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LABORATORY INVESTIGATIONS

Volatile anaesthetics antagonize nitrous oxide and morphine-induced analgesia in the rat

T. GOTO, J. J. A. MAROTA AND G. CROSBY

Summary

We reported previously that nitrous oxide induces pre-emptive analgesia that is partially antagonized by naloxone and totally antagonized by halothane. The aims of this study were to determine if halothane and isoflurane are similar in this respect and to examine if volatile anaesthetics antagonize the analgesic effect of exogenous opioids. We found that 75 % nitrous oxide prolonged tail-flick latency by 37 % and this analgesia was dose-dependently inhibited by halothane and, less effectively, by isoflurane. In contrast, morphine 1.25 mg kg^{-1} i.v. also prolonged tail-flick latency by 35 % but, unlike nitrous oxide-induced analgesia, this effect was attenuated only by high doses of halothane and was unaffected by isoflurane. Neither halothane nor isoflurane alone altered the tail-flick response. We conclude that both halothane and isoflurane dose-dependently antagonized nitrous oxide analgesia but antagonized morphine-induced analgesia to a lesser extent. (*Br. J. Anaesth*. 1996; **76**: 702–706)

Key words

Anaesthetics gases, nitrous oxide. Anaesthetics volatile, halothane. Anaesthetics volatile, isoflurane. Analgesics opioid, morphine. Interactions (drugs). Rat.

The analgesic actions of anaesthetic agents have been studied extensively over the past several years, but interactions between drugs have not been well characterized. We reported previously that 75 % nitrous oxide produces pre-emptive analgesia in the rat formalin test which is antagonized partially by naloxone and completely abolished by 0.5 MAC of halothane [1]. Another study using a similar model demonstrated that isoflurane produced modest antinociception whereas the combination of 1 % isoflurane and 70 % nitrous oxide did not [2], suggesting that nitrous oxide-induced pre-emptive analgesia may also be attenuated by isoflurane.

Based on these data and evidence that nitrous oxide analgesia is mediated at least in part by the endogenous opioid system [1, 3], it follows that these volatile anaesthetics might also antagonize analgesia produced by an exogenous opioid analgesic. In this study we tested this hypothesis by examining and comparing the influence of halothane and isoflurane on antinociception produced by nitrous oxide or morphine. The tail-flick test was chosen to assess analgesia because, as a spinal reflex [4], the tail-flick response is highly resistant to the hypnotic–sedative effects of anaesthetics [5].

Materials and methods

ANIMAL PREPARATIONS

Studies were performed with the approval of the Institutional Subcommittee on Research Animal Care in 81 male Sprague–Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA) weighing 300–325 g. Rats were maintained in a 12-h light–dark cycle (lights on at 07:00) and allowed free access to food and water. Animals assigned to receive morphine had an indwelling i.v. catheter inserted before the experiments. For this purpose, rats were anaesthetized with 1 % halothane in oxygen and placed in the supine position. After local infiltration with 0.25 % bupivacaine 0.2 ml, the skin was incised in the supraclavicular area, 2–3 mm lateral to the midline. The right external jugular vein was exposed and cannulated with a sterile catheter (PE-50 tubing, Clay Adams, Parsippany, NJ, USA) filled with normal saline containing heparin 100 u. ml^{-1}. The catheter was tunnelled subcutaneously and exteriorized to the interscapular area in the back. After operation rats were housed in individual cages and allowed to recover for at least 48 h before they were used for the experiments. To control for known diurnal fluctuations in responsiveness to nociceptive stimuli [6], experiments were performed between the hours of 10:00 and 22:00 in random order.

TAIL-FLICK TEST

Rats were allocated to one of 17 anaesthetic groups as follows: (1) 75 % nitrous oxide ($n = 14$); (2) 0.5 MAC of halothane (0.9 % inspired concentration; $n = 5$) (3) 0.5 MAC of isoflurane (1.1 %; $n = 6$); (4) 100 % oxygen without anaesthetic ($n = 5$); (5–7) 75 % nitrous oxide with 0.1, 0.2 or 0.5 MAC of halothane ($n = 6$, 4, 5 per group, respectively);

TAKAHISA GOTO*, MD, JOHN J. A. MAROTA, PHD, MD, GREGORY CROSBY†, MD, Anesthesia Services, Massachusetts General Hospital and Department of Anaesthesia, Harvard Medical School, Boston, MA 02114, USA. Accepted for publication: December 13, 1995.

*Present address: Department of Anaesthesia, Teikyo University Ichihara Hospital, 3426-3 Anesaki, Ichihara, Chiba 299-01 Japan.

†Address for correspondence: Department of Anesthesiology, University of Wisconsin, B6/319 Clinical Sciences Center, 600 Highland Avenue, Madison, Wisconsin 53747, USA.

(8–10) 75 % nitrous oxide with 0.1, 0.2 or 0.5 MAC of isoflurane ($n = 6$ per group); (11) morphine 1.25 mg kg^{-1} i.v.; (12–14) morphine 1.25 mg kg^{-1} i.v. with 0.2, 0.5 or 0.7 MAC of halothane; (15–17) morphine 1.25 mg kg^{-1} with 0.2, 0.5 or 0.7 MAC of isoflurane ($n = 5$ per group). The inspired concentrations of halothane and isoflurane were calculated on the basis of reported MAC values in the rat of 0.95–1.11 % for halothane [7–9] and 1.38–1.58 % for isoflurane [7–9], and an estimated ratio of end-tidal to inspired concentration of halothane and isoflurane of 0.5–0.6 and 0.7–0.8, respectively, in spontaneously breathing rats after 20 min [7]. The dose of morphine (1.25 mg kg^{-1} i.v.) was chosen because in preliminary experiments it provided the same degree of analgesia as 75 % nitrous oxide.

Anaesthesia was induced by placing the animals in a plexiglass box prefilled and flushed continuously with 3 litre min^{-1} of the anaesthetic in oxygen. Oxygen 100 % was given to animals that received morphine alone. The concentrations of nitrous oxide (Ohmeda 5200 CO_2 analyzer, Madison, WI, USA), halothane, isoflurane (Datex 222 anesthetic agent analyzer, Puritan Bennett, Tewksbury, MA, USA) and oxygen (Ohmeda 5100 oxygen analyzer), and the temperature inside the box were measured continuously. Animals were left undisturbed for 20 min so that they would reach a steady state of anaesthesia. They were then removed briefly from the box ($<$ 30 s) and the tail-flick test was performed. Immediately after one measurement was made, animals were returned to the box and maintained under anaesthesia for another 3 min. Then the tail-flick test was repeated and these two measurements were averaged to yield the tail-flick latency (TFL) during anaesthesia. In addition, the control tail-flick response for each rat was determined by averaging duplicate measurements made in the pre-anaesthetic awake state.

The tail-flick test was performed by placing the tail of each rat 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 pre-anaesthetic TFL was typically in the range 1.5–1.8 s. Results of the test are expressed as maximum percentage effect (MPE) according to the formula:

$$\text{MPE} = \frac{(\text{TFL during anaesthesia}) - (\text{pre-anaesthetic TFL})}{(\text{cut-off time}) - (\text{pre-anaesthetic TFL})} \times 100(\%)$$

For this part of the study, each animal was used twice with at least a 5-day interval between the first and second experiments.

In order to determine the effect of the anaesthetic regimens on physiological variables such as tail skin temperature, Pa_{CO_2} and arterial pressure that can affect tail-flick latency [10–12], additional rats were prepared with femoral artery and vein catheters during approximately 15 min of 1 % halothane–75 % nitrous oxide anaesthesia. Rats were then partially immobilized with a pelvic plaster cast and allowed at least 3 h to recover from surgery and anaesthesia. Rats subsequently received one of the following anaesthetic regimens: (1) 75 % nitrous oxide ($n = 8$); (2, 3) 75 % nitrous oxide with 0.5 MAC of halothane or isoflurane ($n = 4$ per group); (4) morphine 1.25 mg kg^{-1} i.v. ($n = 8$); (5, 6) morphine with 0.5 MAC of halothane or isoflurane ($n = 4$ per group). At the end of 20 min of anaesthesia, tail temperature, mean arterial pressure (MAP) and arterial blood-gas tensions were measured. For measurement of tail temperature, a hypodermic needle temperature probe (Model HYP2; Omega, Stamford, CT, USA) was inserted 2 mm into the skin between the middle and distal thirds of the ventral aspect of the tail (i.e. the portion of the tail where thermal stimuli were applied during the tail-flick test) and temperature recorded by a microprocessor thermometer (Model HH21 : Omega, Stamford, CT, USA). For this part of the study, each animal was used only once.

DATA ANALYSIS

To evaluate the effects of volatile anaesthetics on nitrous oxide or morphine analgesia, a regression line of the log doses of a volatile anaesthetic *vs* tail-flick latency MPE values was first constructed by the least square method for each combination of anaesthetics. The IC_{50} (the inspired concentration of a volatile anaesthetic which produces 50 % attenuation of nitrous oxide or morphine-induced analgesia) and 95 % confidence intervals were then calculated. Analysis of variance (ANOVA) was used to compare the effect of halothane, isoflurane or 100 % oxygen alone on TFL. ANOVA and Dunnett's test were used to compare physiological data from animals that received volatile anaesthetics with nitrous oxide or morphine with data from those that received nitrous oxide or morphine alone. $P < 0.05$ was considered significant.

Results

Nitrous oxide 75 % increased tail-flick latency (mean MPE 37 (SEM 5) %) but 0.5 MAC of halothane or isoflurane did not alter the response (MPE -4.1 (2) % and -1.5 (1) %, respectively; $F = 0.89$, df = 2 and 13, $P > 0.20$). Both volatile anaesthetics dose-dependently antagonized nitrous oxide analgesia (fig. 1A) and halothane was more potent than isoflurane in this regard. Halothane doses as low as 0.1 and 0.2 MAC produced 76 % and 107 % antagonism of nitrous oxide analgesia, respectively; as the antagonism produced by the lowest concentration was greater than 50 %, we could only estimate an IC_{50} of $<$ 0.1 MAC (confidence level 95 %). Isoflurane 0.5 MAC also induced almost complete (85 %) antagonism, but lower doses were less effective (IC_{50} = 0.23 MAC; 95 % confidence interval 0.18–0.30 MAC).

By design, morphine 1.25 mg kg^{-1} alone prolonged tail-flick latency to the same extent as 75 % nitrous oxide (MPE 35 (9)%). Halothane dose-dependently antagonized this morphine analgesia, with 0.7 MAC producing 80 % antagonism (IC_{50} = 0.43 MAC; 95 % confidence interval 0.36–0.51 MAC) (fig. 1B).

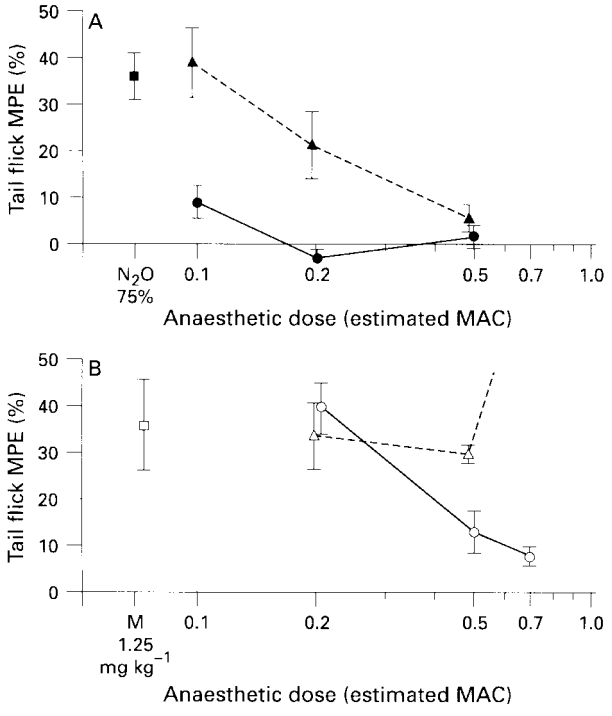

Figure 1 Effect of volatile anaesthetics on nitrous oxide (N_2O) or morphine-induced analgesia. Tail-flick testing was performed twice each before and 20 min after the start of anaesthesia, and the measured latencies were converted to maximum percentage effect (MPE) according to the formula described in the text. (A) N_2O–halothane (●) and N_2O–isoflurane (▲) interactions. (B) Morphine–halothane (○) and morphine–isoflurane (△) interactions. Anaesthetic dose is the dose of volatile anaesthetic added to N_2O or morphine, respectively. The combination of morphine and 0.7 MAC of isoflurane prolonged the tail-flick latency above the cut-off time (i.e. MPE >100 %) in all animals. Data are mean, SEM for 4–14

In contrast, isoflurane did not attenuate morphine analgesia at either 0.2 or 0.5 MAC and, at 0.7 MAC, prolonged tail-flick latency beyond the cut-off time (MPE >100 %; fig. 1B). However, 0.7 MAC of isoflurane combined with morphine also produced some degree of motor dysfunction (i.e. mild flaccidity with some impairment of weight-bearing), suggesting that the prolonged tail-flick response may not be caused entirely by the antinociceptive effects of these agents.

Halothane did not alter tail temperature, whereas isoflurane in combination with nitrous oxide reduced it slightly ($P < 0.05$; table 1). MAP was lower in rats that received a volatile anaesthetic but remained within the physiological range. Morphine-treated rats became hypercapnic and halothane, but not isoflurane, increased Pa_{CO_2} slightly in both nitrous oxide and morphine-treated animals (table 1).

Discussion

We have demonstrated that sub-MAC concentrations of volatile anaesthetics dose-dependently antagonized the analgesic action of nitrous oxide, and that halothane was more potent than isoflurane. In addition, halothane antagonized morphine-induced analgesia in a dose-dependent manner, whereas isoflurane has no effect. As higher doses of halothane were required to antagonize morphine compared with nitrous oxide analgesia, and isoflurane antagonized the effect of nitrous oxide but not morphine, we conclude that nitrous oxide was more sensitive than morphine to analgesic antagonism by volatile anaesthetics.

In previous work using the formalin test to assess nociception [1], we demonstrated that nitrous oxide analgesia can be antagonized by halothane, and these results have been reproduced recently by others [13]. Antagonism between nitrous oxide and volatile anaesthetics has also been reported for MAC in the rat [8, 14] and suppression of learning in humans [15], although interpretation of the MAC studies is a subject of much debate [16–18]. Similarly, a marked morphine–halothane antagonism has been reported for the escape reaction threshold to tail pressure [19] and the cardiac acceleration response to tail clamp [20] in the rat, but the agents were additive in suppressing noxious stimulation-induced purposeful movements [20]. Thus the nature of these drug interactions may vary with the end-point.

It appears unlikely that alterations in physiological variables account for the observed antagonism between the volatile anaesthetics and nitrous oxide or morphine. The isoflurane-induced decrease in tail skin temperature cannot account for attenuation of nitrous oxide analgesia as tail hypothermia has the opposite effect (i.e. prolongs tail-flick latency) [10]. However, the fact that isoflurane, but not halothane, reduced tail skin temperature may be at least partially responsible for the weaker antagonism of nitrous oxide or morphine analgesia produced by isoflurane than halothane. Similarly, moderate hypercapnia (Pa_{CO_2} 8.0–13.3 kPa) prolongs tail-flick latency [11], and therefore slight increases in Pa_{CO_2} produced by the addition of halothane would be expected to intensify, rather than antagonize, nitrous oxide and morphine analgesia. With respect to arterial pres-

Table 1 Physiological data (mean (SEM)) for eight (nitrous oxide (N_2O) or morphine alone groups) or four (combination groups) animals. Data of animals that received the combination of volatile anaesthetic and N_2O or morphine were compared with those that received N_2O or morphine, alone respectively, by ANOVA and Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$

Anaesthesia	MAP (mm Hg)	Tail temp (°C)	pH	Pa_{CO_2} (kPa)	Pa_{O_2} (kPa)
N_2O	138 (1)	28.5 (0.5)	7.42 (0.01)	5.3 (0.1)	16.8 (0.4)
N_2O + halothane	123 (4)**	28.1 (0.6)	7.38 (0.01)*	5.8 (0.1)**	16.7 (0.9)
N_2O + isoflurane	111 (2)**	25.8 (0.2)**	7.43 (0.02)	4.9 (0.4)	17.2 (0.7)
Morphine	136 (2)	26.3 (0.4)	7.36 (0.01)	6.3 (0.1)	60.3 (1.2)
Morphine + halothane	125 (5)*	26.8 (0.7)	7.29 (0.02)**	7.0 (0.2)	64.3 (0.9)
Morphine + isoflurane	110 (2)**	25.7 (0.2)	7.38 (0.01)	6.2 (0.2)	61.9 (1.2)

sure, hypertension is known to produce antinociception [12], but we know of no data on the effect of hypotension. If a mild decrease in arterial pressure were the mechanism underlying antagonism of nitrous oxide and morphine analgesia by volatile anaesthetics, isoflurane should produce greater antagonism than halothane because arterial pressure was lower with isoflurane. This again contradicts our results.

Although neither halothane nor isoflurane alone altered tail-flick latency, these anaesthetics could have intrinsic hyperalgesic properties. In fact, halothane has been reported to slightly decrease the pain threshold to pressure in humans [21]. In our study, the short baseline tail-flick latency caused by intense heat stimuli might have obscured such effects. However, studies with tail-flick testing with a longer baseline latency (5–6 s) have shown that sub-MAC concentrations of halothane and isoflurane modestly prolong tail-flick latency in rodents [22, 23]. In addition, the volatile agents inhibit noxious heat-evoked firing of spinal wide dynamic range nociceptive neurones *in vivo* [24] and suppress electrical stimulation-induced nociceptive transmission in the spinal cord *in vitro* [25]. Hence, it is unlikely that the hyperalgesic properties of the volatile anaesthetics explain the observed antagonism between volatile agents and nitrous oxide or morphine. It is also not likely that volatile anaesthetics possess opioid antagonist properties as, although both morphine and nitrous oxide activate opioid receptors either directly or indirectly [1, 3], halothane does not displace specific binding of radiolabelled ligands to either μ or κ receptor subtypes *in vitro* [26, 27]. We have postulated previously, however, that volatile anaesthetics may antagonize analgesia by a metabolic mechanism [1]. Nitrous oxide and morphine analgesia require active neural processes, including activation of a descending inhibitory pathway [28, 29]. Thus by decreasing the cerebral or spinal metabolic rate, halothane or isoflurane could prevent such neural activation. Although unproved, this hypothesis could explain why nitrous oxide is more susceptible than morphine to antagonism by volatile anaesthetics; spinal transection studies suggest that a descending inhibitory pathway plays a more important role in mediating nitrous oxide than morphine analgesia [28, 29].

Although tail-flick testing is a widely accepted measure of analgesia in animals, extrapolation of these results to the clinical setting requires caution. First, as the MAC of nitrous oxide in rats is 148–235 % [18, 30, 31], we have used only 0.3–0.5 MAC of nitrous oxide, which is lower than that usually administered clinically. It is natural to speculate that the analgesic effect of higher doses of nitrous oxide or morphine may be more resistant to antagonism by volatile anaesthetics. In fact, profound analgesia produced by morphine 10 mg kg^{-1} i.v. (the tail-flick MPE >100 %) was not affected by up to 1 MAC of halothane [unpublished observation]. Second, the tail-flick response is a spinal reflex [4] and such measures are not used clinically to evaluate the adequacy of anaesthesia, analgesia, or both. Third, as discussed previously, the nature of the interaction among anaesthetics appears to vary for different end-points [1, 8, 14, 19, 20]; integrated, complex pain processes may be affected differently than a spinal reflex. Nevertheless, our experiments demonstrate that a simple relationship between anaesthesia and analgesia cannot be assumed because addition of a volatile anaesthetic to nitrous oxide or morphine, while deepening anaesthesia, resulted in less profound analgesia. Thus anaesthesia and analgesia are different end-points and should be considered separately.

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