Advances in Understanding the Actions of Nitrous Oxide

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Nitrous oxide (N_2O) has been used for well over 150 years in clinical dentistry for its analgesic and anxiolytic properties. This small and simple inorganic chemical molecule has indisputable effects of analgesia, anxiolysis, and anesthesia that are of great clinical interest. Recent studies have helped to clarify the analgesic mechanisms of N_2O , but the mechanisms involved in its anxiolytic and anesthetic actions remain less clear. Findings to date indicate that the analgesic effect of N_2O is opioid in nature, and, like morphine, may involve a myriad of neuromodulators in the spinal cord. The anxiolytic effect of N_2O , on the other hand, resembles that of benzodiazepines and may be initiated at selected subunits of the γ -aminobutyric acid type A (GABA_A) receptor. Similarly, the anesthetic effect of N_2O may involve actions at GABA_A receptors and possibly at N-methyl-D-aspartate receptors as well. This article reviews the latest information on the proposed modes of action for these clinical effects of N_2O .

Key Words: Nitrous oxide; Pharmacology; Anesthesia; Analgesia; Anxiolysis.

Nitrous oxide (N_2O) was discovered in 1793 by the English scientist Joseph Priestley, who also discovered oxygen (O_2). In 1799, Sir Humphrey Davy administered N_2O to visitors at the Pneumatic Institute and gave it for the first time the term “laughing gas.” He astutely noted the analgesic effects of the gas and even predicted its application in suppression of pain during surgical procedures. However, for the next 40 years or so, the primary use of N_2O was for recreational enjoyment and public shows. N_2O found a more scientific use as an anesthetic in clinical dentistry and medicine in the early 1840s. Horace Wells, an American dentist, had one of his own teeth extracted while inhaling N_2O —the first demonstration of clinical anesthesia. There followed a period of wide clinical use of N_2O for dental anesthesia, which, unfortunately, was followed by a period of abandonment when it was recognized that the high concentrations required for anesthesia frequently placed the patient at risk of severe hypoxia and death from as-

phyxiation. Although the specific manner of use of N_2O has changed considerably in the past 150 years, it remains today the most widely used of all general anesthetic agents and plays an all-important role in both dental and medical practice. The introduction of anesthesia through N_2O is considered a great achievement in dentistry, comparable to the discovery of local anesthesia and the fluoridation of water.^{1,2}

Today the common medical uses of N_2O include balanced anesthesia during surgery, wherein N_2O is frequently combined with other general anesthetic drugs and nonanesthetic preoperative drugs, and analgesia for the relief of pain in clinical as well as emergency situations. There are significant nonmedical uses of N_2O as well. In industry, N_2O is used as an oxidizer in atomic absorption spectrometry and in the manufacture of semiconductors. In the dairy industry, N_2O is used as a bacteriostatic, tasteless, odorless food processing propellant. N_2O is also injected into the air intake of car engines by racing enthusiasts to boost horsepower. N_2O is also used to prepare divers for deep dives because it mimics the disorientation and behavioral changes of decompression illness (the “bends”) when a diver surfaces from the depths too rapidly.

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For nearly 150 years since its discovery, N₂O has been used by clinicians without a clear knowledge of its mechanisms of action. Only over the last 20–30 years has there been any significant research elucidating the mechanisms of the analgesic, antianxiety, and anesthetic effects of N₂O. In 1985, Eger published the first comprehensive book on N₂O, gathering all available information up to that time and providing an excellent summary of clinical and research issues.³ Since then, a large amount of research has been added, clarifying a number of issues about the actions of N₂O. A Medline search for research manuscripts on the pharmacology of N₂O revealed 2439 articles between 1940 and 1980; a similar search conducted recently uncovered 5801 articles between 1981 and 2006.

This timely review will update readers on the current state of knowledge of the pharmacokinetic and pharmacodynamic aspects of N₂O. The relevant clinical uses of N₂O as an analgesic, anxiolytic, and anesthetic drug will be explored.

ANALGESIA

Subanesthetic concentrations of N₂O produce only analgesic and anxiolytic effects without unconsciousness.⁴ Analgesic N₂O has a long history of use in obstetrics for relief of labor pain.⁵ N₂O is also used for self-administered analgesia in cancer patients,⁶ preemptive analgesia,⁷ and to alleviate pain and discomfort associated with a number of medical procedures, including intra-articular drug injection,⁸ peripheral intravenous cannulation,⁹ sigmoidoscopy,¹⁰ colonoscopy,¹¹ ophthalmologic procedures,¹² and biopsy procedures.¹³ In Europe, Entonox (BOC Group), a 50% N₂O:50% oxygen mixture, is widely used in emergency medical care of patients in accident scenes and during ambulance transportation.^{14–16}

The mechanism of the analgesic effect of N₂O is gradually being clarified through elucidation of the antinociceptive effect of N₂O in animals. Because animals are unable to report a reduction in sensation of pain, analgesia is more properly determined in animals as antinociception or a diminished responsiveness to a noxious stimulus.

The Opioid Hypothesis of N₂O Antinociceptive Action

It was as early as 1943 when the analgesic effect of N₂O was judged to be comparable to that of opioid analgesic drugs—30% of N₂O was deemed to be equieffective as 10–15 mg of morphine.¹⁷ It was not until the mid-1970s that it was first reported that N₂O-induced antinociception in mice and rats was sensitive to blockade

by the narcotic antagonist naloxone.^{18,19} N₂O-induced analgesia in human subjects was also antagonized by naloxone.^{20–23}

A large number of studies have established an important role for opioid receptors in the periaqueductal gray (PAG) area of the midbrain in pain modulation.²⁴ The N₂O-induced analgesic effect could be completely ablated after lesioning the PAG in the rat²⁵ or following microinjection of opioid antagonists into the PAG.^{26–28}

Although these results clearly implicate opioid receptors in mediation of N₂O-induced antinociception and analgesia, it is appreciated today that opioid receptors are not a monolithic species. There are multiple opioid receptors that are capable of mediating pain relief, and the specific subtypes of opioid receptors that mediate the antinociceptive effects of N₂O appear to depend on various factors including the species and/or strain, the regions of the brain, and the experimental noxious stimulus.²⁹ In the mouse abdominal constriction test, N₂O antinociception was unaffected by either μ or δ opioid antagonists but was sensitive to blockade by drugs with antagonist properties at κ opioid receptors.^{30,31} A κ opioid ligand also protected N₂O antinociception in mice from antagonism by an irreversible, nonselective opioid antagonist.³² The κ opioid receptor subtype appears to be involved at both the supraspinal and spinal cord levels in suppression of chemical noxious stimulation,³³ which is supported by observations that N₂O-induced antinociception was antagonized by supraspinal and spinal pretreatment with antisera against the endogenous κ opioid ligand, dynorphin (DYN).^{34,35} In the rat hot plate test, μ and ϵ opioid receptor subtypes appear to perform a main function at the supraspinal level, as demonstrated by the effectiveness of μ and ϵ opioid antagonists to reduce N₂O-induced antinociception.²⁶

The opioid connection was further strengthened by reports of morphine-tolerant animals being cross-tolerant to N₂O.^{19,36} The fact that cross-tolerance was unilateral in that N₂O-tolerant animals were not cross-tolerant to morphine led Berkowitz et al to hypothesize that N₂O might work through stimulating the neuronal release of endogenous opioid peptides.³⁶ Chronic treatment with morphine results in desensitization of opioid receptors and/or signal transduction mechanisms, hence resulting in cross-tolerance to N₂O, which relies on the same opioid receptors. Chronic treatment with N₂O results in a tolerance that is attributable to excessive depletion of endogenous opioid peptide stores, such that a subsequent exposure to N₂O is unable to release sufficient quantities of opioid peptides to cause antinociception. The chronic exposures to N₂O in these tolerance investigations were not sufficient for inducing the same changes at the receptor and/or signal levels that were observed in the chronic morphine studies.

The first chemical evidence for N₂O-induced release of opioid peptides did not emerge until nearly 10 years following the first report of naloxone antagonism of N₂O-induced antinociception. Exposure to 75% N₂O for 60 minutes increased by 2-fold the amount of immunoreactive methionine-enkephalin (ME) in fractions of perfusate collected from ventricular-cisternally perfused rats.³⁷ At the end of N₂O exposure, the levels of immunoreactive ME returned to baseline. This led to the conclusion that N₂O was capable of inducing the neuronal release of either ME itself or a ME-like peptide in the rat brain. Later studies also showed that N₂O increased β -endorphin concentrations in the arcuate propriomelanocortin neuronal system in rats,³⁸ a result that was reproduced in an *in vitro* system.³⁹

The most extensive studies have been conducted in the mouse abdominal constriction test. The opioid peptide released by N₂O was identified as DYN in experiments utilizing rabbit antisera against rat opioid peptides. N₂O antinociception was antagonized by intracerebroventricular pretreatment with antisera against DYN_{1–8} and DYN_{1–13} but not ME or β -endorphin.³⁴ In a subsequent study, it was discovered that N₂O antinociception was also sensitive to antagonism by intrathecal pretreatment with antisera to DYN_{1–8}, DYN_{1–13}, and ME.³⁵ These findings are consistent with studies reporting that activation of supraspinal κ opioid receptors causes a release of ME in the spinal cord.⁴⁰ Therefore, in this one experimental model, it appears that N₂O evokes its antinociceptive effect through the supraspinal release of various DYNs, which are the endogenous ligands of κ opioid receptors, and spinal release of DYNs and ME.

Involvement of Nitric Oxide in N₂O Antinociception

Nitric oxide (NO) is a naturally occurring gas that only recently has been recognized as an endogenous biological regulator of great significance. Science magazine declared NO as “The Molecule of the Year” for 1992.⁴¹ There is evidence that NO released from nitrergic neurons seems to regulate the release of a variety of transmitters (acetylcholine, catecholamines, excitatory and inhibitory amino acids, serotonin, histamine, and adenosine) in the brain.⁴² N₂O antinociception was antagonized in dose-related fashion by a series of L-arginine analogs that competitively inhibit NO synthase (NOS), including L-N^G-nitro arginine. This antagonism was stereoselectively reversed by administration of L-arginine but not D-arginine. L-N^G-nitro arginine had no such interaction with morphine or the κ opioid agonist U-50,488H.⁴³ Later studies demonstrated that N₂O-induced antinociception was more specifically antago-

nized by pretreatment with a selective inhibitor of neuronal NOS⁴⁴ or an antisense oligodeoxynucleotide directed against neuronal NOS.⁴⁵ Nitric oxide also appears to play a key role in opioid peptide release. A tangential study was designed to test this hypothesis. If N₂O antinociception in the abdominal constriction model is caused by stimulated release of DYNs, which then activate κ opioid receptors, and if NO appears not to influence κ opioid receptor or signal transduction, then it is possible that NO influences the stimulated release of DYNs.⁴⁶ (See Figure 1)

Further evidence of the importance of NO in N₂O antinociception emerged from experiments in inbred mouse strains. N₂O evoked a concentration-dependent antinociception in various mouse strains, including the C57BL/6 inbred strain, but DBA/2 inbred mice exposed to identical levels of N₂O responded with only a weak antinociceptive effect.^{47,48} When mice were exposed to N₂O, there was increased whole-brain NOS activity—as quantified by radioconversion of [¹⁴C]L-arginine to [¹⁴C]L-citrulline—in the C57BL/6 mouse but not the DBA/2 mouse.⁴⁹ This apparent correlation between antinociceptive responsiveness and increase in NOS enzyme activity was recently confirmed in mice selectively bred for low sensitivity to N₂O-induced antinociception.⁵⁰

Quantitative trait loci analysis in C57BL/6 and DBA/2 mice, their B6D2F₁ offspring, 22 BXD recombinant inbred strains (derived from the progenitors), and 600 offspring bred from the F₂ generation identified 2 markers from chromosomes 2 and 5 that were significantly correlated with N₂O antinociception and 1 marker from chromosome 18 that was suggestive.^{48,51} It is significant that the genetic control of neuronal NOS is localized to mouse chromosome 5 in the same vicinity as one of the significant markers in the quantitative trait loci analysis.⁵² Determination of other NOS-related factors may also be located in the same area of the chromosome.

Therefore, it seems likely that in the mouse abdominal constriction model, NO provokes the release of endogenous opiates (DYN peptides) playing a mediatory role in the antinociceptive effect of N₂O. The location of the nerve terminals from which DYN is released and the location of the κ opioid receptors have not been determined.

Descending Pathways Activated by N₂O

Fujinaga and Maze⁵³ hypothesized that the release of endogenous opioid peptides and the subsequent stimulation of opioid receptors activate descending pathways that modulate nociceptive processing in the spinal cord. There are several steps to this process, as demonstrated by a series of elegant studies conducted in rats. A nor-

Figure 1. Mechanism of N₂O-induced analgesia. N₂O is thought to stimulate the neuronal release of endogenous opioid peptide or dynorphins (DYNs); the molecular aspects of how N₂O initiates this process are as yet unknown. The presynaptic nerve terminal takes up L-arginine (L-Arg), which is converted by the enzyme nitric oxide synthase (NOS) to L-citrulline (L-Cit) and nitric oxide (NO). NO appears to be involved in the stimulated release of DYNs. DYNs traverse the synaptic cleft and activate postsynaptic opioid receptors, which belong to the 7-transmembrane-spanning, G protein-coupled superfamily of receptors.

adrenergic pathway is the most important descending pathway; however, it is under tonic inhibition by a γ -aminobutyric acid (GABA)-ergic pathway in the A7 area of the pons. The stimulation of opioid receptors by N₂O-released opioid peptides inhibits the inhibitory GABA-ergic pathway, thus causing disinhibition of the descending noradrenergic pathway.

This hypothesis is borne out by the experimental evidence. Transection of the spinal cord prevents the antinociceptive response to N₂O.⁵⁴ Microinjection of opioid receptor antagonists into the PAG effectively blocks the antinociceptive effect of N₂O,^{26–28} whereas opioid antagonists administered intrathecally have no effect.⁵⁵ Conversely, intrathecal but not supraspinal administration of α_2 adrenergic receptor blockers antagonizes N₂O-induced antinociception.⁵⁵ Studies in transgenic mice indicate that α_{2B} and/or α_{2C} but not α_{2A} adrenergic receptors are responsible for the antinociceptive effect of N₂O.⁵⁶ Alpha₁ adrenergic receptors have also been implicated in N₂O-induced antinociception.⁵⁷ Depletion of norepinephrine in the spinal cord antagonizes the antinociceptive effect of N₂O.⁵⁴

Descending noradrenergic inhibitory neurons are not functional at birth and take at least 3 weeks to fully develop in rats.⁵⁸ It has been suggested that the central nervous system of a 3-week-old rat is equivalent to that of a human at the toddler stage.⁵⁹ This may explain experimental observations that rats do not exhibit sensitivity to the antinociceptive effects of N₂O before 4 weeks of age.^{60,61} Although the sequential neurological developments in rat and human central nervous systems are not totally comparable, these results suggest that N₂O may not be efficacious as an analgesic agent in early childhood.

The disinhibited noradrenergic pathway appears to modulate spinal nociceptive processing by 2 divergent pathways (Figure 2). One population of α_2 adrenergic receptors is located on second-order neurons in the pain pathway, whereas the other is located on inhibitory GABA interneurons in the spinal cord. This dual involvement of GABA as pronociceptive supraspinally and antinociceptive spinally is consistent with experimental findings. N₂O-induced antinociception was antagonized by intracerebroventricular administration of muscimol, a GABA type A (GABA_A) agonist, and intrathecal administration of gabazine, a GABA_A antagonist.⁶²

Immunohistochemical and in situ hybridization identification of the immediate early gene *c-fos* or the FOS protein that it encodes can be used to map functional activation in discrete brain regions of rats following physiological, pharmacological, or psychological stimulation. N₂O exposure increases c-Fos expression in the pontine noradrenergic nuclei as well as the spinal cord.⁶³ N₂O-induced c-Fos expression in the spinal cord was colocalized to cells containing the rate-limiting enzyme in the synthesis of GABA.⁶⁴ Expression of c-Fos in these regions was antagonized by opioid receptor blockade and also by stimulation of GABA_A receptors in the PAG. Microinjection of opioid antagonist and GABA_A agonist into the pontine A7 nuclei also inhibited N₂O-induced expression of c-Fos in the spinal cord as well as attenuate N₂O-induced antinociception.⁶³

Tolerance to N₂O Antinociception

As with many centrally mediated drug effects, continuous administration of N₂O results in development of tolerance to the antinociceptive effect of N₂O in experimental animals³⁹ and to the analgesic effect of N₂O in human subjects.⁶⁵ Studies in different rat strains have provided valuable insight into the development of tolerance to N₂O. The Fischer rat strain exhibits a robust antinociceptive response to N₂O but does not show acute tolerance, whereas the Lewis rat strain is poorly responsive to N₂O-induced antinociception.⁶⁶ In addi-

tion to differential sensitivity to N_2O , the Fisher and Lewis rats also differ in neurochemistry and behavioral reactions to other centrally active drugs.^{67,68} Compared to the Fischer rat, the poorly responsive Lewis strain has lower basal levels of endogenous opioid peptides and does not respond with an increase in opioid peptide levels following the administration of morphine.⁶⁸ This is also consistent with findings that maintenance of high levels of opioid peptide by inhibiting enkephalinase enzyme can prevent the development of acute tolerance to N_2O in rats.⁶⁹

ANXIOLYSIS

In dentistry, subanesthetic concentrations of N_2O are routinely used to produce moderate sedation for dental surgery in anxious patients.⁷⁰ Minimal and moderate sedation (or conscious sedation, as was the previous terminology used) is mediated by the administration of agents causing alterations in the level of consciousness, cognition, motor coordination, degree of anxiety, and physiological parameters. It is not defined by specific medications or their doses but instead by the patient's response: the patient must retain the ability to respond purposefully to verbal commands either alone or accompanied by light tactile stimulation.⁷¹

In pediatric dentistry, N_2O is an invaluable tool in managing the mildly to moderately anxious child. The ease of its administration, its wide margin of safety, its analgesic and anxiolytic effects, and, most of all, its rapid reversibility make it an ideal drug for use in children.^{72–75} The most recent survey of the active members of the American Academy of Pediatric Dentistry by Houpt⁷⁶ reported that 61% of 1758 respondents used N_2O/O_2 with other sedative agents. The American Academy of Pediatric Dentistry recognizes nitrous oxide/oxygen inhalation as a safe and effective technique to reduce anxiety, produce analgesia, and enhance effective communication between a patient and health care provider.⁷¹

There is evidence that the relaxation and relief from anxiety during inhalation of N_2O is a specific anxiolytic effect that is independent of the analgesic action of N_2O . The mechanisms involved are not yet completely understood.

The Benzodiazepine/GABA Receptor Hypothesis of N_2O Anxiolysis

N_2O evokes patterns of behavioral response that are reminiscent of the effects of benzodiazepines in different animal models of experimental anxiety, including the mouse staircase test,^{77–79} the mouse elevated plus

Figure 2. Influence of N_2O on descending inhibitory pathways. N_2O induces release of endogenous opioid peptides (EOP) that activate opioid receptors on γ -aminobutyric acid (GABA)-ergic pontine nuclei. This pathway, in turn, activates descending noradrenergic system in the dorsal horn of the spinal cord that directly inhibits or indirectly inhibits (through a GABA interneuron) nociceptive processing at the level of the primary afferent and second-order neurons that transmit sensory signals up the ascending nociceptive pathway.

maze,⁸⁰ the mouse light/dark exploration test,⁸¹ the mouse hole board,⁸² the rat social interaction test,⁸³ and the rat conditioned defensive burying test.⁸⁴ N_2O - and benzodiazepine-induced anxiolytic-like behaviors were equally sensitive to antagonism by the benzodiazepine binding site blocker flumazenil.^{79,83,84} Mice that are rendered tolerant to benzodiazepines by daily treatment with escalating doses of chlordiazepoxide are cross-tolerant to the anxiolytic-like behavioral response to N_2O .^{79,80} These findings strongly implicate that the anxiolytic effect of N_2O is associated with brain benzodiazepine mechanisms.

Signaling Pathway That Mediates Anxiolytic-like Activity

Because benzodiazepines work through facilitation of GABA-ergic inhibitory neurotransmission, research was conducted to determine involvement of GABA_A receptors in N₂O anxiolysis. In the light/dark exploration test, N₂O- and chlordiazepoxide-induced increases in time spent in the light compartment as well as the number of transitions were blocked by the benzodiazepine antagonist flumazenil and the GABA_A antagonist SR-95531 (2-[3-carboxypropyl]-3-amino-6-[4-methoxyphenyl]-pyridazinium bromide).⁸¹ Consistent with the known interaction between benzodiazepine and GABA_A receptors, these findings indicate that GABA_A receptors mediate the anxiolytic-like effects caused by chlordiazepoxide and N₂O activation of benzodiazepine receptors. This is also supported by observations that N₂O-induced depression of visual evoked potentials is antagonized by a benzodiazepine inverse agonist.⁸⁵

N₂O- and benzodiazepine-induced anxiolytic-like effects in animal models of anxiety are also sensitive to antagonism by inhibition of NOS, a family of enzymes responsible for the synthesis of NO. Studies in the elevated plus maze revealed that the increased open-arm activity produced by N₂O and chlordiazepoxide was blocked by a nonselective NOS inhibitor; this antagonism was stereoselectively reversed by L-arginine.^{86,87} In the light/dark exploration test, selective neuronal NOS inhibitors antagonized both N₂O- and chlordiazepoxide-induced increases in the time spent in the light compartment.^{81,88} The antagonism of N₂O was duplicated by NO scavenger hemoglobin⁸⁹ as well as an antisense oligodeoxynucleotide against neuronal NOS.⁸⁹ These findings suggest that NO plays a key role in the anxiolytic signaling mechanism downstream from the benzodiazepine/GABA_A receptor complex.⁸⁸ The key role of NO in anxiolysis was also evidenced by the anxiolytic-like effects of a centrally administered NO donor.⁹⁰

The soluble 3',5'-cyclic guanosine monophosphate (cGMP)-dependent pathway has been identified by many studies as the main signal transduction pathway of NO.⁴² In experiments in the light/dark exploration test, N₂O anxiolysis was blocked by the guanylyl cyclase inhibitor ODQ (1H-[1,2,4]oxadiazolo[4,3- α]quinoxalin-1-one) and the GMP-dependent protein kinase (PKA)/cGMP-dependent protein kinase (PKG) inhibitor H8 (N-[2-(methyl-amino)ethyl]-5-isoquinoline sulfonamide HCl) and was potentiated by the cyclic GMP phosphodiesterase inhibitor zaprinast.⁹¹ cGMP is known to act upon several different targets: cGMP-dependent protein kinases (PKG), cGMP-gated cation channels, or cGMP-regulated phosphodiesterase.⁹² N₂O-induced anxiolytic-like behavior was significantly attenuated by inhibitors

of PKG but not cAMP-dependent protein kinase (PKA).⁹³ (See Figure 3)

Although there is evidence that stimulation of GABA_A receptors activates an anxiolytic signaling pathway that includes an enzyme sequence of NOS, soluble guanylyl cyclase, and PKG, how N₂O acts at the molecular level to stimulate the BZ binding site and GABA_A receptor is not yet known. In a manner similar to how N₂O activates opioid receptors, it is plausible that N₂O may induce neuronal release of endogenous benzodiazepine factors that then stimulate the GABA_A receptor.

ANESTHESIA

N₂O has a well-known role in medical history because it was the first drug used for surgical anesthesia. Despite its limited anesthetic potency, N₂O is the most widely used general anesthetic agent. With a minimum alveolar concentration of 104% in humans, N₂O by itself would require high volume percentage and hyperbaric conditions to achieve anesthesia in 50% of subjects.⁹⁴ Therefore, because of its low potency, in clinical practice, N₂O is generally used to reduce the minimum alveolar concentration of a second inhalation agent for anesthesia and increase the rate of induction (ie, the second gas effect⁹⁵) and to provide or augment the analgesic component of general anesthesia.

General anesthetics like N₂O have long been hypothesized to act in a nonspecific manner on neuronal membranes, alter membrane fluidity, and/or influence ion channels. But more recently, it has been suggested that general anesthetics might act on one or more superfamilies of ligand-gated ion channels that include GABA_A, glycine, nicotinic acetylcholine, 5-hydroxytryptamine₃, and glutamate receptors.^{96,97} Among the ligand-gated ion channels, the GABA_A receptor is considered to be a prime target of volatile and intravenous anesthetics. Several anesthetics are known to potentiate the activity of GABA at inhibitory GABA_A receptor. N₂O itself has been reported to affect various ligand-gated ion channels.^{98–100}

N-methyl-D-aspartate (NMDA)-type glutamate receptors have recently emerged as a possible target of inhalation anesthetic drugs. Studies in cultured rat hippocampal neurons revealed that N₂O inhibited NMDA-activated currents in a dose-dependent manner but had no effect on GABA-activated currents.¹⁰¹ Consistent with NMDA antagonism, N₂O is reported to up-regulate binding of NMDA radioligand in the cerebral cortex.¹⁰² N₂O also inhibited excitotoxic neurodegeneration that is mediated through NMDA receptors. Similar to other NMDA antagonists, the neurotoxic effect of N₂O is age-dependent and is sensitive to attenuation by GABA-erg-

Figure 3. Mechanism of N_2O -induced anxiolysis. N_2O is thought to cause activation of the benzodiazepine (BZ) binding site as its effects are blocked by flumazenil. This action facilitates γ -aminobutyric acid (GABA) activation of its binding site, resulting in chloride ion influx. The increased chloride ion concentration in the neuron might cause activation of calmodulin (CaM), which then activates the enzyme nitric oxide synthase (NOS). NOS converts the amino acid L-arginine (L-Arg) to L-citrulline (L-Cit) and NO, which stimulates the enzyme soluble guanylyl cyclase producing the second messenger cyclic guanosine monophosphate (cyclic GMP). The cyclic GMP, in turn, stimulates a cyclic GMP-dependent protein kinase (PKG) that leads to the anxiolytic drug effect.

ic drugs.^{103,104} It is suggested that a common property of NMDA receptor antagonism may underlie the similar pharmacological profiles of N_2O and ketamine, an intravenous dissociative anesthetic. The 2 drugs, in fact, produce synergistic neurotoxicity when used together.¹⁰⁵

SUMMARY

It is apparent from the above discussion that N_2O has multiple mechanisms of action that underlie its varied pharmacological properties. Current research indicates that the analgesic effect of N_2O appears to be initiated by stimulated neuronal release of endogenous opioid peptides, with subsequent activation of opioid receptors and descending GABA and noradrenergic pathways that modulate nociceptive processing at the spinal level. The anxiolytic effect of N_2O involves activation of the $GABA_A$ receptor through the benzodiazepine binding site, although whether N_2O acts directly or indirectly upon the latter targets remains uncertain. The anxiolytic pathway that is stimulated includes a segment that involves a sequence of 3 key enzymes, NOS, soluble guanylyl cyclase, and PKG. The anesthetic effect of N_2O appears to be caused by inhibition of NMDA glutamate receptors and removing its excitatory influence in the nervous system.

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