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RAPID COMMUNICATION

Contrasting Influences of 5-Hydroxytryptamine Receptors in Nitrous Oxide Antinociception in Mice

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MUELLER, J. L. AND R. M. QUOCK. *Contrasting influences of 5-hydroxytryptamine receptors in nitrous oxide antinociception in mice.* PHARMACOL BIOCHEM BEHAV **41**(2) 429–432, 1992. — 5-Hydroxytryptamine (5-HT) mechanisms may play a role in opioid-mediated antinociception. Since opioid mechanisms have been implicated in nitrous oxide antinociception, this study was conducted to determine the possible role of 5-HT receptors in nitrous oxide antinociception. Male Swiss Webster mice were pretreated with one of two 5-HT receptor blockers and then tested in the acetic acid abdominal constriction test for their antinociceptive response to nitrous oxide, the κ-opioid agonist U-50,488H, or the μ-opioid agonist sufentanil. Results indicate that the 5-HT_3 receptor blocker ICS-205,930 antagonized both nitrous oxide and U-50,488H effects but not that of sufentanil. Mianserin, a 5-HT_{1c}/5-HT_2 receptor blocker, potentiated effects of both nitrous oxide and U-50,488H but not that of sufentanil. These findings show similarities in nitrous oxide and U-50,488H antinociception and further support our hypothesis that nitrous oxide works through central κ-opioid mechanisms in mice. The results also suggest different roles for 5-HT receptor subtypes in mediating or modulating the antinociceptive effect of nitrous oxide.

Nitrous oxide Antinociception 5-Hydroxytryptamine receptors Mice

THE discovery of multiple opioid receptors triggered a massive search for new analgesic drugs dissimilar to morphine and, hopefully, devoid of the more undesirable properties of classical opioid drugs. One drug that resulted from this search was trans(±)3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U-50,488H). U-50,488H antinociception is mediated through an opioid receptor with lower affinity for naloxone—the κ-opioid receptor—than does the site that mediates morphine antinociception—the μ-opioid receptor (19). More recent research has implicated 5-hydroxytryptamine (5-HT) mechanisms in mediation of the antinociceptive effect of U-50,488H (6–8,10,20).

In previous research, we provided evidence that the antinociceptive effect of the anesthetic gas nitrous oxide in mice appears to be mediated by κ- rather than μ-opioid receptors (12–15). Accordingly, the present investigation was conducted to compare the influence of different 5-HT receptor blockers on the antinociceptive effects of nitrous oxide, U-50,488H, and, by comparison, the μ-opioid agonist sufentanil.

METHOD

Antinociceptive Testing

Male 20 to 25-g Swiss Webster mice (Sasco Inc., Omaha, NE) were assessed for their antinociceptive responsiveness to nitrous oxide using the abdominal constriction test. Mice were treated (IP) with 0.1 ml per 10 g body weight of 0.6% acetic acid; 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.

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The responsiveness of various treatment groups of mice to nitrous oxide was calculated as % antinociception =

$$100 \times \frac{\left(\begin{array}{c}\text{mean \# constrictions}\\ \text{in control mice}\end{array}\right) - \left(\begin{array}{c}\text{mean \# constrictions}\\ \text{in exposed mice}\end{array}\right)}{(\text{mean \# constrictions in control mice})}.$$

Nitrous Oxide Exposure

Cages of five mice each were inserted into a small, inflatable, polyethylene AtmosBag® glovebag (Aldrich, Milwaukee, WI) immediately following IP treatment with 0.6% acetic acid. Nitrous oxide and oxygen were delivered into the glovebag, using a standard nitrous oxide/oxygen dental sedation system (Porter, Hatfield, PA). The proportions of nitrous oxide and oxygen were varied within a total inflow rate of 10 l/min to achieve the different test concentrations of nitrous oxide (25% nitrous oxide: 2.5 l/min nitrous oxide plus 7.5 l/min oxygen; 50% nitrous oxide: 5.0 l/min nitrous oxide plus 5.0 l/min oxygen; and 70% nitrous oxide: 7.0 l/min nitrous oxide plus 3.0 l/min oxygen). The test concentration of nitrous oxide within the glovebag was confirmed using a POET II anesthetic monitoring system (Criticare, Milwaukee, WI). Exhausted gases were vented from the glovebag to a fumehood. Control experiments were conducted in a sealed glovebag filled with compressed air.

Drugs

The following drugs were used in this study: Nitrous Oxide, USP, Oxygen, USP, and Compressed Air, USP (Rockford Industrial, Rockford, IL); U-50,488H (Upjohn, Kalamazoo, MI); sufentanil citrate (Janssen, Beerse, Belgium); ICS-205,930 (Cambridge Biochemicals, Cambridge, MA); and mianserin hydrochloride (Organon International, Oss, The Netherlands). Mixtures of nitrous oxide and oxygen were administered by inhalation as described above. All other drugs were prepared in 0.9% saline. ICS-205,930 and mianserin were administered IP in a volume of 0.1 ml per 10 g body weight; control animals were treated IP with vehicle (saline). Drug pretreatments (0.3 mg/kg ICS-205,930 or 0.3 mg/kg mianserin) were administered 30 min prior to exposure to 25, 50, or 70% nitrous oxide or 10 min prior to SC administration of either U-50,488H or sufentanil.

Statistical Analysis of Data

Dose-response curves were constructed for drug-induced antinociception in different treatment groups. Analgesic dose 50% (AD_{50}) values and their 95% confidence intervals were calculated for each pretreatment group and compared using the method of Litchfield and Wilcoxon (9).

RESULTS

Dose-response curves for nitrous oxide, U-50,488H, and sufentanil antinociception in various treatment groups are compared in Fig. 1. As shown in Table 1, the AD_{50} values for nitrous oxide and U-50,488H antinociception are both significantly elevated by ICS-205,930 and reduced by mianserin. However, the AD_{50} value for sufentanil antinociception was not appreciably affected by either drug pretreatment.

DISCUSSION

Our previous studies have gathered evidence that the antinociceptive effect of nitrous oxide in mice is not mediated by μ-opioid receptors but rather by κ-opioid receptors. First, if the effect were μ-opioid-mediated, it should be sensitive to antagonism by a μ-selective opioid receptor blocker, yet nitrous oxide antinociception was reduced not by β-funaltrexamine but by the κ-selective antagonist norbinaltorphimine (12,13). Second, if the effect were μ-opioid-mediated, it should be greatly attenuated in a recombinant inbred mouse strain deficient in brain μ-opioid receptors, yet nitrous oxide evoked prominent antinociception in CXBK/By mice (15). Third, if the effect were μ-opioid-mediated, it should remain intact following selective protection of μ-opioid receptors from alkylation by β-chlornaltrexamine, yet it was spared after protection of κ-opioid receptors by U-50,488H rather than protection of μ-opioid receptors by D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) or sufentanil (14,15).

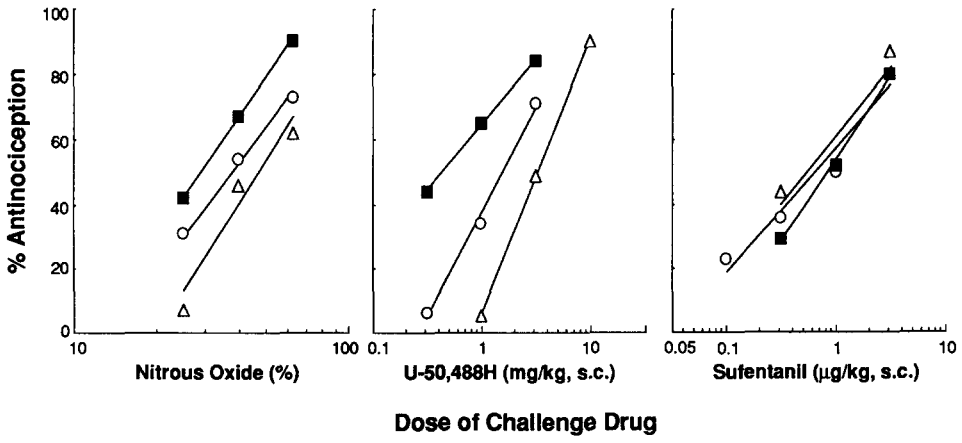

FIG. 1. Dose-response curves (AD_{50} values and 95% confidence intervals) for nitrous oxide antinociception in different groups of mice pretreated with: ○, saline; △, ICS-205,930 (0.3 mg/kg); ■, mianserin (0.03 mg/kg). Values were determined from 30–60 mice per treatment group.

TABLE 1

INFLUENCE OF ICS-205,930 AND MIANSERIN ON NITROUS OXIDE, U-50,488H, AND SUFENTANIL ANTINOCICEPTION IN MICE

Antinociceptive Challenge	Vehicle Control	ICS-205,930 Pretreatment	Mianserin Pretreatment
Nitrous oxide	39.0%	55.5%	25.6%
	(28.4–53.4%)	(48.2–63.8%)*	(21.4–30.7%)†
U-50,488H	1.56 mg/kg	3.33 mg/kg	0.40 mg/kg
	(0.95–2.55 mg/kg)	(2.43–4.58 mg/kg)*	(0.21–0.76 mg/kg)†
Sufentanil	0.60 μg/kg	0.49 μg/kg	0.77 μg/kg
	(0.30–1.20 μg/kg)	(0.29–0.83 μg/kg)	(0.50–1.17 μg/kg)

*$p < 0.05$, significant antagonism.
†$p < 0.05$, significant potentiation.

Additional supportive evidence might also be similarities between nitrous oxide and U-50,488H in 5-HT involvement in mediation or modulation of their antinociceptive effects. In our hands, ICS-205,930, a 5-HT_3 receptor blocker (17), significantly antagonized effects of both nitrous oxide and U-50,488H but not sufentanil, while mianserin, a 5-HT_{1c}/5-HT_2 receptor blocker (11), significantly potentiated nitrous oxide and U-50,488H antinociception but not that of sufentanil.

Von Voigtlander et al. (20) reported that U-50,488H antinociception in mice was antagonized by SC pretreatment with the 5-HT receptor blockers cyproheptadine, ketanserin, and pirenperone but not metergoline or mianserin. More recently, Ho and Takemori (6,7) reported that ICV U-50,488H antinociception was antagonized by ICV pindolol, methysergide, mianserin, ketanserin, pirenperone, and ICS-205,930. However, ICV U-50,488H antinociception was antagonized only by IT pindolol or methysergide but not mianserin, ketanserin, pirenperone, or ICS-205,930. When U-50,488H was given IT, its antinociceptive effect was antagonized by IT pindolol or methysergide, potentiated by IT mianserin, ketanserin, or pirenperone, and unaffected by IT ICS-205,930. They concluded that 5-HT plays an important role in U-50,488H antinociception and that supraspinal and spinal 5-HT_1, 5-HT_2, and 5-HT_3 receptors are all involved albeit different modes of interaction.

Many studies have demonstrated attenuation of morphine effects after depletion of 5-HT by *p*-chlorophenylalanine (PCPA) (1,4,16) or blockade of 5-HT receptors (3,5). However, other studies failed to find any disruption of morphine antinociception by PCPA (18) or by the 5-HT blockers methysergide, mianserin, or metergoline (2). Thus, an indispensable role for 5-HT in morphine antinociception remains debatable and the involvement of 5-HT in μ-opioid antinociception is, as yet, equally unclear.

The findings of this investigation are in agreement with those of earlier studies (6,7,20) that 5-HT mechanisms are involved in U-50,488H antinociception; it also appears that 5-HT mechanisms likewise play a role in nitrous oxide antinociception. However, these results lend more doubt to their involvement in μ-opioid antinociception, at least with 5-HT_3 and 5-HT_{1c}/5-HT_2 receptors. The parallel interactions of ICS-205,930 and mianserin with nitrous oxide and U-50,488H, but not sufentanil, suggest additional similarities in nitrous oxide and U-50,488H antinociception, providing further evidence that nitrous oxide antinociception in mice is mediated via κ-opioid receptors. It is also apparent that different 5-HT receptor subtypes may subserve opposing functions in mediation of the κ-opioid-mediated antinociceptive responses to nitrous oxide and U-50,488H.

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REFERENCES

1. Berge, O. G.; Gasmer, O. B.; Hole, K. Serotonin receptor antagonists induce hyperalgesia without preventing morphine antinociception. Pharmacol. Biochem. Behav. 19:873–878; 1983.
2. Berge, O. G.; Hole, K.; Ogren, S. O. Attenuation of morphine induced analgesia by p-chlorophenylalanine and p-chloroamphetamine: test-dependent effects and evidence for brainstem 5-hydroxytryptamine involvement. Brain Res. 271:51–64; 1983.
3. Crisp, T.; Perrotti, J. M.; Eller, R. W.; Smith, D. Opiate analgesia in the spinal cord involves a local serotonergic component. Pain 4(Suppl.):44; 1987.
4. Fennessy, M. R.; Lee, J. R. Modification of morphine analgesia by drugs affecting adrenergic and tryptaminergic mechanisms. J. Pharm. Pharmacol. 22:930–935; 1970.
5. Gorlitz, B. D.; Frey, H. H. Central monoamines and antinociceptive drug action. Eur. J. Pharmacol. 20:171–180; 1972.
6. Ho, B. Y.; Takemori, A. E. Serotonergic involvement in the antinociceptive action and the development of tolerance to the kappa-opioid receptor agonist, U-50,488H. J. Pharmacol. Exp. Ther. 250:508–514; 1989.
7. Ho, B. Y.; Takemori, A. E. Attenuation of the antinociceptive action of the selective κ-opioid receptor agonist, U-50,488H, by ICS-205,930. Eur. J. Pharmacol. 178:371–373; 1990.
8. Ho, B. Y.; Takemori, A. E. Release by U-50,488H of [^{3}H] serotonin from brain slices and spinal cord synaptosomes of U-50,488H-tolerant and nontolerant mice. J. Pharmacol. Exp. Ther. 254:8–12; 1990.
9. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96:99–108; 1949.
10. Nakazawa, T.; Yamanishi, Y.; Kaneko, T. A comparative study of monoaminergic involvement in the antinociceptive action of E-2078, morphine and U-50,488E. J. Pharmacol. Exp. Ther. 257: 748–753; 1991.
11. Peroutka, S. J. 5-Hydroxytryptamine receptor subtypes: Molecular, biochemical and physiological characterization. Trends Neurosci. 11:496–500; 1988.
12. Quock, R. M.; Best, J. A.; Chen, D. C.; Vaughn, L. K.; Portoghese, P. S.; Takemori, A. E. Mediation of nitrous oxide

analgesia in mice by spinal and supraspinal κ-opioid receptors. Eur. J. Pharmacol. 175:97–100; 1990; corrigendum 187:564; 1990.
13. Quock, R. M.; Graczak, L. M. Influence of narcotic antagonist drugs upon nitrous oxide analgesia in mice. Brain Res. 440:35–41; 1988.
14. Quock, R. M.; Mueller, J. Protection by U-50,488H against β-chlornaltrexamine antagonism of nitrous oxide antinociception in mice. Brain Res. 549:162–164; 1991.
15. Quock, R. M.; Mueller, J.; Vaughn, L. K. Mediation of nitrous oxide analgesia in mice by kappa but not mu opioid receptors. Pharmacologist 32:151; 1990.
16. Reigle, T. G.; Barker, W. L. p-Chloroamphetamine antagonism of the analgesia and increase in brain noradrenaline metabolism produced by morphine. J. Pharm. Pharmacol. 35:324–325; 1983.
17. Richardson, B. P.; Engel, G.; Donatsch, P.; Stadler, P. A. Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316:126–131; 1985.
18. Sugrue, M. F. Effect of depletion of rat brain 5-hydroxytryptamine on morphine-induced antinociception. J. Pharm. Pharmacol. 31:253–255; 1979.
19. von Voigtlander, P. F.; Lahti, R. A.; Ludens, J. H. U-50,488: A selective and structurally novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224:7–12; 1983.
20. von Voigtlander, P. F.; Lewis, R. A.; Neff, G. L. Kappa opioid analgesia is dependent on serotonergic mechanisms. J. Pharmacol. Exp. Ther. 231:270–274; 1984.