Nitrous oxide persistently alleviates pain hypersensitivity in neuropathic rats: A dose-dependent effect

Meric Ben Boujema MSc[1], Emilie Laboureyras MSc[1], Jan Pype PhD[2], Baptiste Bessière PhD[2], Guy Simonnet PhD[1]

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BACKGROUND: Despite numerous pharmacological approaches, there are no common analgesic drugs that produce meaningful relief for the majority of patients with neuropathic pain. Although nitrous oxide (N_2O) is a weak analgesic that acts via opioid-dependent mechanisms, it is also an antagonist of the *N*-methyl-D-aspartate receptor (NMDAR). The NMDAR plays a critical role in the development of pain sensitization induced by nerve injury.
OBJECTIVE: Using the chronic constriction injury of the sciatic nerve in male rats as a preclinical model of neuropathic pain, the first aim of the present study was to evaluate the lowest N_2O concentration and the shortest time of N_2O postinjury exposure that would produce persistent relief of neuropathic pain. The second aim was to compare the effects of N_2O with gabapentin, a reference drug used in human neuropathic pain relief.
METHODS: Changes in the nociceptive threshold were evaluated using the paw pressure vocalization test in rats.
RESULTS: Among the various N_2O concentrations tested, which ranged from 25% to 50%, only 50% N_2O single exposure for 1 h 15 min induced a persistent (minimum of three weeks) and significant (60%) reduction in pain hypersensitivity. A single gabapentin dose (75 mg/kg to 300 mg/kg, intraperitoneally) induced an acute (1 h to 1 h 30 min) dose-dependent effect, but not a persistent effect such as that observed with N_2O.
CONCLUSIONS: These preclinical results suggest that N_2O is advantageous for long-lasting neuropathic pain relief after sciatic nerve injury compared with other drugs used in humans such as gabapentinoids or NMDAR antagonists. The present preclinical study provides a rationale for developing comparative clinical studies.

Key Words: *Central sensitization; Neuropathic pain; Nitrous oxide*

Le soulagement persistant de l'hypersensibilité à la douleur par le protoxyde d'azote chez des rats neuropathiques : un effet proportionnel à la dose

HISTORIQUE : Malgré les nombreuses interventions pharmacologiques, aucun analgésique courant n'apporte un soulagement significatif à la majorité des patients souffrant de douleurs neuropathiques. Même si le protoxyde d'azote (N_2O) est un analgésique léger qui agit par des mécanismes opioïdergiques, c'est également un antagoniste du récepteur de l'acide *N*-méthyl-D-aspartique (RNMDA). Le RNMDA joue un rôle essentiel dans l'apparition de la sensibilisation à la douleur induite par une lésion nerveuse.
OBJECTIF : La présente étude faisait appel au modèle préclinique de douleur neuropathique par constriction chronique du nerf sciatique chez des rats mâles pour évaluer la plus faible concentration de N_2O et la plus courte période d'exposition au N_2O après la blessure pour produire un soulagement persistant de la douleur neuropathique. L'objectif secondaire consistait à comparer les effets du N_2O avec la gabapentine, un médicament de référence utilisé pour soulager la douleur neuropathique chez les humains.
MÉTHODOLOGIE : Les chercheurs évaluaient les changements de seuil nociceptif au moyen du test de vocalisation induite par pression de la patte des rats.
RÉSULTATS : Parmi les diverses concentrations de N_2O mises à l'essai, qui se situaient entre 25 % et 50 %, l'exposition au N_2O à 50 %pendant 1 h 15 était la seule à induire une réduction persistante (d'au moins trois semaines) et importante (60 %) de l'hypersensibilité à la douleur. Une seule dose de gabapentine (75 mg/kg à 300 mg/kg, par voie intrapéritonéale) induisait un effet aigu proportionnel à la dose (1 h à 1 h 30), mais pas un effet persistant comme celui observé grâce au N_2O.
CONCLUSIONS : D'après les résultats précliniques, le N_2O est avantageux pour soulager une douleur neuropathique de longue durée après une lésion du nerf sciatique par rapport aux autres médicaments utilisés chez les humains, tels que les gabapentinoïdes ou les antagonistes des RNMDA. La présente étude préclinique justifie l'élaboration d'études cliniques comparatives.

Neuropathic pain involves not only a nociceptive process but also a transitional process (1), which is persistent and increases synaptic gain, thereby leading to persistent pain (2). Based on this concept, the recommendations of the European Federation of Neurological Societies guidelines do not include antinociceptive drugs as proposed by the WHO for cancer pain but, instead, focus on drugs acting as antiepileptics, antidepressants and lidocaine plasters as the first line of treatment (3). A promising therapeutic strategy is the use of *N*-methyl-D-aspartate receptor (NMDAR) antagonists based on evidence that the overactivation of NMDARs plays a critical role in the development of long-lasting sensitization of pain pathways induced by injury (4,5). However, NMDAR antagonists, such as ketamine or related compounds, often result in unacceptable side effects (6). Although novel NMDAR antagonists that selectively target the NR_2B subunit have a superior therapeutic index with more limited side effects (7,8), they require long-term or repetitive treatments for sustained analgesic effect (6,9); this approach leads to patient discomfort and high costs because hospitalization is necessary for such a treatment.

Nitrous oxide (N_2O) is a common analgesic acting via endogenous opioid release (10,11). However, several in vitro (12,13) and in vivo (14,15) studies have reported that N_2O also acts as an NMDAR antagonist that may prevent or reduce pain sensitization (16). We have previously shown (17) that a single 50% N_2O exposure for 1 h 15 min induced a persistent reduction in hyperalgesia-allodynia in a rat neuropathic pain model associated with a chronic constriction injury (CCI) at the sciatic nerve (18). Although several concerns regarding the deleterious effects of N_2O have been raised in recent years, qualitative reviews of current controversies (19,20) have concluded that N_2O

[1]*Team "Homéostasie-Allostasie-Pathologie-Réhabilitation", UMR CNRS 5287, INCIA, Université de Bordeaux, Bordeaux;* [2]*Air Liquide Santé International, Centre de Recherche Paris-Saclay, Jouy-en-Josas, France*
Correspondence: Pr Guy Simonnet, Department: Team «Homéostasie-Allostasie-Pathologie-Réhabilitation», UMR CNRS 5287, Université de Bordeaux, 146 rue Léo Saignat, F- 33076, Bordeaux, France. Telephone 335-5757-1406, fax 335-5757-1204, e-mail gsimonnet@yahoo.com

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should remain an option in contemporary anesthesia. In patients receiveing general anesthesia for major noncardiac surgery in the Gas mixture for Anaesthesia (ENIGMA-II) trial, addition of N_2O to the gas mixture did not increase the risk for death, cardiovascular complications or the risk for surgical site infection (21). Moreover, the intraoperative N_2O led to a reduction in the risk for persistent postsurgical pain (PPSP) (22). However, this long-term beneficial effect in humans was obtained with a high concentration such as 70% N_2O/30% oxygen mixture; this dose induces profound sedative effects, limiting its use outside a hospital environment. Therefore, the first aim of our study was to determine the lowest N_2O concentration and the shortest time of N_2O post-spinal nerve tissue injury capable of inducing persistent relief in the CCI male rat model. The second aim was to compare the effects of N_2O with gabapentin, a reference drug used in humans to treat neuropathic pain.

METHODS

Animals

Experiments were performed on adult male Sprague Dawley rats (Charles River Laboratories, France) weighing 250 g to 300 g. The rats were housed in groups of four per cage with a 12 h light/12 h dark cycle (lights on at 07:00) at a constant mean (± SD) room temperature of 23±2°C. The animals had ad libitum access to food and water. All experiments were performed during the light period. Experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Ethics Committee in Animal Experimentation of Bordeaux (CEEA50, Project Number 5012066-A), in an authorized laboratory (No. B-33-063-6) and under the supervision of the authorized researcher Ben Boujema (No. 3310009, delivered by the *Ministère de l'Alimentation, de l'Agriculture et de la Pêche*).

Neuropathic pain model

A peripheral mononeuropathy was produced on day 0 (D_0), using the CCI model (18). Rats were anesthetized with 1% to 3% isoflurane vapourized via a nose cone. The left common sciatic nerve was exposed by blunt dissection at the mid-thigh level, and four loose ligatures (4-0 chromic catgut) were tied around the nerve (identified as the injured hind paw). The muscle and skin were closed in layers and the wound site was covered with an antibiotic mixture of 2% fucidine (Léo, France) and Primyxine (oxytetracycline hydrochloride and polymyxin B sulfate; Chemineau, France). No surgery was performed on the right hind paw (the uninjured hind paw). From an ethical viewpoint and in the purpose of limiting the number of animals in pain experiments, no sham-operated control rats (surgery and sciatic nerve exposed but not injury) were performed in the present study because the authors previously reported (17) that no significant change in nociceptive threshold was observed in these sham-operated animals. To minimize differences in the procedure, all operations were performed by the same experimenter. Animals were given 24 h to recover after the operation.

Drugs

Gabapentin (75 mg/kg, 150 mg/kg or 300 mg/kg [Sigma-Aldrich, France]) was dissolved in physiological saline (0.9%) and administered by intraperitoneal injection (3 mL/kg body weight). Control animals received an equal volume of saline injections.

The different N_2O concentrations were delivered (Air Liquide, France) via bottles containing premixed nitrous oxide, oxygen and nitrogen: $N_2O/N_2/O_2$ 12.5%/37.5%/50% (12.5% N_2O), $N_2O/N_2/O_2$ 25%/25%/50% (25% N_2O), $N_2O/N_2/O_2$ 35%/15%/50% (35% N_2O) and N_2O/O_2 50%/50% (50% N_2O). A bottle containing N_2/O_2 50%/50% (Air) was used for the control group (Air Liquide, France). All gas exposure was performed in a Plexiglas chamber (42 cm × 26 cm × 26 cm) as previously described (16). Four rats were placed in each chamber. Fresh gases was fed into the chamber (4 L/min) through an inlet port and purged by a vacuum set. N_2O concentrations were monitored continuously to confirm premixed gas concentrations (VEO Multigas Monitor, Phasein Medical Technologies, Sweden).

Mechanical test

The nociceptive threshold (NT) in handled rats was determined using a modification of the Randall-Selitto method (23): a paw pressure vocalization test consisted of constantly increasing pressure that was applied to the hind paw until the rat squeaked. A Basile analgesimeter was used (Apelex, France; stylus tip diameter 1 mm). A 600 g cut-off value was chosen to prevent tissue damage.

General procedures

Animals were acclimated to the animal care unit for four days on arrival to the laboratory. To avoid perturbation from experimental conditions that could affect measurement of the NT, the experiments were performed by the same experimenter under quiet conditions in a testing room located near the animal care unit. For two weeks before the experiment, the animals were weighed daily and handled gently for 5 min; animals were then placed into the test room for 2 h (from 09:00 to 11:00), where they were left to become accustomed to the various apparatuses. All experiments began at 10:00 during the light period. Rats were also acclimated to the plexiglas chamber for one week (15 min per day), with the gas inflow rate set at 4 L/min. NT measurements were taken for two days preceding the surgery (ie, on D_{-2} and D_{-1}) and repeated on D_0 before tissue injury. Experiments were initiated only when no statistical change in the basal NT was observed for three successive days (D_{-2}, D_{-1} and D_0, one-way ANOVA, $P>0.05$). The reference value of NT was chosen as the basal value before tissue injury for each hind paw on D_0. The rats were randomly assigned to different experimental groups. Nitrous exposures or gabapentin intraperitoneal injections were performed seven days post-CCI in all experiments because, as previously reported (17), a stable and homogenous hyperalgesia was obtained over this time.

Experimental protocols

Experiment 1: Delayed effects of a single N_2O exposure for 1 h 15 min at various concentrations (12.5%, 25%, 35% and 50% N_2O) on neuropathic pain: In this experiment, five groups of rats (each group n=8) were used: CCI rats exposed to air, 12.5% N_2O, 25% N_2O, 35% N_2O and 50% N_2O for 1 h 15 min. Gas exposures were performed seven days (D_7) after hind paw injury (D_0). The mechanical NT was evaluated for both the injured and uninjured hind paws once daily from D_{-2} to D_{12} or D_{14}.

Experiment 2: Delayed effects of repeated daily N_2O exposures (1 h 15 min) for three days at various concentrations (12.5%, 25%, 35% and 50% N_2O) on neuropathic pain: In this experiment, five groups of rats (each group n=8) were used: CCI rats exposed to air, 12.5% N_2O, 25% N_2O, 35% N_2O and 50% N_2O for 1 h 15 min. Gas exposures were performed once daily on D_7, D_8 and D_9 after the hind paw injury (D_0). The mechanical NT was evaluated for both the injured and uninjured hind paws once daily from D_{-2} to D_{21}.

Experiment 3: Effects of a single (45 min) or repeated (4 × 45 min) daily 50% N_2O exposure on neuropathic pain: Two experiments were performed to evaluate the influence of time exposure on the NT. The first experiment included two groups of rats (each group n=8): CCI rats were exposed to either air or 50% N_2O for 45 min. Gas exposures were performed seven days (D_7) after the hind paw injury (D_0). The second experiment included two groups of rats (n=8): CCI rats received four consecutive exposures to air or 50% N_2O for 45 min. Gas exposures were performed daily on D_7, D_8, D_9 and D_{10} after the hind paw injury (D_0). The mechanical NT was evaluated for both the injured and uninjured hind paws once daily from D_{-2} to D_{14}.

Experiment 4: Dose-effect study of gabapentin (75 mg/kg, 150 mg/kg, and 300 mg/kg) injection on neuropathic pain: Four groups of rats were used in this experiment. One week after the nerve injury (D_7), rats were injected with either saline (n=8), 75 mg/kg of gabapentin (n=8), 150 mg/kg of gabapentin (n=8) or 300 mg/kg of gabapentin (n=8). The mechanical NT was evaluated every 30 min, 60 min and 90 min after gabapentin injection on D_7. The NT was also evaluated once daily until D_{14}.

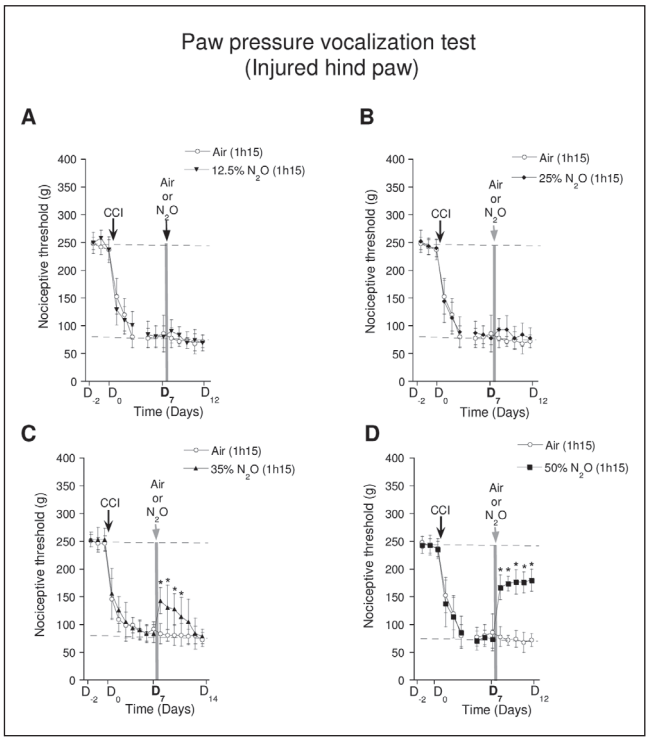

Figure 1) *Delayed effects of a single nitrous oxide (N_2O) exposure at various concentrations (***A** *12.5%,* **B** *25%,* **C** *35% and* **D** *50%) on the mechanical nociceptive threshold (NT) in a male rat neuropathic pain model. One week after chronic constriction injury (CCI) of the sciatic nerve performed on day 0 (D_0), CCI rats were exposed to various N_2O concentrations for 1 h 15 min. The NT was evaluated once daily until D_{12} or D_{14} on the injured hind paw. The NT was expressed as the mean ± SD. Dunnett test *P<0.05 for comparison with D_7 value. White circles: air group (n=8); black inverted triangle: 12.5% N_2O group (n=8); black diamond: 25% N_2O group (n=8); black triangle: 35% N_2O group (n=8); and black square: 50% N_2O group (n=8). The shaded areas indicate the day of the N_2O exposure*

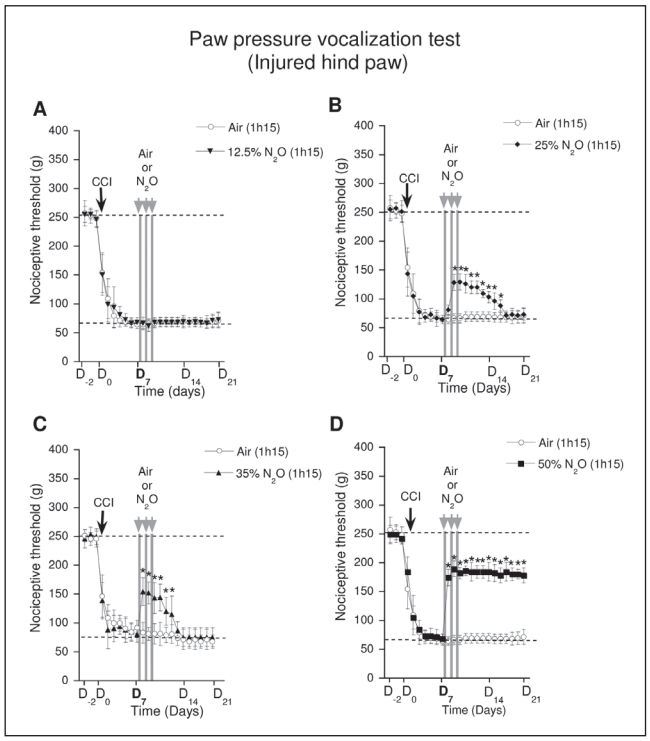

Figure 2) *Delayed effects of repeated nitrous oxide (N_2O) exposures at various concentrations (***A** *12.5%,* **B** *25%,* **C** *35% and* **D** *50%) on the mechanical nociceptive threshold (NT) in a male rat neuropathic pain model. One week after chronic constriction injury (CCI) of the sciatic nerve was performed on day 0 (D_0), CCI rats were subjected to daily repeated exposures (× 3) at various N_2O concentrations for 1 h 15 min. The NT on injured hind paw was evaluated once daily until D_{21}. The NT was expressed as the mean ± SD. Dunnett test *P<0.05 for comparison with D_7 value. White circles: air group (n=8); black inverted triangle: 12.5% N_2O group (n=8); black diamond: 25% N_2O group (n=8); black triangle: 35% N_2O group (n=8); and black square: 50% N_2O group (n=8). The shaded areas indicate the day of the N_2O exposures*

Statistical analysis

All data are expressed as mean ± SD. One- and two-way ANOVA was used to assess the time effects of treatments on the NT and individual group comparisons. The Dunnett post hoc test was used to assess the differences between time points versus the reference value on D_7 (ie, before the first gas exposure). The Newman-Keuls post hoc test was used for multiple comparisons among groups; P<0.05 was considered to be statistically significant.

RESULTS

As expected, sciatic nerve damage induced a significant NT decrease in all male rats (Dunnett's test P<0.05 for comparison with the D_0 basal value) on the injured hind paw (left hind paw). A more moderate NT decrease was observed on the uninjured hind paw (right hind paw).

Experiment 1: Delayed effect of a single N_2O exposure for 1 h 15 min at various concentrations (12.5%, 25%, 35% and 50% N_2O) on neuropathic pain

No change in the NT decrease induced by sciatic nerve injury was observed in rats that were exposed to a single 12.5% or 25% N_2O exposure (P>0.05) (Figures 1A and 1B).

A single 35% N_2O exposure on D_7 induced a 43% reduction in the NT decrease on the injured hind paw after 24 h on D_8 (Dunnett's test P<0.05, Figure 1C), and a complete reduction on the uninjured hind paw (Dunnett's test P<0.05). These effects progressively disappeared, ie, they were limited to four and five days for both the injured and uninjured hind paws during the N_2O postexposure period.

A single 50% N_2O exposure on D_7 induced a sustained reduction (57% to 66%) in the NT decrease on the injured hind paw from D_8 to D_{12} (Dunnett's test P<0.05, Figure 1D) and a complete reduction on the uninjured hind paw (Dunnett's test P<0.05).

Experiment 2: Delayed effects of repeated daily N_2O exposures (1 h 15 min) for three days at various concentrations (12.5%, 25%, 35% and 50% N_2O) on neuropathic pain

No change in the NT decrease was observed in rats subjected to air or 12.5% N_2O exposure once daily on D_7, D_8 and D_9 (Dunnett's test P>0.05 Figure 2A).

A series of three daily 25% N_2O exposures for 1 h 15 min induced a partial reduction in the NT decrease on both the injured (Dunnett's test P<0.05, Figure 2B) and uninjured hind paws (Dunnett's test P<0.05). This reduction was maximal (34%) after the second exposure on D_9. This beneficial effect progressively decreased during the N_2O postexposure period, and disappeared nine and 14 days after exposure in both the injured and uninjured hind paws, respectively.

In contrast, a series of three daily 35% N_2O exposures for 1 h 15 min induced a reduction in the NT decrease on the injured hind paw (Dunnett test P<0.05, Figure 2C). This reduction was maximal

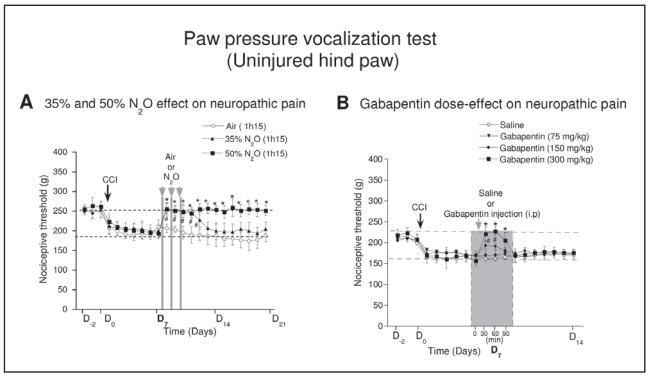

Figure 3) *Dose effects of nitrous oxide (N_2O) or gabapentin on the uninjured hind paw in a male rat neuropathic pain model. One week after chronic constriction injury (CCI) of the sciatic nerve was performed on day 0 (D_0), CCI rats were subjected to daily repeated exposures (× 3) at two N_2O concentrations (35% or 50%) for 1 h 15 min* (**A**) *or were injected one time with various gabapentin concentrations (75 mg/kg, 150 mg/kg or 300 mg/kg)* (**B**). *The nociceptive threshold (NT) was evaluated once daily until D_{21} or until D_{14}, respectively. The NT was expressed as the mean ± SD. Dunnett test *P<0.05 for comparison with D_7 value for 50% N_2O group* (**A**) *or 300 mg/kg gabapentin group* (**B**). *#P<0.05 for comparison with the D_7 value for the 35% N_2O group* (**A**) *or the 150 mg/kg gabapentin group* (**B**). *White circles:* (**A**) *or saline group* (**B**); *black triangle: 35% N_2O group* (**A**); *black inverted triangle: 75 mg/kg gabapentin group* (**B**); *black diamond: 150 mg/kg gabapentin group* (**B**); *and black square: 50% N_2O* (**A**) *or 300 mg/kg gabapentin group* (**B**); *(n = 8 per group). The shaded areas indicate the day of the N_2O exposure or the day of the intraperitoneal gabapentin injection*

(44%) after the first exposure (D_8) and was maintained during the three days of N_2O exposure (D_8 to D_{10}). This beneficial effect progressively disappeared during the post-N_2O exposure period. A complete reduction in the NT decrease on the uninjured hind paw was observed during the N_2O exposure period (Figure 3A), and this effect progressively disappeared.

A series of three daily 50% N_2O exposures for 1 h 15 min induced a sustained reduction in the NT decrease (58% to 66% on D_8 to D_{21}) on the injured hind paw (Figure 2D) and completely eliminated the NT decrease on the uninjured hind paw (Figure 3A) (Dunnett's test P<0.05).

Experiment 3: Effects of a single (45 min) or repeated (4 × 45 min) daily 50% N_2O exposure on neuropathic pain

A single 50% N_2O exposure limited to 45 min on D_7 induced a reduction in the NT decrease on the injured hind paw after 24 h on D_8 (Dunnett test P<0.05, Figure 4A) and a partial (53.8%) reduction on the uninjured hind paw (Dunnett's test P<0.05). This reduction was not persistent during the postexposure period; it completely disappeared on D_{10} on the injured hind paw (Dunnett test P>0.05, Figure 4A) and on D_9 on the uninjured hind paw (Dunnett test P>0.05).

When daily 50% N_2O exposures were repeated for four days, the reduction in the NT on the injured hind paw decrease was maintained during the N_2O exposure period from D_7 to D_{10} (Dunnett's test *P*<0.05, Figure 4B) but disappeared one day later on D_{11} (Dunnett test P>0.05).

Experiment 4: Dose effects of gabapentin (75 mg/kg, 150 mg/kg and 300 mg/kg) injection on neuropathic pain

No change in the NT decrease was observed in rats that received saline or the lowest gabapentine dose (75 mg/kg) on D_7 on either the injured (Dunnett test P>0.05, Figure 5A) or uninjured hind paws (Dunnett test P>0.05, Figure 3B).

A single intraperitoneal injection of 150 mg/kg of gabapentin on D_7 induced a partial reduction in the NT decrease on both the injured and uninjured hind paws for 1 h after gabapentin injection (Dunnett test

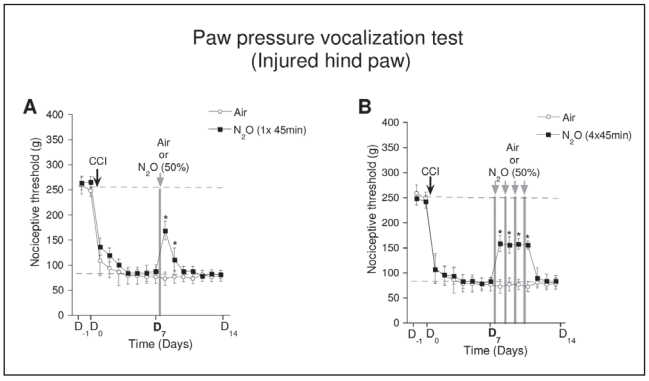

Figure 4) *Effects of a single (45 min) or repeated (4 × 45 min) daily 50% nitrous oxide (N_2O) exposure in a male rat neuropathic pain model. One week after chronic constriction injury (CCI) of the sciatic nerve was performed on day 0 (D_0), CCI rats were exposed to 50% N_2O concentration for 45 min one time* (**A**) *or four times* (**B**). *The nociceptive threshold (NT) was evaluated once daily until D_{14} on injured hind paw. The NT was expressed as the mean ± SD. Dunnett test *P<0.05 for comparison with D_7 value. White circles: air group (n=8); black squares: 50% N_2O group (n=8). The shaded areas indicate the day of the N_2O exposures*

P<0.05, Figures 5B and 3B). In contrast to the N_2O results, no delayed effect was observed 24 h after the gabapentin injection (D_8 to D_{14}).

A single intraperitoneal injection of 300 mg/kg of gabapentin on D_7 induced a reduction in the NT decrease on the injured hind paw for 1 h 30 min (Dunnett test P<0.05, Figure 5C) and for 1 h in the uninjured hind paw (Dunnett test P<0.05, Figure 3B). No effect was observed during the postgabapentin injection period (D_8 to D_{14}) on either the injured or uninjured hind paws.

DISCUSSION

Two main findings can be drawn from the present preclinical dose-dependent effect study. The first shows that a single 50% N_2O exposure for 1 h 15 min in male rats is necessary and sufficient for inducing a persistent alleviation of neuropathic pain induced by a sciatic nerve injury. The second finding indicates that a single gabapentin injection only induced an acute analgesic effect, not a persistent effect on pain hypersensitivity as observed following a single 50% N_2O exposure.

A systemic literature review indicates that PPSP is common and is often reported as neuropathic (5,24). It is difficult to manage PPSP because the response to most drugs remains unpredictable despite attempts to develop a more rational therapeutic approach (25,26). Here, our approach using the CCI model mimicked neuropathic pain in patients who are often treated weeks or months after nerve injury, particularly after a surgical lesion. The safety of N_2O has been questioned (19,27-30) in recent years. Animal and in vitro studies have shown that toxic effects only occur at extreme doses of N_2O exposure (31), up to three times the maximum concentration used in this study at hyperbaric conditions (120% to 150% N_2O), or for long duration, such as 50% for 72 h (32). NMDAR antagonists, such as MK-801, phencyclidine, and ketamine, or N_2O exposure (31) have been used to demonstrate that the longer excitatory injury will be more likely to convert from reversible to irreversible neurotoxic reactions in neurons because NMDAR blockage is maintained for a longer period of time. Thus, it is critical to determine the lowest N_2O concentration and the shortest time of N_2O postinjury exposure capable of inducing persistent relief in the neuropathic pain model. Our study indicates that a 50% N_2O exposure for 1 h 15 min induced a persistent (minimum of three weeks) and significant (60%) reduction in pain hypersensitivity in this experimental model of neuropathic pain. Some caution must be exercised with these results because no blinded approach and sham-operated rats were used in the present study. However, we previously reported (17) that no significant change in NT was observed in sham-operated animals (surgery and sciatic nerve

exposed but not injured). This indicates that the long-lasting NT decrease observed in these studies is the result from nerve injury, not from surgery. A shorter 50% N_2O exposure such as 45 min of exposure, only induced a transient effect, whereas repeated daily exposure to 50% N_2O for 1 h 15 min for three days did not improve the relief of neuropathic pain hypersensitivity compared with the single 1 h 15 min exposure. Although repeated exposures to 25% or 35% N_2O induced a 30% to 40% reduction in neuropathic pain within a few days, there was no persistent effect, ie, the NT values progressively returned to the values observed before N_2O exposure.

These preclinical findings suggest that a single exposure to N_2O may be an efficient strategy for alleviating neuropathic pain in humans. A useful index to make interspecies comparisons, particularly between rats and humans, is the minimum alveolar anesthetic concentration, ie, the concentration that prevents purposeful movement to supramaximal noxious stimulation in 50% of subjects. To achieve the minimum alveolar anesthetic concentration, N_2O exposure must be used at 105% in humans and approximately 150% in rats (33,34). If 105% in humans is also equivalent to 150% in rats for analgesia, then 50% N_2O in rats is proportional to 35% N_2O in humans. This result means that a reduction in the risk of persistent postsurgical pain does not required a high N_2O concentration exposure, such as the 70% used in the ENIGMA trial; in that trial (22), intraoperative N_2O led to a reduction in the risk for chronic pain in patients by more than one-half, with a median follow-up of 4.5 years. The margin of safety may be increased by limiting patients to a lower %N_2O exposure for 1 h 15 min.

The beneficial effects of a single exposure to 50% N_2O on neuropathic pain prompted us to compare the effects induced by a single gabapentin administration. This comparison was performed given that gabapentinoid drugs have been proposed by the European Federation of Neurological Societies guidelines (3) as the first line of treatment despite various side effects (35). Because spinal plasticity and sensitization play pivotal roles in neuropathic pain after peripheral nerve injury, most laboratory studies have focused on the actions of gabapentin in the spinal cord (36-42). However, some studies proposed that gabapentin also acts on supraspinal structures to stimulate the bulbospinal descending inhibition to alleviate neuropathic pain (41,43). Although long-term clinical use of gabapentin is required for alleviating chronic neuropathic pain, a single administration of this drug is also used in acute postoperative pain management (44-46). Similarly, a single dose of 150 mg of pregabalin is highly effective against neuralgia associated with thoracotomy (47).

These findings led us to evaluate the effects of a unique systemic administration via an intraperitoneal injection. Our study indicates that a single gabapentin intraperitoneal injection induced an acute dose-dependent effect within 2 h. However, in contrast to results obtained with a single 50% N_2O exposure for 1 h 15 min, there was no persistent effect. The limited effect of gabapentin is in agreement with results of a recent study (48) showing that an intrathecal pregabalin infusion for four weeks in rats produced analgesia only as long as the drug was administered, without blocking the emergence of persistent pain once the infusion ended. Moreover, the use of gabapentin induces a high risk for dizziness, edema and somnolence in humans (49). From a translational perspective, these results suggest that a single 50% N_2O exposure may be more advantageous than a single gabapentin administration to induce a sustained relief of neuropathic pain in humans as it is used in surgical patients. Clinical trials must be performed to confirm such a therapeutic effect, especially in postoperative patients because peripheral nerve lesions are a major cause of chronic pain after surgery.

The mechanisms of these two different treatments warrant discussion. As previously reported, N_2O induced two types of effects on neuropathic pain (17). The first and well-known effect was an acute opioid-dependent analgesic effect that disappeared as soon as the N_2O exposure ended (10). It has been proposed that the acute N_2O-induced antinociceptive effect is mediated by indirect inhibition of

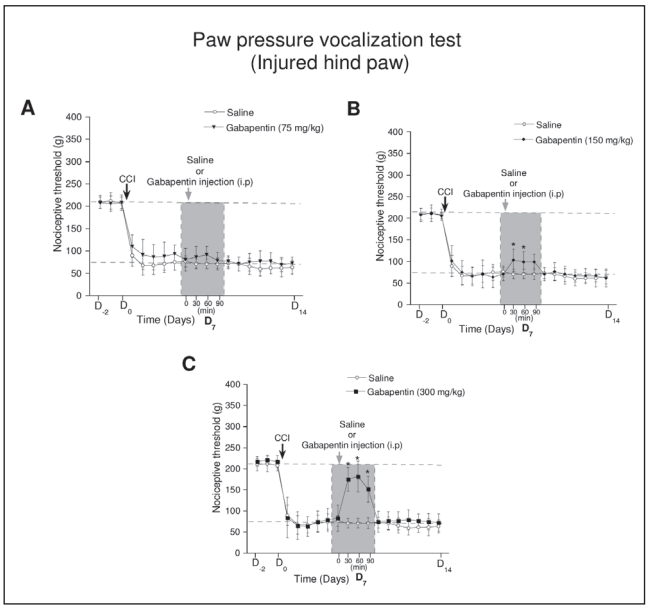

Figure 5) *Dose-effects of a single intraperitoneal gabapentin injection (***A** *75 mg/kg,* **B** *150 mg/kg and* **C** *300 mg/kg), in a male rat neuropathic pain model. One week after chronic constriction injury (CCI) of the sciatic nerve was performed on day 0 (D_0), CCI rats were injected with various gabapentin concentrations. The nociceptive threshold (NT) was evaluated every 30 min for 1 h 30 min after gabapentin injection. The NT on the injured hind paw was evaluated once daily until D_{14}. The NT was expressed as the mean ± SD. Dunnett test *P<0.05 for comparison with the D_7 value. White circles: air group (n=8); black inverted triangle: 75 mg/kg gabapentin group (n=8); black diamond: 150 mg/kg gabapentin group (n=8); and black square: 300 mg/kg gabapentin group (n=8). The shaded areas indicated the day of the intraperitoneal gabapentin injection*

the nociceptive afferent neurons and/or postsynaptic inhibition of the second-order neurons via an opioid release in the periaqueductal brainstem; this leads to the activation of the descending noradrenergic inhibitory pathways and the subsequent activation of GABAergic interneurons through α_1 adrenoreceptors (10,11).

Recently, a second effect of N_2O has been described, a delayed and persistent non-opioid-dependent alleviation of neuropathic pain hypersensitivity (17). One hypothesis is that NMDAR antagonistic properties of N_2O may be responsible for this reduction in pain hypersensitivity because it mimics the pharmacological effects of NMDAR antagonists, such as ketamine or memantine, in preclinical models (26). In the same neuropathic pain model, we previously reported (17) that ketamine did not induce the acute analgesic effects observed with gabapentin or N_2O: it only induced a delayed reduction in pain hypersensitivity that was limited to two days. Other mechanisms involving AMPA receptors (50), G-Protein-gated inward rectifying K^+ channels (51), and the two-pore-domain K^+ channel TREK-1 cannot be excluded (52); these alternative mechanisms should be further explored.

On the part of gabapentin, it is well acknowledged that its efficacy mainly depends on its action at the $\alpha_2\beta$ subunit of calcium channels that are up-regulated in primary afferents and the spinal cord after nerve injury (38). However, at the supraspinal level, it has been demonstrated that gabapentin and other $\alpha_2\beta$ ligands decrease presynaptic GABA release in the locus coeruleus, consistent with gabapentin-induced activation of noradrenergic neurons in the locus coeruleus and, thus, an increase in noradrenaline release in the spinal cord (40,53). Interestingly, gabapentin induces more spinal noradrenaline release in spinal nerve ligation animals compared with control animals, likely due to noradrenergic sprouting in the spinal cord after

spinal cord ligation (54). These results suggest that gabapentin reduces presynaptic GABA release by disinhibiting the descending noradrenergic inhibitory pathways. Although speculative, one hypothesis is that both the acute N_2O analgesic effects and short-term gabapentin effects observed in neuropathic pain have some common mechanisms via the activation of the descending noradrenergic inhibitory pathways.

Our study has demonstrated that exposure to 50% N_2O for 1 h 15 min completely reduced the sustained contralateral pain hypersensitivity observed in the unlesioned hind paw in this neuropathic pain model. Interestingly, N_2O exposure always re-established the basal NT. This result strongly suggests that long-lasting N_2O effects on neuropathic pain were not analgesic effects per se, but resulted from an inhibition of central neuroplasticity mechanisms; these mechanisms may have led to a pain hypersensitivity responsible for the hyperalgesia in both the lesioned and unlesioned hind paws triggered by the unilateral nerve injury.

CONCLUSIONS

The present study demonstrates that a single exposure to 50% N_2O may represent a new and interesting therapeutic approach for inducing persistent neuropathic pain relief, at least after spinal nerve injury, compared with other compounds used in clinical setting (ie, gabapentinoids, sodium channel inhibitors or NMDAR antagonists). It would be interesting to evaluate effects of N_2O exposure after trigeminal injury. The main advantage of N_2O is that it induces a persistent relief of neuropathic pain for several weeks as early as the first 50% N_2O exposure. As compared with other drug treatments used for relieving neuropathic pain, this gas exposure does not require long-term or repetitive treatments for obtaining sustained pain relief. This may represent a favorable benefit:risk:cost ratio. These results provide a rationale for testing this compound in clinical studies aimed at improving neuropathic pain.

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