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Nitrous Oxide Induces Preemptive Analgesia in the Rat That Is Antagonized by Halothane

Takahisa Goto, M.D., John J. A. Marota, Ph.D, M.D.,† Gregory Crosby, M.D.‡*

Background: **Noxious stimulation-induced sensitization of the central nervous system has been proposed as a key element in the development of subsequent protracted pain. Accordingly, the authors used the formalin model of pain to test the hypothesis that general anesthesia can produce preemptive analgesia and thereby interfere with noxious stimulation-induced central sensitization.**

Methods: **Rats received 0.9% or 1.8% halothane, 30% or 75% nitrous oxide (N_2O), or 75% N_2O plus 0.9% halothane (n = 4 or 5 per group). Control rats (n = 5) received only 100% oxygen. Fifteen minutes after the induction of anesthesia, formalin was injected subcutaneously into a hind paw of each rat, and anesthesia was maintained for 5 more min. Because the behavioral pain response to formalin (*i.e.*, flinching of the injected paw) is biphasic, these treatment groups were anesthetized only during phase 1 (acute phase). Another group (n = 5) received 75% N_2O only during phase 2 (delayed phase). Reversibility of the N_2O effect was tested by the administration of naloxone before phase 1 or naltrexone during phase 2 (n = 5 per group). Finally, additional rats anesthetized as described above (n = 4 or 5 per group) underwent tail-flick testing during anesthesia.**

Results: **All anesthetics reduced phase 1 pain behavior, but only N_2O produced antinociception on tail-flick testing. Thirty percent and 75% N_2O, administered during phase 1, suppressed phase 2 flinching 29% and 49%, respectively, whereas nitrous oxide administered after phase 1 did not suppress phase 2 pain behavior. This effect of nitrous oxide was reversed by an opioid antagonist given during phase 1 but not phase 2. Halothane administered during phase 1 had no effect on phase 2 flinching, and it antagonized the effect of 75% N_2O.**

Conclusions: **Nitrous oxide induces dose-dependent preemptive analgesia in this model that is reversed partially by naloxone, thus suggesting the involvement of endogenous opioids in this action. In contrast, halothane has no preemptive analgesic properties and even antagonizes the analgesic effect of nitrous oxide. Hence, the hypnotic potency of an anesthetic is a poor indication of its preemptive analgesic potential. (Key words: Analgesia, preemptive. Anesthesia: general. Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane. Pain: neuroplasticity. Sensitization.)**

HUMAN[1,2] and animal[3,4] studies have demonstrated that noxious stimulation produces long-lasting changes in the central nervous system (CNS) that result in a hyperexcitable state. This noxious stimulation-induced central sensitization has been proposed as a key factor in the development of protracted pain that persists after the initial stimulus has abated.[5] In animals models[4] and some clinical studies,[6] analgesia given before the onset of a painful stimulus (*i.e.*, preemptive analgesia) has been shown to reduce or even prevent subsequent pain by preventing this pain-induced "neuroplasticity". In contrast, the same analgesic treatment administered even a few minutes after the initial introduction of a painful stimulus either cannot prevent the development of central excitability and pain behavior or does so with greatly reduced efficacy.[4,6]

The rat formalin test has been used extensively to study the mechanisms underlying preemptive analgesia.[7–12] This well characterized model involves prolonged, tonic pain generated by tissue injury from injection of formalin. Because tonic pain appears to be modulated differently in the CNS than phasic pain (*e.g.*, produced by thermal stimuli used in the tail-flick and hot-plate tests), the formalin model is thought to approximate clinical pain better than tests that use phasic stimuli.[7] In this model, a small amount of diluted formalin is injected subcutaneously into the hind paw of an awake rat. This stimulus evokes a progressive, *biphasic* pain-related behavioral response that includes flinching and licking of the injected paw.[7,8] The early phase behaviors (phase 1) begin immediately after injection and last only about 5 min; the more prolonged late-phase responses (phase 2) begin about 15 min after injection and last 60–90 min. Recent studies suggest that phase 1 is caused predominantly by activation of C-fiber afferents by the peripheral stimulus.[9] Phase 2,

* Fellow in Anaesthesia and Critical Care.

† Instructor in Anaesthesia.

‡ Associate Professor of Anaesthesia.

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Address reprint requests to Dr. Crosby: Department of Anesthesia, Massachusetts General Hospital, Boston, Massachusetts 02114.

however, is the result of central sensitization of nociceptive neurons induced by phase 1 activity[9] and is thought to be mediated in part by excitatory amino acids such as glutamate.[10,11] Therefore, blockade of phase 1 stimulation and/or disruption of central neurochemical processes responsible for sensitization attenuate the phase 2 hyperalgesic response.

Opioid analgesics[9,10] and local anesthetics[12] have been shown to prevent central sensitization in this model. The ability of general anesthetics to influence such processes has not been investigated thoroughly, however. Inasmuch as a principal function of general anesthetics is to disrupt the normal process by which peripheral stimuli are perceived by and registered on the CNS, one would predict that these agents influence nociceptive processes. Indeed, the fact that nitrous oxide (N_2O)[13] and halothane[14] have electrophysiologic effects on spinal nociceptive neurons that are similar to those of morphine provides evidence that these anesthetics affect central transmission of noxious stimuli. Nitrous oxide, in particular, has accepted analgesic properties that may be mediated by endogenous opioid peptides.[15,16] Anesthetics also alter the responsiveness of neurons to excitatory amino acid neurotransmitters[17,18] and, consequently, may perturb the central sensitization process. Based on such considerations, we predicted that general anesthetics would prevent noxious stimulation-induced central facilitation. Accordingly, we examined the hypothesis that nitrous oxide or halothane administered *only* during the brief acute phase of noxious stimulation would alter pain behavior in the postanesthetic period.

Materials and Methods

Studies were performed on 74 male Sprague-Dawley rats weighing between 300–325 g with the approval of the Subcommittee on Research Animal Care. Rats were maintained in a 12-h light–dark cycle (lights on at 07:00 h) and allowed free access to food and water. To control for known diurnal fluctuations in responsiveness to nociceptive stimuli,[19] experiments were performed between 10:00 and 22:00 h in randomized order.

Experimental Paradigm

Rats were divided into five treatment groups as follows: (1) 30% N_2O, (2) 75% N_2O, (3) 0.9% halothane, (4) 1.8% halothane, or (5) 75% N_2O plus 0.9% halothane. A control group received only 100% oxygen but otherwise was handled in an identical fashion. Each group consisted of five animals except for the 0.9% and 1.8% halothane groups, which contained only four animals each.

In all cases, the total duration of anesthesia was 20 min (fig. 1). Anesthesia was induced by placing the animals in a plexiglass box prefilled and flushed continuously at 3 l/min with one of the anesthetics in a balance of oxygen. Animals were left undisturbed for 15 min so that they would reach a steady state of anesthesia. Rats then were removed briefly from the box (< 15 s) so formalin could be injected into the left hind paw. Five percent formalin was prepared from a 37% formaldehyde solution by 1:19 dilution with 0.9% normal saline and administered subcutaneously in a volume of 50 μl into the plantar surface of the left hind paw with a 27-G needle. Animals were returned immediately to the box and maintained under anesthesia for 5 more min, *i.e.,* to provide anesthesia *only* during phase 1 (fig. 1). Rats then were removed from the anesthesia chamber, transferred to an clear cage bedded thinly with wood chips, and allowed to awaken. Thus, animals were awake and conscious when phase 2 pain-related behavior was assessed.

The concentrations of nitrous oxide (Ohmeda 5200 CO_2 analyzer, Madison, WI), halothane (Datex 222 an-

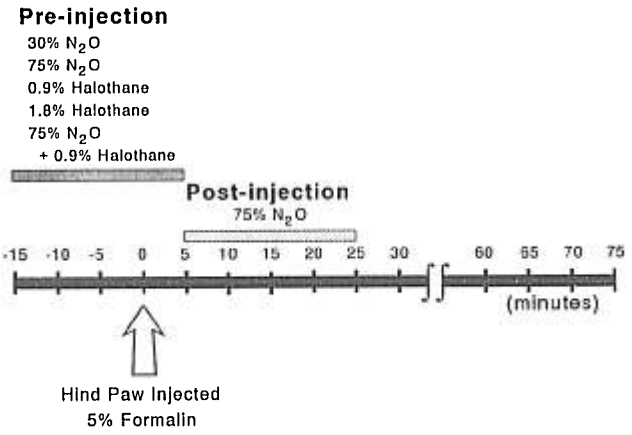

Fig. 1. The time schedule of anesthetic administration for formalin test animals. Except for one group of animals (postinjection 75% N_2O group), the anesthetic was administered for 15 min before and 5 min after formalin injection to provide anesthesia during *only* the phase 1 portion of the formalin pain response. The 75% N_2O postinjection group received anesthesia 5–25 min after formalin injection. Thus, in all cases the phase 2 portion of the formalin pain response (30–75 min after formalin injection) was observed after the animals had recovered from anesthesia.

esthetic agent analyzer, Puritan Bennett, Tewksbury, MA), and oxygen (Ohmeda 5100 oxygen analyzer) inside the box were measured continuously. The inspired concentrations of nitrous oxide and halothane (75% and 0.9%, respectively) were chosen to provide approximately 0.5 minimum alveolar concentration (MAC) anesthesia. These doses were calculated on the basis of reported MACs in the rat of 148–155% for nitrous oxide[20,21] and 0.95–1.11% for halothane,[22,23] and an estimated ratio of end-tidal–to–inspired concentration of halothane of 0.5–0.6 in spontaneously breathing rats after 20 min.[23]

Based on the results of these initial studies, three additional experiments were conducted. To assess the possibility that the effects of nitrous oxide in this model were opioid-mediated, a seventh group of animals (n = 5) received naloxone (20 mg/kg; dissolved in 0.9% normal saline to a final concentration of 10 mg/ml) intraperitoneally 15 min before the foot injection and coincident with the start of 75% N_2O. Similarly, to determine if nitrous oxide's effect on phase 2 behavior could be related to ongoing actions of endogenous opioids even after nitrous oxide was discontinued, an eighth group of animals (n = 5) received naltrexone (20 mg/kg) intraperitoneally (concentration, 10 mg/ml in normal saline) 5 min after the foot injection, when 75% N_2O was discontinued (N_2O → naltrexone group). In separate preliminary experiments, these doses of naloxone and naltrexone completely reversed the antinociceptive effect of intravenous morphine (10 mg/kg) on the tail-flick test for 30 min and > 2 h, respectively. Finally, to test the hypothesis that blockade of phase 1 is critical for prevention of phase 2 pain behavior, a ninth group of rats (nitrous oxide postinjection group) received 75% N_2O for 20 min beginning 5 min *after* the foot injection (fig. 1). Hence, these animals experienced phase 1 response without anesthesia or analgesia.

Formalin-induced pain responses are primarily supraspinally mediated behaviors.[8] Therefore, to examine the antinociceptive effects of these anesthetics at the spinal level, we also used a behavior that is known to be a spinal reflex response, namely, the tail-flick test.[24] For this portion of the study, 31 additional rats were divided into seven groups (n = 4 or 5 per group) and anesthetized exactly as described above, except that these animals were not injected with formalin, and analgesia was evaluated only during anesthesia (therefore it was not necessary to include the postinjection nitrous oxide and nitrous oxide → naltrexone groups). The test was performed in the preanesthetic, awake state to obtain a baseline and then was repeated 15 and 20 min after the rat was placed in the anesthesia box. Each animal was removed from the box long enough for one measurement to be performed at each time point.

Behavioral Observations

In our analysis, flinching was used as a measure of formalin-induced pain. Flinching is one of the pain-related behaviors of the formalin model and is characterized by a spontaneous, rapid, brief shaking or lifting of the paw. Accordingly, each episode of shaking, vibrating, or lifting of the paw was counted as one flinch; the total number of flinches of the injected hind paw were counted and recorded every 5 min for 75 min after the foot injection. Flinching was chosen as a measure of pain because it is more robust and spontaneous than other formalin pain-related behaviors (*e.g.*, licking) and, consequently, is thought to be more reliable for this purpose.[8]

The tail-flick test was performed by placing the tail of each rat (awake animals were partially restrained) over a slit 1.5 cm from a 150-W focused projector bulb. The end-point of the test was removal of the tail; a cut-off time of 6 s was imposed to avoid permanent tissue damage. The preanesthetic tail-flick latency was typically in the 1.5–1.8 s range. Results of the test are expressed as maximum percentage effect according to the formula:

$$\text{MPE} = \frac{(\text{TFL under anesthesia}) - (\text{preanesthesia TFL})}{(\text{cut-off time}) - (\text{preanesthesia TFL})} \times 100(\%)$$

Data Analysis

Data from phase 1 (0–5 min after formalin injection) and phase 2 (30–75 min after formalin injection) responses of the formalin test were considered separately. To minimize the influence of residual anesthetic on phase 2 flinching, phase 2 was defined as the interval 30–75 min after formalin injection (although some flinching was seen as early as 15 min after injection). The mean of the total number of flinches during each phase was calculated for each group and compared to data from the unanesthetized control group with analysis of variance (ANOVA) and Dunnett's test for multiple comparisons. Tail-flick data (based on the maximum percentage effect) were analyzed similarly.

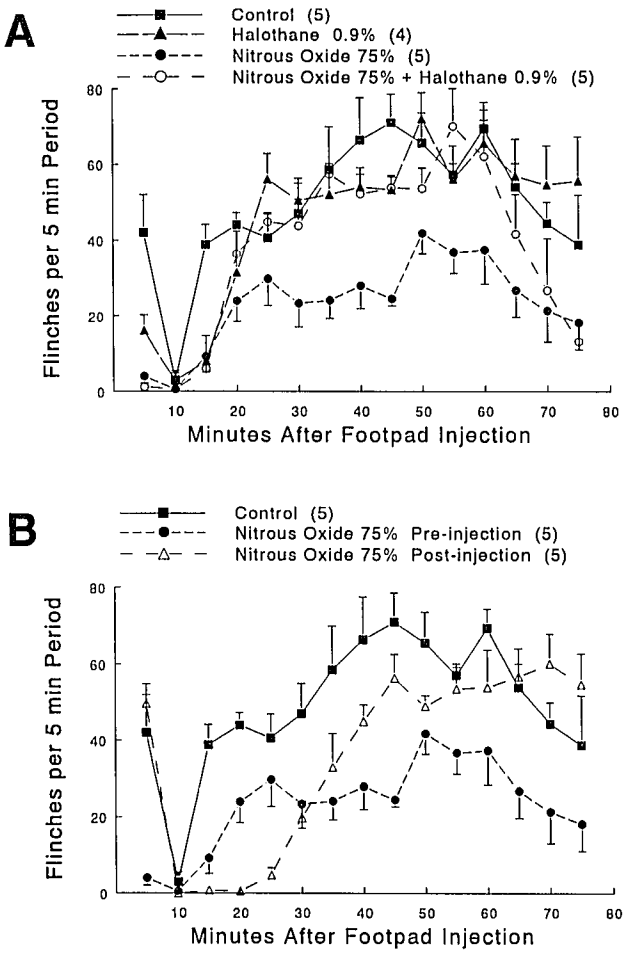

Fig. 2. The time course of anesthetic effects on formalin-induced flinching behavior. ***(A)*** **Effects of the type of anesthetic agent. Anesthesia was administered before and for 5 min after footpad injection in all groups. Although 30% N_2O and 1.8% halothane groups are not included in this figure, the pattern of the curves for these groups is similar to that of those shown.** ***(B)*** **Effects of the timing of nitrous oxide administration. Seventy-five percent nitrous oxide was administered either before and for 5 min after footpad injection (nitrous oxide preinjection group) or between 5 and 25 min after injection (nitrous oxide postinjection group). The control and the 75% N_2O preinjection groups are the same as those illustrated in *A*. In both figures, data represent mean ± SEM for the number of animals indicated in parentheses.**

Results

Animals that received 75% N_2O or 0.9% halothane lost spontaneous movements within 5–10 min after the start of anesthesia, while those treated with 1.8% halothane or the combination of 75% N_2O and 0.9% halothane also lost the righting reflex. None of the anesthetized animals vocalized or became agitated during formalin injection. Rats that received 1.8% halothane required 12–17 min for full clinical recovery, but all others recovered within 1–3 min of discontinuing the anesthetic. At the time phase 2 behavior was assessed, animals previously anesthetized were clinically indistinguishable from controls.

Subcutaneous injection of formalin to unanesthetized rats resulted in a highly reproducible, biphasic increase in flinching behavior of the injected paw (fig. 2A). The characteristic phase 1 (0–5 min) and phase 2 (30–75 min) responses were clearly present. Halothane or nitrous oxide suppressed phase 1 flinching behavior in a dose-dependent manner (table 1), with 1.8% halothane and the combination of 75% N_2O plus 0.9% halothane essentially completely suppressing the response.

Halothane or nitrous oxide administered only during phase 1 had very different effects on phase 2 flinching behavior, however (fig. 2A and table 1). Neither dose of halothane affected phase 2 behavior (fig. 2A). In marked contrast, nitrous oxide, although administered only during phase 1, produced dose-dependent

Table 1. Effects of Anesthesia on Formalin-induced Pain and Tail-flick Latency

Anesthesia	Formalin Test Flinches: Phase 1 (0–5 min)	Formalin Test Flinches: Phase 2 (30–75 min)	Tailflick MPE
Control	46 ± 5	513 ± 32	−2 ± 0.4
Halothane			
0.9%	16 ± 4* (65)	519 ± 27 (−1)	−4 ± 2
1.8%	0 ± 0* (100)	465 ± 12 (9)	−9 ± 3
Nitrous oxide			
30%	21 ± 6 (54)	365 ± 49† (29)	11 ± 4†
75%	4 ± 2* (91)	259 ± 31* (49)	32 ± 4*
75% + NAL	19 ± 4* (58)	394 ± 38 (23)	8 ± 3
75% → NTX	5 ± 2* (89)	320 ± 14* (38)	—
75% Post	50 ± 5 (−8)	461 ± 27 (10)	—
Nitrous oxide plus halothane	1 ± 1* (97)	431 ± 50 (16)	1 ± 3

Data are mean ± SEM for four or five animals per group (see methods).

Number in parentheses represent the percentage suppression of flinching from the control. Tailflick latency was converted to maximum percentage effect (MPE) according to the formula described in the text. Because flinch data are presented as percentage suppression and tailflick as MPE, negative numbers represent, respectively, an increase in flinches or a decrease in tailflick latency. All data were compared to the appropriate control group by analysis of variance and Dunnett's test.

NAL = naloxone coincident with the start of nitrous oxide; NTX = naltrexone given after nitrous oxide was discontinued.

* $P < 0.01$.

† $P < 0.05$.

suppression of phase 2 flinching (fig. 2A); 30% and 75% N_2O decreased flinching by 29% ($P < 0.05$) and 49% ($P < 0.01$), respectively. This analgesic effect was reversed by simultaneous administration of naloxone, whereas rats given naltrexone after the termination of nitrous oxide anesthesia still had fewer phase 2 flinches than did the control animals ($P < 0.01$; table 1). Moreover, halothane antagonized the analgesic effect of nitrous oxide on phase 2 behavior. Thus, whereas phase 2 flinching was suppressed 49% by 75% N_2O alone, there was no difference in the rate or time-course of phase 2 flinching between controls and those anesthetized with the combination of 75% N_2O and 0.9% halothane (fig. 2A and table 1).

Administration of nitrous oxide during phase 1 was critical to the development of phase 2 analgesia, because 75% N_2O begun after the phase 1 response to formalin did not suppress phase 2 behavior. That is, although flinching behavior was reduced while nitrous oxide was being administered (*i.e.*, 5–25 min after the foot injection), as soon as it was discontinued, the frequency of flinching increased to the level seen in unanesthetized control rats (fig. 2B and table 1).

Anesthetic effects on tail-flick latency paralleled those on phase 2 behavior in the formalin model but did not correlate with suppression of the phase 1 response (table 1). Thus, halothane 0.9% and 1.8%, while decreasing phase 1 but not phase 2 flinching, did not prolong tail-flick latency, whereas 30% and 75% N_2O, which reduced phase 2 flinching, also produced modest dose-dependent antinociception as determined by tail-flick (maximum percentage effect 11% [$P < 0.05$] and 32% [$P < 0.01$], respectively). Furthermore, naloxone also reversed the effect of 75% N_2O in this test and, whereas 75% N_2O alone prolonged tail-flick latency by 32%, the combination of 75% N_2O and 0.9% halothane had no effect (table 1).

Discussion

This study demonstrates that halothane, even at 1 MAC doses, has no effect on the facilitatory state that develops after noxious stimulation, whereas nitrous oxide suppresses the behavioral manifestations of central sensitization in a dose-dependent and naloxone-reversible manner. In the formalin model, therefore, a brief period of nitrous oxide anesthesia can have lasting effects on pain behavior provided that it is administered before the critical, acute phase (phase 1) of noxious stimulation. Thus, nitrous oxide, but not halothane, creates a preemptive analgesic state. Moreover, because the combination of 75% N_2O and 0.9% halothane did not reduce phase 2 behavior, we conclude that halothane actually antagonizes nitrous oxide-induced preemptive analgesia.

Phase 2 pain behavior in the formalin model is a manifestation of a central facilitated state and correlates electrophysiologically with enhanced responsiveness of spinal nociceptive neurons to innocuous and noxious stimuli (so-called "windup").[7,9] This state is triggered by the repetitive C-fiber barrage that occurs immediately after formalin is injected;[9] blockade of this brief (~ 5 min) first phase prevents the development of the subsequent facilitated state. Consequently, anesthetics could disrupt injury-induced central sensitization either by preventing the entry of noxious stimuli into the CNS or by interfering with events within the nervous system that are responsible for development or maintenance of a facilitated state. Morphine[8–10] and local anesthetics[12] act by the first mechanism (*i.e.*, they prevent entry of noxious stimuli), whereas excitatory amino acid antagonists such as MK-801, which block the phase 2 response without suppressing phase 1 activity, probably have a primary effect on central neurochemical processes-mediating facilitation.[10,11]

Because in all but one group, anesthesia was administered only during the first 5 min after the formalin injection, this study allows no conclusions to be made concerning whether general anesthetics can interfere with central mechanisms that consolidate or maintain sensitization. On the other hand, one can make a strong case from these experiments that nitrous oxide suppresses phase 2 pain behavior by blocking the entry and/or impact of noxious stimuli on the CNS during phase 1. This hypothesis is based on the observation that nitrous oxide, but not halothane, prolongs tail-flick latency and suppresses phase 2 flinching behavior. Studies in spinal cord-transected rats have shown that the tail-flick response is essentially a spinal reflex with little supraspinal component,[24] whereas formalin-induced flinching involves a supraspinal as well as a spinal component.[8] Because anesthetics and other sedatives/hypnotics clearly disrupt supraspinally mediated behaviors, it is not surprising that phase 1 flinching behavior was markedly reduced by the administration of nitrous oxide or halothane during that period.

Lack of a behavioral response to formalin is not, however, conclusive evidence that noxious stimuli were not reaching the spinal cord. Indeed, the fact that tail-flick latency was prolonged modestly by nitrous oxide

but was unchanged by halothane or halothane plus nitrous oxide (at a time when these regimens profoundly suppressed phase 1 flinching behavior) is presumptive evidence that both the afferent and efferent limbs of this spinal reflex arc were intact during anesthesia with halothane or halothane plus nitrous oxide, but not with nitrous oxide alone. It follows, therefore, that of these anesthetic regimens only nitrous oxide alone is capable of reducing the entry or impact of peripheral nociceptive impulses on the spinal cord, while halothane alone or in combination with nitrous oxide allows spinal neurons to receive and respond to afferent noxious stimuli. This is consistent with other experimental observations: thermally-evoked firing of wide dynamic range nociceptive neurons in the spinal cord dorsal horn persists under 0.5–1.5% halothane anesthesia.[14] Based on such reasoning, we speculate that nitrous oxide, but not halothane, produces preemptive analgesia in part because it interferes at the spinal level with the entry of noxious stimuli into the CNS and, therefore, prevents subsequent central facilitatory changes from being triggered.

The hypothesis that nitrous oxide exerts some of its effects via an action on the endogenous opioid system is both old and controversial. Although some studies show no evidence of nitrous oxide-induced opioid activity,[25] others reveal cross tolerance between morphine and nitrous oxide [15] and partial reversal of nitrous oxide-induced antinociception by naloxone.[15,16,26] Furthermore, although nitrous oxide does not interact directly with opioid receptors,[27] it increases the brain tissue concentrations of opioid peptides such as beta-endorphin[28] and Met-enkephalin.[29] Because the preemptive analgesic action of nitrous oxide was reversed partially by the simultaneous administration of naloxone during phase 1, and naloxone itself does not affect formalin-induced pain behaviors,[30,31] our data support the notion that nitrous oxide does indeed exert its analgesic effects in part by altering the activity of endogenous opioids. In this regard, it is interesting that morphine also produces preemptive analgesia in this model.[8–10] In contrast to the effect of naloxone administered during phase 1, we could not demonstrate the reversal of nitrous oxide-induced preemptive analgesia by naltrexone, a long-acting opioid receptor antagonist, administered after nitrous oxide was discontinued (*i.e.*, during phase 2). Although this suggests that the analgesic state created by nitrous oxide is not secondary to ongoing opioid activity, the statistical power of this observation is weak because the small number of animals in the naltrexone group makes it difficult to detect significant differences. Accordingly, we conclude that endogenous opioids are involved in initiating the preemptive analgesic effect of nitrous oxide but cannot be certain whether they also are involved in sustaining it.

Failure of a combination of 75% N_2O and 0.9% halothane to reduce phase 2 flinching behavior in the formalin test was unexpected because 75% N_2O alone provided substantial preemptive analgesia in this model. To our knowledge, this is the first demonstration that an analgesic effect of nitrous oxide can be antagonized by halothane. There is evidence, however, for an antagonistic effect between nitrous oxide and volatile anesthetics on MAC because the concentration of nitrous oxide and a volatile anesthetic required to achieve 1 MAC is greater for the mixture than one would expect on the basis of simple addition of the MACs of each agent separately.[22,32] Further support for the notion of antagonism between nitrous oxide and a volatile anesthetic comes from a recent study demonstrating that the combination of 1% isoflurane and 70% N_2O administered during phase 1 of the formalin test does not suppress phase 2 behavior, whereas isoflurane alone (1% and 2.5%) reduced phase 2 flinching by 34%.[33] Our observations regarding halothane-nitrous oxide anesthesia are similar and, consequently, confirm that inhalation anesthesia does not block postinjury facilitation. The effect of nitrous oxide alone was not examined in that study,[33] however, and the observation that a modest analgesic effect of isoflurane was eliminated by the addition of nitrous oxide was unexpected and unexplained. It remains so because the preponderance of evidence in the literature indicates that nitrous oxide is an analgesic[15,16] and, for the first time, our data show it to be an effective preemptive analgesic. Differences between the studies relating to the anesthetic state of control animals at the time of formalin injection (brief isoflurane anesthesia *vs.* none), site of injection (dorsal *vs.* plantar surface of the hind paw), and definition of the phase 2 interval (10–60 min *vs.* 30–75 min) exist, but these differences are minor and cannot explain how isoflurane and nitrous oxide, which singly are capable of blocking postinjury facilitation, are ineffective when administered jointly. Accordingly, the mechanism by which nitrous oxide-induced preemptive analgesia is antagonized by volatile anesthetics is unknown. We postulate, however, that it may occur on a metabolic basis; if nitrous oxide-induced preemptive analgesia requires active neural processes

(*e.g.*, the activation of a descending inhibitory pathway, which has been shown to mediate the antinociceptive action of nitrous oxide),[26,34] halothane and presumably other volatile anesthetics could interfere by decreasing the spinal or cerebral metabolic rate, thereby preventing neural activation.

A potential limitation of this study is that the investigator who counted flinches was not blinded to the treatment the animal had received. If this introduces a meaningful bias, then virtually all studies using this model are suspect because none of the dozens recently published[8,12] (some in this journal)[10,33] have been blinded. Perhaps this is because the flinching behavior is quite robust and easy to recognize. In fact, control data obtained by a new member of our laboratory who had had no previous experience with the formalin test and no idea what to expect were indistinguishable from those obtained by our most experienced person. Therefore, although blinding is a theoretic consideration in these studies, it is unlikely to be of any practical importance.

Although formalin-induced pain is presumably analogous to postoperative pain, extrapolation of these results to the clinical setting requires caution. First, the stimuli are different: Formalin pain is due primarily to peripheral tissue inflammation,[7] whereas surgical pain has both inflammatory and neuropathic components.[5] Second, species differences may exist.[7] Third, postsurgical pain generally follows a far more protracted time course than that of formalin-induced pain, whereas the duration of preemptive analgesia may be short. For instance, in a recent human study that compared the effects of lidocaine infiltration of the skin either before or after cutaneous thermal injury, preemptive analgesia lasted for only the first 70 min after injury.[35] Nevertheless, it is clear from our experiments that both the type of anesthetic agent and timing of its administration relative to noxious stimulation can have substantial impact on subsequent pain. Moreover, the hypnotic potency of an agent and the lack of responsiveness during anesthesia are evidently not reliable indicators of preemptive analgesic properties, because nitrous oxide, a poor hypnotic, is a good preemptive analgesic, whereas halothane, a potent hypnotic, is not analgesic. Thus, the hypnotic and analgesic properties of general anesthetics should be considered separately, because not all analgesics are anesthetics, and not all anesthetics are preemptive analgesics.

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