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Nitrous Oxide: Mechanism of Its Antinociceptive Action

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Nitrous oxide (N₂O) is an anesthetic gas known to produce an analgesic effect at subanesthetic concentrations. This analgesic property of N₂O can be clinically exploited in a broad range of conditions where pain relief is indicated. The mechanism of this analgesic effect was long thought to be nonspecific in nature, but a landmark study by Berkowitz and others in 1976 first implicated an opioid mechanism of action, possibly via N₂O-stimulated neuronal release of endogenous opioid peptides to activate opioid receptors. N₂O-induced release of opioid peptide has been demonstrated in both in vivo and in vitro preparations. Reversal of N₂O-induced antinociception in animals by narcotic antagonists has been reported by a number of laboratories. Subsequent studies have utilized more selective opioid antagonists to identify the opioid receptor subtypes involved in the antinociceptive effect of N₂O. Extensive pharmacological testing in the mouse abdominal constriction and rat hot plate paradigms have established that N₂O-induced antinociception is mediated by δ-opioid receptors in the former and by µ- and ε-opioid receptors in the latter. Current studies focus on two recent developments. The poor responsiveness of the DBA/2J mouse strain to N₂O has led to pharmacogenetic studies that hope to identify the underlying genetic basis for antinociceptive responsiveness to N₂O. Other research suggests an involvement of nitric oxide (NO) in mediating the antinociceptive effects of N₂O in both rats and mice.

Introduction

N₂O is one of the simpler inorganic chemicals that is utilized clinically. First identified by Joseph Priestley some 200 years ago, N₂O possesses three outstanding clinical properties. First, it produces analgesia. The analgesic effect caused by inhalation of 20% N₂O in oxygen is reportedly equivalent to that evoked by 15 mg morphine sulfate (9). N₂O-induced analgesia has been utilized to relieve pain associated with childbirth (40), myocardial infarction (36,73), diagnostic or interventional procedures (6,29,35), postsurgical recovery (23,50,51), intractable pain (38), refractory pain (16), and accident and trauma (2,12,72). Second, N₂O produces an anesthetic effect. N₂O enjoys a prominent role in medical history because it was the first drug used for surgical anesthesia. Despite its low anesthetic potency, it continues to be widely used in anesthesiology because of its ability to reduce the minimum alveolar concentration of other inhalation agents. Third, N₂O produces a significant anxiolytic effect. In clinical dentistry, it is popularly used for producing conscious sedation, a state in which patients experience reduced anxiety and pain (67). Recent research indicates that this antianxiety effect is distinct from the analgesic action of N₂O and may be mediated by central benzodiazepine mechanisms (13,57,64).

The Opioid Nature of N₂O-Induced Antinociception

The ability of inhaled N₂O to suppress pain was first reported in 1800 by Sir Humphry Davy, who suggested that it might be used to relieve pain during surgical operations (68). Surgical anesthesia was first demonstrated by Horace Wells in 1844, who had one of his own teeth extracted painlessly while he was under N₂O. Though N₂O was used for dental extractions and other surgical procedures for many years, its mechanism of action was ascribed to be a nebulous “nonspecific” action on the brain, most probably the result of a generalized depression of central nervous system function (69).

Most studies of N₂O-induced antinociception in mice have been carried out using the abdominal constriction
test. In this procedure, mice receive an intraperitoneal (i.p.) injection of a dilute concentration of a chemical irritant such as phenylquinone or glacial acetic acid. Exactly 5 min later, the number of abdominal contractions (i.e., lengthwise stretches of the torso with concave arching of the back) are counted for a fixed period. Exposure to increasing concentrations of N₂O causes a dose-related suppression in the number of abdominal contractions induced by i.p. injection of phenylquinone or dilute acetic acid (5,58). The first evidence suggesting a specific mechanism of action was provided by Berkowitz and coworkers, who reported that the antinociceptive effect of N₂O in mice was significantly reduced by subcutaneous (s.c.) pretreatment with 5.0 mg/kg naloxone or naltrexone (4,5). Quock and associates have conducted more extensive dose–response studies, in which increasing s.c. pretreatment doses (1.0–10 mg/kg) of naloxone caused a progressive shift to the right of the dose–response curve for N₂O-induced antinociception (56). Pretreatment with 10 mg/kg (+)-naloxone, s.c., was completely ineffective in influencing N₂O-induced antinociception, demonstrating that the ability of naloxone to attenuate N₂O was a stereospecific drug effect at the opioid receptor and not the consequence of some non-specific action of naloxone (58).

Systemic pretreatment with 10 mg/kg naltrexone methylbromide was also ineffective in altering N₂O-induced antinociception (58). However, following intracerebroventricular (i.c.v.) pretreatment with this quaternary ammonium form of naltrexone, there was an attenuation in N₂O-induced antinociception. Because naltrexone methylbromide does not penetrate the blood–brain barrier, these findings implicate opioid receptors in the central nervous system as being responsible for mediating N₂O-induced antinociception. Spinal cord opioid receptors are also involved in N₂O-induced antinociception because intrathecal (i.t.) pretreatment with 0.5–5.0 µg naloxone causes a dose-dependent reduction in N₂O-induced antinociception (55).

Although most N₂O studies in mice have been conducted in the abdominal constriction test, some investigations have utilized the hot plate paradigm. In this test, N₂O-induced antinociception was antagonized by pretreatment with 20 mg/kg naloxone s.c. (66) but not by doses of (+)-naloxone as high as 40 mg/kg (45).

N₂O-induced antinociception and antagonism of that antinociceptive effect have been most prominently demonstrated in rats using the hot plate paradigm, tail withdrawal from warm water, and the radiant heat tail flick test. In these models, antinociception is indicated by a significant prolongation in the latency time to reaction (hindpaw lick or escape attempt, tail withdrawal, and tail flick, respectively) to the thermal noxious stimulus. A general characteristic of N₂O-induced antinociception in rats was the steep dose dependency of the drug effect. The most consistent antinociceptive effect was in response to 70–75% N₂O, whereas 50% N₂O evoked a highly variable response and lower concentrations were generally ineffective (32,48,81).

The antinociceptive effect of N₂O in rats is also sensitive to antagonism by naloxone and naltrexone. As in mice, the antagonism of N₂O in the tail flick test required higher systemic doses (5–10 mg/kg) than required for antagonism of morphine (4,81). In rats implanted with central microinjection cannulae, the antinociceptive effect of 70% N₂O in the hot plate test was significantly antagonized by i.c.v. doses of 5.0 and 10 µg of naltrexone; increasing the i.c.v. dose of naltrexone to 20 µg failed to increase the antagonism (32). When 20 ng naloxone was administered into the cisternum, the antinociceptive effect of N₂O in the tail flick test was immediately terminated (48).

Tolerance and cross-tolerance studies conducted in the mouse abdominal constriction and rat tail flick paradigms suggest that N₂O might act indirectly on opioid receptors via stimulated release of opioid peptides. Chronic injections of up to 400 mg/kg morphine daily or s.c. implantation of 75-mg morphine pellets rendered animals tolerant to morphine-induced antinociception and also cross-tolerant to N₂O-induced antinociception. However, after exposure to 75% N₂O for 16–18 h, animals became tolerant to the antinociceptive effect of 80% N₂O but they were not cross-tolerant to morphine-induced antinociception (3,4). Therefore, N₂O tolerance induced by chronic exposure to N₂O resulted mainly from depletion of the releasable pool of endogenous opioid peptides with no concomitant change in responsiveness of opioid receptors to morphine. Consequently, in N₂O-tolerant animals, morphine continued to produce antinociception. On the other hand, morphine tolerance reduced responsiveness to the antinociceptive effects of both morphine and opioid peptides released by N₂O.

N₂O-induced release of opioid peptides has been demonstrated in both in vivo and in vitro preparations. Exposure to 75% N₂O increased the amount of immunoreactive (IR) methionine-enkephalin (ME) in fractions of artificial cerebrospinal fluid (CSF) collected from urethane-anaesthetized, ventricular-cisternal perfused rats (59). Based on kinetic modeling, the rate of neuronal release of ME in this in vivo system was increased by 140% in the presence of 75% N₂O (71). Comparable findings were reported in chronically cannulated dogs exposed to 66–75% N₂O (15), where there were significantly elevated levels of IR ME and [Arg⁷-Phe³]ME in CSF samples collected from the third ventricle. In in vitro studies, exposure to increasing concentrations of N₂O was shown to increase quantities of IR β-endorphin (β-EP) released from superfused rat basal hypothalamic cells (82).

Results from pharmacological studies also support the hypothesis of an indirect action of N₂O on opioid recep-
MECHANISM OF N₂O ANTINOCICEPTION

N₂O-induced antinociception in rats was attenuated in a dose-related manner by i.c.v. pretreatment with 50–200 µg of rabbit antiserum selective for β-EP. In addition, i.c.v. pretreatment with the endopeptidase 24.11-inhibitor phosphoramidon significantly potentiated N₂O-induced antinociception in rats (21). Paradoxically, N₂O-induced antinociception was not blocked by a ME antibody in i.c.v. doses as high as 400 µg (26).

An alternative explanation is that N₂O acts directly on the opioid receptor (22). This hypothesis is based largely on in vitro radioligand binding studies, in which N₂O, bubbling through tissue homogenate, reduced [³H]dihydromorphine ([³H]DHM) binding in whole mouse brain (1) and resulted in appearance of an additional super-high-affinity binding site for [³H]naloxone in rat forebrain (10). In a more recent study of brain µ-opioid and κ-opioid receptors, 100% N₂O or 2% halothane in oxygen was bubbled through tissue homogenate prepared from whole guinea pig brain (49). N₂O decreased the [³H]DHM binding affinity without affecting the density of binding sites and decreased the density of [³H]ethylecyclohexylketone ([³H]EKC) binding sites without affecting binding affinity. By comparison, halothane decreased both [³H]DHM and [³H]EKC binding affinities and reduced the density of binding sites for [³H]EKC. These results, suggest the authors, indicate the existence of specific membrane targets for anesthetic drugs.

The Role of Multiple Opioid Receptor Subtypes in N₂O-Induced Antinociception

The observation that N₂O-induced antinociception was sensitive to antagonism by opioid receptor blockers was the initial indicator and strongest evidence for mediation by endogenous opioid mechanisms. However, the s.c. doses of both naloxone and naltrexone required for antagonism of N₂O-induced antinociception were greater than those needed to antagonize morphine. Also, the antagonism was always partial, never complete. There are two possible explanations for this incomplete antagonism of N₂O antinociception by opioid receptor blockers. One, N₂O-induced antinociception might be only partly determined by opioid mechanisms and may involve a nonopioid component. Two, N₂O-induced antinociception might be mediated by opioid receptors other than the classical μ-opioid receptor. It is now acknowledged that naloxone and naltrexone can block μ-opioid receptors at low doses, but the dose of each must be increased in order to occupy non-μ-opioid receptor types (53). The introduction of newer, subtype-selective antagonists has made it possible to more precisely identify the central opioid receptors that mediate N₂O-induced antinociception.

One difficulty in determining the opioid receptors that mediate N₂O-induced antinociception is that identification of opioid receptor subtypes depend on the type and intensity of noxious stimulus applied. It was previously suggested that different opioid receptor subtypes participate in supraspinal and spinal antinociceptive responses depending upon whether the test utilized chemical or thermal stimuli to evoke pain (78). For instance, antinociception in the rat abdominal constriction test (chemical stimulus) is mediated by μ- and κ-opioid, but not δ-opioid, receptors (65), whereas spinal antinociception in the hot plate test (thermal stimulus) is mediated by μ and δ, but not κ, receptors (65). More current research has demonstrated that κ-opioid receptor-mediated antinociception is intensity dependent in thermal nociceptive tests (43,52). On the other hand, studies to date show that even “selective” δ-opioid agonists possess a residual cross-reactivity at μ-opioid receptors (11), possibly providing an explanation to those reports of an antinociceptive effect of δ-opioid agonists in tests utilizing chemical noxious stimuli (54). Interpretation is further complicated by findings of modulatory interactions between different opioid receptor subtypes (31,33).

N₂O-Induced Antinociception in Mice

To investigate whether κ-opioid receptors might be involved, mice were pretreated with 5.0 mg/kg MR-2266, i.p. (58). MR-2266 [(-)-5,9-diethyl-α,5,9-dialkyl-2-hydroxy-6,7-benzomorphan] is a κ-opioid receptor blocker with significant μ-opioid antagonist properties. MR-2266 significantly antagonized N₂O-induced antinociception, suggesting that μ- and/or κ-opioid receptors might be involved in N₂O-induced antinociception. Further evidence suggesting a role for κ-opioid receptors in N₂O-induced antinociception was demonstrated by the marked antagonism of the N₂O effect by the κ-selective opioid antagonist norbinaltorphimine (nor-BNI) (55). Effective antagonist doses were 50 nmol (36.7 µg), i.c.v., and 5.0 nmol (3.7 µg), i.t. Hence, evidence is strong that spinal and perhaps supraspinal κ-opioid receptors mediate N₂O-induced antinociception in the mouse abdominal constriction test. Pretreatment with 5.0 µg of the μ-selective opioid antagonist β-funaltrexamine, administered either i.c.v. or i.t., failed to influence N₂O-induced antinociception, suggesting that μ-opioid receptor blockers are not involved in N₂O-induced antinociception in the abdominal constriction paradigm. This is also supported by the robust antinociceptive effect of N₂O in μ-opioid receptor-deficient CXBK mice (61). The δ-opioid antagonists ICI-174,864 (N,N-diallyl-Tyr-Alb-Aib-Phe-Leu-ΟΗ, where Alb is α-aminoisobutyric acid) and naltrindole were ineffective in reducing N₂O-induced antinociception in the mouse abdominal constriction test (Quock, unpublished findings).

Additional support for the κ-opioid hypothesis of N₂O-induced antinociception comes from receptor pro-
tection studies involving pretreatment with \( \beta \)-chlornaltrexamine (\( \beta \)-CNA), a nonselective alkylation of opioid receptor subtypes (79). Mice were pretreated with 2.0 \( \mu \)g \( \beta \)-CNA, i.c.v., then tested for antinociceptive responsiveness to 50% \( N_2 O \) 24 h later; the antinociceptive effect of \( N_2 O \) was significantly reduced (60). But when \( \kappa \)-opioid receptors were spared from \( \beta \)-CNA alkylation by i.c.v. coadministration of 30 \( \mu \)g of the \( \kappa \)-opioid agonist U-50,488H \( \{ \text{trans} \pm \} -3,4\text{-dichloro-N-methyl-N-[2-1(pyrr-rolidinyl)cyclohexyl]benzene-acetamide methanesulfonate} \}, \) the antinociceptive response to \( N_2 O \) was undiminished. On the other hand, when \( \mu \)-opioid receptors were protected from \( \beta \)-CNA by coadministration of 0.1 \( \mu \)g of the \( \mu \)-opioid ligand CTOP (d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH\(_2\)), \( N_2 O \)-induced antinociception remained antagonized. Thus, brain \( \kappa \)-opioid, but not \( \mu \)-opioid, receptors appear to play a more critical role in the antinociceptive effect of \( N_2 O \) in the mouse abdominal constriction test.

Experimental evidence suggests the involvement of an additional opioid mechanism in the action of \( N_2 O \). Although higher doses of naloxone administered s.c., i.c.v., or i.t. produce a dose-related antagonism of \( N_2 O \)-induced antinociception in the mouse abdominal constriction test, ultraslow doses exert quite the opposite influence. Naloxone in pg/kg doses administered s.c. or fg doses administered i.t. causes a significant potentiation of \( N_2 O \)-induced antinociception (56). Pretreatment i.c.v. with fg doses of naloxone had neither an antagonistic nor potentiatory influence on \( N_2 O \)-induced antinociception. Consistent with the hypothesized \( \kappa \)-opioid nature of \( N_2 O \)-induced antinociception, ultraslow doses of naloxone administered s.c. or i.t., but not i.c.v., had the same potentiating outcome on the antinociceptive effect of U-50,488H. These results support the concept of a descending opioid system that can inhibit antinociception (17–19). Spinal opioid receptors in this system are ultra sensitive to antagonism by naloxone at doses so extremely low that antinociception induced by administration of classical \( \mu \)- and \( \kappa \)-opioid agonists is not antagonized. Hence, ultraslow doses of naloxone inactivate this antinociception–inhibitory system and potentiate the antinociceptive effects of both \( N_2 O \) and U-50,488H.

\( N_2 O \)-Induced Antinociception in Rats

Subtype-selective opioid antagonists have also been used to study \( N_2 O \)-induced antinociception in the rat hot plate paradigm. CTOP is a somatostatin analogue that can preferentially block \( \mu \)-opioid receptors (37) and selectively antagonize the effects of morphine and the \( \mu \)-selective opioid agonist DAMGO (\( \{ \text{o-Ala}^2 \text{MePhe}^4, \text{Gly}-\text{ol}\} \)-enkephalin) in rats (14). Administration i.c.v. of 1 \( \mu \)g CTOP significantly attenuated the antinociceptive effect of 70% \( N_2 O \); increasing the dose to 5 \( \mu \)g CTOP failed to increase the degree of antagonism. When CTOP was microinjected directly into the periaqueductal gray (PAG), a known site of action of morphine antinociception (30,80), \( N_2 O \)-induced antinociception was antagonized in dose-dependent manner up to an intracerebral dose of 1.0 \( \mu \)g (32). This localization of \( \mu \)-opioid receptors involved in \( N_2 O \)-induced antinociception to the PAG is consistent with findings that kainic acid lesions of the PAG significantly reduce \( N_2 O \)-induced antinociception in the tail flick test (81).

\( N_2 O \)-Induced antinociception may also be mediated by the \( \epsilon \)-opioid receptor. \( \beta \)-Endorphin (1-27) \( \{ \beta - \text{EP} (1-27) \} \) is a molecule of \( \beta \)-EP that is truncated by four amino acids at the C-terminal. This peptide selectively antagonizes the effects of \( \beta \)-EP and is a putative \( \epsilon \)-opioid receptor blocker (70). At i.c.v. doses below 5.0 \( \mu \)g, \( \beta \)-EP (1-27) produced dose-related antagonism of \( N_2 O \)-induced antinociception in the rat hot plate test. At i.c.v. doses greater than 5.0 \( \mu \)g, the antagonistic activity of \( \beta \)-EP (1-27) was gradually reduced and \( \beta \)-EP (1-27) produced a dose-related increase in the antinociceptive response to 70% \( N_2 O \). Rats pretreated with 7.0 \( \mu \)g \( \beta \)-EP (1-27) i.c.v. demonstrated the greatest variability of responsiveness to 70% \( N_2 O \), suggesting that 7.0 \( \mu \)g is near the threshold dose separating antagonistic and potentiatory influences upon \( N_2 O \)-induced antinociception (32). These data are consistent with earlier findings that \( \beta \)-EP (1-27) has partial agonist activity and can cause antinociception at higher doses (25,70). Although \( \beta \)-EP (1-27) administered i.c.v. reduced \( N_2 O \)-induced antinociception, comparable doses administered directly into the PAG had no effect on \( N_2 O \)-induced antinociception. \( \beta \)-EP (1-27) was not effective in reducing \( N_2 O \) antinociception when administered directly into the PAG. Though the \( \epsilon \)-opioid receptors involved in \( N_2 O \)-induced antinociception do not seem to reside in the PAG, possible sites might include the raphe obscurus nucleus and raphe pallidus nucleus in the caudal medulla oblongata or the medial posterior nucleus accumbens, medial preoptic area, and arcuate hypothalamic nucleus in the forebrain, which are active sites where administration of \( \beta \)-EP inhibits the tail flick response in rats (76,77).

In contrast to the \( \mu \)-opioid and \( \epsilon \)-opioid receptors that appear to mediate \( N_2 O \) antinociception in the hot plate test, other opioid receptor subtypes seem to be involved in other tests. When 55–58°C water was used as the noxious thermal stimulus, \( N_2 O \)-induced antinociception was significantly antagonized by i.c.v. pretreatment with 5.0 \( \mu \)g of the \( \kappa/\mu \)-opioid receptor blocker MR-2266, partly reduced by 5.0 \( \mu \)g of the \( \delta \)-opioid receptor blocker ICI-174,864, and unaltered by 10–50 \( \mu \)g of the \( \mu \)-selective opioid antagonist \( \beta \)-fumaltrexamine (63). These results suggest possible involvement of \( \kappa \)- and \( \delta \)-opioid, but not \( \mu \)-opioid, receptors in \( N_2 O \)-induced antinociception in the tail withdrawal paradigm.
The Mechanism of N₂O Antinociception

Because of the genetic homogeneity within individual mouse strains, pharmacological differences between strains are indicative of allelic variability between independent inbred strains. Comparative studies have in fact demonstrated strain-dependent differences in responsiveness to N₂O-induced antinociception (61). N₂O-induced antinociception was assessed in eight inbred strains, and results show the following order of responsiveness: A/J (the most sensitive) ≥ C57BL/6ByJ = C57BL/6J = BALB/cByJ = C3H/HeJ > CXBK/ByJ = CBA/J >> DBA/2J (the least sensitive). The weak antinociceptive response to N₂O in DBA/2J mice, which are sensitive to morphine- and U-50,488H-induced antinociception, indicates some underlying neurobiological difference in the DBA/2J.

Subsequent pharmacogenetic studies on N₂O-induced antinociception have focused on C57BL/6J and DBA/2J strains. These strains are particularly useful for the study of the genetic determinants of N₂O responsiveness for several reasons. First, they have large differences in responsiveness to N₂O with the C57BL/6J strain being much more sensitive to N₂O-induced antinociception than the DBA/2J strain. Second, these strains have been compared extensively for differences in morphine-induced antinociception (the DBA/2J is more sensitive), morphine-induced locomotor activation (the C57BL/6J is more sensitive), and susceptibility to development of morphine tolerance and physical dependence (8,24). Third, crosses of the C57BL/6J and DBA/2J strains have resulted in 26 B × D recombinant inbred (RI) strains. Each RI strain is a random recombination of the progenitor chromosomes in a homozygous state as a result of redistribution of the original F₂ genetic variance so that it exists almost entirely between strains and is almost completely absent within strains. Finally, a large number of marker gene loci have now been mapped (>1200).

Preliminary studies conducted in 22 B × D RI strains revealed a hierarchy of responsiveness to 70% N₂O ranging from a < 10% antinociceptive response in the B × D-27/Ty to a 100% response in the B × D-14 and -25/Ty lines. Cluster analysis showed one cluster of 16 strains approximating the C57BL/6 progenitor (61.9–100% antinociceptive response to 70% N₂O) and another of six strains approximating the DBA/2 progenitor (9.1–40% antinociceptive response to 70% N₂O) (62). The robust strain differences permitted screening the strain antinociceptive means with marker gene loci previously mapped in B × D RI strains. Significant associations at the 0.002 level were found on seven chromosomes, suggesting the presence of quantitative trait loci (QTL). As the number of test subjects in each RI strain is increased, the number of promising QTL will be further restricted and confirmed.

The Role of NO in N₂O-Induced Antinociception

A related but far more reactive oxide of nitrogen is NO, which consists of a single nitrogen atom coupled to a single oxygen atom. In recent years, NO has been recognized as being an important biological regulator in macrophage cytotoxic activity, physiological and pharmacological vasodilatation, and inhibition of platelet aggregation (34,44). Current neurobiological research has also identified NO as an intracellular messenger and even a new type of neurotransmitter in both the peripheral and central nervous systems (7,20). Central to investigations of NO function are drugs that inhibit the key enzyme NO synthase (NOS); these include the substituted arginines L-NAME, L-N¹-monomethyl arginine methyl ester (L-NAME), L-N¹-nitro arginine methyl ester (L-NAME), L-N¹-monomethyl nitro arginine (L-NMMMA), and 7-nitro indazole.

There is recent evidence of a modulatory role played by NO in N₂O-induced antinociception. In the mouse abdominal constriction paradigm, s.c. pretreatment with 3.0–10 mg/kg L-NAME caused a dose-related antagonism of N₂O-induced antinociception (41). Pretreatment with 10 mg/kg L-NAME or 10 mg/kg L-NMMMA, s.c., also significantly attenuated the antinociceptive effect of N₂O. Pretreatment with 0.5 μg L-NAME, i.c.v., also antagonized N₂O-induced antinociception. These doses of L-NAME, L-NAME, and L-NMMMA alone failed to produce any antinociceptive effect. Antagonism of N₂O-induced antinociception by s.c.-administered L-NAME and i.c.v.-administered L-NAME were both reversed by i.c.v. treatment with 20 μg l-arginine but not 20 μg d-arginine. Because only the L form of arginine is a substrate for NOS, these results implicate NO in the antinociceptive effect of N₂O.

Similar results were obtained in experiments conducted in the rat hot plate test (41). Pretreatment with 10–100 μg L-NAME, i.c.v., produced a dose-related antagonism of N₂O-induced antinociception. The antagonism of the N₂O effect by either 50 μg L-NAME, i.c.v., or 50 μg L-NAME, i.c.v., was stereospecifically reversed by i.c.v. treatment with 10 μg l-arginine but not 10 μg d-arginine.

Pretreatment with 10 mg/kg L-NOARG, s.c., failed to influence the antinociceptive effects of two other opioid drugs, the predominantly µ-opioid agonist morphine and the κ-selective opioid agonist U-50,488H (41). The failure of NOS inhibition to reduce the antinociceptive effects of these direct-acting opioid receptor agonists mitigates against the idea that NO is a general mediator in central pathways that mediate opioid antinociception. Furthermore, in the absence of NOS inhibition, i.c.v. treatment with 20 μg l-arginine significantly potentiated...
N₂O-induced antinociception in the mouse abdominal constriction test. Yet under similar circumstances, i.c.v. treatment with 10 µg L-arginine (which reversed L-NOARG and L-NAME antagonism of N₂O) failed to influence the antinociceptive effect of exposure to 70% N₂O in the rat hot plate test. Pretreatment with d-arginine was without effect in either paradigm. The fact that increasing brain levels of NO with L-arginine enhanced N₂O-induced antinociception in mice but not in rats might suggest that the contribution of NO to N₂O action in the two species is not identical.

What then is the role of NO in N₂O-induced antinociception? One possible explanation is that NO might play a role in the mechanism of neuronal release of opioid peptides. Tseng and his associates have shown that β-EP-induced antinociception is related to stimulated release of ME in the spinal cord and activation of spinal δ-opioid receptors by ME (74,75). In urethane-ane- 

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ethetized, i.t.-perfused rats, i.c.v. β-EP-induced increase of ME levels in the i.t. perfusate was significantly sup- 

pressed by perfusing with artificial CSF containing 100 

µM L-NOARG (27,28,39). The addition of 50 mM L-arginine, but not d-arginine, into the CSF perfusate reversed the suppression of the ME increase by L-NOARG. On the other hand, increasing the perfusate concentration of L-NOARG to 250 µM failed to produce a greater suppression of the β-EP-induced increase in ME. These findings suggest that NO may mediate the β-EP-induced release of ME in the spinal cord. If β-EP is the key opioid mediator in N₂O in rats, as suggested by in vitro release (82) and antibody antagonism studies (26), such a role for NO might explain the antagonism of N₂O antinociception in rats by NOS inhibitors.

Other research has uncovered an apparent role for NO in hyperalgesia at the level of the spinal cord. Synaptic plasticity may be intimately involved in the mechanism of chronic pain; in a manner analogous to the role it plays in long-term potentiation, NO may act as a retro- 

grade messenger to maintain a high level of presynaptic activity in hyperalgesia (42). A proactive role of NO in hyperalgesia is supported by observations of an antinoci- 

ceptive effect of NOS inhibitors in mouse tail flick, hot plate, abdominal constriction, and formalin-induced paw lick tests (46,47). However, the standard challenge doses of L-NAME were generally 50–100 mg/kg, which was 5–10 times greater than the 10 mg/kg dose used in our studies, and the resulting antinociceptive effects were not sensitive to antagonism by naloxone.

Summary

Twenty years ago, N₂O was merely one of a number of inhalation anesthetics that was distinguished by an unfavorable minimum alveolar concentration and the necessity of combining it with a more potent anesthetic agent to produce surgical anesthesia. Recent research, however, has revealed additional central pharmacologi- 

cal actions (analgesia, anxiolysis) that broaden the clinical applications of N₂O. There is increasing evidence of an opioid basis to the analgesic effect of N₂O. Yet N₂O appears to be strikingly unlike any other pharmacologi- 

cal agent with opioid analgesic properties. The mecha- 

nism by which N₂O interacts with endogenous opioid systems remains uncertain. Various in vitro and in vivo investigations have demonstrated N₂O-induced release of ME and β-EP, suggesting an indirect mode of action of N₂O with endogenous opioid systems. Pharmacologi- 

cal antagonism of N₂O-induced antinociception in ani- 

mals has been achieved using first naloxone and then various selective receptor blockers to identify the opioid receptor subtypes involved in mediating the antinociceptive effect of N₂O in mice and rats. The antinociceptive effect of N₂O in the mouse abdominal constriction test appears to be mediated by κ-opioid receptors. In con- 

trast, N₂O-induced antinociception in the rat hot plate test apparently involves supraspinal µ- and ε-opioid re- 

ceptors. Future research in inbred mouse strains prom- 

ises to help elucidate the neurobiological prerequisites for N₂O action by uncovering what is aberrant in DBA/ 

2J and B×D RI strains characterized by poor antinoci- 

ceptive responsiveness to N₂O. Exciting new findings of antagonism of N₂O-induced antinociception by inhibition of brain NO production suggests a possible role for NO in the mechanism of N₂O action, possibly as a regu- 

lator of the neuronal release of opioid peptide. It has be- 

come increasing apparent that N₂O is not only a clini- 

cally useful anesthetic and analgesic agent but also a unique investigative tool for studying opioid mecha- 

nisms. Elucidation of its uncommon mechanism of in- 

teraction with opioid systems will produce a better un- 

derstanding of the opioid systems themselves.

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