

1-1-1995

# Nitrous Oxide: Mechanism of Its Antinociceptive Action

Raymond M. Quock  
*University of Illinois College of Medicine*

Linda K. Vaughn  
*Marquette University, linda.vaughn@marquette.edu*

## MINIREVIEW

# Nitrous Oxide: Mechanism of Its Antinociceptive Action

RAYMOND M. QUOCK AND LINDA K. VAUGHN

*Department of Biomedical Sciences, University of Illinois College of Medicine  
at Rockford, Rockford, IL 61107-1897*

*Department of Basic Sciences, Marquette University School of Dentistry, Milwaukee, WI 53233-2188*

Submitted: November 14, 1994

Final Revision: December 14, 1994

Accepted: February 7, 1995

Nitrous oxide ( $N_2O$ ) is an anesthetic gas known to produce an analgesic effect at subanesthetic concentrations. This analgesic property of  $N_2O$  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  $N_2O$ -stimulated neuronal release of endogenous opioid peptides to activate opioid receptors.  $N_2O$ -induced release of opioid peptide has been demonstrated in both in vivo and in vitro preparations. Reversal of  $N_2O$ -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  $N_2O$ . Extensive pharmacological testing in the mouse abdominal constriction and rat hot plate paradigms have established that  $N_2O$ -induced antinociception is mediated by  $\kappa$ -opioid receptors in the former and by  $\mu$ - and  $\epsilon$ -opioid receptors in the latter. Current studies focus on two recent developments. The poor responsiveness of the DBA/2J mouse strain to  $N_2O$  has led to pharmacogenetic studies that hope to identify the underlying genetic basis for antinociceptive responsiveness to  $N_2O$ . Other research suggests an involvement of nitric oxide (NO) in mediating the antinociceptive effects of  $N_2O$  in both rats and mice.

Nitrous oxide    Opioid peptides    Antinociception    Mechanism of action

### Introduction

$N_2O$  is one of the simpler inorganic chemicals that is utilized clinically. First identified by Joseph Priestley some 200 years ago,  $N_2O$  possesses three outstanding clinical properties. First, it produces analgesia. The analgesic effect caused by inhalation of 20%  $N_2O$  in oxygen is reportedly equivalent to that evoked by 15 mg morphine sulfate (9).  $N_2O$ -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,  $N_2O$  produces an anesthetic effect.  $N_2O$  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,  $N_2O$  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  $N_2O$  and may be mediated by central benzodiazepine mechanisms (13,57,64).

### The Opioid Nature of $N_2O$ -Induced Antinociception

The ability of inhaled  $N_2O$  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  $N_2O$ . Though  $N_2O$  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  $N_2O$ -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 constrictions (i.e., lengthwise stretches of the torso with concave arching of the back) are counted for a fixed period. Exposure to increasing concentrations of N<sub>2</sub>O causes a dose-related suppression in the number of abdominal constrictions 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 N<sub>2</sub>O 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 N<sub>2</sub>O-induced antinociception (56). Pretreatment with 10 mg/kg (+)-naloxone, s.c., was completely ineffective in influencing N<sub>2</sub>O-induced antinociception, demonstrating that the ability of naloxone to attenuate N<sub>2</sub>O 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 N<sub>2</sub>O-induced antinociception (58). However, following intracerebroventricular (i.c.v.) pretreatment with this quaternary ammonium form of naltrexone, there was an attenuation in N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception. Spinal cord opioid receptors are also involved in N<sub>2</sub>O-induced antinociception because intrathecal (i.t.) pretreatment with 0.5–5.0 μg naloxone causes a dose-dependent reduction in N<sub>2</sub>O-induced antinociception (55).

Although most N<sub>2</sub>O studies in mice have been conducted in the abdominal constriction test, some investigations have utilized the hot plate paradigm. In this test, N<sub>2</sub>O-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).

N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception in rats was the steep dose dependency of the drug effect.

The most consistent antinociceptive effect was in response to 70–75% N<sub>2</sub>O, whereas 50% N<sub>2</sub>O evoked a highly variable response and lower concentrations were generally ineffective (32,48,81).

The antinociceptive effect of N<sub>2</sub>O in rats is also sensitive to antagonism by naloxone and naltrexone. As in mice, the antagonism of N<sub>2</sub>O in the rat 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% N<sub>2</sub>O 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 mg naloxone was administered into the cisternum, the antinociceptive effect of N<sub>2</sub>O 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 N<sub>2</sub>O 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 N<sub>2</sub>O-induced antinociception. However, after exposure to 75% N<sub>2</sub>O for 16–18 h, animals became tolerant to the antinociceptive effect of 80% N<sub>2</sub>O but they were not cross-tolerant to morphine-induced antinociception (3,4). Therefore, N<sub>2</sub>O tolerance induced by chronic exposure to N<sub>2</sub>O 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 N<sub>2</sub>O-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 N<sub>2</sub>O.

N<sub>2</sub>O-induced release of opioid peptides has been demonstrated in both in vivo and in vitro preparations. Exposure to 75% N<sub>2</sub>O increased the amount of immunoreactive (IR) methionine-enkephalin (ME) in fractions of artificial cerebrospinal fluid (CSF) collected from urethane-anesthetized, 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% N<sub>2</sub>O (71). Comparable findings were reported in chronically cannulated dogs exposed to 66–75% N<sub>2</sub>O (15), where there were significantly elevated levels of IR ME and [Arg<sup>6</sup>-Phe<sup>7</sup>]ME in CSF samples collected from the third ventricle. In in vitro studies, exposure to increasing concentrations of N<sub>2</sub>O 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 N<sub>2</sub>O on opioid recep-

tors. N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception in rats (21). Paradoxically, N<sub>2</sub>O-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 N<sub>2</sub>O acts directly on the opioid receptor (22). This hypothesis is based largely on in vitro radioligand binding studies, in which N<sub>2</sub>O, bubbling through tissue homogenate, reduced [<sup>3</sup>H]dihydromorphine ([<sup>3</sup>H]DHM) binding in whole mouse brain (1) and resulted in appearance of an additional super-high-affinity binding site for [<sup>3</sup>H]naloxone in rat forebrain (10). In a more recent study of brain μ-opioid and κ-opioid receptors, 100% N<sub>2</sub>O or 2% halothane in oxygen was bubbled through tissue homogenate prepared from whole guinea pig brain (49). N<sub>2</sub>O decreased the [<sup>3</sup>H]DHM binding affinity without affecting the density of binding sites and decreased the density of [<sup>3</sup>H]ethylketocyclazocine ([<sup>3</sup>H]EKC) binding sites without affecting binding affinity. By comparison, halothane decreased both [<sup>3</sup>H]DHM and [<sup>3</sup>H]EKC binding affinities and reduced the density of binding sites for [<sup>3</sup>H]EKC. These results, suggest the authors, indicate the existence of specific membrane targets for anesthetic drugs.

#### The Role of Multiple Opioid Receptor Subtypes in N<sub>2</sub>O-Induced Antinociception

The observation that N<sub>2</sub>O-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 N<sub>2</sub>O-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 N<sub>2</sub>O antinociception by opioid receptor blockers. One, N<sub>2</sub>O-induced antinociception might be only partly determined by opioid mechanisms and may involve a nonopioid component. Two, N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception.

One difficulty in determining the opioid receptors that mediate N<sub>2</sub>O-induced antinociception is that identifica-

tion 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).

#### N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception, suggesting that μ- and/or κ-opioid receptors might be involved in N<sub>2</sub>O-induced antinociception. Further evidence suggesting a role for κ-opioid receptors in N<sub>2</sub>O-induced antinociception was demonstrated by the marked antagonism of the N<sub>2</sub>O 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 N<sub>2</sub>O-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 N<sub>2</sub>O-induced antinociception, suggesting that μ-opioid receptors are not involved in N<sub>2</sub>O-induced antinociception in the abdominal constriction paradigm. This is also supported by the robust antinociceptive effect of N<sub>2</sub>O in μ-opioid receptor-deficient CXBK mice (61). The δ-opioid antagonists ICI-174,864 (*N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH, where Aib is α-aminoisobutyric acid) and naltrindole were ineffective in reducing N<sub>2</sub>O-induced antinociception in the mouse abdominal constriction test (Quock, unpublished findings).

Additional support for the κ-opioid hypothesis of N<sub>2</sub>O-induced antinociception comes from receptor pro-

tection studies involving pretreatment with  $\beta$ -chloral-trexamine ( $\beta$ -CNA), a nonselective alkylator 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%  $N_2O$  24 h later; the antinociceptive effect of  $N_2O$  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 {*trans*( $\pm$ )-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidiny)cyclohexyl]benzene-acetamide methane sulfonate}, the antinociceptive response to  $N_2O$  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<sub>2</sub>),  $N_2O$ -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  $N_2O$  in the mouse abdominal constriction test.

Experimental evidence suggests the involvement of an additional opioid mechanism in the action of  $N_2O$ . Although higher doses of naloxone administered s.c., i.c.v., or i.t. produce a dose-related antagonism of  $N_2O$ -induced antinociception in the mouse abdominal constriction test, ultralow 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  $N_2O$ -induced antinociception (56). Pretreatment i.c.v. with fg doses of naloxone had neither an antagonistic nor potentiatory influence on  $N_2O$ -induced antinociception. Consistent with the hypothesized  $\kappa$ -opioid nature of  $N_2O$ -induced antinociception, ultralow 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 ultrasensitive 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, ultralow doses of naloxone inactivate this antinociception-inhibitory system and potentiate the antinociceptive effects of both  $N_2O$  and U-50,488H.

### $N_2O$ -Induced Antinociception in Rats

Subtype-selective opioid antagonists have also been used to study  $N_2O$ -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 ([D-Ala<sup>2</sup>MePhe<sup>4</sup>, Glyol<sup>5</sup>]enkephalin) in rats (14). Administration i.c.v. of 1  $\mu$ g CTOP significantly attenuated the antinociceptive effect of 70%  $N_2O$ ; 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),  $N_2O$ -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  $N_2O$ -induced antinociception to the PAG is consistent with findings that kainic acid lesions of the PAG significantly reduce  $N_2O$ -induced antinociception in the tail flick test (81).

$N_2O$ -induced antinociception may also be mediated by the  $\epsilon$ -opioid receptor.  $\beta$ -Endorphin(1-27) [ $\beta$ -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  $N_2O$ -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%  $N_2O$ . Rats pretreated with 7.0  $\mu$ g  $\beta$ -EP(1-27) i.c.v. demonstrated the greatest variability of responsiveness to 70%  $N_2O$ , suggesting that 7.0  $\mu$ g is near the threshold dose separating antagonistic and potentiatory influences upon  $N_2O$ -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  $N_2O$ -induced antinociception, comparable doses administered directly into the PAG had no effect on  $N_2O$ -induced antinociception.  $\beta$ -EP(1-27) was not effective in reducing  $N_2O$  antinociception when administered directly into the PAG. Though the  $\epsilon$ -opioid receptors involved in  $N_2O$ -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  $N_2O$  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,  $N_2O$ -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  $\beta$ -funaltrexamine (63). These results suggest possible involvement of  $\kappa$ - and  $\delta$ -opioid, but not  $\mu$ -opioid, receptors in  $N_2O$ -induced antinociception in the tail withdrawal paradigm.

### The Pharmacogenetics of N<sub>2</sub>O-Induced 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 N<sub>2</sub>O-induced antinociception (61). N<sub>2</sub>O-induced antinociception was assessed in eight inbred strains, and results show the following order of responsiveness: A/J (the most sensitive)  $\geq$  C57BL/6ByJ = C57BL/6J = BALB/cByJ = C3H/HeJ > CXBK/ByJ = CBA/J >> DBA/2J (the least sensitive). The weak antinociceptive response to N<sub>2</sub>O 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 N<sub>2</sub>O-induced antinociception have focused on C57BL/6J and DBA/2J strains. These strains are particularly useful for the study of the genetic determinants of N<sub>2</sub>O responsiveness for several reasons. First, they have large differences in responsiveness to N<sub>2</sub>O with the C57BL/6J strain being much more sensitive to N<sub>2</sub>O-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  $\times$  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 F<sub>2</sub> 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  $\times$  D RI strains revealed a hierarchy of responsiveness to 70% N<sub>2</sub>O ranging from a < 10% antinociceptive response in the B  $\times$  D-27/Ty to a 100% response in the B  $\times$  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% N<sub>2</sub>O) and another of six strains approximating the DBA/2 progenitor (9.1–40% antinociceptive response to 70% N<sub>2</sub>O) (62). The robust strain differences permitted screening the strain antinociceptive means with marker gene loci previously mapped in B  $\times$  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 N<sub>2</sub>O-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 vasodilation, 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-N<sup>G</sup>-nitro arginine (L-NOARG), L-N<sup>G</sup>-nitro arginine methyl ester (L-NAME), L-N<sup>G</sup>-monomethyl nitro arginine (L-NMMA), and 7-nitro indazole.

There is recent evidence of a modulatory role played by NO in N<sub>2</sub>O-induced antinociception. In the mouse abdominal constriction paradigm, s.c. pretreatment with 3.0–10 mg/kg L-NOARG caused a dose-related antagonism of N<sub>2</sub>O-induced antinociception (41). Pretreatment with 10 mg/kg L-NAME or 10 mg/kg L-NMMA, s.c., also significantly attenuated the antinociceptive effect of N<sub>2</sub>O. Pretreatment with 0.5  $\mu$ g L-NAME, i.c.v., also antagonized N<sub>2</sub>O-induced antinociception. These doses of L-NOARG, L-NAME, and L-NMMA alone failed to produce any antinociceptive effect. Antagonism of N<sub>2</sub>O-induced antinociception by s.c.-administered L-NOARG and i.c.v.-administered L-NAME were both reversed by i.c.v. treatment with 20  $\mu$ g L-arginine but not 20  $\mu$ g D-arginine. Because only the L form of arginine is a substrate for NOS, these results implicate NO in the antinociceptive effect of N<sub>2</sub>O.

Similar results were obtained in experiments conducted in the rat hot plate test (41). Pretreatment with 10–100  $\mu$ g L-NOARG, i.c.v., produced a dose-related antagonism of N<sub>2</sub>O-induced antinociception. The antagonism of the N<sub>2</sub>O effect by either 50  $\mu$ g L-NOARG, i.c.v., or 50  $\mu$ g L-NAME, i.c.v., was stereospecifically reversed by i.c.v. treatment with 10  $\mu$ g L-arginine but not 10  $\mu$ g D-arginine.

Pretreatment with 10 mg/kg L-NOARG, s.c., failed to influence the antinociceptive effects of two other opioid drugs, the predominantly  $\mu$ -opioid agonist morphine and the  $\kappa$ -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  $\mu$ g L-arginine significantly potentiated

$N_2O$ -induced antinociception in the mouse abdominal constriction test. Yet under similar circumstances, i.c.v. treatment with 10  $\mu$ g L-arginine (which reversed L-NOARG and L-NAME antagonism of  $N_2O$ ) failed to influence the antinociceptive effect of exposure to 70%  $N_2O$  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  $N_2O$ -induced antinociception in mice but not in rats might suggest that the contribution of NO to  $N_2O$  action in the two species is not identical.

What then is the role of NO in  $N_2O$ -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  $\beta$ -EP-induced antinociception is related to stimulated release of ME in the spinal cord and activation of spinal  $\delta$ -opioid receptors by ME (74,75). In urethane-anesthetized, i.t.-perfused rats, i.c.v.  $\beta$ -EP-induced increase of ME levels in the i.t. perfusate was significantly suppressed by perfusing with artificial CSF containing 100  $\mu$ M 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  $\mu$ M failed to produce a greater suppression of the  $\beta$ -EP-induced increase in ME. These findings suggest that NO may mediate the  $\beta$ -EP-induced release of ME in the spinal cord. If  $\beta$ -EP is the key opioid mediator in  $N_2O$  in rats, as suggested by in vitro release (82) and antibody antagonism studies (26), such a role for NO might explain the antagonism of  $N_2O$  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 retrograde 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 antinociceptive 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,  $N_2O$  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 pharmacological actions (analgesia, anxiolysis) that broaden the clinical applications of  $N_2O$ . There is increasing evidence of an opioid basis to the analgesic effect of  $N_2O$ . Yet  $N_2O$  appears to be strikingly unlike any other pharmacological agent with opioid analgesic properties. The mechanism by which  $N_2O$  interacts with endogenous opioid systems remains uncertain. Various in vitro and in vivo investigations have demonstrated  $N_2O$ -induced release of ME and  $\beta$ -EP, suggesting an indirect mode of action of  $N_2O$  with endogenous opioid systems. Pharmacological antagonism of  $N_2O$ -induced antinociception in animals 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  $N_2O$  in mice and rats. The antinociceptive effect of  $N_2O$  in the mouse abdominal constriction test appears to be mediated by  $\kappa$ -opioid receptors. In contrast,  $N_2O$ -induced antinociception in the rat hot plate test apparently involves supraspinal  $\mu$ - and  $\epsilon$ -opioid receptors. Future research in inbred mouse strains promises to help elucidate the neurobiological prerequisites for  $N_2O$  action by uncovering what is aberrant in DBA/2J and B  $\times$  D RI strains characterized by poor antinociceptive responsiveness to  $N_2O$ . Exciting new findings of antagonism of  $N_2O$ -induced antinociception by inhibition of brain NO production suggests a possible role for NO in the mechanism of  $N_2O$  action, possibly as a regulator of the neuronal release of opioid peptide. It has become increasingly apparent that  $N_2O$  is not only a clinically useful anesthetic and analgesic agent but also a unique investigative tool for studying opioid mechanisms. Elucidation of its uncommon mechanism of interaction with opioid systems will produce a better understanding of the opioid systems themselves.

### Acknowledgements

This research was supported in part by research grants from the National Institutes of Health (DE-06271, DE-06894, and DE-09998), the Marquette University Committee on Research, the University of Illinois at Chicago (BRSR 207RR 05369), and Tokyo Medical College.

### References

1. Ahmed, M.S. and Byrne, W.L. Opiate receptor binding studies influence of a reversible sulfhydryl reagent. In *Endogenous Opiate Agonists and Antagonists*, ed. by E.L. Way. Pergamon, New York, pp. 51–54, 1980.
2. Baskett, P.J.F. and Whitnell, A. Use of Entonox in the ambulance service. *Br. Med. J.* 2: 41–43, 1970.
3. Berkowitz, B.A., Finck, A.D., Hynes, M.D., and Ngai, S.H. Tolerance to nitrous oxide analgesia in rats and mice. *Anesthesiology* 51: 309–312, 1979.
4. Berkowitz, B.A., Finck, A.D., and Ngai, S.H. Nitrous ox-

- ide analgesia: Reversal by naloxone and development of tolerance. *J. Pharmacol. Exp. Ther.* **203**: 539-547, 1977.
5. Berkowitz, B.A., Ngai, S.H., and Finck, A.D. Nitrous oxide 'analgesia': Resemblance to opiate action. *Science* **194**: 967-968, 1976.
  6. Braun, S.D., Miller, G.A., Jr., Ford, K.K., Newman, G.E., Moore, A.V., Jr., Toy, R., Brock, R., and Dunnick, N.R. Nitrous oxide: Effective analgesic for vascular and interventional procedures. *Am. J. Roentgenol.* **145**: 377-379, 1985.
  7. Brecht, D.S. and Snyder, S.H. Nitric oxide: A novel neuronal messenger. *Neuron* **8**: 8-11, 1992.
  8. Castellano, C. and Oliverio, A. A genetic analysis of morphine-induced running and analgesia in the mouse. *Psychopharmacology (Berlin)* **41**: 197-200, 1975.
  9. Chapman, W.P., Arrowood, J.A., and Beecher, H.K. The analgetic effects of low concentrations of nitrous oxide compared in man with morphine sulphate. *J. Clin. Invest.* **22**: 871-875, 1943.
  10. Daras, C., Cantrill, R.C., and Gillman, M.A. [<sup>3</sup>H]Naloxone displacement: Evidence for nitrous oxide as opioid receptor agonist. *Eur. J. Pharmacol.* **89**: 177-178, 1983.
  11. Delay-Goyet, P., Ruiz-Gayo, M., Baamonde, A., Gacel, G. Morgat, J.-L., and Roques, B.P. Brain passage of BUBU, a highly selective and potent agonist for  $\delta$  opioid receptors: In vivo binding and  $\mu$  versus  $\delta$  receptors occupancy. *Pharmacol. Biochem. Behav.* **38**: 155-162, 1991.
  12. Donen, N., Tweed, W.A., White, D., Guttormson, W., and Enns, J. Pre-hospital analgesia with Entonox. *Acta Anaesthesiol. Scand.* **29**: 453-455, 1985.
  13. Emmanouil, D.E., Johnson, C.H., and Quock, R.M. Nitrous oxide anxiolytic effect in mice in the elevated plus maze: Mediation by benzodiazepine receptors. *Psychopharmacology (Berlin)* **115**: 167-172, 1994.
  14. Fanselow, M.S., Calcagnetti, D.J., and Helmstetter, F.J. Role of mu and kappa opioid receptors in conditioned fear-induced analgesia: The antagonistic actions of norbinaltorphimine and the cyclic somatostatin octapeptide, Cys<sup>2</sup>Tyr<sup>3</sup>Orn<sup>5</sup>Pen<sup>7</sup>-amide. *J. Pharmacol. Exp. Ther.* **250**: 825-830, 1989.
  15. Finck, A.D., Samaniego, E., and Ngai, S.H. Nitrous oxide selectively releases met(5)-enkephalin and met(5)-enkephalin-arg(6)-phe(7) into canine third ventricular cerebrospinal fluid. *Anesth. Analg.* **80**: 664-670, 1995.
  16. Fosburg, M.T. and Crone, R.K. Nitrous oxide analgesia for refractory pain in the terminally ill. *J. Am. Med. Assoc.* **250**: 511-513, 1983.
  17. Fujimoto, J.M., Arts, K.S., Rady, J.J., and Tseng, L.F. Intracerebroventricular analgesia enhanced by intrathecal dynorphin A (1-7) antibody. In International Narcotics Research Conference (INRC) '89, ed. by R. Quirion, K. Jhamandas, and C. Gianoulakis. Alan R. Liss, Inc., New York, pp. 433-436, 1990.
  18. Fujimoto, J.M. and Holmes, B. Systemic single dose morphine pretreatment desensitizes mice to the spinal antianalgesic action of dynorphin A (1-17). *J. Pharmacol. Exp. Ther.* **254**: 1-7, 1990.
  19. Fujimoto, J.M. and Rady, J.J. Intracerebroventricular physostigmine-induced analgesia: Enhancement of naloxone,  $\beta$ -funaltrexamine and nor-binaltorphimine and antagonism by dynorphin A (1-17). *J. Pharmacol. Exp. Ther.* **251**: 1045-1052, 1989.
  20. Garthwaite, J., Charles, S.L., and Chess-Williams, R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336**: 385-388, 1988.
  21. Gillespie, T., Simonds, J.B., and Quock, R.M. Potentiation of nitrous oxide antinociception by the endopeptidase 24.11-inhibitor phosphoramidon in the rat hot plate paradigm. *FASEB J.* **7**: A488, 1993.
  22. Gillman, M.A. Analgesic (subanesthetic) nitrous oxide interacts with the endogenous opioid system. *Life Sci.* **39**: 1209-1222, 1986.
  23. Goddard, J.M. Postoperative nitrous oxide analgesia administration from air entrainment oxygen masks with a primary flow of Entonox. *Anaesthesia* **41**: 915-918, 1986.
  24. Gwynn, G.J. and Domino, E.F. Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. II. Antinociceptive tolerance and physical dependence. *J. Pharmacol. Exp. Ther.* **231**: 312-316, 1984.
  25. Hammonds, R.G., Nicolas, P., and Li, C.H.  $\beta$ -Endorphin-(1-27) is an antagonist of  $\beta$ -endorphin analgesia. *Proc. Natl. Acad. Sci. USA* **81**: 1389-1390, 1984.
  26. Hara, S., Gagnon, M.J., Quock, R.M., and Shibuya, T. Effect of opioid peptide antisera on nitrous oxide antinociception in rats. *Pharmacol. Biochem. Behav.* **48**: 699-702, 1994.
  27. Hara, S., Kuhns, E.R., Ellenberger, E.A., Mueller, J.L., Shibuya, T., Endo, T., and Quock, R.M. Involvement of nitric oxide in intracerebroventricular  $\beta$ -endorphin-induced neuronal release of methionine-enkephalin. *Brain Res.* **675**: 190-194, 1995.
  28. Hara, S., Mueller, J.L., Ellenberger, E.A., Kuhns, E.R., Shibuya, T., Endo, T., and Quock, R.M. Involvement of nitric oxide in neuronal release of spinal cord methionine-enkephalin after intracerebroventricular  $\beta$ -endorphin in rats. *Can. J. Physiol. Pharmacol.* **72**(Suppl. 1): 352, 1994.
  29. Henderson, J.M., Spence, D.G., Komocar, L.M., Bonn, G.E., and Stenstrom, R.J. Administration of nitrous oxide to pediatric patients provides analgesia for venous cannulation. *Anesthesiology* **72**: 269-271, 1990.
  30. Herz, A., Albus, K., Metys, J., Schubert, P., and Teschemacher, H.J. On the central sites for the analgesic action of morphine and fentanyl. *Neuropharmacology* **9**: 539-551, 1970.
  31. Heyman, J.S., Vaught, J.L., Mosberg, H.I., Haaseth, R.C., and Porreca, F. Modulation of  $\mu$ -mediated antinociception by  $\delta$  agonists in the mouse: Selective potentiation of morphine and normorphine by [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]enkephalin. *Eur. J. Pharmacol.* **165**: 1-10, 1989.
  32. Hodges, B.L., Gagnon, M.J., Gillespie, T.R., Breneisen, J.R., O'Leary, D.F., Hara, S., and Quock, R.M. Antagonism of nitrous oxide antinociception in the rat hot plate test by site-specific  $\mu$ - and  $\epsilon$ -opioid receptor blockade. *J. Pharmacol. Exp. Ther.* **269**: 596-600, 1994.
  33. Horan, P., Tallarida, R.J., Haaseth, R.C., Matsunaga, T.O., Hruby, V.J., and Porreca, F. Antinociceptive interactions of opioid delta receptor agonist with morphine in mice: Supra- and sub-additivity. *Life Sci.* **50**: 1535-1541, 1992.



34. Ignarro, L.J. Endothelium-derived nitric oxide: Actions and properties. *FASEB J.* **3**: 31-36, 1989.
35. Katzen, B.T. and Edwards, K.C. Nitrous-oxide analgesia for interventional radiologic procedures. *Am. J. Roentgenol.* **140**: 145-148, 1983.
36. Kerr, F., Irving, J.B., Ewing, D.J., and Kirby, B.J. Nitrous-oxide analgesia in myocardial infarction. *Lancet* **1**: 63-66, 1972.
37. Kramer, T.H., Shook, J.E., Kazmierski, W., Ayers, E.A., Wire, W.S., Hruba, V.J., and Burks, T.F. Novel peptidic mu opioid antagonists: Pharmacologic characterization in vitro and in vivo. *J. Pharmacol. Exp. Ther.* **249**: 544-551, 1989.
38. Kripke, B.J. and Hechtman, H.B. Nitrous oxide for pentazocine addiction and for intractable pain. *Anesth. Analg.* **51**: 520-527, 1972.
39. Kuhns, E.R., Hara, S., Ellenberger, E.A., Muller, J.L., and Quock, R.M. Effect of L-N<sup>G</sup>-nitro arginine on perfusate levels of methionine-enkephalin after central  $\beta$ -endorphin treatment in intrathecally-perfused rats. *FASEB J.* **8**: A628, 1994.
40. Marx, G.F. and Bassell, G.M. Nitrous oxide in obstetrics. In *Nitrous Oxide/N<sub>2</sub>O*, ed. by E.I. Eger, II. Elsevier, New York, pp. 185-201, 1985.
41. McDonald, C.E., Gagnon, M.J., Ellenberger, E.A., Hodges, B.L., Ream, J.K., Tousman, S.A., and Quock, R.M. Inhibitors of nitric oxide synthesis antagonize nitrous oxide antinociception in mice and rats. *J. Pharmacol. Exp. Ther.* **269**: 601-608, 1994.
42. Meller, S.T. and Gebhart, G.F. Nitric oxide and nociceptive processing in the spinal cord. *Pain* **52**: 127-136, 1993.
43. Millan, M.J. Kappa-opioid receptor-mediated antinociception in the rat. I. Comparative actions of mu- and kappa-opioids against noxious thermal, pressure and electrical stimuli. *J. Pharmacol. Exp. Ther.* **251**: 334-341, 1989.
44. Moncada, S., Palmer, R.M.J., and Higgs, E.A. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43**: 109-142, 1991.
45. Moody, E.J., Mattson, M., Newman, A.H., Rice, K.C., and Skolnick, P. Stereospecific reversal of nitrous oxide analgesia by naloxone. *Life Sci.* **44**: 703-709, 1989.
46. Moore, P.K., Oluyomi, A.O., Babbedge, R.C., Wallace, P., and Hart, S.L. L-N<sup>G</sup>-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. *Br. J. Pharmacol.* **102**: 198-202, 1991.
47. Mustafa, A.A. Mechanisms of L-N<sup>G</sup>-nitro arginine methyl ester-induced antinociception in mice: A role for serotonergic and adrenergic neurons. *Gen. Pharmacol.* **23**: 1177-1182, 1992.
48. Novelli, G.P., Pieraccioli, E., and Peduto, V.A. N<sub>2</sub>O analgesia and dependence: Effects of intracisternal administration of naloxone or physostigmine. In *Pharmacological Basis of Anesthesiology: Clinical Pharmacology of New Analgesics and Anesthetics*, ed. by M. Tiengo and M.J. Cousins. Raven Press, New York, pp. 225-236, 1983.
49. Ori, C., Ford-Rice, F., and London, E.D. Effects of nitrous oxide and halothane on  $\mu$  and  $\kappa$  opioid receptors in guinea pig brain. *Anesthesiology* **70**: 541-544, 1989.
50. Parbrook, G.D. and Kennedy, B.R. Value of premixed nitrous-oxide and oxygen mixtures in the relief of post-operative pain. Double-blinded trial of 25% nitrous oxide in oxygen, 15% nitrous oxide in oxygen, and oxygen. *Br. Med. J.* **2**: 1303-1305, 1964.
51. Parbrook, G.D., Rees, G.A.D., and Robertson, G.S. Relief of post-operative pain: Comparison of a 25% nitrous-oxide and oxygen mixture with morphine. *Br. Med. J.* **2**: 480-482, 1964.
52. Parsons, C.G. and Headley, P.M. Spinal antinociceptive actions of  $\mu$ - and  $\kappa$ -opioids: The importance of stimulus intensity in determining 'selectivity' between reflexes to different modalities of noxious stimuli. *Br. J. Pharmacol.* **98**: 523-532, 1989.
53. Paterson, S.J., Robson, L.E., and Kosterlitz, H.W. Classification of opioid receptors. *Br. Med. Bull.* **39**: 31-36, 1983.
54. Porreca, F., Mosberg, H.O., Omnaas, J.R., Burks, T.R., and Cowan, A. Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. *J. Pharmacol. Exp. Ther.* **240**: 890-894, 1987.
55. Quock, R.M., Best, J.A., Chen, D.C., Vaughn, L.K., Portoghesi, P.S., and Takemori, A.E. Mediation of nitrous oxide analgesia by spinal and supraspinal  $\kappa$ -opioid receptors. *Eur. J. Pharmacol.* **175**: 97-100, 1990; *Corrigendum* **187**: 564, 1990.
56. Quock, R.M., Curtis, B.A., Reynolds, B.J., and Mueller, J.L. Dose-dependent antagonism and potentiation of nitrous oxide antinociception by naloxone in mice. *J. Pharmacol. Exp. Ther.* **267**: 117-122, 1993.
57. Quock, R.M., Emmanouil, D.E., Vaughn, L.K., and Pruhs, R.J. Benzodiazepine receptor mediation of behavioral effects of nitrous oxide in mice. *Psychopharmacology (Berlin)* **107**: 310-314, 1992.
58. Quock, R.M. and Graczak, L.M. Influence of narcotic antagonist drugs upon nitrous oxide analgesia in mice. *Brain Res.* **440**: 35-41, 1988.
59. Quock, R.M., Kouchich, F.J., and Tseng, L.-F. Does nitrous oxide induce release of brain opioid peptides? *Pharmacology* **30**: 95-99, 1985.
60. Quock, R.M. and Mueller, J. Protection by U-50,488H against  $\beta$ -chlornaltrexamine antagonism of nitrous oxide antinociception in mice. *Brain Res.* **549**: 162-164, 1991.
61. Quock, R.M., Mueller, J.L., and Vaughn, L.K. Strain-dependent differences in responsiveness of mice to nitrous oxide (N<sub>2</sub>O) antinociception. *Brain Res.* **614**: 52-56, 1993.
62. Quock, R.M., Mueller, J.L., Vaughn, L.K., and Belknap, J.K. Nitrous oxide antinociception in B x D recombinant inbred mouse strains and identification of quantitative trait loci. *FASEB J.* **8**: A628, 1994.
63. Quock, R.M., Walczak, C.K., Henry, R.J., and Chen, D.C. Effect of subtype-selective opioid receptor blockers on nitrous oxide antinociception in rats. *Pharmacol. Res.* **22**: 351-357, 1990.
64. Quock, R.M., Wetzell, P.J., Maillefer, R.H., Hodges, B.L., Curtis, B.A., and Czech, D.A. Benzodiazepine receptor-mediated behavioral effects of nitrous oxide in the rat social interaction test. *Pharmacol. Biochem. Behav.* **46**: 161-165, 1993.
65. Schmauss, C., Yaksh, T.L., Shimohigashi, Y., Harty, G., Jensen, T., and Rodbard, D. Differential association of

- spinal  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors with cutaneous thermal and visceral chemical nociceptive stimuli in the rat. *Life Sci.* **33(Suppl. D)**: 653-656, 1983.
66. Smith, E.H. and Rees, J.H.M. The effects of naloxone on the analgesic activities of general anesthetics. *Experientia* **37**: 289-290, 1981.
67. Smith, R.A. and Beirne, O.R. The use of nitrous oxide by dentists. In *Nitrous Oxide/N<sub>2</sub>O*, ed. by E.I. Eger, II. Elsevier, New York, pp. 281-304, 1985.
68. Smith, W.D.A. A history of nitrous oxide and oxygen anaesthesia. Part I. Joseph Priestley to Humphry Davy. *Br. J. Anaesth.* **37**: 790-798, 1965.
69. Sonnenschein, R.R., Jamison, R., Loveseth, L.J., Casseles, W.H., and Ivy, A.C. A study on the mechanism of nitrous oxide analgesia. *J. Appl. Physiol.* **1**: 254-258, 1948.
70. Suh, H.H., Tseng, L.F., and Li, C.H.  $\beta$ -Endorphin-(1-27) antagonizes  $\beta$ -endorphin- but not morphine-, D-Pen<sup>2</sup>-D-Pen<sup>5</sup>-enkephalin- and U50,488H-induced analgesia in mice. *Neuropharmacology* **27**: 957-963, 1988.
71. Swartz, C.M. and Quock, R.M. Kinetic modeling of hormone release: Application to time-averaged met-enkephalin release. *Psychoneuroendocrinology* **12**: 377-384, 1987.
72. Thal, E.R., Montgomery, S.J., Atkins, J.S., and Roberts, B.G. Self-administered analgesia with nitrous oxide. *J. Am. Med. Assoc.* **242**: 2418-2419, 1979.
73. Thompson, P. and Locon, B. Nitrous oxide as an analgesic in acute myocardial infarction. *J. Am. Med. Assoc.* **235**: 924-927, 1976.
74. Tseng, L.F., Higgins, M.J., Hong, J.S., Hudson, P.M., and Fujimoto, J.M. Release of immunoreactive Met-enkephalin from the spinal cord by intraventricular  $\beta$ -endorphin but not by morphine in anesthetized rats. *Brain Res.* **343**: 60-69, 1985.
75. Tseng, L.F., King, R.C., and Fujimoto, J.M. Release of immunoreactive Met-enkephalin by intraventricular  $\beta$ -endorphin in anesthetized rats. *Regul. Pept.* **14**: 181-192, 1986.
76. Tseng, L.L.F., Tang, R., Stackman, R., Camara, A., and Fujimoto, J.M. Brainstem sites differentially sensitive to  $\beta$ -endorphin and morphine for analgesia and release of met-enkephalin in anesthetized rats. *J. Pharmacol. Exp. Ther.* **253**: 930-937, 1990.
77. Tseng, L.F. and Wang, Q. Forebrain sites differentially sensitive to  $\beta$ -endorphin and morphine for analgesia and release of met-enkephalin in the pentobarbital-anesthetized rat. *J. Pharmacol. Exp. Ther.* **261**: 1028-1036, 1992.
78. Tyers, M.B. A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmacol.* **69**: 503-512, 1980.
79. Ward, S.J., Portoghese, P.S., and Takemori, A.E. Pharmacological profiles of  $\beta$ -funaltrexamine ( $\beta$ -FNA) and  $\beta$ -chlornaltrexamine ( $\beta$ -CNA) on the mouse vas deferens preparation. *Eur. J. Pharmacol.* **80**: 377-384, 1982.
80. Yaksh, T.L. and Rudy, T.A. Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* **4**: 299-359, 1978.
81. Zuniga, J.R., Joseph, S.A., and Knigge, K.M. Nitrous oxide analgesia: Partial antagonism by naloxone and total reversal after periaqueductal gray lesions in the rat. *Eur. J. Pharmacol.* **142**: 51-60, 1987.
82. Zuniga, J.R., Joseph, S.A., and Knigge, K.M. The effects of nitrous oxide on the secretory activity of pro-opiomelanocortin peptides from basal hypothalamic cells attached to cytodex beads in a superfusion in vitro system. *Brain Res.* **420**: 66-72, 1987.

---

Send reprint requests to: Raymond M. Quock, Ph.D., Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford, 1601 Parkview Avenue, Rockford, IL 61107-1897.

---