cause disinhibition of pain transmission at the supraspinal level. The result could depend on the relative amounts of agonism by the drug at each of these sites.

In conclusion, we have demonstrated that the commonly used volatile anesthetic agents significantly attenuate spinal sensitization in the rat paw formalin test. We confirmed a lack of effect by the combination of halothane and nitrous oxide. We also demonstrated suppression of spinal sensitization by intravenous propofol and a lack of effect by intravenous thiopental. These results may have important implications regarding the development of postoperative pain. We postulate that GABA-agonist action may explain the inhibitory effects of inhalation and intravenous anesthetic agents on sensitization of the spinal cord.

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