Anesthesiology
78:713–721, 1993
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Morphine, But Not Inhalation Anesthesia, Blocks Post-injury Facilitation

The Role of Preemptive Suppression of Afferent Transmission

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Background: **The subcutaneous injection of formalin in the rat paw results in several minutes of flinching (phase 1) followed by cessation of activity then resumption of flinching (phase 2), which depends on facilitation of spinal transmission evoked by C-fiber activity generated immediately after the noxious stimulus. It was hypothesized that suppression of dorsal horn activity during and immediately after formalin injection by inhalation anesthetics or intrathecal opiates would block spinal facilitation and inhibit phase 2 flinching, even if the anesthetic or opiate were eliminated before phase 2.**

Methods: **Flinches/min were observed 1 and 5 min after formalin injection (phase 1) and at 5-min intervals thereafter for 60 min (phase 2) for five groups of rats: control (group 1); 1% isoflurane before and for 6 min after formalin (group 2); 2.5% isoflurane before and for 6 min after formalin (group 3); 1% isoflurane and 70% N_2O before and for 6 min after the formalin (group 4); and 30 μg intrathecal morphine given 20 min before formalin and 30 μg intrathecal naloxone given 6 min after formalin, combined with 1% isoflurane as in group 2 (group 5).**

Results: **All groups, except control, exhibited essentially complete suppression of phase 1 flinching. The changes in phase 2 flinching, expressed as a percent of total phase 2 flinches for the control animals, were: control (100%) = group 4 (109 ± 17%) > group 2 (66 ± 13%) = group 3 (66 ± 14%) > group 5 (19 ± 12%).**

Conclusions: **Isoflurane, even at high concentrations, administered during and shortly after a noxious stimulus produces only a modest reduction in facilitation of afferent processing. The addition of intrathecal morphine during the period of nociceptor activity results in marked attenuation of the facilitated state. (Key words: Analgesics, opioid: morphine. Anesthetics, gases: nitrous oxide. Anesthetics, volatile: isoflurane. Receptors: opiate. Spinal cord.)**

Received from the Departments of Anesthesiology, *Medical College of Wisconsin, Milwaukee, Wisconsin, and †University of California, San Diego, LaJolla, California. Accepted for publication December 7, 1992. Supported in part by DA02110 (TLY) and by support from the Department of Anesthesiology, Medical College of Wisconsin, and from Mr. and Mrs. Joseph Uihlein, Jr. (SEA).

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THERE is a growing body of literature indicating that local or regional anesthesia administered before the onset of surgery can significantly reduce the severity of postoperative pain and the postoperative opioid requirement.[1–4] There is somewhat less clinical evidence suggesting that systemic[1,5] or epidural[6] opioid administration before surgery results in reduced postoperative analgesic demand.

Intense nociceptor activation, leading to C-fiber evoked activity in the dorsal horn, results in the development of a facilitated state, such that spinal cord neurons display an exaggerated response to subsequent noxious stimuli.[7–9] This facilitation appears mediated in part by the local release of glutamate which, acting through an NMDA receptor, produces long-term changes in neuronal excitability. The behavioral correlates of the facilitated processing induced by a protracted C-fiber afferent input have been studied by the use of models in which a chemical irritant, such as formalin, is injected subcutaneously into a single paw. This treatment results in an acute barrage of C-fiber activity, followed by a slow ongoing presence of afferent spikes. In the rat, such injections result in an acute first phase, followed by a delayed, long-lasting second phase of flinching and licking behavior. Using this model, it has been shown that the intrathecal administration of NMDA antagonists before but not after formalin can significantly diminish the phase 2 response.[10,11] These data suggest that the facilitated state requires the presence of activity at the NMDA receptor to *initiate* the facilitated state but not sustain it. Opioids administered either systemically or spinally before a noxious stimulus have been shown to block C-fiber evoked sensitization.[12,13] This is not surprising in light of the ability of opioids to act presynaptically to block transmitter release from C-fibers.[14,15] However, it is not clear what effects volatile anesthetics exert on such facilitatory processes. While not systematically studied,

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it is important to note that some of the electrophysiologic studies of C-fiber-induced spinal sensitization have been carried out in animals under a surgical plane of anesthesia, using volatile agents such as halothane.[8,13] These observations suggest that, in contrast to opioids or NMDA antagonists, spinal facilitation may not be diminished by many inhalation anesthetics.

To determine the effect of inhalation anesthesia and opioids given before stimulation on post-injury facilitation, we prepared a model using the rat formalin test that approximated the clinical situation as closely as possible, *i.e.,* volatile anesthetic alone (isoflurane or isoflurane and nitrous oxide) or isoflurane in combination with morphine pretreatment during and immediately after formalin injection (phase 1).

Methods

The following studies were carried out under a protocol approved by the Institutional Animal Care Committee of the University of California, San Diego. Male Sprague-Dawley rats weighing 250–350 g were utilized for these studies.

Animal Preparation

Animals that received intrathecal saline or morphine and naloxone were implanted with chronic lumbar intrathecal catheters introduced *via* an incision in the atlanto-occipital membrane under halothane anesthesia as previously described by Yaksh and Rudy.[16] Animals showing neurologic deficits after implantation were excluded. All testing was carried out 4–9 days after intrathecal implantation.

Formalin Test

The formalin test was carried out as previously described.[11] In brief, the animals were individually allowed to breathe 3% isoflurane until immobile. Control animals without intrathecal catheters (group 1a) were removed quickly from the anesthesia and given a subcutaneous injection of 50 μl 5% formalin into the dorsum of the right hind paw using a 30-G needle. They then were placed in a clear plexiglass chamber for observation. Coordinated spontaneous movement was typically noted <30 s after injection. Animals routinely displayed a flinching, withdrawal movement of the injected hind paw. Flinches/min were then recorded at 1 and 5 min after injection and at 5-min intervals thereafter for 60 min. The animals were then killed with an overdose of barbiturate.

Experimental Paradigms

A series of discrete studies were carried out to assess the effects of anesthetics on second phase formalin test behavior. A summary of the treatments groups is presented in table 1.

Isoflurane alone: Animals were anesthetized with isoflurane. They were individually placed in a clear plexiglass anesthetic box and anesthetized with 3% isoflurane in 30% oxygen. As soon as they were immobile, the isoflurane concentration was reduced to 1% (group 2a) or 2.5% isoflurane (group 3) or changed to 1% isoflurane and 70% N_2O (group 4). After 5 min of exposure to the adjusted anesthetic concentrations, the animal received the subcutaneous injection of 50 μl 5% formalin. Six minutes after the formalin injection, the rat was removed from the anesthetic and placed in a plexiglass chamber for observation. Flinches/min

Table 1. Summary Table of Experimental Manipulations in Formalin Test

Group	N	Preinduction Treatment	Induction Anesthesia (T = −10)	Anesthesia at Form Inject (T −10 to 6 min)	Phase 2 Treatment
1a	5	None	Iso (3%)*	None	None
1b	7	IT Sal	Iso (3%)*	None	IT Sal
2a	4	IT Sal	Iso (3%)	Iso (1%)	IT Sal (T = 6 min)
2b	4	IT Sal	Iso (3%)	Iso (1%)	IT Nal (T = 6 min)
3	6	None	Iso (3%)	Iso 2.5%	IT Sal (T = 6 min)
4	6	None	Iso (3%)	Iso (1%) + 70% N_2O	IT Sal (T = 6 min)
5	6	IT Mor (30 μg)	Iso (3%)	Iso (1%)	IT Nal (T = 6 min)
6	5	IT Mor (30 μg)	Iso (3%)	Iso (1%)	IT Nal (T = 6 min)
7	4	None	Iso (3%)	None	IT Nal (T = 36 min) Iso (0.25%)

* Isoflurane (3%) administered only 2 min before formalin.

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were recorded at 5-min intervals until 60 min after formalin injection.

To determine the effects of subanesthetic concentrations of isoflurane on the phase 2 formalin response, four rats were injected with formalin as described for control animals (group 1a). However, beginning 6 min later, they were exposed to 0.25% isoflurane, which was continued through 60 min (group 7). Two animals underwent the same paradigm but with 0.5% isoflurane administered during phase 2.

Isoflurane and morphine: Animals were anesthetized with 1% isoflurane as described above for group 2. In addition, the rats were given 30 μg intrathecal morphine 20 min before injection of formalin. To permit assessment of the phase 2 formalin response in the absence of an ongoing opioid effect, 30 μg intrathecal naloxone was administered 6 min after formalin injection (group 5) or 6 and 30 min after formalin injection (group 6), just before discontinuing isoflurane.

For control purposes, two additional groups were examined. To determine the effects of multiple intrathecal injections on the isoflurane/formalin response, 20 μl intrathecal saline was given 20 min before formalin testing plus 6 min after formalin injection (group 1b, given intrathecal saline). To determine the effects of naloxone alone on the isoflurane/formalin response, 20 μl intrathecal saline was given 20 min before formalin testing and 30 μg intrathecal naloxone was given 6 min after formalin injection (group 2b). These animals received 1% isoflurane during phase 1 as described for group 2a. The treatment protocols and numbers of animals in each group are summarized in table 1.

To determine the efficacy and duration of the naloxone reversal of intrathecal morphine, antinociception was tested using the 52° C hot plate test in a separate group of animals. After two baseline measurements, animals were injected intrathecally with 30 μg morphine, and a repeat measurement was recorded 20 min later. Immediately after that measurement, animals were injected with 30 μg intrathecal naloxone, and repeat measurements were taken after 5, 15, 30, 45, and 60 min.

Drugs

Inspired isoflurane (Anaquest) concentration was monitored with a Puritan Bennett (Westmont, IL) anesthetic agent monitor (model 222). Intrathecal morphine sulfate (Merck, West Point, PA) or naloxone HCl (Dupont, Manati, PR) were given using a micrometer-driven microinjector system. All drugs were delivered in 10 μl preservative-free normal saline followed by an equal volume of saline to clear the catheter dead space. Intrathecal saline injections were all 20 μl in volume.

General Behavior

In all animals that underwent general anesthesia during phase 1, a brief evaluation of neurologic function and response to noxious stimulation was performed after induction, just before formalin injection. This testing consisted of assessing the corneal reflex (blink induced by light touch applied to cornea), pinna reflex (twitch of the ear in response to tactile stimulation of the auditory canal), response to paw pinch, and the thermal tail dip. In the tail dip, the tail was rapidly immersed in 4 cm of water maintained at 52° C. Latency to a tail movement was recorded. In the absence of a response, the tail was removed at 6 s and that latency recorded.

Data Analysis

The total number of flinching behaviors was determined for all of the phase 2 (10–60 min) observations for each animal, and these data were compared by one-way analysis of variance (StatView II). *Post hoc* comparisons were done using Sheffe's test. The hot plate data were normalized to percent of maximal response, and the means for each of the post-naloxone measurements were compared to the mean baseline (pre-morphine) measurements by paired Student's *t* test. For display purposes, means of phase 2 data from the formalin test were expressed as percentages of control. When this was done, the ratios of the means and standard errors were calculated according to the method described by Tallarida and Murray.[17]

Results

The subcutaneous injection of formalin after the brief administration of isoflurane resulted in a reliable biphasic incidence of flinching of the injected hind paw.

Control Studies

The data from group 1a and group 1b were found to be essentially identical, and data from these groups were pooled and used as the baseline control (group 1; fig. 1). Likewise, there was no difference between the group anesthetized with 1% isoflurane that received saline after phase 1 (group 2a) and the group anesthetized with 1% isoflurane that received 30 μg naloxone at the end of phase 1 (group 2b), and these groups

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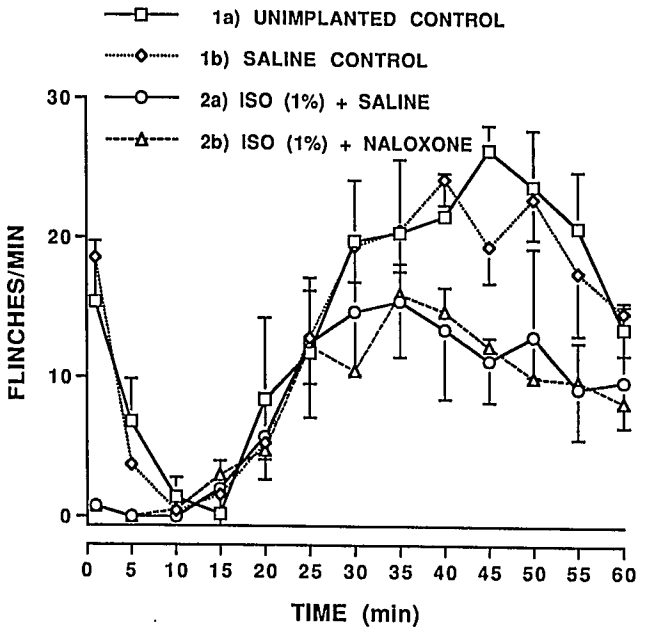

Fig. 1. Mean number of flinches/min plotted as a function of time after injection of formalin. Treatment groups consist of 1a, unimplanted control animals; 1b, animals with implanted intrathecal catheters injected with saline 20 min before and 6 min after formalin; 2a, 1% isoflurane-anesthetized animals treated with saline 20 min before and 6 min after formalin; and 2b, 1% isoflurane-anesthetized animals treated with saline 20 min before and naloxone 6 min after formalin.

also were pooled for comparison with the other treatment groups (group 2; fig. 1).

The animals exposed to 0.25% isoflurane (group 7) beginning 6 min after formalin and continued throughout phase 2 did not have a significantly different mean phase 2 response from baseline control (table 2). Animals exposed to 0.5% isoflurane during phase 2 showed considerable somnolence and failed to exhibit any flinching behavior when sleeping. When aroused, they exhibited an obviously reduced response rate (data not shown).

The intrathecal injection of 30 μg naloxone completely reversed the analgesic effect of 30 μg intrathecal morphine on the hot plate test (table 3). At 30 min after naloxone response, latencies remained essentially the same as pre-morphine baseline. By 45 min, however, there was a noticeable though not statistically significant return of analgesia, suggesting some diminution of the naloxone effect. Because of these findings, in animals given morphine pretreatment plus one dose of naloxone, only the first 30 min of phase 2 data were used when comparing responses to those of control animals.

Table 2. Phase 2 Activity, Controls

Group	N	No. of Flinches, Phase 2
1a, Unimplanted control	5	178.0 ± 17.0
1b, Saline control	7	159.0 ± 17.2
2a, 1% Isoflurane, saline	4	107.3 ± 26.3
2b, 1% Isoflurane, naloxone	4	90.25 ± 36.9
7, 0.25% Isoflurane, postformalin	4	184.5 ± 27.5

Values are mean ± SD.

Sensory Testing

Sensory testing of anesthetized animals just before formalin injection produced the following results: Corneal reflexes were well preserved in animals treated with 1% isoflurane alone or morphine plus 1% isoflurane, but were uniformly abolished in animals anesthetized with 2.5% isoflurane or 1% isoflurane plus nitrous oxide. Pinna reflexes were variably suppressed in the 1% isoflurane and 1% isoflurane plus morphine groups but were abolished in all of the 2.5% isoflurane- and 1% isoflurane plus nitrous oxide-anesthetized animals. Withdrawal to paw pinch was uniformly present in the 1% isoflurane group, but was completely abolished in all other inhalation anesthesia groups and in all animals pretreated with morphine. Tail dip withdrawal latencies were 3 s or less in all animals in the 1% isoflurane group and >6 s in all animals in the other anesthetized groups and in all animals receiving morphine plus 1% isoflurane.

Phase 1 effect: The administration of isoflurane at 1% or 2.5%, 1% isoflurane and nitrous oxide, or 1% isoflurane and morphine resulted in essentially complete suppression of the phase 1 response (figs. 1, 2, and 3).

Phase 2 effects: Maintenance of low concentration (1%) isoflurane during and for 6 min after formalin injection (group 2) produced a modest but significant

Table 3. Time Course of Intrathecal Naloxone (30 μg) Reversal of Intrathecal Morphine (30 μg)

Time (min)	Mean Latency	% Max Effect
Baseline	12.0 ± 1.3	
20 min post MS	60.0 ± 0.0	100
5 min post naloxone	12.2 ± 1.7	0
15 min post naloxone	8.4 ± 0.6	0
30 min post naloxone	13.4 ± 1.9	3
45 min post naloxone	29.0 ± 7.1	35
60 min post naloxone	40.4 ± 8.1	59*

* Different from baseline ($P < 0.05$).

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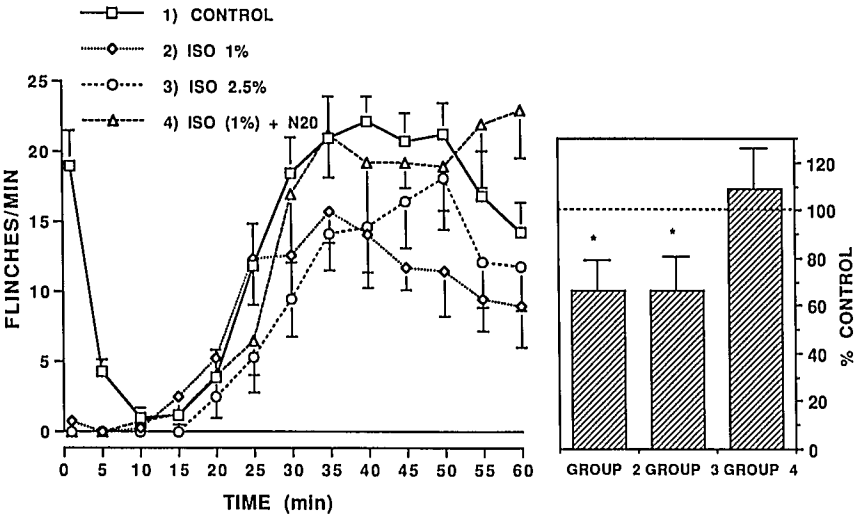

Fig. 2. *(Left)* **Mean number of flinches/min plotted as a function of time after injection of formalin. Treatment groups consist of 1, control animals (groups 1a and 1b combined; see figure 1); 2, animals treated with 1% isoflurane; 3, animals treated with 2.5% isoflurane; and 4, animals treated with 1% isoflurane plus 70% N_2O. All inhalation anesthetics were administered during the period from 10 min before to 6 min after formalin.** *(Right)* **Bar graphs show the means of the total number of flinches recorded during phase 2 for the treatment groups expressed as percent of control. Error bars denote SEM.[17] *Significantly different from control (one-way analysis of variance; $P < 0.05$).**

reduction in the mean number of phase 2 flinches as compared to the unanesthetized control animals. Maintenance of a high concentration (2.5%) during phase 1 (group 3) produced essentially the same degree of response suppression. Responses in animals receiving 1% isoflurane plus nitrous oxide (group 4) unexpectedly showed no suppression and were similar to control animals. While the number of flinches/min peaked at around 40 min for all other groups, the activity of the animals receiving nitrous oxide continued to rise throughout phase 2. Rats receiving the lowest isoflurane dose plus intrathecal morphine and naloxone showed a near maximal reduction in flinching behavior (fig. 3). Thus, expressed as a percent of the control, results were: control (100%) = group 4 (109 ± 17%) > group 2 (66 ± 13%) = group 3 (66 ± 14%) > group 5 (19 ± 12%) = group 6 (19 ± 7%; table 4).

Since the animals that received morphine plus one dose of naloxone may have had return of pharmacologic effects of morphine before the end of phase 2, they were analyzed separately, using only the first 30 min of phase 2 data. As can be seen from figure 3, there was substantial suppression of flinching behavior during the first 30 min after naloxone administration (mean 11.2 ± 6.6 *vs.* control 57.5 ± 8.0; $P < 0.001$) before the naloxone effect had subsided. The reduction in re-

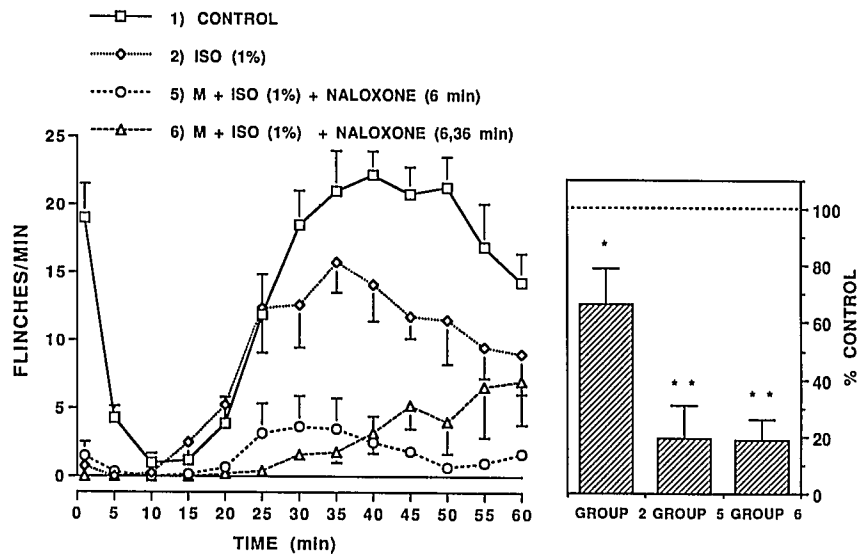

Fig. 3. *(Left)* **Mean number of flinches/min plotted as a function of time after injection of formalin. Treatment groups consist of 1, control animals (groups 1a and 1b combined; see fig. 1); 2, animals treated with 1% isoflurane; 5, animals treated with 30 μg intrathecal morphine pretreatment plus 1% isoflurane plus 30 μg intrathecal naloxone 6 min after formalin; and 6, animals treated with 30 μg intrathecal morphine pretreatment plus 1% isoflurane plus 30 μg intrathecal naloxone 6 and 36 min after formalin.** *(Right)* **Bar graphs show the means of the total flinches recorded during phase 2 for the treatment groups expressed as percent of control (for group 5, only the 10–35-min data were used to compare response to groups 1 and 2). Error bars denote SEM.[17] *Significantly different from control (one-way analysis of variance; $P < 0.05$). **Significantly different from control ($P < 0.001$); significantly different from group 2 ($P < 0.05$).**

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Table 4. Effects of Inhalation Anesthetics and Morphine Pretreatment/Isoflurane/Naloxone (at 6 and 36 min) on Phase 2 Response

Group	N	Mean Phase 2
1, Control (1a + 1b)	12	162.8 ± 14.5
2, 1% Isoflurane (2a + 2b)	8	104.6 ± 18.6*
3, 2.5% Isoflurane	6	104.8 ± 20.7*
4, 1% Isoflurane, 70% N_2O	6	171.7 ± 22.9
5, Morphine, 1% Isoflurane, naloxone × 2	5	30.0 ± 11.2†

* Different from group 1 ($P < 0.05$).

† Different from group 1 ($P < 0.001$), groups 2 and 3 ($P < 0.05$), and group 4 ($P < 0.001$).

sponse to near zero after 40 min may relate in part to a late loss of naloxone antagonism.

Discussion

Formalin Test and Anesthetics

The results of this study with the formalin test, a model of focal acute injury, indicate that 1% isoflurane (0.7 MAC for rats)[18] administered during the period of phase 1 produced only a modest attenuation of the subsequent phase 2 sensitization. This sensitization was not reduced further by raising the isoflurane concentration to levels as high as 2.5% (the anesthetic level at which autonomic responses are suppressed [MACBAR] for rats).[19,20] Surprisingly, the suppressive effect of isoflurane was blocked by the addition of nitrous oxide, an anesthetic agent with accepted analgesic properties.[20–22] There was, however, dramatic and significant reduction of phase 2 sensitization by the addition of morphine to the isoflurane, even though the pharmacologic effects of the opiate were present only during phase 1 of the formalin test.

We recognize and exclude several potential sources of error that might influence the results of this study.

1. The possible effect of the intrathecal catheter was excluded by the observation that phase 1 and phase 2 responses were identical when implanted control animals were compared to unimplanted control animals.
2. The continued depression of the phase 2 behavior after naloxone in animals treated with morphine during phase 1 could have been due to either naloxone exerting an effect by itself or to an inadequate reversal of morphine. We showed, however, that intrathecal naloxone had no effect alone. Moreover, the time course of the antagonism of morphine by naloxone on the hot plate clearly indicated that the naloxone doses were adequate and the timing was appropriate.
3. Residual effects of anesthetic still present during phase 2 might have been responsible for isoflurane's modest suppressive effect. The lack of effect of 0.25% isoflurane administered during phase 2 makes such an error unlikely. It was only at a concentration (0.5%) that produced obvious behavioral depression that reduction in phase 2 activity was seen. Failure of 0.25% isoflurane to alter the phase 2 behavior also emphasizes that low concentrations of isoflurane do not paradoxically augment the phase 2 behavior.
4. Since only inspired gas concentrations were measured, it is possible that the animals were in fact not at a surgical plane of anesthesia. We exclude this for several reasons. Animals under 2.5% isoflurane or 1% isoflurane plus nitrous oxide showed *no* corneal, pinnae, or withdrawal responses at the time the formalin was injected. This emphasizes, by stringent behavioral criteria, that the animals were anesthetized at the time of the stimulus.

Opiates and Spinal Facilitation

In the formalin model, single unit recording from peripheral axons has emphasized that there is an initial activation by the injection of irritant, followed by a prolonged ongoing elevation in spontaneous afferent activity.[23] Such a repetitive afferent barrage has been shown to evoke an augmented response pattern in dorsal horn wide dynamic range neurons, first referred to by Mendell[24] as "windup." Subsequent studies have indicated that this augmented activity has a unique pharmacology.

NMDA antagonists administered before the stimulus will not block the initial activation of the cell but will prevent the development of the augmented discharge pattern.[9] Such observations, in concert with the observation that formalin injection evokes significant increases in glutamate release from spinal cord,[25] suggest that such C-fiber stimulation can initiate processes leading to a significant augmentation in dorsal horn reactivity, in part through the activation of an NMDA site. Previous work in the formalin behavioral model has confirmed the role of the NMDA receptor in this process.[10] The intrathecal delivery of NMDA receptor channel blockers, such as MK801 and ketamine, before

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but not after formalin significantly reduces the phase 2 behavior in the unanesthetized animal.[11] The inability of NMDA antagonists given between phase 1 and phase 2 to block the phase 2 response emphasizes the fact that, while windup requires the NMDA site for its initiation, the NMDA receptor is not required for its sustenance.

Other studies have demonstrated the ability of opioids to block nociceptor-induced spinal sensitization. Dickenson and Sullivan[13] observed that the injection of formalin resulted in a profound augmentation in the discharge of dorsal horn wide dynamic range neurons in rats. The spinal administration of mu opioid agonist DAGO (Tyr-D-AlaGlyMePheGly-ol) *before* the formalin injection blocked the augmentation, reducing neuronal activity as compared to control. This block persisted in the face of the administration of naloxone 2 min *after* the formalin. Woolf and Wall[12] evaluated the effect of systemic morphine pretreatment on C-fiber-induced facilitation of the flexor reflex in decerebrate-spinal rats. They showed that 0.5 mg/kg prevented the prolonged facilitation but that 10 times that dose was required to suppress the facilitated activity once it was established. Significant data indicate that opiates at the spinal level may act presynaptically to diminish the release of transmitters from C-fiber afferents.[14] Thus, as with pretreatment with local anesthetics, the selective effects of opiates on the release of transmitters from certain C-fiber populations appears to remove the component of the afferent input that serves as the initiating stimulus.

Inhalation Anesthetics and Spinal Sensitization

The weak effect of isoflurane, even at MAC-BAR concentrations, in blocking initiation of the second phase facilitation is consistent with electrophysiologic studies showing that spinal windup and facilitation also will occur in the rat anesthetized with volatile anesthetics.[13] There is considerable evidence to show that the processes leading to spinal facilitation are not substantially obtunded by volatile anesthetics:

1. It is well known that the behavioral and electrophysiologic indices of spinal facilitation depend upon the release of glutamate and the activation of spinal NMDA receptors.[9,10] It also has been shown that release of peptide and amino acid neurotransmitters from primary afferents is not abolished by inhalation anesthetics.[26,‡] Even though excitation of dorsal horn wide dynamic range neurons is powerfully suppressed by high anesthetic concentrations,[27] it would appear, based on the above release data, that the depressed excitatory response of the cell is due to a direct suppression of cellular excitability and not to a reduced release of afferent neurotransmitter.
2. NMDA receptor-mediated facilitation in other areas of the nervous system is not blocked by inhalation anesthetics. In the hippocampus, an example of activity-dependent facilitation, called long-term potentiation, also can be prevented by treatment before but not after the stimulus with NMDA antagonists.[28] MacIver *et al.*[29] showed that methoxyflurane failed to block long-term potentiation, while Pearce *et al.*[30] showed that halothane, isoflurane, and enflurane all failed to suppress long-term potentiation, even at concentrations as high as 2.1 MAC, implying that processes responsible for the initiation of the facilitatory mechanisms remain unblocked.

Based on the present experiments and the above comments, we conclude that spinal facilitatory processes mediated by the occupancy of spinal receptors, including those for glutamate, set in play intracellular processes that are not obtunded by concentrations of volatile anesthetics that block the activity of spinal neuronal nets. Upon removal of the depressant actions of the anesthetic, the augmented cellular activity consistent with these biochemical changes is manifested in the form of an exaggerated neuronal response and behavioral hyperalgesia.

The suppressive effect of nitrous oxide plus 1% isoflurane on the phase 2 response was significantly less than that of 1% isoflurane alone. The lack of effect of the combined anesthetics was unexpected, particularly in light of evidence that nitrous oxide purportedly exerts a portion of its analgesic effect though an opiate mechanism.[20–22] The findings of this study would suggest that nitrous oxide does not exert an appreciable analgesic effect through a spinal μ-opiate mechanism. It should be noted that these data do not controvert the idea that nitrous oxide has a "MAC-sparing" action; but it further supports the concept that inhalation anesthesia does not prevent the processes leading to spinal facilitation. Further study is needed to deter-

‡ Sorkin LS: Unpublished data. 1992.

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mine whether nitrous oxide facilitates nociceptive processing.

Clinical Significance

These studies raise several issues that we believe are relevant to clinical anesthesia practice. The present studies emphasize that the anesthetized state, as defined by loss of consciousness or lack of motor response (inherent in the concept of MAC), may be dissociated from processes leading to post-injury facilitation. The level of anesthesia required to prevent the post-injury state of facilitated processing, which we have named MAC-FAC, remains to be defined, clearly exceeds MAC-BAR and may not be achievable with inhalation agents alone. In contrast to the inhalation anesthetics, spinal opiates appear to prevent excitation of dorsal horn cells by C-fiber activation. Their preoperative administration may prove to be a reasonable method of achieving MAC-FAC with relatively little physiologic compromise.

We appreciate that the conditions of surgery and the formalin test are not strictly analogous. In the formalin test, the sensitizing nociceptive barrage is confined to a brief period during and immediately after formalin injection. During surgery, the afferent barrage is present during incision and may continue throughout the surgical procedure. Therefore, a brief period of profound analgesia at the beginning of surgical stimulation may not be adequate, and the pharmacologic effect of the opioid throughout the intraoperative and early postoperative period is probably necessary if dorsal horn sensitization is to be minimized.

This study also may have implications with respect to the development of chronic hyperalgesia and allodynia. Prolonged dorsal horn sensitization may lead to chronic or perhaps even irreversible chronic pain states, possibly through damage to inhibitory interneurons traumatized by excessive NMDA receptor excitation resulting from afferent activity.[31] It is now speculated that nitric oxide generated by NMDA receptor activation may be a mediator of such cellular toxicity.[32] While the clinical evidence that preemptive analgesia can prevent the development of chronic pain is limited,[2] this notion deserves further study.

We would like to thank Annika Malmberg and Dr. Linda Sorkin for their comments and assistance.

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