Nitrous Oxide Revisited

Evidence for Potent Antihyperalgesic Properties

Philippe Richebé, M.D., Ph.D.,* Cyril Rivat, Ph.D.,† Cyril Creton, M.Sc.,† Jean-Paul Laulin, Ph.D.,‡ Pierre Maurette, M.D., Ph.D.,§ Marc Lemaire, M.D.,¶ Guy Simonnet, Ph.D.¶

Background: Although opioids are unsurpassed analgesics for surgery, they also induce an N-methyl-D-aspartate–dependent enhancement of postoperative hyperalgesia. Because nitrous oxide (N₂O) has anti-N-methyl-D-aspartate properties, the purpose of this study was to evaluate nitrous oxide ability to prevent such an opioid-induced hyperalgesia in rats.

Methods: First, preventive effects of 50/50% N₂O–O₂ on the development of delayed hyperalgesia observed after inflammatory pain (hind paw carrageenan injection on D₀) were examined for several days. Second, the ability of nitrous oxide (10–40%) to limit opioid-induced hyperalgesia induced by fentanyl was evaluated in nonsuffering rats. Third, antihyperalgesic effects of various nitrous oxide concentrations (20–50%) were assessed in both inflammatory and incisional pain models in fentanyl-treated rats (4 × 100 μg/kg subcutaneously). Finally, the analgesic effect of a single dose of morphine was evaluated 24 h after fentanyl administration and nitrous oxide (D₀) to assess its preventive effect on acute morphine tolerance in both nonsuffering and hind paw–incised rats.

Results: When applied on D₀, nitrous oxide reduced delayed hyperalgesia induced by inflammation. Exposure to nitrous oxide on D₀ also reduced opioid-induced hyperalgesia in nonsuffering rats in a dose-dependent manner. In fentanyl-treated rats with inflammatory or incisional pain, nitrous oxide strongly limited both magnitude and duration of hyperalgesia. Moreover, nitrous oxide exposure on D₀ opposed development of acute tolerance to analgesic effects of morphine administered on D₀ in both nonsuffering and incised fentanyl-treated rats.

Conclusions: Nitrous oxide, an N-methyl-D-aspartate receptor antagonist, prevented the enhancement of pain sensitivity induced by both nociceptive inputs and fentanyl and opposed acute morphine tolerance. Results suggest that perioperative nitrous oxide use reduces exaggerated postoperative pain and morphine consumption.

* Staff Anesthesiologist, Department of Anesthesia and Intensive Care II, Centre Hospitalier et Universitaire de Bordeaux, Bordeaux, France. Ph.D. Student, Laboratoire Homeostasie-Allostasie-Pathologie EA3666, Université Victor Segalen-Bordeaux 2. † Staff Researcher, # Professor, Laboratoire Homeostasie-Allostasie-Pathologie EA3666, Université Victor Segalen-Bordeaux 2. ‡ Assistant Professor Université Bordeaux 1, Bordeaux, France. § Professor, Department of Anesthesiology and Intensive Care III, Centre Hospitalier et Universitaire de Bordeaux, Bordeaux, France. ¶ M.D., Medical Gases Domain Director, Research and Development, Claude-Delorme Research Center, Air Liquide, Jouy-en-Josas, France.


Address reprint requests to Dr. Simonnet: Laboratoire Homeostasie-Allostasie-Pathologie EA3666, Université Victor Segalen-Bordeaux 2, 146, rue Léo Saignat, 33076 Bordeaux Cedex, France. Address electronic mail to: gsimonnet@yahoo.com. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

NITROUS oxide is commonly used in humans for pain relief. Experimental animal studies have revealed that nitrous oxide induces opioid peptide release in the periaqueductal brainstem, leading to disinhibition (activation) of the descending noradrenergic inhibitory pathways via inhibition of γ-aminobutyric acid–mediated interneurons. This results in a negative modulation of the nociceptive processes at the spinal cord level.¹–³

A challenging hypothesis is that the nitrous oxide analgesic effect is not limited to its antinociceptive effect via endogenous opioids systems but may also be due to the preventive blockade of pain sensitization induced by nociceptive inputs. It is generally recognized that tissue damage associated with surgical lesions or inflammations often produces peripheral and central sensitization that may outlast the stimuli leading to sustained hyperalgesia, allodynia, and persistent pain.⁴–⁷ At the central level, many experimental studies have shown a critical role for excitatory amino acids to injury-induced pain sensitization via N-methyl-D-aspartate (NMDA) receptors. Because the aim of preemptive analgesia is to reduce central sensitization that arises from surgical noxious inputs, many clinical studies have evaluated the effectiveness of several NMDA receptor antagonists for improving postoperative pain management. Clinical studies using intravenous low doses of NMDA receptor antagonists have reported controversial results in humans.⁸ However, ketamine and dextromethorphan have demonstrated promising antihyperalgesic effects in several clinical trials leading to a reduction in both postoperative pain and morphine consumption.⁹–¹¹

Because nitrous oxide was recently shown to be an NMDA receptor antagonist,¹² one hypothesis is that nitrous oxide should have beneficial antihyperalgesic properties mimicking the ketamine ones, especially when large opioid doses are used during surgery. Experimental¹³–¹⁸ and clinical studies¹⁹–²¹ have reported that opioids may paradoxically facilitate the activation of NMDA-dependent pronociceptive systems leading to exaggerated postoperative pain.

The purpose of the current study was to evaluate the nitrous oxide potency for preventing pain sensitization induced by nociceptive inputs and high doses of fentanyl.¹⁶–¹⁸ To assess such a hypothesis, we first evaluated the effect of a 50/50% N₂O–O₂ mixture in rats with inflammatory pain induced by a unilateral hind paw injection of the proinflammatory drug carrageenan. Second, we tested the effects of different nitrous oxide concentrations on the development of hyperalgesia in-
duced by high doses of fentanyl in nonsuffering rats. Third, we evaluated preventive effects of different nitrous oxide concentrations on the fentanyl enhancement of hyperalgesia induced by inflammatory or incisional nociceptive stimuli. Fourth, the 50/50% \text{N}_2\text{O} - \text{O}_2 mixture perioperative use was tested for evaluating its effectiveness to prevent acute morphine tolerance observed after such a hind paw surgery associated with perioperative high doses of fentanyl.\textsuperscript{18}

**Materials and Methods**

**Animals**

Experiments were performed on adult male Sprague-Dawley rats (Charles River Laboratories, l’Abresle, France) weighing 300–350 g, housed in groups of five per cage with a 12-h light–12-h dark cycle (lights on at 7:00 AM) at a constant room temperature of 23° ± 2°C. The animals had access to food and water ad libitum. Pharmacologic tests and care of the animals were conducted in accordance with the Animals Care and Use manual of the National Institutes of Health (Bethesda, MD, National Institutes of Health, 1999). This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. When the experiments were done, the rats were killed with carbon dioxide. These experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (J.-P. L.).

**Drugs**

Fentanyl citrate, morphine, naloxone, and carrageenan \(\lambda\) (Sigma-Aldrich, Saint-Quentin Fallavier, France) were dissolved in physiologic saline (0.9%). Fentanyl (60 and 100 \(\mu\)g/kg), morphine (2 and 3 mg/kg), and naloxone (1 mg/kg) were administered subcutaneously (1 ml/kg body weight). Control animals received an equal volume of saline injections. Carrageenan (0.2 ml of a 1% carrageenan solution in saline) was prepared 24 h before each experiment. With regard to the incisional pain model, an ointment with 2% Fucidine (Léo, St. Quentin-en-Yvelines, France) and Primyxine (oxytetracycline hydrochloride and polymyxin B sulfate; Chemineau, Vouvray, France) was placed on the wound after the surgery. Nitrous oxide (Air Liquide Santé France, Paris, France) was delivered \textit{via} bottles containing premixed nitrous oxide, oxygen, and nitrogen. Different concentrations were used for nitrous oxide, oxygen, and nitrogen: oxygen was set at 50% in all cases, nitrous oxide varied from 10 to 50%, and nitrogen varied from 0 to 40%.

**Exposures to Gas**

All exposures to gas were performed in a plexiglas chamber (42 cm long, 26 cm wide, 26 cm high) with a sliding door on one side to insert rats. Five rats were introduced in each chamber. Fresh gases were fed into the chamber through an inlet port (4 l/min) and purged by a vacuum set for sucking out the gas at the same rate as the fresh gas inflow. Oxygen and nitrous oxide concentrations were continuously monitored to confirm the gases’ concentrations. All gas exposures were initiated 15 min before the beginning of each experiment and were followed for 4 h of exposure. The total gas exposure time was 4 h 15 min.

**Measurement of Nociceptive Threshold**

Nociceptive thresholds in handheld rats were determined by a modification of the Randall-Selitto method.\textsuperscript{22} the paw-pressure vocalization test, in which a constantly increasing pressure is applied to the hind paw until the rat squeaks. The Basile analgesimeter (Aplex, Massy, France; stylus tip diameter, 1 mm) was used. A 600-g cutoff value was determined to prevent tissue damage.

**Carrageenan Injection**

On D\textsubscript{0}, the basal value of the nociceptive threshold was evaluated, and rats were placed in a plastic cage and then anesthetized with 3% halothane for 3 min. Carrageenan (0.2 ml of a 1% carrageenan solution in saline) was then injected into one rat plantar hind paw subcutaneously. Injections were performed with a 25-gauge needle.

**Surgical Procedure**

Just before the surgery, rats were anesthetized with 1–3% halothane vaporized \textit{via} a nose cone. The plantar aspect of the operated hind paw was prepared in a sterile manner with 5% povidone iodine solution, and the foot was placed through a hole in a sterile drape. As previously described,\textsuperscript{23} a 1-cm long incision, starting 0.5 cm from the heel and extending toward the toes, was made with a No. 11 blade, through skin and fascia of the plantar aspect of the left hind paw including the underlying muscle. The plantaris muscle was then raised and incised longitudinally, leaving the muscle origin and insertion intact. After hemostasis with gentle pressure, the skin was apposed with two mattress sutures of 5-0 nylon on a curved needle. The wound site was covered with an antibiotic mixture of polymyxin B, oxytetracycline, and fusidate. At the end of the surgery, halothane was stopped, and rats were allowed to recover in the plastic box breathing the nitrous oxide–oxygen–nitrogen mixture according to the group to which they were allocated.

**General Procedure**

After arrival in the laboratory, animals were acclimated to the animal care unit for 4 days. To avoid stress resulting from the experimental conditions that might affect measurement of the nociceptive threshold, the experiments were performed by the same experimenter in quiet conditions in a testing room close to the animal.
Nitrous oxide is an antihyperalgesic drug

Experimental Protocols

In a preliminary experiment, the 50/50% N₂O–O₂ treatment administered on D₀ was compared with air in naive rats. This experiment was conducted to evaluate whether 50% O₂ concentration used in the following five sets of experiment had any effect per se on the nociceptive threshold.

Experiment 1: Administration of the 50/50% N₂O–O₂ Treatment in Rats with a Carrageenan Inflammation. Rats were allocated to one of the following groups: (1) N₂O–O₂ 50/50%, (2) air, for 4 h after starting the exposure to gas. Nociceptive threshold measurements were performed 2, 4, and 6 h after carrageenan injection (D₀) and once daily during the 7 subsequent days (D₁–D₇). Nociceptive threshold was observed for 3 successive days (D₂–D₄) and repeated on D₅ (one-way analysis of variance, P > 0.05). The reference value of the nociceptive threshold was chosen as the basal value on D₀. The experimenter was unaware of the administered treatment.

Experiment 2: Administration of Different Concentrations of Nitrous Oxide–Oxygen–Nitrogen in Fentanyl-treated Rats. Two hours after the basal nociceptive threshold measurement on D₀, one fentanyl (100 µg/kg) injection (or saline) was performed four consecutive times every 15 min, resulting in a total dose of 400 µg/kg. Exposure to 50% O₂ concentration used in the following five sets of experiment had any effect per se on the nociceptive threshold during the 7 subsequent days (D₈–D₁₄). When rats had returned to the basal nociceptive threshold value, one naloxone injection (1 mg/kg subcutaneously) was performed on D₀, and the nociceptive threshold was measured 5 min later.

Experiment 3: Administration of Various Concentrations of Nitrous Oxide–Oxygen–Nitrogen Treatment in Rats with a Carrageenan Inflammation and Treated by Fentanyl on D₀. Fentanyl was administered as described in the second set, resulting in a total dose of 400 µg/kg. Five min after the first fentanyl injection, rats received a carrageenan injection. Exposure to gas began 15 min before the first fentanyl administration. Rats breathed air or different concentrations of nitrous oxide–oxygen–nitrogen (20/50/30, 30/50/40, 40/50/10, and 50/50/0%, respectively) for 4 h after the first fentanyl injection. Nociceptive threshold measurements were performed 2, 4, 6, and 8 h after fentanyl injection (D₀) and once daily during the 12 subsequent days (D₁–D₁₂). When rats had returned to the basal nociceptive threshold value, one naloxone injection (1 mg/kg subcutaneously) was performed on D₁₂, and the nociceptive threshold was measured 5 min later.

Experiment 4: Administration of 50/50% N₂O–O₂ in Rats Scheduled for Left Foot Plantar Incision and Treated by Fentanyl on D₀. Fentanyl was administered as described in the second set, resulting in a total dose of 400 µg/kg. Exposure to nitrous oxide–oxygen began in the Plexiglas chamber 15 min before the first fentanyl injection. All rats received a left foot plantar incision under halothane anesthesia 15 min after the first fentanyl injection. Then, they recovered in the Plexiglas chamber breathing for 4 h either the equimolar mixture of nitrous oxide–oxygen or air according to the original group to which they were allocated. Nociceptive thresholds were estimated according the design of the third set of experiments, except that the naloxone test was performed on D₀.

Experiment 5: Morphine Administration on D₁ after Exposure to the 50/50% N₂O–O₂ Treatment in the Incisional Pain Model. In a first phase (experiment 5A), the analgesic efficiency of 2 mg/kg subcutaneous morphine was estimated 24 h after fentanyl injection (4 × 60 µg/kg) in rats that breathed air or the 50/50% N₂O–O₂ for 4 h during the analgesic effect of fentanyl (D₀). Control of morphine effectiveness was estimated in rats receiving saline instead of fentanyl on D₀. Nociceptive threshold was estimated every 30 min for 6 h after fentanyl injection on D₀ and for 3 h after morphine injection on D₁. Nociceptive thresholds were also estimated every day for the 7 subsequent days. A naloxone test was performed on D₇, as described previously.

In a second phase (experiment 5B), analgesic efficiency of morphine (3 mg/kg) was estimated on D₁, 24 h after one plantar incision (D₀) in fentanyl-treated rats (4 × 100 µg/kg) breathing air or the 50/50% N₂O–O₂ for 4 h during the analgesic effect of fentanyl (D₀). Nocicep-
tive threshold was estimated every 2 h for 8 h after fentanyl injection on D0, and every 30 min for 1.5 h after morphine injection on D1. Nociceptive thresholds were also estimated daily for the 8 subsequent days. A naloxone test was performed on D8 as described previously.

Calculation and Statistical Analysis

To evaluate the time course effects of treatments on nociceptive threshold (basal reference value: precarrageenan or presurgery value on D0 for all experiments and initial reference value for the premorphine value on D1 for morphine analgesia), an analysis of variance followed by post hoc analysis using the Dunnett test was performed on D0, and another one was performed on the days after the treatments in each group. The Mann-Whitney test was used to compare the morphine analgesic indexes. Analgesic indexes for morphine-induced analgesia represented by the area under the curve were expressed as a mean percentage (± SD) of the reference threshold in rats breathing air (data not shown) (open circles = 50/50% N2O–O2-treated rats (n = 10); filled circles = air-treated rats (n = 10).

Results

Effect of 50% O2 Concentration on the Nociceptive Threshold (Preliminary Experiment)

No effect of oxygen was observed on the nociceptive threshold in rats breathing 50% O2 as compared with rats breathing air (data not shown) (P > 0.05).

Effect of 50/50% N2O–O2 on Nociceptive Threshold in Rats with a Hind Paw Inflammation Induced by One Plantar Carrageenan Injection (Experiment 1)

In rats exposed to air, the plantar carrageenan injection in one foot induced a decrease of the nociceptive threshold on the ipsilateral and contralateral paws on D0, which lasted 4 and 2 days, respectively (figs. 1A and B; Dunnett test, P < 0.05). In rats exposed to 50/50% N2O–O2, a smaller decrease of the nociceptive threshold was observed on D0 and on D1 for both hind paws (figs. 1A and B; Dunnett test, P < 0.05). When injected on D1, naloxone induced a marked decrease of the nociceptive threshold, which was smaller for both hind paws in nitrous oxide–oxygen-breathing rats as compared with the reduction observed in air-breathing rats (Dunnett test, P < 0.05).

Effect of Different Concentrations of Nitrous Oxide–Oxygen–Nitrogen on Delayed Fentanyl-induced Hyperalgesia in Rats (Experiment 2)

As described previously, fentanyl administration induced analgesia followed by both immediate (hours) and delayed hyperalgesia for several days (figs. 2A–D). Exposure to nitrous oxide (10, 20, 30, or 40%) completely reduced the immediate hyperalgesia observed after analgesia on D0 (Dunnett test, P > 0.05). Exposure to nitrous oxide on D0 also induced a dose-dependent reduction in the delayed nociceptive threshold decrease observed for several days in air-treated rats. When nitrous oxide was used at only 10% concentration, the nociceptive threshold decrease was still significant for 2 days (Dunnett test, P < 0.05; fig. 2A) and completely suppressed for exposures to the highest nitrous oxide concentrations (Dunnett test, P > 0.05). Comparison between air and nitrous oxide–treated rats indicated a significant difference for 3 days with 10 and 20% concentrations and for 5 days with 30 and 40% concentrations (Dunnett test, P < 0.05). Naloxone injected on D2 induced a significant decrease of the nociceptive threshold in rats preexposed to air or 10% N2O (Student t test, P < 0.05) but not in rats preexposed to 20, 30, and 40% N2O (Student t test, P > 0.05).
Effect of Various Concentrations of Nitrous Oxide–Oxygen–Nitrogen on Both Fentanyl-induced Analgesia and Long-lasting Hyperalgesia in Rats with Hind Paw Inflammation (Experiment 3)

As shown in figure 3, exposure to nitrous oxide enhanced fentanyl analgesic effect for the highest gas concentrations (Dunnett test, \( P < 0.05 \)). Fentanyl analgesic effect was followed by a large and sustained decrease of the nociceptive threshold for several days in air-treated rats (Dunnett test, \( P < 0.05 \)). When nitrous oxide was administered on D0 at 20% concentration, no change in the nociceptive threshold decrease was noticed with rats breathing air (Dunnett test, \( P > 0.05 \); fig. 3A). Preexposure of rats to 30% \( N_2O \) induced a significant difference for 1 day as compared with rats breathing air (Dunnett test, \( P < 0.05 \); fig. 3B). When used at 40 and 50% concentrations, nitrous oxide reduced the nociceptive threshold decrease for several days as compared with rats breathing air (Dunnett test, \( P < 0.05 \); figs. 3C and D). When injected on D12, naloxone induced a significant decrease in the nociceptive threshold in rats preexposed to air or 20–40% \( N_2O \) (Student \( t \) test, \( P < 0.05 \)) but not in rats preexposed to 50% \( N_2O \) (Student \( t \) test, \( P > 0.05 \)).
Effect of 50/50% N\textsubscript{2}O–O\textsubscript{2} on Both Fentanyl-induced Analgesia and Delayed Fentanyl-induced Hyperalgesia in Rats with Plantar Incision (Experiment 4)

As shown in figure 4, exposure to 50/50% N\textsubscript{2}O–O\textsubscript{2} during the analgesic effect of fentanyl induced an enhancement of analgesia (Dunnett test, \( P < 0.05 \)). In air-treated rats, fentanyl analgesic effect was followed by a large and sustained decrease of the nociceptive threshold for 4 days (Dunnett test, \( P < 0.05 \)). In rats treated with nitrous oxide on D\textsubscript{0}, decrease of nociceptive threshold was limited to 3 days (Dunnett test, \( P < 0.05 \); fig. 4).

When injected on D\textsubscript{8} in rats that had returned to the basal nociceptive threshold, naloxone induced a smaller decrease in the nociceptive threshold as compared with rats preexposed to air (Dunnett test, \( P < 0.05 \)).

Effect of 50/50% N\textsubscript{2}O–O\textsubscript{2} on Acute Tolerance to Morphine Analgesic Effect (Experiment 5)

In a first experiment (experiment 5A), tolerance to morphine analgesic effect was assessed in rats on D\textsubscript{1} during hyperalgesia induced by fentanyl administration performed on the day before (D\textsubscript{0}). As compared with the analgesic effect observed in non–fentanyl-treated rats,
NITROUS OXIDE IS AN ANTIHYPERALGESIC DRUG

Fig. 4. Effect of premixed 50/50% N₂O–O₂ treatment on fentanyl-incipision-induced hyperalgesia. The fentanyl injection was performed on D₀. The first fentanyl injection was performed 15 min before the plantar incision and every 15 min for a total dose of 4 × 100 µg/kg. The nitrous oxide or air was administered to rats starting from 15 min before the first fentanyl injection and for 4 h. Nociceptive threshold was assessed on D₀, D₁, D₂ and D₃ then every 2 h after the first fentanyl injection until 8 h on D₆ and subsequently once daily for 8 days. When the rats had returned to the basal nociceptive threshold (D₈), naloxone was injected (1 mg/kg subcutaneously), and the nociceptive threshold was evaluated 5 min later. Nociceptive threshold is expressed as mean ± SD. * Dunnett test, P < 0.05 compared with the D₁ basal value. # Dunnett test, P < 0.05 for comparison between groups. Open circles = N₂O–O₂–fentanyl-treated rats (n = 10); filled circles = air-treated rats (n = 10).

Fig. 5. Effect of premixed 50/50% N₂O–O₂ treatment on fentanyl-induced hyperalgesia and acute morphine tolerance. The fentanyl or saline injections were performed on D₀ (total dose: 4 × 60 µg/kg or same volume of saline). The nitrous oxide or air was administered to rats from 15 min before the first fentanyl injection and for 4 h. Morphine (2 mg/kg subcutaneously) was injected on D₁ 24 h later. Nociceptive threshold was assessed at the hind paw level on D₁, D₄, and D₇ every 30 min after the first fentanyl injection and to 6 h on D₈; every 30 min after morphine injection on D₁ for 3 h; and subsequently once daily for 7 days. On D₈, all rats were injected with naloxone (1 mg/kg subcutaneously), and the nociceptive threshold was evaluated 5 min later. Nociceptive threshold is expressed as mean ± SD. * Dunnett test, P < 0.05 compared with the D₁ basal value. # Dunnett test, P < 0.05 for comparison between groups. Open circles = N₂O–O₂–fentanyl-treated rats (n = 10); filled circles = air–fentanyl-treated rats; white square = air–saline-treated rats; black square = air–saline–fentanyl-treated rats; dashed square = N₂O–O₂–fentanyl-treated rats. Tolerance to morphine analgesic effect as indicated by the reduction of nociceptive inputs and its enhancement by fentanyl, coadministration of 50/50% N₂O–O₂ with fentanyl reduced acute tolerance to the analgesic effect of postoperative morphine.

Discussion

This experimental investigation on animals shows that nitrous oxide, an NMDA receptor antagonist, is able to reduce fentanyl-induced hyperalgesia observed after analgesia in a dose-dependent manner. Moreover, by preventing the development of pain hypersensitivity induced by nociceptive inputs and its enhancement by fentanyl, coadministration of 50/50% N₂O–O₂ with fentanyl reduced acute tolerance to the analgesic effect of postoperative morphine.

As expected, when applied for 4 h in rats with unilateral inflammation, the 50/50% N₂O–O₂ mixture induced an antinociceptive effect as indicated by the reduction of nociceptive threshold decrease at the inflamed paw level. Interestingly, reduction of nociception largely outlasted the 4 h 15 min exposure time to nitrous oxide because reduction of nociceptive threshold decrease was still observed at the inflamed hind paw level 24 h after stopping nitrous oxide treatment. Noteworthy is our observation that the 50/50% N₂O–O₂ treatment also strongly reduced the nociceptive threshold decrease observed for 2 days after injury at the non-carrageenan-injected hind paw that had not received any nociceptive input. This indicates that a time-limited exposure to nitrous oxide may oppose development of secondary hyperalgesia or allodynia, which have been previously described as mainly resulting from a central pain sensitization process.¹⁷,²⁴,²⁵ We have previously reported that...
During the past decade, acute tolerance has been reported in animal preclinical studies, this potent NMDA-like antihyperalgesic effect of nitrous oxide could partly explain the controversial results observed in clinical studies about the preemptive potency of NMDA receptor antagonists. Although the published meta-analysis did not take into account whether nitrous oxide was used during the anesthetic procedure,9–11 this suggests that NMDA receptor antagonist properties of nitrous oxide play a critical role in its antihyperalgesic effect. However, comparison of the results showed that the preventive antihyperalgesic effect was stronger on the inflammatory pain model than on the incisional pain model. The meaning of such a difference has to be explained.

Although the current results have been gathered from animal preclinical studies, this potent NMDA-like antihyperalgesic effect of nitrous oxide could partly explain the controversial results observed in clinical studies about the preemptive potency of NMDA receptor antagonists. Because the published meta-analysis did not take into account whether nitrous oxide was used during the anesthetic procedure,9–11 this critical point should be studied in the future for better assessing in humans the therapeutical interest of NMDA receptor blockade in a preemptive strategy for postoperative pain management.

During the past decade, acute tolerance has been re-
ported as a new adverse effect related to short-term opioid use for surgery. Clinical studies have shown that fentanyl or remifentanil administration for abdominal or orthopedic surgeries increased morphine requirement, suggesting short-term tolerance. As reported for postoperative hyperalgesia, it seems that the larger the intraoperative fentanyl or remifentanil dose is, the greater the postoperative morphine requirement is. Interestingly, short-term tolerance has also been observed in human volunteers 1–2 h after the beginning of low-dose remifentanil infusion. In the rat incisional pain model, we recently demonstrated that the postoperative decrease of morphine effectiveness is closely related to the hyperalgesia level observed 24 h after incision and fentanyl administration. By reducing the hyperalgesia level, ketamine, when administered before both surgery and fentanyl administration, improved the postoperative effectiveness of morphine. Because nitrous oxide is effective in preventing postinjury hyperalgesia in fentanyl-treated rats, we finally evaluated the effectiveness of 50/50% \( \text{N}_2\text{O}–\text{O}_2 \) treatment in preventing acute morphine tolerance in both nonsuffering and painful fentanyl-treated rats. As previously shown for heroin, we reported that both time course and area under the curve related to the morphine analgesic effect were unchanged when morphine was injected 24 h after fentanyl administration during the hyperalgesic period. In fact, during this period, the impression of less analgesia, i.e., apparent tolerance, as seen by the decrease in morphine maximum analgesic effect, was a consequence of the nociceptive threshold shift to lower values. This confirms our original hypothesis that short-term tolerance observed during the postoperative period is not mainly due to an actual decrease in the analgesic morphine potency per se as described classically but is related to sustained pain hypersensitivity induced by an initial opioid exposure. By totally reducing hyperalgesia, the 50/50% \( \text{N}_2\text{O}–\text{O}_2 \) pretreatment completely restored morphine effectiveness in nonsuffering fentanyl-treated rats. Although it was not possible to demonstrate such a type of result in painful rats because no analgesic reference effect may be evaluated as in nonsuffering rats, our study showed that the apparent enhancement of morphine effectiveness by nitrous oxide pretreatment is mainly due to the reduction of the nociceptive threshold decrease i.e., pain hypersensitivity induced by the fentanyl exposure 24 h previously. Considering our previous data regarding the NMDA receptor antagonist ketamine on the same incisional pain model, this also suggests that NMDA receptor antagonist properties of nitrous oxide play a critical role for preventing acute tolerance to analgesic effects of opioid agonists.

These beneficial and prolonged effects of nitrous oxide on postoperative pain management led us to determine whether such a treatment may protect against long-term pain vulnerability as described after some types of surgeries. As reported previously, our results showed that naloxone precipitated hyperalgesia when this opioid receptor antagonist was administered, after return to basal nociceptive threshold, in rats treated by a previous heroin or fentanyl administration. The fact that administration of an opioid-receptor antagonist induced no effect in naive rats but induced a pharmacologic effect such as hyperalgesia in rats without apparent pain has led us to suggest that rats with previous incisional pain and opioid histories did not return to their initial equilibrium (homeostasis) between opioid-dependent antinociceptive systems and NMDA-dependent pronociceptive systems. We previously proposed they were in a new equilibrium (allostasis) with a high level balance between these two opposite pain-controlling systems that mask one another. By sharply blocking opioid-dependent antinociceptive systems, naloxone-precipitated hyperalgesia would allow the level of pronociceptive system functioning in animal experimental models to be unmasked. This might explain the pain vulnerability observed in rats with pain and opioid histories. Noteworthy is our observation that preliminary treatment with nitrous oxide, as with ketamine, prevented naloxone-precipitated hyperalgesia when the opioid receptor antagonist was administered after return to normal nociceptive threshold. Interestingly, all these beneficial effects of nitrous oxide on experimental models were observed for low concentrations of nitrous oxide substantially below to the minimal alveolar concentration in rats.

Because opioids are widely used for surgery, the results of this study suggest that nitrous oxide, an NMDA receptor antagonist, could reduce the occurrence of exaggerated postoperative pain and tolerance observed in major surgeries with opioid-based anesthesia. Consequently, this may facilitate postoperative rehabilitation and perhaps limit the development of pain chronicization.

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