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

亜酸化窒素はラットにおいて先行鎮痛を生じ、その効果はハロセンによって拮抗される

後藤隆久

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

亜酸化窒素はラットにおいて先行鎮痛を生じ、
その効果はハロセンによって拮抗される

後藤　隆久

BACKGROUND

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 animal 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 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, which conforms to the guidelines of the United States National Institute of Health, the International Association for the Study of Pain, and the Society for Neuroscience[13], involves prolonged, tonic pain generated by tissue injury from injection of formalin. Because it has recently become increasingly clearer that tonic pain is modulated differently in the CNS than phasic, transient pain (*e.g.*, produced by thermal stimuli used in the tail-flick and hot-plate tests), the formalin model is thought to better approximate clinical pain 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 minutes; the more prolonged late-phase responses (phase 2) begin about 15 minutes after injection and last 60-90 minutes. Recent studies suggest that phase 1 is caused predominantly by activation of C-fiber afferents by the peripheral stimulus[9]. Phase 2,

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)[14] and halothane[15] 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[16, 17]. Anesthetics also alter the responsiveness of neurons to excitatory amino acid neurotransmitters[18,19] and, consequently, may perturb the central sensitization process. Based on such considerations, I predicted that general anesthetics would prevent noxious stimulation-induced central facilitation. Accordingly, I examined the hypothesis that N_2O or halothane administered *only* during the brief acute phase of noxious stimulation would alter pain behavior in the postanesthetic period. I chose the formalin test for this purpose because, as described above, the electrophysiological and behavioral responses to this particular stimulation has been extensively investigated[7-12]. Especially, unlike other animal models of long-term pain such as carrageenan or Freund's adjuvant injection, formalin-induced pain consists of two distinct periods of hyperalgesia (i.e., produced by direct stimulation and by central sensitization) that are clearly distinguishable from each other. This unique feature provides me an opportunity to administer anesthetics *only* during the period of direct stimulation and investigate their

effects on central sensitization. Finally, I felt this choice appropriate from the ethical stand point because formalin-induced pain inflicts less protracted discomfort on animals than do other animal models tonic pain.

MATERIALS AND METHODS

Studies were performed with approval of the Subcommittee on Research Animal Care in 74 male Sprague-Dawley rats weighing between 300-325 g. 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[20], experiments were performed between the hours of 10:00 and 22:00 in randomized order.

EXPERIMENTAL PARADIGM

Rats were divided into 5 anesthetic groups as follows: 30% N_2O, 75% N_2O, 0.9% halothane, 1.8% halothane, or 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 5 animals except for the 0.9% and 1.8% halothane groups, which contained only 4 animals each.

In all cases, the total duration of anesthesia was 20 minutes (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 were then removed briefly from the box (< 15 s) so formalin could be injected into the left hind paw. Five percent formalin was prepared from 37% formaldehyde solution by 1:19 dilution with 0.9% normal saline (the final concentration of formaldehyde was 1.85%) 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 were then removed from the anesthesia chamber, transferred to a 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 N_2O (Ohmeda 5200 CO_2 analyzer), halothane (Datex 222 anesthetic agent analyzer, Puritan Bennett), and oxygen (Ohmeda 5100 oxygen analyzer) inside the box were measured continuously. The inspired concentrations of N_2O 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 N_2O[21,22] and 0.95-1.11% for halothane[23,24], and an estimated ratio of end-tidal to inspired concentration of halothane of 0.5-0.6 in spontaneously breathing rats after 20 min[24].

Based on the results of these initial studies, three additional experiments were conducted. To assess the possibility that the effects of N_2O 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 (i.p.) 15 min before the foot injection and coincident with the start of 75% N_2O. Similarly, to determine if N_2O's effect on phase 2 behavior could be related to ongoing actions of endogenous opioids even after N_2O was discontinued, an eighth group of animals (n = 5) received naltrexone 20 mg/kg i.p. (concentration = 10 mg/ml in normal saline) 5 min after the foot injection, when 75% N_2O was discontinued (N_2O-->NTX 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 examine whether the analgesic effect of N_2O is diminished once central sensitization is triggered, a ninth group of rats (N_2O post-injection group) received 75% N_2O for 20 minutes 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, I also used a behavior that is known to be a spinal reflex response, namely, the tail-flick test[25]. For this portion of the study, 31 additional rats were divided into 7 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 post-injection N_2O and N_2O-->NTX 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.

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-watt focused projector bulb. The end point of the test was removal of the tail; a cut-off time of 6 sec was imposed to avoid permanent tissue damage. The pre-anesthetic tail-flick latency (TFL) was typically in the 1.5 - 1.8 s range. Results of the test are expressed as maximum percentage effect (MPE) according to the formula:

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

DATA ANALYSIS

Data from phase 1 (0-5 min after formalin injection) and phase 2 (30-75 min) 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 MPE) were analyzed similarly.

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 N_2O suppressed phase 1 flinching behavior in a dose-dependent manner (Table), with 1.8% halothane and the combination of 75% N_2O plus 0.9% halothane essentially completely suppressing the response.

Halothane or N_2O administered only during phase 1 had very different effects on phase 2 flinching behavior, however (fig.2A and table). Neither dose of halothane affected phase 2 behavior (fig.2A). In marked contrast, N_2O, although administered only during phase 1, produced dose-dependent 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. Moreover, halothane antagonized the analgesic effect of N_2O 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).

The analgesic effect of N_2O was partially reversed by simultaneous administration of naloxone since animals given naloxone combined with N_2O displayed phase 2 flinching not significantly different from that of controls. On the other hand, rats given naltrexone after the termination of N_2O anesthesia still had fewer phase 2 flinches than did the control animals ($P < 0.01$; table). Whether post-N_2O naltrexone was less effective than simultaneous naloxone treatment cannot be firmly concluded based solely on these data, however, because the difference in the number of flinches between these two antagonist-treatment groups were relatively small. Moreover, since Dunnett's test was used for statistical analysis in this study, by definition, no direct comparison was made between these two groups.

Administration of N_2O during phase 1 was critical to the development of phase 2 analgesia since 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 N_2O was being administered (*i.e.*, 5-25 min after foot injection), as soon as it was

discontinued, the frequency of flinching increased to the level of unanesthetized control rats (fig.2B and table).

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). 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 (MPE 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).

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, since the combination of 75% N_2O and 0.9% halothane did not reduce phase 2 behavior, I 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 sensitization is triggered by the repetitive barrage of the primary afferent C fibers that occurs immediately after formalin is injected[9]; blockade of this brief (~5 min) phase 1 prevents the development of the subsequent hyperexcitable state and thereby suppresses phase 2. Indeed, pre-formalin treatments with morphine[8-10] or local anesthetics[12] have been shown to suppress phase 2 by this mechamism.

The current results strongly suggest that nitrous oxide exers similar actions because nitrous oxide proportionally inhibited phase 1 and phase 2 flinching. However, suppression of flinching during phase 1 is not conclusive evidence that nitrous oxide prevented afferent noxious inputs from reaching the spinal cord because formalin-induced flinching is mediated at least in part supraspinally[8] and is therefore susceptible to suppression by non-specific anesthetizing or sedating actions of anesthetics. In fact, propofol , an intravenous agent with potent hypnotic and sedative properties, suppresses phase 1 flinching although it has no analgesic effects[25]. To circumvent this problem and more accurately evaluate the anesthetics' ability to prevent the entry of noxious inputs into the spinal cord, the tail-flick test was used in the current study. Formalin-induced phase 1 pain behaviors and tail-flick response are similar in that both are evoked by direct afferent noxious stimuli. However, being a spinal reflex with little supraspinal component[26], the tail-flick response has been shown to be highly resistant to the hypnotic/sedative effects of anesthetics[27]. In this study, nitrous oxide modestly prolonged tail-flick latency, strongly indicating that it is indeed analgesic, *i.e.*, capable of blocking the entry and/or impact of peripheral nociceptive impulses on the spinal cord. Based on such reasoning, I conclude that nitrous oxide inhibits phase 2 flinching in part because it interferes at the spinal level with entry of noxious stimuli into the CNS and thereby prevents subsequent central sensitization from being triggered.

In contrast to the nitrous oxide effect, halothane alone or a combination of halothane and nitrous oxide produced no inhibition of phase 2 flinching although they strongly suppressed phase 1. To explain this disparity between phase 1 and 2, at least two possibilities need to be considered. First, formalin-generated noxious barrage

might have entered the spinal cord during phase 1 even though the animals were made immobile by halothane or nitrous oxide plus halothane. In this case, phase 1 stimuli would trigger central sensitization, resulting in subsequent manifestation of phase 2 behavioral responses. Second, anesthetics might have perturbed the neurochemical processes mediating central sensitization, and thereby disrupted the dependency of phase 2 sensitized state on phase 1 activity.

The first possibility is supported by the fact that tail-flick latency was unaffected by either halothane alone or in combination with nitrous oxide. This suggests that these anesthetics are indeed incapable of blocking the entry and/or impact of noxious inputs on the spinal cord. This is also 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]. Thus, unlike nitrous oxide alone, halothane alone or in combination with nitrous oxide allows the spinal neurons to receive and respond to afferent noxious stimuli, which trigger the facilitated state leading to the formation of phase 2 responses. Lack of a behavioral response to formalin during phase 1 does not necessarily contradict with the failure of these anesthetics to attenuate tail-flick response because, as discussed above, formalin-induced nociceptive behaviors are far more susceptible than a tail-flick response to suppression by non-specific actions of anesthetics.

Regarding the second possibility, *i.e.*, perturbation of the neurochemical mechanisms mediating central sensitization, this study allows no firm conclusions to be made because anesthesia was administered only during the first 5 min after the formalin injection. However, because halothane is known to exert multiple and complex actions on the neurotransmitter and second messenger systems within the CNS, it is quite conceivable that this volatile agent might affect injury-induced facilitatory process. Key factors in the development of central sensitization is activation of excitatory amino acid (EAA) receptors, especially the *N*-methyl-*D*-aspartate receptor subtype[10,11], and a subsequent increase in intracellular calcium[5] leading to a cascade of events including

activation of protein kinase C[5] and nitric oxide synthase (NOS)[28]. Halothane could theoretically affect each of these steps, although these actions may exert opposing influences on the final sensitized state. Halothane reduces the release of the EAA glutamate[29] and suppresses the depolarization of central neurons in response to glutamate[18,19]. Halothane also attenuates the glutamate-induced increase in intracellular calcium level[30], although the baseline calcium level may be unaffected[30] or increased[31]. This increase was thought to be due to release of intraneuronally stored calcium because it was sensitive to dantrolene[31]. In addition, halothane activates protein kinase C[32] and may or may not inhibit neuronal NOS[33,34]. Finally, noxious stimulation activates the descending inhibitory system which negatively modulates injury-induced hyperexcitability of the spinal neurons[35,36]. However, subanesthetic concentrations of volatile anesthetics, such as those used in this study, may attenuate such inhibition. Thus, halothane-induced modulation of central sensitization is determined by an ultimate balance of many opposing factors. The results of the current study suggest that the net effect is almost neutral, i.e., halothane probably has little effect on central sensitization.

Halothane is generally believed to be an analgesic because it is a potent anesthetic and because analgesia is thought to be the essential component of anesthesia[37]. Lack of analgesic and preemptive analgesic properties of halothane demonstrated in this study may appear inconsistent with this traditional concept. This discrepancy may be accounted for by at least two reasons. The first is the difference in end-point to evaluate analgesia. 'Analgesia' as a component of general anesthesia refers to a state where the adequately anesthetized subject neither remembers pain nor responds to stimuli by moving or by changing the blood pressure or heart rate[37]. In contrast, the end-point used the current study was inhibition of the noxious stimulation-induced spinal reflex (tail-flick response) and central sensitization (phase 2 formalin flinching). It is quite reasonable to assume that different end-points of analgesia can be differently modulated by drugs; a simple spinal reflex of tail-flick, multifactorial,

integrated processes of central sensitization, and suppression of consciousness, mobility, and hemodynamic responses may all be affected differently because they are different phenomenon involving different neurotransmitters and/or neural pathways. Second, analgesic properties of halothane may depend on the dose administered. In fact, humans given subanesthetic doses of halothane experience unaltered or even slightly augmented pain as compared to their non-anesthetic state[38,39] while anesthetic doses clearly depress their ability to 'report' pain and thereby create a so-called analgesic state. It is also well known that different doses of halothane are required to prevent various consequences evoked by noxious afferent stimuli such as arousal, movements, hemodynamic responsiveness, and catecholamine release [37,40].

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[41], others reveal cross tolerance between morphine and nitrous oxide[16] and partial reversal of nitrous oxide-induced antinociception by naloxone[16,17,42]. Furthermore, although nitrous oxide does not interact directly with opioid receptors[43], it increases the brain tissue concentrations of opioid peptides such as beta-endorphin[44] and Met-enkephalin[45]. Since the preemptive analgesic action of nitrous oxide was partially reversed by simultaneous administration of naloxone during phase 1, and naloxone itself does not affect formalin-induced pain behaviors[46,47], 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]. On the other hand, I could not demonstrate 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 each group makes it difficult to detect significant differences. Accordingly, I conclude

that endogenous opioids are probably 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 my knowledge, this is the first demonstration that an analgesic effect of nitrous oxide can be antagonized by halothane, and these results have been reproduced by others[48] and reconfirmed in my laboratory[49]. Antagonism between nitrous oxide and volatile anesthetics has also been reported for MAC in the rat[23,50] and suppression of learning in humans[51], although interpretation of the MAC studies is a subject of much debate[52-54].

It is unlikely that the observed antagonism between nitrous oxide and volatile anesthetics can be explained by the hyperalgesic properties of volatile anesthetics because halothane alone did not alter formalin-induced flinching behavior or tail-flick response. Nor is it likely that, although nitrous oxide activates opioid receptors as discussed above, the volatile anesthetics possess opioid antagonist properties since halothane does not displace specific binding of radiolabelled ligands to either μ or k receptor subtype *in vitro* [55,56]. I postulate that the observed antagonism between nitrous oxide and volatile anesthetics may occur on a metabolic basis; if nitrous oxide-induced preemptive analgesia requires active neural processes (*e.g.*, activation of descending inhibitory pathway, which has been shown to mediate antinociceptive action of nitrous oxide[42,57]), halothane and presumably other volatile agents could interfere by decreasing spinal or cerebral metabolic rate and thereby preventing neural activation. Unfortunately, there is no direct evidence in the literature that small doses of halothane (*e.g.*, 0.5 MAC as in this study) suppress nitrous oxide-induced augmentation of CNS metabolism. However, it has been indirectly demonstrated that slightly larger, although still subanesthetic, doses of isoflurane or enflurane (0.6-0.8 MAC) can counteract metabolic effects of nitrous oxide because, while nitrous oxide alone increases cerebral

and spinal metabolic rate[58,59], it is not altered by a substitution of 60-65% nitrous oxide for equal-MAC fraction of volatile anesthetics during 1.2 -1.4 MAC enflurane or isoflurane anesthesia[60,61].

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[8,10,12] 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 my laboratory who had no previous experience with the formalin test and no idea what to expect were indistinguishable from those obtained by the most experienced person. Therefore, although blinding is a theoretical 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 primarily due 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, pre-emptive analgesia lasted for only the first 70 min after injury[62]. Nevertheless, it is clear from these 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, hypnotic potency of an agent and lack of responsiveness during anesthesia are evidently not reliable indicators of preemptive analgesic properties since 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.

SUMMARY

(1) Nitrous oxide induced dose-dependent preemptive analgesia in the rat formalin test, and thereby suppressed central sensitization-dependent hyperalgesia even after it was discontinued.

(2) This effect was partially reversed by naloxone, suggesting the involvement of endogenous opioids in this action of nitrous oxide.

(3) Halothane, in contrast, demonstrated no preemptive analgesic properties and even antagonized the analgesic effect of nitrous oxide. These results suggests that the hypnotic potency of an anesthetic is a poor indicator of its preemptive analgesic potential.

REFERENCES

1. LaMotte RH, Shain CN, Simone DA, Tsai EFP: Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms. J Neurophysiol 1991; 66:190-211.
2. Torebjök HE, Lundberg LER, LaMotte RH: Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol 1992; 448:765-780.
3. Woolf CJ: Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306:686-688.
4. Woolf CJ, Wall PD: Morphine-sensitive and morphine-insensitive actions of C-fiber input on the rat spinal cord. Neurosci Lett 1986; 64:221-225.
5. Woolf CJ, Chong MS: Preemptive analgesia--treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 1993; 77:362-379.
6. Katz J, Kavanagh BP, Sandler AN, Nierenberg H, Boylan JF, Friedlander M, Shaw BF: Preemptive analgesia: Clinical evidence of neuroplasticity contributing to postoperative pain. Anesthesiology 1992; 77:439-446.
7. Tjølsen A, Berge O, Hunskaar S, Rosland JH, Hole K: The formalin test: an evaluation of the method. Pain 1992; 51:5-17.
8. Wheeler-Aceto H, Cowan A: Standardization of the rat paw formalin test for the evaluation of analgesics. Psychopharmacology 1991; 104:35-44.
9. Dickenson AH, Sullivan AF: Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain 1987; 30:349-360.
10. Yamamoto T, Yaksh TL: Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology 1992; 77:757-763.

11. Murray CW, Cowan A, Larson AA: Neurokinin and NMDA antagonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model. Pain 1991; 44:179-185.

12. Coderre TJ, Vaccarino AL, Melzack R: Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. Brain Res 1990; 535:155-158.

13. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals (editorial). Pain 1983; 16 :109-10.

14. Taub A, Hoffert M, Kitahata LM: Lamina-specific suppression and acceleration of dorsal-horn unit activity by nitrous oxide: A statistical analysis. Anesthesiology 1974; 40:24-31.

15. Namiki A, Collins JG, Kitahata LK, Kikuchi H, Homma E, Thalhammer JG: Effects of halothane on spinal neuronal responses to graded noxious heat stimulation in the cat. Anesthesiology 1980; 53:475-480.

16. Berkowitz BA, Ngai SH, Finck AD: Nitrous oxide "Analgesia": Resemblance to opiate action. Science 1976; 194:967-968.

17. Yang JC, Clark WC, Ngai SH: Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology 1980; 52:414-417.

18. Richards CD, Smaje JC: Anaesthetics depress the sensitivity of cortical neurones to l-glutamate. Br J Pharmacol 1976; 58:347-357.

19. Puil E, El-Beheiry H: Anaesthetic suppression of transmitter actions in neocortex. Br J Pharmacol 1990; 101:61-66.

20. Frederickson RC, Burgis V, Edwards JD: Hyperalgia induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. Science 1977; 198:756-758.

21. Russel GB, Graybeal JM: Direct measurement of nitrous oxide MAC and neurologic monitoring in rats during anesthesia under hyperbaric conditions. Anesth Analg 1992; 75:995-999.

22. Wardley-Smith B, Halsey MJ: Mixtures of inhalation and i.v. anaesthetics at high pressure. Br J Anaesth 1985; 57:1248-56.

23. Cole DJ, Kalichman MW, Shapiro HM, Drummond JC: The nonlinear potency of sub-MAC concentrations of nitrous oxide in decreasing the anesthetic requirement of enflurane, halothane, and isoflurane in rats. Anesthesiology 1990; 73:93-99.

24. White PF, Johnston RR, Eger EI II: Determination of anesthetic requirement in rats. Anesthesiology 1974; 40:52-57.

25. Goto T, Marota JJA, Crosby G: Pentobarbitone, but not propofol, produces pre-emptive analgesia in the rat formalin model. Br J Anaesth 1994;72:662-667.

26. Irwin S, Houde RW, Bennett DR, Hendershot LC, Seevers MH: The effects of morphine, methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. J Pharmacol Exp Ther 1951; 101:132-143.

27. Ding XH, Ji XQ, Tsou K: Pentobarbital selectively blocks supraspinal morphine analgesia. Evidence for $GABA_A$ receptor involvement. Pain 1990;43:371-376.

28. Malmberg AB, Yaksh TL: Spinal nitric oxide synthase inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in formalin test in rats. Pain 1993;54:291-300.

29. Schlame M, Hemmings HC Jr: Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. Anesthesiology 1995;82:1406-1416.

30 Puil E, El-Beheiry H, Baimbridge KG: Anesthetic effects of glutamate-stimulated increase in intraneuronal calcium. J Pharmacol Exp Ther 1990;255:955-961.

31 Mody I, Tanelian DL, MacIver MB: Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. Brain Research 1991;538:319-323.

32 Hemmings HC Jr, Adamo AI: Activation of endogenous protein kinase C by halothane in synaptosomes. Anesthesiology 1996;84:652-662.

33. Tobin JR, Martin LD, Breslow MJ, Traystman RJ: Selective anesthetic inhibition of brain nitric oxide synthase. Anesthesiology 1994;81:1264-1269.

34. Gengasamy A, Ravichandran LV, Reikersdorfer CG, Johns RA: Inhalational anesthetics do not alter nitric oxide synthase activity. J Pharmacol Exp Ther 1995;273:599-604.

35. Bouhassira D, Gall O, Chitour D Le Bars D: Dorsal horn convergent neurones; negative feedback triggered by spatial summation of nociceptive afferents. Pain 1995;62:195-200.

36. Wilder-Smith OHG, Tassonyi E, Senly C, Otten Ph, Arendt-Nielsen L: Surgical pain is followed not only by spainal sensitization but also by supraspinal antinociception. Br J Anaesth 1996;76:816-821.

37. Stanski DR: Monitoring depth of anesthesia, Anesthesia 4th ed. Edited by Miller RD. New York, Churchill Livingstone, 1994, pp1127-1159.

38. Dundee JW, Nicholl RM, Black GW: Alterations in response to somatic pain associated with anaesthesia. X: Further studies with inhalational agents. Br J Anaesth 1962;34:158-160.

39. Tomi K, Mashimo T, Tashiro C, Yagi, Pak M, Nishimura S, Nishimura M, Yoshiya I: Alterations in pain threshold and psychomotor response associated with subanaesthetic concentrations of inhalation anaesthetics in humans. Br J Anaesth 1993;70:684-686.

40. Roizen MF, Horrigan RW, Frazer BM: Anesthetic doses blocking adrenergic (stress) and cardiovascular responses to incision--MAC BAR. Anesthesiology 1981;54:390-398.

41. Levine JD, Gordon ND, Fields HL: Naloxone fails to antagonize nitrous oxide analgesia for clinical pain. Pain 1982; 13:165-170.

42. Zuniga JR, Joseph SA, Knigge KM; Nitrous oxide analgesia: partial antagonism by naloxone and total reversal after periaqueductal gray lesions in the rat. Eur J Pharmacol 1987; 142:51-60.

43. Lawrence D, Livingston A: Opiate-like analgesic activity in general anaesthetics. Br J Pharmacol 1981; 73:435-442.

44. Zuniga JR, Joseph SA, Knigge KM: The effects of nitrous oxide on the central endogenous pro-opiomelanocortin system in the rat. Brain Res 1987; 420:57-65.

45. Finck AD, Samaniego E, Ngai SH: Nitrous oxide selectively release met^5-enkephalin and met^5-enkephalin-arg^6-phe^7 into canine third ventricular cerebrospinal fluid. Anesth Analg 1995;80:664-670.

46. North MA: Naloxone reversal of morphine analgesia but failure to alter reactivity to pain in the formalin test. Life Sci 1977; 22:295-302.

47. Pertovaara A, Mecke E, Carlson S: Attempted reversal of cocaine-induced antinociception effects with naloxone, an opioid antagonist. Eur J Pharmacol 1991; 192:349-353.

48. O'Connor TC, Abram SE: Inhibition of nociception-induced spinal sensitization by anesthetic agents. Anesthesiology 1995; 82:259-266.

49. Goto T, Marota JJA, Crosby G: Volatile anaesthetics antagonize nitrous oxide and morphine-induced analgesia in the rat. Br J Anaesth 1996; 76:702-706.

50. DiFazio CA, Brown RE, Ball CG, Heckel G, Kennedy SS: Additive effects of anesthetics and theories of anesthesia. Anesthesiology 1972; 36:57-63.

51. Chorkoff BS, Bennett HL, Eger EI II. Does nitrous oxide antagonize isoflurane-induced suppression of learning? Anesthesiology 1993; 79:724-732.

52. Eger EI II. Does 1 + 1 = 2? (editorial) Anesth Analg 1989; 68:551-555.

53. Cole DJ, Kalichman MW, Shapiro HM, Eger EI II. Does 1 + 1 = 2? --a continuing debate. Anesth Analg 1990; 70:126-127.

54. Gonsowski CT, Eger EI II. Nitrous oxide minimum alveolar anesthetic concentration in rats is greater than previously reported. Anesth Analg 1994; 79:710-712.

55. Inoki R, Kim SY, Maeda S, Nakamae J, Mashimo T, Yoshiya I. Effect of inhalational anesthetics on the opioid receptors in the rat brain. Life Sci 1983; 33 (Supp I):223-226.

56. Lawrence D, Livingston A: Opiate-like analgesic activity in general anaesthetics. Br J Pharmacol 1981; 73:435-442.

57. Komatsu T, Shingu K, Tomemori N, Urabe N, Mori K: Nitrous oxide activates the supraspinal pain inhibition system. Acta Anaesthesiol Scand 1981; 25:519-522.

58. Pelligrino DA, Miletich DJ, Hoffman WE, Albrecht RF: Nitrous oxide markedly increases cerebral cortical metabolic rate and blood flow in the goat. Anesthesiology 1984;60:405-412.

59. Crosby G, Crane AM, Sokoloff L: A comparison of local rates of glucose utilization in spinal cord and brain in couscious and nitrous oxide- or pentbarbital-treated rats. Anesthesiology 1984;61:434-438.

60. Cole DJ, Shapiro HM: Different 1.2 MAC combinations of nitrous oxide-enflurane cause unique cerebral and spinal cord metabolic responses in the rat. Anesthesiology 1989;70:787-792.

61. Algotsson L, Messeter K, Rosén I, Holmin T: Effects of nitrous oxide on cerebral haemodynamic and metabolism during isoflurane anaesthesia in man. Acta Anaesthesiol Scand 1992;36:46-52.

62. Dahl JB, Brennum J, Arendt-Nielsen A, Jensen TS, Kehlet H: The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin. Pain 1993; 53:43-51.

FIGURE AND TABLE LEGENDS

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

Figure 2A,B. The time course of anesthetic effects on formalin-induced flinching behavior. (Fig.2A) 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 these curves is similar to that of those shown. (Fig.2B) Effects of the timing of N_2O administration. 75% N_2O was administered either before and for 5 min after footpad injection (N_2O pre-injection group) or between 5 and 25 min after injection (N_2O post-injection group). The control and 75% N_2O pre-injection groups are the same as those illustrated in Fig.2A. In both figures, data represent mean ± SEM for the number of animals in parentheses.

Table. Effect of anesthesia on formalin-induced pain and tail-flick latency. Flinch data are presented as the mean ± SEM for 4 or 5 animals per group (see Methods and Materials). Numbers in parentheses represent the percentage suppression of flinching from the control. Tail-flick latency was converted to maximum percentage effect (MPE) according to the formula described in the text, and data are presented as mean ± SEM. Since flinch data are presented as percentage suppression and tail-flick as MPE, negative numbers represent, respectively, an increase in flinches or a decrease in tail-

flick latency. All data were compared to the appropriate control group by ANOVA and Dunnett's test.

*$P < 0.01$, +$P < 0.05$.

FIGURE 1

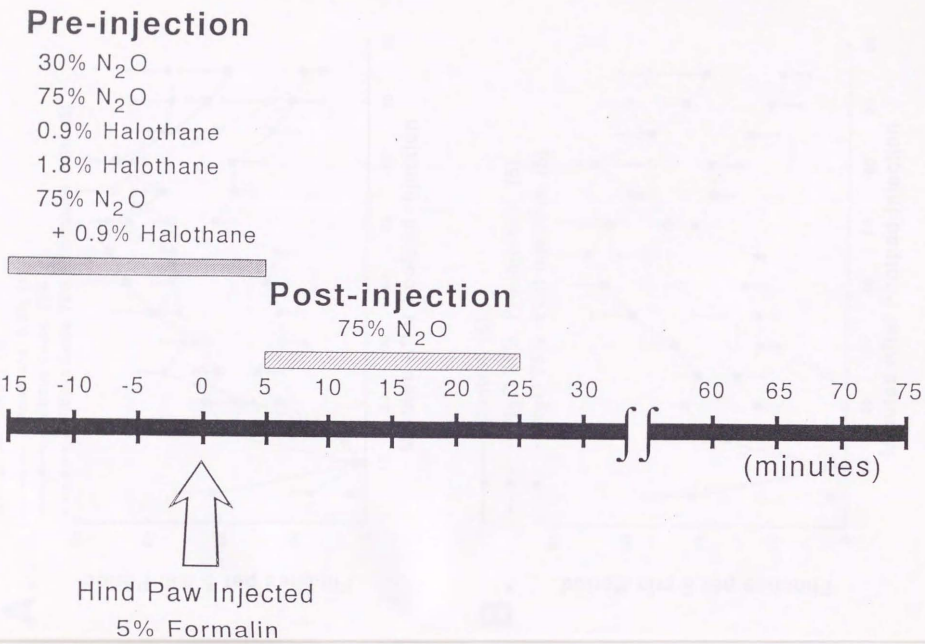
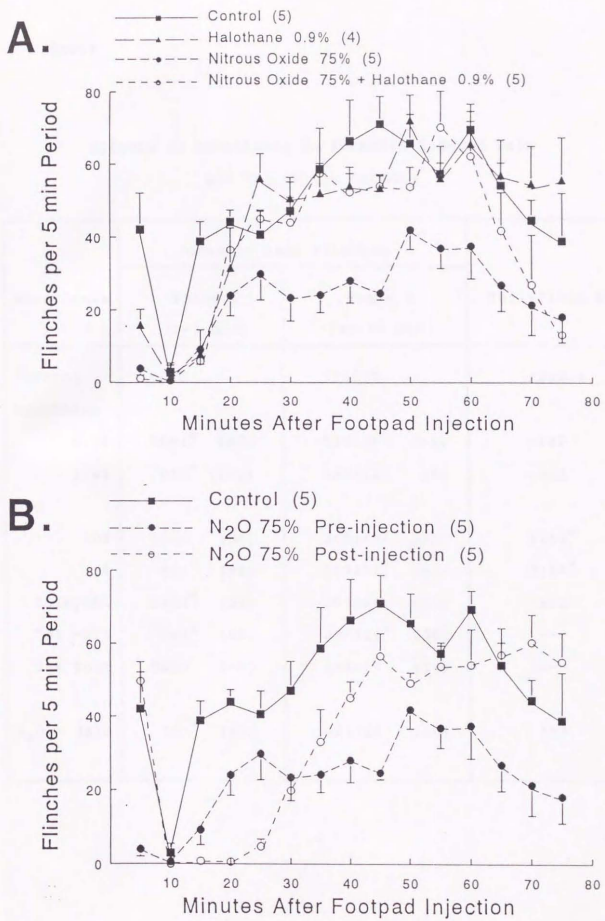

Pre-injection
30% N_2O
75% N_2O
0.9% Halothane
1.8% Halothane
75% N_2O
+ 0.9% Halothane
Post-injection
75% N_2O
-15 -10 -5 0 5 10 15 20 25 30 60 65 70 75
(minutes)
Hind Paw Injected
5% Formalin

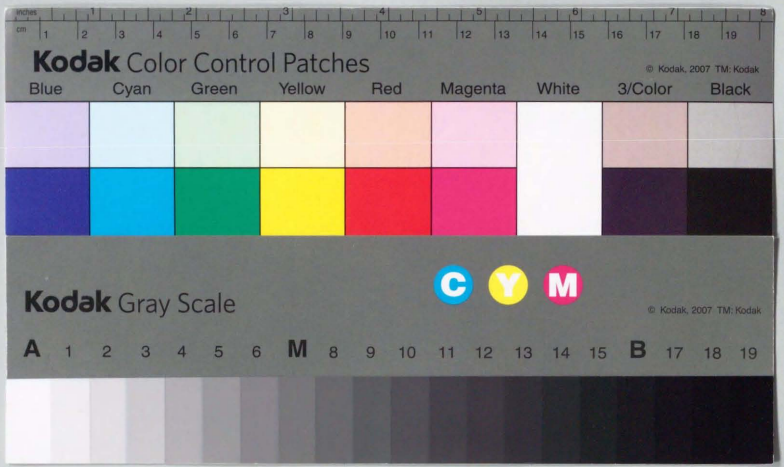
FIGURE 2


A.
Control (5)
Halothane 0.9% (4)
Nitrous Oxide 75% (5)
Nitrous Oxide 75% + Halothane 0.9% (5)
Flinches per 5 min Period
Minutes After Footpad Injection
B.
Control (5)
N2O 75% Pre-injection (5)
N2O 75% Post-injection (5)
Flinches per 5 min Period
Minutes After Footpad Injection

TABLE

Effects of Anesthesia on Formalin-Induced Pain and Tail-Flick Latency

Anesthesia	Formalin Test Flinches		Tail-Flick MPE
	Phase 1 (0-5 min)	Phase 2 (30-75 min)	
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
N_2O			
30%	21±6 (54)	365±49+ (29)	11±4+
75%	4±2* (91)	259±31* (49)	32±4*
75%w/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)	---
N_2O + Halo	1±1* (97)	431±50 (16)	1±3


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19