Published in final edited form as:

Eur Neuropsychopharmacol. 2008 March ; 18(3): 194–199.

Nitrous oxide-antinociception is mediated by opioid receptors and nitric oxide in the periaqueductal gray region of the midbrain

Dimitris E. Emmanouil^a, Andrea S. Dickens^b, Rick W. Heckert^{b,c}, Yusuke Ohgami^b, Eunhee Chung^b, Shujie Han^{b,c}, and Raymond M. Quock^{b,*}

^aDepartment of Pediatric Dentistry, School of Dentistry, University of Athens, Athens, Greece

^bDepartment of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164–6534, USA

^cGraduate Program in Pharmacology and Toxicology, Washington State University, Pullman, WA 99164–6534, USA

Abstract

Previous studies have shown that nitrous oxide (N₂O)-induced antinociception is sensitive to antagonism by blockade of opioid receptors and also by inhibition of nitric oxide (NO) production. The present study was conducted to determine whether these occur within the same brain site. Mice were stereotaxically implanted with microinjection cannulae in the periaqueductal gray (PAG) area of the midbrain. In saline-pretreated mice, exposure to 70% N₂O resulted in a concentration-dependent antinociceptive effect in the mouse abdominal constriction test. Pretreatment with an opioid antagonist in the PAG significantly antagonized the antinociceptive effect. Pretreatment with an inhibitor of NO production in the PAG also significantly antagonized the antinociceptive effect. These findings suggest that N₂O acts in the PAG via an NO-dependent, opioid receptor-mediated mechanism to induce antinociception.

Keywords

Nitrous oxide; antinociception; periaqueductal gray; opioid receptors; nitric oxide

1. Introduction

Nitrous oxide, also known as “laughing gas” or simply N₂O, has gained clinical acceptance because of its anesthetic, analgesic and anxiolytic properties, which have been utilized in medicine and dentistry. How such a simple inorganic chemical structure can evoke this wide range of pharmacological effects is intriguing.

Exposure of test animals to N₂O results in an antinociceptive effect that is sensitive to antagonism by naloxone and other opioid receptor blockers (Berkowitz et al., 1976; Quock and Graczak, 1988), indicating a key role for opioid receptors in the drug effect. We have previously reported that the antinociceptive effect of N₂O is also antagonized by inhibition of nitric oxide (NO) production (McDonald et al., 1994; Ishikawa and Quock, 2003a; Li et al.,

* Corresponding author: Raymond M. Quock Department of Pharmaceutical Sciences College of Pharmacy Washington State University P.O. Box 646534 Pullman, WA 99164–6534, USA Tel.: +1 509 335 5956; fax: +1 509 335 5902 E-mail address: quockr@wsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2004), implicating an involvement of NO in the drug effect as well. The present study was conducted to ascertain whether the opioid receptors and NO function that are involved in N₂O-induced antinociception are co-localized in the periaqueductal gray (PAG) of the midbrain, which has been shown to be involved in N₂O-induced antinociception (Zuniga et al., 1987; Hodges et al., 1994; Fang et al., 1997).

2. Experimental procedures

2.1. Subjects

A total of 265 male NIH Swiss mice, 18–22 g body weight, were obtained from Harlan Laboratories (Indianapolis, IN) and were housed five per cage in an ALAAAC-accredited animal facility. Food and water were available *ad libitum*. The facility was maintained on a 12-h light/dark cycle (lights on 0700–1900) under standard conditions (22 ± 1°C room temperature, 33% humidity). Mice were kept in the holding room for at least four days following arrival in the facility prior to use. The animals were tested in control and experimental groups of 8–12 mice per group.

The experimental protocol was approved by the Institutional Review Committee for the Use and Care of Animal Subjects of Washington State University and is in compliance with *The Guide for the Care and Use of Laboratory Animals* (US National Research Council, 1996). All care was taken to minimize pain and discomfort in the experimental animals.

2.2. Surgery

Under isoflurane anesthesia, mice were mounted in a digital stereotaxic system (Cartesian Research, Inc., Sandy, OR). Using aseptic technique, 26-G stainless steel external guide cannulae (Plastics One, Roanoke, VA) were stereotaxically directed at the PAG of the midbrain at coordinates – 3.0 mm AP, ± 0.2 mm ML, and – 3.0 mm DV (Franklin and Paxinos, 2001). The tips of the external guide cannulae were positioned 1.0 mm dorsal to the target sites. Cannulae were secured to the calvarium using stainless steel screws and dental cement. Each cannula was plugged with a solid 33-gauge dummy cannula. After surgery, mice were allowed a minimum of five days recovery time before testing.

2.3. Histological verification of microinjection site

At the end of the experiments, animals were anesthetized with 2.5% isoflurane, and 0.5 l 10% India ink dye was microinjected into the PAG over 15 min to mark the microinjection site. Thirty minutes after dye injections, mice were perfused with cold phosphate-buffered saline followed by 4% formaldehyde. The brains were dissected out and cryoprotected in 25% sucrose. Forty-µm coronal sections were cut on a cryostat and processed for staining with 0.5% cresyl violet. The sections were later observed under a stereomicroscope and photographed (Fig. 1).

2.4. Agents

Nitrous Oxide, U.S.P. and Oxygen, U.S.P. (A&L Welding, Spokane, WA) were mixed and delivered using a dental-sedation system (Porter, Hatfield, PA) at a total flow rate of 10 l/min. Mice were individually exposed in a clear Plexiglas® exposure chamber (35 cm L × 20 cm W × 15 cm H) with gas inlet and outlet ports. The concentrations of N₂O and O₂ delivered into the box were monitored using a POET II® anesthetic monitoring system (Criticare, Milwaukee, WI). Exhausted gases were routed by polyethylene tubing to a nearby fume hood.

β-Chlornaltrexamine was purchased from the Sigma Chemical Company (St. Louis, MO) and TRIM (1-[2-trifluoromethylphenyl]imidazole) was purchased from the Alexis Biochemical Company (San Diego, CA). Both agents were prepared in 0.9% physiological saline and

microinjected in an intra-PAG (i.PAG) volume of 0.5 μ l. The pre-treatment times for β -chlornaltrexamine and TRIM were 24 h and 30 min, respectively.

2.5. Antinociception assessment

Antinociceptive responsiveness to N₂O was assessed by the abdominal constriction test. Mice were treated i.p. with 0.6% acetic acid (0.1 ml/10 g body weight); exactly 5 min later, the number of abdominal constrictions—lengthwise stretches of the torso with concave arching of the back—in each animal was counted for a 6-min period while in the clear Plexiglas® exposure chamber under conditions of room air or 25%, 50% or 70% N₂O in oxygen (O₂).

The degree of antinociception produced by N₂O in various treatment groups of mice was calculated as:

$$\% \text{ antinociceptive effect} = 100 \times \frac{\# \text{ constrictions in control mice} - \# \text{ constrictions in exposed mice}}{\# \text{ constrictions in control mice}}$$

Raters were trained extensively in evaluation of glacial acetic acid-induced abdominal constrictions during preliminary experiments. While raters were not blinded to drug conditions, multiple raters were used for some but not all experiments; however, the number of abdominal constrictions counted was very consistent between the raters.

2.6. Experimental design

To ascertain dose-dependent antagonism of N₂O by β -CNA, different groups of mice were pretreated i.PAG with 0 (vehicle control), 0.1 or 0.5 μ g β -CNA 24 hr prior to being assessed for antinociceptive responsiveness to 70% N₂O (Fig. 2).

To determine the influence of β -CNA pretreatment on the dose-response curve for N₂O-induced antinociception, different groups of mice were pretreated i.PAG with 0 (vehicle control) or 0.1 μ g β -CNA 24 hr prior to being assessed for antinociceptive responsiveness to 0% (room air control), 25%, 50% or 70% N₂O (Fig. 3).

To ascertain dose-dependent antagonism of N₂O by TRIM, different groups of mice were pretreated i.PAG with 0 (vehicle control), 10 or 30 μ g TRIM 30 min prior to being assessed for antinociceptive responsiveness to 70% N₂O (Fig. 4).

To determine the influence of TRIM pretreatment on the dose-response curve for N₂O-induced antinociception, different groups of mice were pretreated i.PAG with 0 (vehicle control) or 10 μ g TRIM 30 min prior to being assessed for antinociceptive responsiveness to 0% (room air control), 25%, 50% or 70% N₂O (Fig. 5).

2.7. Statistical analysis

The percent antinociceptive responses of control and experimental groups were compared using one-way analysis of variance (ANOVA) and post-hoc Bonferroni test. Dose-response curves were constructed for N₂O-induced antinociception for each treatment group. The probit of the percent antinociceptive response (percent inhibition of abdominal constrictions) was plotted against the log concentration of N₂O, and the antinociceptive dose, 50% (AD₅₀) values and 95% confidence intervals were determined by the method of Litchfield and Wilcoxon (1949), as described by Dewey et al [1970].

3. Results

On average, i.p. treatment with 0.6% glacial acetic acid evoked 25.6 ± 2.3 abdominal constrictions over a 6-min period starting 5 min following injection (N=12). Following exposure to increasing concentrations of N₂O (25%, 50% and 70%), the mean number of glacial acetic acid-induced abdominal constrictions was reduced to 23.2 ± 1.8 (9.0% antinociceptive response), 14.9 ± 1.4 (41.8% antinociceptive response) and 4.6 ± 1.3 (70.1% antinociceptive response), respectively.

In animals that were pretreated i.PAG with saline vehicle 24 h earlier, exposure to 70% N₂O evoked a 70.1% antinociceptive response. In mice that were pretreated with one of two doses of β -CNA (0.1 or 0.5 μ g) 24 h earlier, the peak antinociceptive response was reduced in a dose-related manner to 33.5% and 15.6%, respectively (Fig. 2). Intra-PAG treatment with β -CNA alone had no influence on the number of glacial acetic acid-induced abdominal constrictions. Comparison of dose-response curves in the absence and presence of 0.1 μ g β -CNA show that opioid receptor blockade in the PAG caused a rightward shift of the dose-response curve for N₂O-induced antinociception (Fig. 3). The AD₅₀ value (with 95% confidence intervals) was 53.7% (37.6–76.8%) for the vehicle control group, whereas, in the β -CNA-pretreated group, the AD₅₀ value was increased to 89.4% (66.9–119.4%).

In another experiment, mice that received an i.PAG microinjection of saline vehicle 30 min beforehand responded to 70% N₂O with a 64.6% antinociceptive response. In mice that were pretreated with one of two doses of TRIM (10 or 30 μ g) 30 min earlier, the intensity of the N₂O antinociceptive response was reduced to 37.6% and 12.2%, respectively (Fig. 4). Intra-PAG treatment with TRIM alone had no influence on the number of glacial acetic acid-induced abdominal constrictions. Comparison of dose-response curves in the absence and presence of 10 μ g TRIM show that inhibition of NO production in the PAG shifted the dose-response curve for N₂O-induced antinociception to the right (Fig. 5). The AD₅₀ value of the vehicle control group was 54.8% (32.5–92.4%), while that of the TRIM pretreatment group was 102.7% (64.4–163.8%).

4. Discussion

The PAG has long been known to be the site of action of opioid-induced antinociception (Jacquet and Lajtha, 1974; Sharpe et al., 1974). It was also reported that kainic acid-induced lesions of the ventral and caudal PAG antagonized the antinociceptive effect of N₂O in rats (Zuniga et al., 1987). We reported that i.PAG microinjection of the μ opioid antagonist CTOP caused a dose-dependent antagonism of N₂O-induced antinociception in the rat hot plate test (Hodges et al., 1994). The antinociceptive response of rats to N₂O in the tail-flick test was also sensitive to antagonism by naloxone administered directly into the ventrolateral part of the PAG (Fang et al., 1997), from which N₂O would activate descending noradrenergic pain-modulatory pathways to the spinal cord (Fujinaga and Maze, 2002). Consistent with these earlier studies, the results of the present investigation demonstrate that i.PAG pretreatment with the non-equilibrium opioid antagonist β -CNA caused a dose-dependent attenuation of the antinociceptive response of mice to N₂O.

We have previously demonstrated that exposure of mice to N₂O increased the level of nitric oxide synthase (NOS) enzyme activity in the brain (Li et al., 2003). NOS activity is apparently critical to the antinociceptive response to N₂O since N₂O-induced antinociception in rats and mice is blocked by pretreatment with any of a variety of NOS-inhibiting arginine analogs (McDonald et al., 1994; Ishikawa and Quock, 2003a) and also by pretreatment with an antisense oligodeoxynucleotide directed against the neuronal NOS enzyme (Li et al., 2004). Moreover, DBA/2 inbred mice that are poorly sensitive to N₂O-induced antinociception failed to exhibit

an increase in NOS enzyme activity during exposure to N₂O when compared to N₂O-sensitive C57BL/6 mice (Ishikawa and Quock, 2003b; Henry et al., 2005). Mice that were selectively bred for poor responsiveness to N₂O likewise did not exhibit an increase in NOS activity during exposure to N₂O (Henry et al., 2005).

The results of the present study demonstrate clearly that microinjection of the NOS-inhibitor TRIM into the PAG also caused a dose-related antagonism of N₂O-induced antinociception. Since we have proposed in earlier investigations that N₂O owes its antinociceptive effect in animals to stimulated neuronal release of endogenous opioid peptides (Branda et al., 2000; Cahill et al., 2000), the importance of the present findings is the localization of both opioid and NO mechanisms in the PAG of the midbrain. In as much as inhibition of NOS can significantly attenuate the increase in release of methionine-enkephalin in the rat spinal cord in response to centrally-administered β -endorphin (Hara et al., 1995), one explanation of these findings is that NO may play a key regulatory role in the N₂O-stimulated neuronal release of endogenous opioid peptides that then activate opioid receptors in the PAG. Consistent with this hypothesis is our recent observation that exposure to N₂O induces an NO-dependent increase in β -endorphin and NO metabolites in diasylate collected from the rat arcuate nucleus (Ohgami et al., 2007).

Other possibilities for NO modulation of N₂O-induced antinociception must also be acknowledged. While there is evidence that NO appears to be involved in the development of opioid tolerance (Kolesnikov et al., 1993; Majeed et al., 1994), most studies report a lack of involvement of NO in morphine-antinociception (Duarte and Ferreira, 1992; Xu and Tseng, 1993; McDonald et al., 1994; Heinzen and Pollack, 2004). There are some exceptions, however, where inhibition of NO production with different NOS-inhibitors altered antinociceptive responsiveness to morphine. Pretreatment with L-N^G-nitro arginine methyl ester (L-NAME) or L-N^G-monomethyl nitro arginine (L-NMMA) reportedly attenuated morphine-induced analgesia in mice (Pataki and Telegdy, 1998; Abacioglu et al., 2001); on the other hand, pretreatment with L-N^G-nitro arginine (L-NOARG) significantly enhanced pain threshold and potentiated morphine-induced antinociception in mice (Ozek et al., 2003) and potentiated morphine-induced antinociception in dogs (Pelligrino et al., 1996). Yet another possibility is NO modulation of the functionality of opioid receptors as increased NO (through L-arginine loading) was found to elevate constitutive activation of the μ opioid receptor (Heinzen et al., 2005).

In summary, these findings indicate that N₂O-induced antinociception in the mouse abdominal constriction test is dependent on both NO and opioid mechanisms that are co-localized in the PAG.

Acknowledgement

This research was supported in part by NIH Grant DA-10047 and by State of Washington Initiative Measure No. 171. A.D. was supported by a Summer Undergraduate Research Fellowship (SURF) from the American Society for Pharmacology and Experimental Therapeutics (ASPET) and the Washington State University College of Pharmacy.

References

- Abacioglu N, Ozmen R, Cakici I, Tunctan B, Kanzik I. Role of L-arginine/nitric oxide pathway in the antinociceptive activities of morphine and mepyrmine in mice. *Arzneimittelforschung* 2001;51:977–983. [PubMed: 11799845]
- Berkowitz BA, Ngai SH, Finck AD. Nitrous oxide “analgesia”: resemblance to opiate action. *Science* 1976;194:967–968. [PubMed: 982058]
- Branda EM, Ramza JT, Cahill FJ, Tseng LF, Quock RM. Role of brain dynorphin in nitrous oxide antinociception in mice. *Pharmacol. Biochem. Behav* 2000;65:217–222. [PubMed: 10672972]

- Cahill FJ, Ellenberger EA, Mueller JL, Tseng LF, Quock RM. Antagonism of nitrous oxide antinociception in mice by intrathecally administered opioid peptide antisera. *J. Biomed. Sci* 2000;7:299–303. [PubMed: 10895052]
- Dewey WL, Harris LS, Howes JF, Nuite J. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J. Pharmacol. Exp. Ther* 1970;175:435–442. [PubMed: 4394803]
- Duarte ID, Ferreira SH. The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway. *Eur. J. Pharmacol* 1992;221:171–174. [PubMed: 1333972]
- Fang F, Guo TZ, Davies MF, Maze M. Opiate receptors in the periaqueductal gray mediate analgesic effect of nitrous oxide in rats. *Eur. J. Pharmacol* 1997;336:137–141. [PubMed: 9384225]
- Franklin, KBJ.; Paxinos, G. The mouse brain in stereotaxic coordinates. 2nd ed.. Academic Press; San Diego: 2001.
- Fujinaga M, Maze M. Neurobiology of nitrous oxide-induced antinociceptive effects. *Mol. Neurobiol* 2002;25:167–189. [PubMed: 11936558]
- Hara S, Kuhns ER, Ellenberger EA, Mueller JL, Shibuya T, Endo T, Quock RM. Involvement of nitric oxide in intracerebroventricular β -endorphin-induced neuronal release of methionine-enkephalin. *Brain Res* 1995;675:190–194. [PubMed: 7796128]
- Heinzen EL, Booth RG, Pollack GM. Neuronal nitric oxide modulates morphine antinociceptive tolerance by enhancing constitutive activity of the mu-opioid receptor. *Biochem. Pharmacol* 2005;69:679–688. [PubMed: 15670586]
- Heinzen EL, Pollack GM. The development of morphine antinociceptive tolerance in nitric oxide synthase-deficient mice. *Biochem. Pharmacol* 2004;67:735–741. [PubMed: 14757173]
- Henry ED, Ohgami Y, Li S, Chung E, Quock RM. Correlation of inbred mouse sensitivity to nitrous oxide antinociception with brain NOS activity following exposure to nitrous oxide. *Pharmacol. Biochem. Behav* 2005;81:764–768. [PubMed: 15993935]
- Hodges BL, Gagnon MJ, Gillespie TR, Breneisen JR, O'Leary DF, Hara S, Quock RM. Antagonism of nitrous oxide antinociception in the rat hot plate test by site-specific μ - and ϵ -opioid receptor blockade. *J. Pharmacol. Exp. Ther* 1994;269:596–600. [PubMed: 8182525]
- Ishikawa M, Quock RM. Role of nitric oxide synthase isoforms in nitrous oxide antinociception in mice. *J. Pharmacol. Exp. Ther* 2003a;306:484–489. [PubMed: 12721331]
- Ishikawa M, Quock RM. N₂O stimulates NOS enzyme activity in C57BL/6 but not DBA/2 mice. *Brain Res* 2003b;976:262–263. [PubMed: 12763262]
- Jacquet YF, Lajtha A. Paradoxical effects after microinjection of morphine in the periaqueductal gray matter in the rat. *Science* 1974;185:1055–1057. [PubMed: 4604871]
- Kolesnikov YA, Pick CG, Ciszewska G, Pasternak GW. Blockade of tolerance to morphine but not to kappa opioids by a nitric oxide synthase inhibitor. *Proc. Natl. Acad. Sci. USA* 1993;90:5162–5166. [PubMed: 7685116]
- Li S, Bieber AJ, Quock RM. Antagonism of nitrous oxide antinociception in mice by antisense oligodeoxynucleotide directed against neuronal nitric oxide synthase enzyme. *Behav. Brain Res* 2004;152:361–363. [PubMed: 15196804]
- Li S, Ohgami Y, Dai Y, Quock RM. Antagonism of nitrous oxide-induced behavior in mice by pharmacologic disruption of endogenous nitric oxide function. *Psychopharmacology* 2003;166:366–372. [PubMed: 12589527]
- Majeed NH, Przewlocka B, Machelska H, Przewlocki R. Inhibition of nitric oxide synthase attenuates the development of morphine tolerance and dependence in mice. *Neuropharmacology* 1994;33:189–192. [PubMed: 7518573]
- McDonald CE, Gagnon MJ, Ellenberger EA, Hodges BL, Ream JK, Tousman SA, Quock RM. Inhibitors of nitric oxide synthesis antagonize nitrous oxide antinociception in mice and rats. *J. Pharmacol. Exp. Ther* 1994;269:601–608. [PubMed: 8182526]
- Ohgami, Y.; Chung, E.; Quock, RM. Pharmacol. Soc. Ann. Mtg. Banff, Alberta; Canada: 2007. Exposure to nitrous oxide causes a nitric oxide-dependent release of β -endorphin in the rat arcuate nucleus. Abstracts of Banff 2007, West.; p. 45

- Ozek M, Uresin Y, Gungor M. Comparison of the effects of specific and nonspecific inhibition of nitric oxide synthase on morphine analgesia, tolerance and dependence in mice. *Life Sci* 2003;72:1943–1951. [PubMed: 12597993]
- Pataki I, Telegdy G. Further evidence that nitric oxide modifies acute and chronic morphine actions in mice. *Eur. J. Pharmacol* 1998;357:157–162. [PubMed: 9797031]
- Pelligrino DA, Laurito CE, VadeBoncouer TR. Nitric oxide synthase inhibition modulates the ventilatory depressant and antinociceptive actions of fourth ventricular infusions of morphine in the awake dog. *Anesthesiology* 1996;85:1367–1377. [PubMed: 8968184]
- Quock RM, Graczak LM. Influence of narcotic antagonist drugs upon nitrous oxide analgesia in mice. *Brain Res* 1988;440:35–41. [PubMed: 2833991]
- Sharpe LG, Garnett JE, Cicero TJ. Analgesia and hyperreactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. *Behav. Biol* 1974;11:303–313. [PubMed: 4411999]
- Xu JY, Tseng LF. Increase of nitric oxide by L-arginine potentiates β -endorphin- but not μ -, δ or κ -opioid agonist-induced antinociception in the mouse. *Eur. J. Pharmacol* 1993;236:137–142. [PubMed: 8391452]
- Zuniga J, Joseph S, Knigge K. Nitrous oxide analgesia: partial antagonism by naloxone and total reversal after periaqueductal gray lesions in the rat. *Eur. J. Pharmacol* 1987;142:51–60. [PubMed: 3691637]

Fig. 1. Photomicrograph of cresyl violet-stained coronal mouse brain section and corresponding atlas plate (Franklin and Paxinos, 2001) indicating the typical site and spread of microinjection as assessed by stain injections. All doses of β -CNA and TRIM were injected at a volume of 0.5 μ l into the PAG.

Fig 2.

Dose-related antagonism of N_2O -induced antinociception by β -CNA. Different groups of mice received i.PAG microinjection of β -CNA or vehicle 24 hr prior to being assessed for antinociceptive responsiveness to 70% N_2O in the abdominal constriction test. The height of each bar indicates the mean percent antinociceptive response $\pm$ s.e.m. of 8–12 mice per group. Significance of difference: *, $p < 0.05$ and **, $p < 0.01$, compared to vehicle (0 μ g) control group (Bonferroni test).

Fig 3. Rightward shift of the dose-response curve for N₂O-induced antinociception by i.PAG microinjection of 0.1 μg β-CNA 24 hr prior to being assessed for antinociceptive responsiveness to N₂O in the abdominal constriction test. Each symbol represents the mean percent antinociceptive response of 8–12 mice per group.

Fig 4.

Dose-related antagonism of N₂O-induced antinociception by TRIM. Different groups of mice received i.PAG microinjection of TRIM or vehicle 30 min prior to being assessed for antinociceptive responsiveness to 70% N₂O in the abdominal constriction test. The height of each bar indicates the mean percent antinociceptive response $\pm$ s.e.m. of 8–12 mice per group. Significance of difference: **, $p < 0.01$, compared to vehicle (0 μ g) control group (Bonferroni test).

Fig 5.

Rightward shift of the dose-response curve for N₂O-induced antinociception by i.PAG microinjection of 10 µg TRIM 30 min prior to being assessed for antinociceptive responsiveness to N₂O in the abdominal constriction test. Each symbol represents the mean percent antinociceptive response of 8–12 mice per group.