Anesthesiology
1999; 91:1401-7


# The Analgesic Action of Nitrous Oxide Is Dependent on the Release of Norepinephrine in the Dorsal Horn of the Spinal Cord

*Chousheng Zhang, M.D.,* M. Frances Davies, Ph.D.,* Tian-Zhi Guo, M.D.,* Mervyn Maze, M.B., Ch.B., F.R.C.P.†*

***Background:*** **The authors and others have demonstrated that supraspinal opiate receptors and spinal $\alpha_2$ adrenoceptors are involved in the analgesic mechanism for nitrous oxide ($N_2O$). The authors hypothesize that activation of opiate receptors in the periaqueductal gray results in the activation of a descending noradrenergic pathway that releases norepinephrine onto $\alpha_2$ adrenoceptors in the dorsal horn of the spinal cord.**

***Methods:*** **The spinal cord was transected at the level of T3–T4 in rats and the analgesic response to 70% $N_2O$ in oxygen was determined by the tail flick latency test. In a separate experiment in rats a dialysis fiber was positioned transversely in the dorsal horn of the spinal cord at the T12 level. The following day, the dialysis fiber was infused with artificial cerebrospinal fluid at a rate of 1.3 μl/min, and the effluent was sampled at 30-min intervals. After a 60-min equilibration period, the animals were exposed to 70% $N_2O$ in oxygen. The dialysis experiment was repeated in animals that were pretreated with naltrexone (10 mg/kg, intraperitoneally) before $N_2O$. In a third series, spinal norepinephrine was depleted with n-(2-chloroethyl)-n-ethyl-2-bromobenzylamine (DSP-4), and the analgesic response to 70% $N_2O$ in oxygen was determined.**

***Results:*** **The analgesic effect of $N_2O$ was prevented by spinal cord transection. After exposure to $N_2O$, there was a fourfold increase in norepinephrine released in the first 30-min period, and norepinephrine was still significantly elevated after 1 h of exposure. The increased norepinephrine release was prevented by previous administration of naltrexone. Depletion of norepinephrine in the spinal cord blocked the analgesic response to $N_2O$.**

***Conclusions:*** **A descending noradrenergic pathway in the spinal cord links $N_2O$-induced activation of opiate receptors in the periaqueductal gray, with activation of $\alpha_2$ adrenoceptors in the spinal cord. $N_2O$-induced release of norepinephrine in the dorsal horn of the spinal cord is blocked by naltrexone, as is the analgesic response. Spinal norepinephrine is necessary for the analgesic response to the $N_2O$. (Key words: Analgesia; descending; inhibition; naltrexone; nitrous oxide; norepinephrine.)**

NITROUS oxide ($N_2O$) is one of the most common agents used in anesthetic practice. Although its first use as an analgesic was described more than 150 yr ago, its mechanism of action has not been defined. Many lines of evidence indicate that $N_2O$ has its analgesic action by activating discrete neuronal pathways. Recently, we reported that $\alpha_2$ adrenoceptors in the spinal cord[1] were necessary to transduce the acute antinociceptive response to $N_2O$. A mechanism of action for $N_2O$ also has been proposed in which opiate receptors are activated through the release of endogenous opiate ligands.[2-5] The periaqueductal gray (PAG) has long been known to be an important site for the analgesic action of opiates (Cf [6]). We[7] and others[8] demonstrated that the analgesic properties of $N_2O$ could be blocked by the discrete introduction of opiate antagonists directly into the PAG.

Several enigmatic issues concerning $N_2O$ remain, including the circuitry involved between the opiate receptors in the PAG and the $\alpha_2$ adrenoceptors in the spinal cord. Clearly, if an adrenergic receptor mediates the action in the spinal cord, then an endogenous pathway that releases norepinephrine or epinephrine should be activated by $N_2O$ exposure. Further evidence for the involvement of a descending noradrenergic pathway in the action of $N_2O$ could be acquired by determining whether its disruption, either by transection or by local administration of the neurotoxin n-(2-chloroethyl)-n-ethyl-2-bromobenzylamine (DSP-4), could eliminate $N_2O$ analgesic action.

* Research Associate.

† Professor. Current position: Professor, Magill Department of Anaesthetics, Imperial College School of Medicine, Chelsea and Westminster Hospital, London, United Kingdom.

Received from the Department of Anesthesia, Stanford University, Palo Alto, California. Submitted for publication December 28, 1998. Accepted for publication June 4, 1999. Supported by the Department of Veterans Affairs, Washington, DC; grant no. 30232 from the National Institute of General Medical Sciences, Bethesda, Maryland; and the Medical Research Council, London, United Kingdom. Presented at the annual meeting of the American Society of Anesthesiologists, Orlando, Florida, October 17–21, 1998.

Address reprint requests to Dr. Maze: Magill Department of Anaesthetics, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, United Kingdom. Address electronic mail to: m.maze@lc.ac.uk

In this series of studies we sought to answer the following questions:

1. Does a pathway descend in the spinal cord to link $N_2O$-induced activation of opiate receptors in the PAG with activation of $\alpha_2$ adrenoceptors in the spinal cord?
2. Does the descending pathway release norepinephrine in the dorsal horn in the spinal cord in response to $N_2O$?
3. Is release of norepinephrine blocked by strategies that prevent $N_2O$-induced analgesia?
4. Is spinal norepinephrine necessary for the analgesic response to $N_2O$?

## Methods

The experimental protocol was approved by the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 250–380 g were used. All tests were performed between 9 AM and 4 PM. A total of 104 rats were used. Each animal was used for only one set of studies to eliminate possible interaction between different doses and routes of drugs.

### *Microdialysis*

The modified methods of Skilling *et al.*,[9] Liu *et al.*,[10] and Peng *et al.*[11] were followed. Briefly, male Sprague-Dawley rats (300–400 g) were anesthetized with isoflurane and the lateral surfaces of vertebra T13 were exposed. Bilateral holes were made through the bone to expose the spinal cord at the level of the dorsal horn.

Except for a 2-mm dialysis zone, dialysis fibers (diameter 200 mm; molecular weight cutoff = 9,000; Spectrum Laboratories, Laguna Hills, CA) were coated with a thin layer of silicon rubber. One end of the fiber was connected to a 90° angled stainless tubing made from a 22-gauge stainless steel needle. A stainless steel dissecting pin was affixed to the lumen of the other end of the fiber. By pushing the pin through the spinal cord and pulling it out the other side, the fiber was positioned so the uncoated portion of the fiber was located within the dorsal horn of the spinal cord. The pin was then cut and the free end of the fiber was attached to a length of PE 20 tubing (Clay Adams, Sparks, MD) with cyanoacrylic glue (Krazy Glue; Elmer's Products, Inc., Columbus, OH). The dialysis fiber, the initial part of the stainless tubing and PE 20 tubing were affixed to the exposed vertebra T13 with dental acrylic, and both tubing ends were externalized in the lumber region. The skin was then sutured around the tubing.

The next day, any animals displaying any signs of limb paralysis were rejected for further study. In the remaining, neurologically intact animals, the two tubing ends were attached to the fluid swivel in the CMA/120 system (Stockholm, Sweden) to allow for free movement, then attached to an infusion pump (Harvard Apparatus, South Natick, MA). The spinal cord was perfused with an artificial cerebrospinal fluid (aCSF) solution (NaCl: 125 mM; $NaH_2PO_4$: 0.5 mM; KCl: 2.5 mM; $Na_2HPO_4$: 2.0 mM; $MgCl_2$: 0.25 mM; and $CaCl_2$: 1.0 mM; pH adjusted to 7.2) at a flow rate of 1.3 μl/min for 180 min to establish a diffusion equilibrium. Samples were collected at 30-min intervals in a vial containing 0.9 μl perchloric acid to achieve a final concentration of 2% and analyzed immediately after collection. All tubing and collection vials were covered with aluminum foil to prevent degradation of norepinephrine by light.

**Chromatographic Conditions.** Norepinephrine was separated by reverse-phase chromatography on an ESA column (catecholamine R-80; ESA Microdialysis, Medford, MA) maintained at 24°C with a column heater. The mobile phase (Cat-A-Phase; ESA) was delivered at a flow rate of 1.0 ml/min using a Beckman 118 Solvent Module (Fullerton, CA). Samples (20 μl) were injected with a BIO-RAD Model AS-100 HPLC Automatic Sampling System (Hercules, CA), and norepinephrine was detected coulometrically (model 5011; ESA). Potentials for the first and second electrodes were set at +100 mV and −300 mV, respectively. A conditioning cell was set at +350mV and was placed before the analytic cell. Retention time, peak area, and concentrations of norepinephrine in the dialysate were measured by comparison with known standards and were determined with the Dynamax MacIntegrator software system (Rainin Instrument Co., Inc., Emeryville, CA). The detection limit for norepinephrine in our assay varied between 0.5–1.6 pg/sample.

**Histologic Examination.** To verify that the fiber traversed the dorsal horn, the spinal cord was removed and fixed in 10% formalin for histologic confirmation of cannula placement. Only animals with the cannula located below lamina I and above the central canal were included in the study.

### *Spinal Cord Transection*

Rats were anesthetized with halothane, and laminectomy was performed at the T3–T4 level. Spinous pro-

cesses and laminae were removed to expose a circular region of dura. The dura was opened and the spinal cord was severed at the T3–T4 level. The muscles were sutured over the laminectomy site and the skin was closed with wound clips. The animals were exposed to $N_2O$ and tested for tail flick response within 6 h after surgery.

*Intrathecal Administration of DSP-4*

Rats were anesthetized with isoflurane, an incision was made over the cervical spine, and a small puncture was made in the dura mater. PE-10 polyethylene tubing (0.28 mm ID) was threaded 8.5 cm into the intrathecal space so the tip of the catheter was positioned at the lumbar level. This tubing was then sutured in place, and the skin was sutured over the tubing. After allowing 7 days for recovery, DSP-4 (100 or 300 μg) was administered in 10 μl normal saline using a perfusion pump at a rate of 10 μl/min followed by a 10-μl flush of normal saline. Behavioral testing or killing for the determination of spinal norepinephrine levels was performed 10 days later.

The levels of norepinephrine in the lumbar enlargement of spinal cord were measured using the high-performance liquid chromatography. Rats were exposed to 100% carbon dioxide for 35 s and then killed. The spinal cord was rapidly extruded from the spinal canal using ice-cold saline; the lumbar enlargement was isolated and weighed. The tissue was put into 600 μl perchloric acid, 2%, and $2 \times 10^{-8}$ M dihydroxybenzylamine, and homogenized and centrifuged at 1,200*g* for 15 min at 4°C. The supernatant was removed and stored at −80°C for later analysis.

*Nociceptive Testing Procedures*

Nociception was assessed by the tail flick response to a noxious thermal stimulus, as previously described.[12] In brief, a high-intensity light beam was focused on the tail, and the time for the rat to move its tail out of the light was recorded as tail flick latency. The latency from three sites on the tail were averaged and a cut-off time of 10 s was predetermined to prevent tissue damage. Baseline measurements consisted of a set of three tail flick determinations at 2-min intervals. Baseline tail flick latencies ranged between 3 and 4 s. In some cases, percent maximal possible effect (%MPE) was calculated as

$$(\text{latency} - \text{baseline})/(\text{cut-off time} - \text{baseline}) \cdot 100$$

*Gas Exposures*

All gas exposures were performed in a clear plastic chamber (92 × 48 × 38 cm) with a sliding door on one side (for insertion of the rats). This airtight chamber was large enough to contain the infusion pump and the analgesimeter device. Fresh test gases (10 l/min) were introduced into the chamber *via* an inflow port, circulated throughout the chamber by a small fan, and purged by vacuum set to aspirate at the same rate as the fresh gas inflow. Oxygen concentration in the chamber was maintained between 22–30%, while $N_2O$ concentration was maintained at 0 or 70% by adjusting the flow rates of $N_2O$, air, and nitrogen (Liquid Carbonic, Houston, TX). Gas concentrations were measured continuously and flow rates were adjusted appropriately to maintain the desired concentrations.

*Statistics*

Release data were analyzed by analysis of variance for repeated measures and *a posteriori* by Scheffé or Bonferroni tests. Nociceptive data were analyzed by unpaired Student *t* test or analysis of variance for repeated measures and *a posteriori* by the Bonferroni multiple comparisons test when appropriate.

## Results

*Spinal Cord Transection Blocked the Analgesic Action of Nitrous Oxide*

Spinal transection at level T3–T4 did not affect the baseline tail flick latency but did block the analgesic action of 70% $N_2O$ (fig. 1).

*Nitrous Oxide Stimulated the Spinal Release of Norepinephrine*

Nitrous oxide caused approximately a fourfold increase in norepinephrine release within the first 30-min collection period (fig. 2). This level of release decreased in subsequent collection periods, reaching baseline values during the 60- to 90-min collection period.

*Naltrexone Suppressed Spinal Norepinephrine Release*

Systemic opiate antagonists, such as naloxone, block the analgesic action of $N_2O$,[13] possibly by antagonizing the action of endogenously released opiates at the level of the PAG.[7] To determine whether an opiate antagonist also suppressed norepinephrine release evoked by $N_2O$, the levels of spinal norepinephrine release were mea-

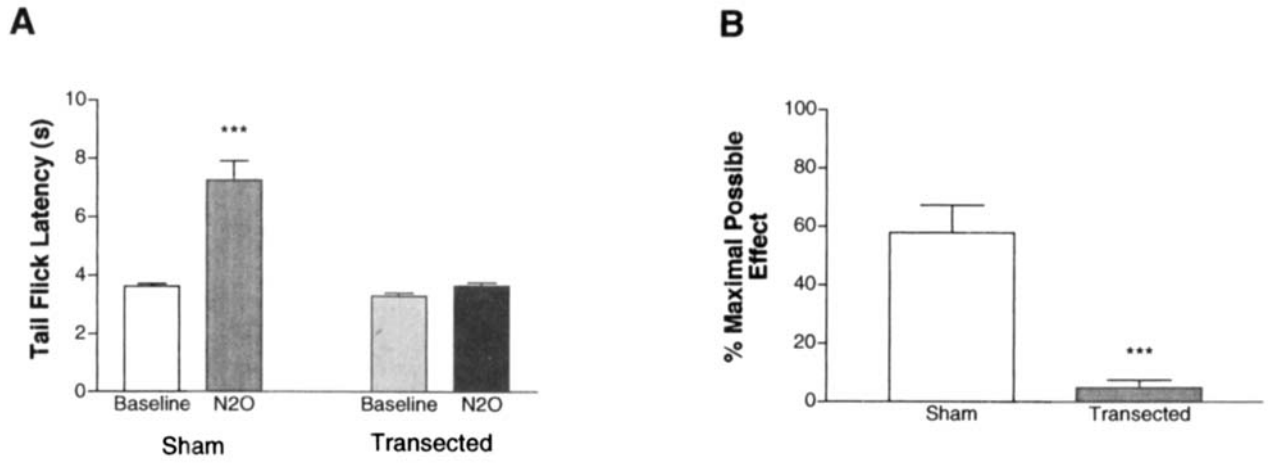


**Fig. 1. Spinal cord transection blocked the analgesic action of $N_2O$. The spinal cords of rats were sectioned at the T3–T4 level before exposure to 70% $N_2O$. The analgesia was measured after 30 min of $N_2O$ exposure. (*A*) Tail flick latencies before and after $N_2O$ in sham and transected animals. Spinal transection did not affect baseline tail flick latencies but did abolish $N_2O$ analgesia. Data are expressed as mean ± SEM. ***$P < 0.001$ Bonferroni multiple comparison test (n = 9). (*B*) The percent maximal possible effect of $N_2O$ was reduced in transected animals. Data are expressed as mean ± SEM. ***$P < 0.001$ unpaired $t$ test (n = 9).**

sured before and after naltrexone, a long-acting opiate antagonist. Naltrexone (10 mg/kg intraperitoneal), a dose that antagonized $N_2O$-induced analgesia for a least 3 h (fig. 3A) but did not affect baseline tail flick latencies (data not shown), had no effect on the basal release of norepinephrine alone but blocked the $N_2O$-evoked release (fig. 3B).

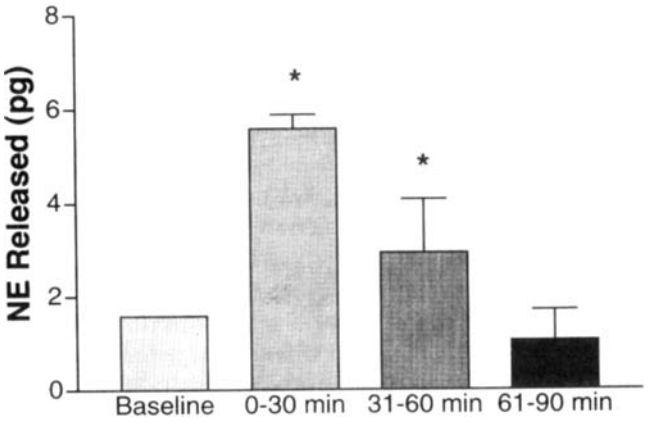


**Fig. 2. $N_2O$ caused norepinephrine release in the dorsal horn of the rat spinal cord. In rats, a dialysis fiber was placed at T12 during isoflurane anesthesia. The dialysis tubing was stabilized with dental acrylic and the ends were externalized. The following day, the inflow of the dialysis tubing was connected *via* a swivel to a pump, which infused artificial cerebrospinal fluid at a rate of 1.3 μl/min. Microdialysate was collected during a 60-min equilibration period, with the rats breathing air, then the rats were exposed to 70% $N_2O$ for 90 min. The effluent was sampled at 30-min intervals with a fraction collector. Norepinephrine was assayed by high-performance liquid chromatography with electrochemical detection. The position of the dialysis portion of the fiber was confirmed histologically at the conclusion of the experiment. Data were analyzed by analysis of variance for repeated measures and *a posteriori* by Scheffé test. Data are expressed as mean ± SEM. *$P < 0.05$ (n = 9).**

### *Depletion of Norepinephrine with DSP-4 Blocked Nitrous Oxide Analgesia*

In animals pretreated with DSP-4 in which the norepinephrine levels were depressed to 22% of control values (fig. 4A), the baseline tail flick latency was not changed (fig. 4B), but the analgesic action of 70% $N_2O$ as measured by percent maximal possible effect was greatly attenuated (fig. 4C).

## Discussion

This study shows that $N_2O$ causes the release of norepinephrine in the spinal cord in the awake freely moving rat. This release decreases with continued $N_2O$ exposure and is dependent on the presence of a functional opiate receptor. In keeping with the hypothesis that $N_2O$ analgesia is mediated by the release of norepinephrine, depletion of norepinephrine stores by DSP-4 or elimination of descending noradrenergic transmission by spinal cord transection attenuates $N_2O$ analgesia. Although spinal cord transection and the insertion of the microdialysis fiber may cause a degree of nerve injury that could modify norepinephrine release characteristics, all effects were temporally related to the onset of $N_2O$ exposure. Although DSP-4 treatment is known to also deplete serotonin,[14] the lack of analgesic effect of

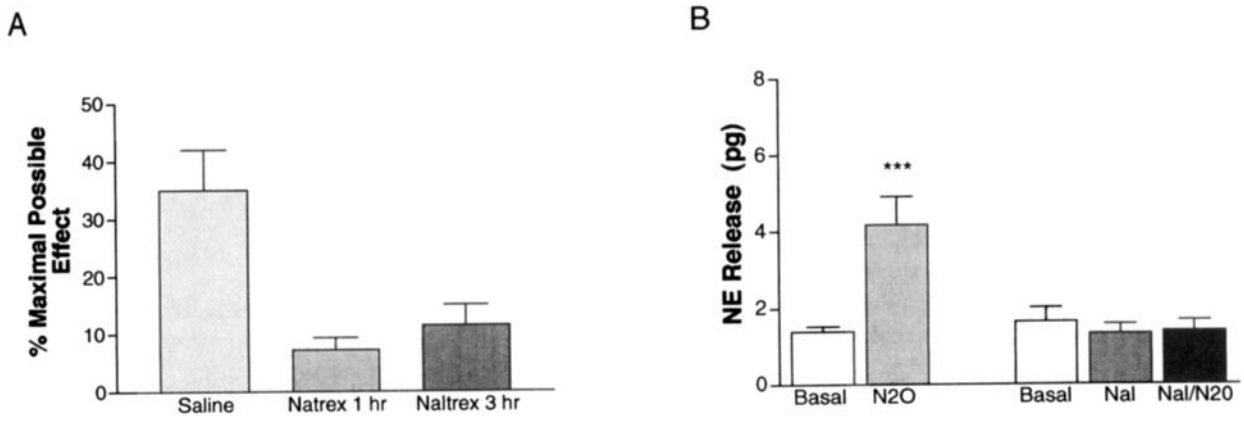


**Fig. 3. Block of opiate receptors with naltrexone suppressed $N_2O$-evoked norepinephrine release. (*A*) In the absence of naltrexone (saline), a 30-min exposure to 70% $N_2O$ had an analgesic action. Pretreatment with naltrexone (10 mg/kg intraperitoneal) given 1 and 3 h before the tail flick test blocked the analgesic effect of 70% $N_2O$. *$P < 0.05$ (n = 6–12). (*B*) Naltrexone (10 mg/kg) was administered 90 min before a 30-min exposure to 70% $N_2O$. Data are expressed as mean ± SEM. ***$P < 0.001$ (n = 6–9).**

$N_2O$ in DSP-4-treated animals coupled with the knowledge that spinal $\alpha_2$ adrenoceptors are necessary for $N_2O$ analgesia[1] indicates that the noradrenergic system is necessary for $N_2O$ analgesia.

By measuring neurotransmitter turnover. others have found that $N_2O$ stimulates norepinephrine turnover in various brain regions.[15] $N_2O$ also has been found to increase brain dopamine turnover,[16] although when turnover in discrete regions of the brain was evaluated, $N_2O$ caused a decreased turnover rate of dopamine in the hippocampus and striatum but an increase in the olfactory bulb.[15] These studies indicate that $N_2O$ causes region-specific alterations in steady state levels and turnover rates of dopamine and norepinephrine within the central nervous system. In addition, $N_2O$ suppression of the activity of wide-dynamic-range neurons, in which activity has been linked to pain transmission, depends on an intact descending inhibitory pathway.[17]

The mechanism by which $N_2O$ affects norepinephrine release is not clear, although current evidence supports a pivotal role for release of endogenous opiate peptides. Previously, we found that an opiate antagonist administered into the PAG reduced the analgesic action of $N_2O$.[7] Coupled with the current finding that $N_2O$-evoked norepinephrine release is blocked by naltrexone, these data are consistent with the hypothesis that $N_2O$ causes the release of endogenous opiate peptides in the PAG, as has been observed by others. Candidate peptides known to be present in the PAG are enkephalin, β-endorphins, or dynorphin.[18] There is some evidence that β-endorphins may be involved because their release is stimulated by $N_2O$ both *in vivo*[3] and *in vitro*.[4] Antiserum against β-endorphin but not

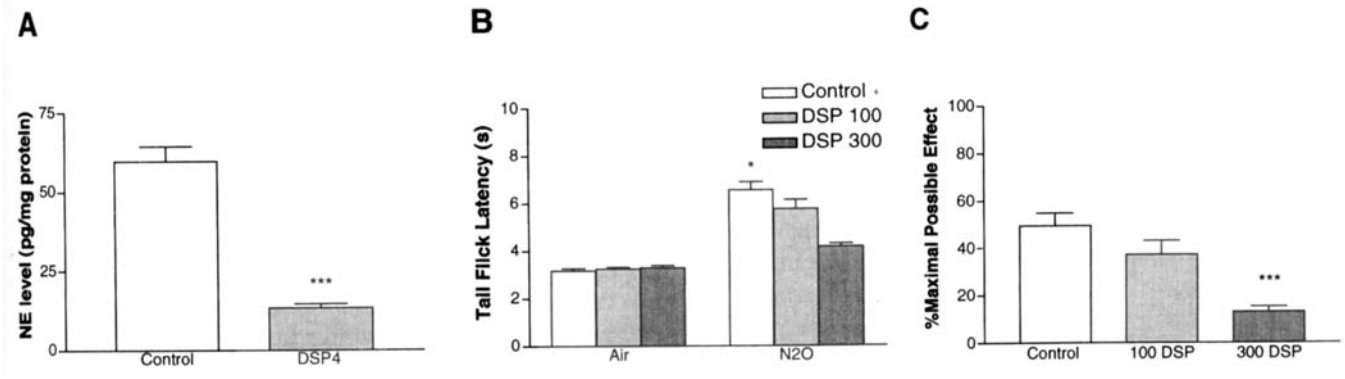


**Fig. 4. Depletion of spinal norepinephrine with DSP-4–blocked $N_2O$ analgesia. (*A*) Intrathecal administration of DSP-4 (300 μg in a volume of 10 μl) 10 days before killing reduced the levels of norepinephrine in the spinal cord. ***$P < 0.001$ (n = 6–7). (*B*, *C*) DSP-4, (100 or 300 μg in a volume of 10 μl) was intrathecally administered 10 days before testing the analgesic action of a 30-min exposure to 70% $N_2O$. There was no change in baseline tail flick latencies with DSP-4 treatment (*B*), but the analgesic action of $N_2O$ was reduced (*C*). Data are expressed as mean ± SEM. *$P < 0.05$, ***$P < 0.001$ (n = 7 or 8).**

against metenkephalin also blocked the $N_2O$-induced antinociception in rats in the hot plate test.[19] However, other studies indicate that metenkephalin may be involved in the analgesic effect of $N_2O$ because cerebrospinal fluid levels of metenkephalin taken from the third ventricle of awake dogs increased significantly; no changes were noted in concentrations of dynorphin A, dynorphin B, or β-endorphin.[5] $N_2O$ also caused an increase in metenkephalin-like immunoreactivity in the brain stem, spinal cord, hypothalamus, and corpus striatum in rats.[20] Enkephalin- and dynorphin-containing cells are present in the PAG but are also found in many other areas of the brain[21]; therefore, $N_2O$ may have an action in brain regions rostral to the PAG. It is known that analgesia can be evoked by electrical stimulation of various sites in the brain, such as the habenular complex,[22,23] arcuate nucleus,[24] and amygdala.[25] Similar to $N_2O$ analgesia, this analgesia is sensitive to blockade by opiate [23-25] and α-adrenergic antagonists.[23,25] Further work is necessary to elucidate the identity of the opiate peptides and mechanism by which $N_2O$ causes their release.

Several lines of evidence indicate that spinally projecting noradrenergic neurons mediate the antinociception produced by the activation of the PAG. Intrathecal injection of $\alpha_2$-adrenergic antagonists can reduce the antinociception produced by either electrical[26] or chemical[27] stimulation of PAG neurons. Intrathecal injection of an $\alpha_2$ antagonist can also attenuate the antinociception produced by microinjection of morphine in the PAG.[28] Additionally, discrete injection of morphine into the PAG produces an increase in norepinephrine metabolites in the spinal cord, and its analgesic effects are attenuated by previous depletion of norepinephrine stores in the spinal cord.[29] Electrophysiologic studies also showed that $\alpha_2$ adrenoceptors in the spinal cord contribute to the mediation of the PAG-induced inhibition of dorsal horn cell activity.[11,30]

There are three nuclei from which noradrenergic neuronal projections to the spinal cord originate. Using the tract tracing methods and combinations of lesions with histochemical methods, numerous studies have shown that noradrenergic neuronal projection to the spinal cord originates from the A5, A6 (locus coeruleus, subcoeruleus), and A7 cell groups.[31,32] In the same substrain of animals used in this study, Basbaum's[33] laboratory showed that the locus ceruleus is the major source of noradrenergic fibers to the dorsal horn region. All these nuclei receive projections from the PAG.[34-37] Subsequent studies will seek to identify which noradrenergic nucleus is responsible for the analgesic action of $N_2O$.

We have not conclusively established whether $N_2O$-induced norepinephrine release is mediated by activation of the descending noradrenergic pathways at a supraspinal or spinal site. In the context of our previous studies that the analgesic response to $N_2O$ is caused by activation of opiate receptors in the PAG,[7] and our current finding that naltrexone blocks $N_2O$-induced norepinephrine release in the dorsal horn of the spinal cord, we believe that the weight of evidence supports a supraspinal site of activation.

There is clear evidence that the acute antinociceptive properties of $N_2O$ decrease over a relatively short time during continuous administration.[38-41] The results of this study show that the release of norepinephrine also diminishes over roughly the same time span, pointing to a site for tolerance proximal to norepinephrine release. Interestingly, the ability of $N_2O$ to affect dopamine turnover also diminishes progressively.[16] However, the precise site of tolerance is not known. Prolonged exposure (18 h) to $N_2O$ decreases opiate receptor density in rat brain stem,[42] and this would be consistent with increased release of endogenous opiate peptides causing a down-regulation of the receptor system. This raises the possibility that, depending on the mechanism involved, "cross-tolerance" may develop to either exogenous opiate or $\alpha_2$-agonist administration, or both, after $N_2O$ exposure. Knowledge of the mechanism for $N_2O$ tolerance may help to identify people who might be less sensitive to the analgesic action of $N_2O$, and also allow the design of strategies to mitigate the development of tolerance to prolong the analgesic effect of $N_2O$.

## References

1. Guo TZ, Poree L, Golden W, Stein J, Fujinaga M, Maze M: Antinociceptive response to nitrous oxide is mediated by supraspinal opiate and spinal alpha 2 adrenergic receptors in the rat. ANESTHESIOLOGY 1996; 85:846-52

2. Quock RM, Kouchich FJ, Tseng LF: Does nitrous oxide induce release of brain opioid peptides? Pharmacology 1985; 30:95-9

3. Zuniga JR, Joseph SA, Knigge KM: The effects of nitrous oxide on the central endogenous pro-opiomelanocortin system in the rat. Brain Res 1987; 420:57-65

4. Zuniga JR, Joseph SA, Knigge KM: 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 1987; 420:66-72

5. Finck AD, Samaniego E, Ngai SH: Nitrous oxide selectively releases $Met^5$-enkephalin and $Met^5$-enkephalin-$Arg^6$-$Phe^7$ into canine third ventricular cerebrospinal fluid. Anesth Analg 1995; 80:664-70

6. Yeung JC, Yaksh TL, Rudy TA: Concurrent mapping of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat. Pain 1977; 4:23-40

7. Fang F, Guo TZ, Davies MF, Maze M: Opiate receptors in the periaqueductal gray mediate analgesic effect of nitrous oxide in rats. Eur J Pharmacol 1997; 336:137-41

8. Hodges BL, Gagnon MJ, Gillespie TR, Breneisen JR, O'Leary DF, Hara S, Quock RM: Antagonism of nitrous oxide antinociception in the rat hot plate test by site-specific mu and epsilon opioid receptor blockade. J Pharmacol Exp Ther 1994; 269:596-600

9. Skilling SR, Smullin DH, Beitz AJ, Larson AA: Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. J Neurochem 1988; 51:127-32

10. Liu DX, Valadez V, Sorkin LS, McAdoo DJ: Norepinephrine and serotonin release upon impact injury to rat spinal cord. J Neurotrauma 1990; 7:219-27

11. Peng YB, Lin Q, Willis WD: Involvement of alpha-2 adrenoceptors in the periaqueductal gray-induced inhibition of dorsal horn cell activity in rats. J Pharmacol Exp Ther 1996; 278:125-35

12. Guo TZ, Jiang JY, Buttermann AE, Maze M: Dexmedetomidine injection into the locus ceruleus produces antinociception. ANESTHESIOLOGY 1996; 84:873-81

13. Berkowitz BA, Finck AD, Ngai SH: Nitrous oxide analgesia: Reversal by naloxone and development of tolerance. J Pharmacol Exp Ther 1977; 203:539-47

14. Theron CN, de Villiers AS, Taljaard JJ: Effects of DSP-4 on monoamine and monoamine metabolite levels and on beta adrenoceptor binding kinetics in rat brain at different times after administration. Neurochem Res 1993; 18:1321-7

15. Karuri AR, Kugel G, Engelking LR, Kumar MS: Alterations in catecholamine turnover in specific regions of the rat brain following acute exposure to nitrous oxide. Brain Res Bull 1998; 45:557-61

16. Murakawa M, Shingu K, Mori K: Effects of nitrous oxide on the brain catecholamines in rats. Ann Acad Med Singapore 1994; 23:452-5

17. Nagasaka H, Taguchi M, Tsuchiya M, Mizumoto Y, Hori K, Hayashi K, Matsumoto I, Hori T, Sato I: Effect of nitrous oxide on spinal dorsal horn WDR neuronal activity in cats [in Japanese]. Masui 1997; 46:1190-6

18. Loughlin SE, Leslie FM, Fallon JH: Endogenous opioid systems, The Rat Nervous System, 2nd edition. Edited by Paxinos G. San Diego, Academic Press, 1995, pp 975-1001

19. Hara S, Gagnon MJ, Quock RM, Shibuya T: Effect of opioid peptide antisera on nitrous oxide antinociception in rats. Pharmacol Biochem Behav 1994; 48:699-702

20. Quock RM, Kouchich FJ, Tseng LF: Influence of nitrous oxide upon regional brain levels of methionine-enkephalin-like immunoreactivity in rats. Brain Res Bull 1986; 16:321-3

21. Fallon JH, Leslie FM: Distribution of dynorphin and enkephalin peptides in the rat brain. J Comp Neurol 1986; 249:293-336

22. Terenzi MG, Guimaraes FS, Prado WA: Antinociception induced by stimulation of the habenular complex of the rat. Brain Res 1990; 524:213-8

23. Terenzi MG, Prado WA: Antinociception elicited by electrical or chemical stimulation of the rat habenular complex and its sensitivity to systemic antagonists. Brain Res 1990; 535:18-24

24. Wang Q, Mao LM, Han JS: Naloxone-reversible analgesia produced by microstimulation of the arcuate nucleus of the hypothalamus in pentobarbital-anesthetized rats. Exp Brain Res 1990; 80:201-4

25. Oliveira MA, Prado WA: Antinociception induced by stimulating amygdaloid nuclei in rats: Changes produced by systemically administered antagonists. Braz J Med Biol Res 1998; 31:681-90

26. Aimone LD, Jones SL, Gebhart GF: Stimulation-produced descending inhibition from the periaqueductal gray and nucleus raphe magnus in the rat: Mediation by spinal monoamines but not opioids. Pain 1987; 31:123-36

27. Jensen TS, Yaksh TL: Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Res 1984; 321:287-97

28. Camarata PJ, Yaksh TL: Characterization of the spinal adrenergic receptors mediating the spinal effects produced by the microinjection of morphine into the periaqueductal gray. Brain Res 1985; 336:133-42

29. Pang IH, Vasko MR: Effect of depletion of spinal cord norepinephrine on morphine-induced antinociception. Brain Res 1986; 371: 171-6

30. Budai D, Harasawa I, Fields HL: Midbrain periaqueductal gray (PAG) inhibits nociceptive inputs to sacral dorsal horn nociceptive neurons through alpha2-adrenergic receptors. J Neurophysiol 1998; 80:2244-54

31. Westlund KN, Bowker RM, Ziegler MG, Coulter JD: Origins and terminations of descending noradrenergic projections to the spinal cord of monkey. Brain Res 1984; 292:1-16

32. Clark FM, Proudfit HK: The projections of noradrenergic neurons in the A5 catecholamine cell group to the spinal cord in the rat: Anatomical evidence that A5 neurons modulate nociception. Brain Res 1993; 616:200-21

33. Rohde DS, Basbaum AI: Activation of coeruleospinal noradrenergic inhibitory controls during withdrawal from morphine in the rat. J Neurosci 1998; 18:4393-402

34. Cameron AA, Khan IA, Westlund KN, Willis WD: The efferent projections of the periaqueductal gray in the rat: A Phaseolus vulgaris-leucoagglutinin study: II. Descending projections. J Comp Neurol 1995; 351:585-601

35. Byrum CE, Guyenet PG: Afferent and efferent connections of the A5 noradrenergic cell group in the rat. J Comp Neurol 1987; 261: 529-42

36. Ennis M, Behbehani M, Shipley MT, Van Bockstaele EJ, Aston-Jones G: Projections from the periaqueductal gray to the rostromedial pericoerulear region and nucleus locus coeruleus: Anatomic and physiologic studies. J Comp Neurol 1991; 306:480-94

37. Bajic D, Proudfit HK: Projections of neurons in the periaqueductal gray to pontine and medullary catecholamine cell groups involved in the modulation of nociception. J Comp Neurol 1999; 405:359-79

38. Zacny JP, Cho AM, Coalson DW, Rupani G, Young CJ, Klafta JM, Klock PA, Apfelbaum JL: Differential acute tolerance development to effects of nitrous oxide in humans. Neurosci Lett 1996; 209:73-6

39. Ramsay DS, Brown AC, Woods SC: Acute tolerance to nitrous oxide in humans. Pain 1992; 51:367-73

40. Avramov MN, Shingu K, Mori K: Progressive changes in electroencephalographic responses to nitrous oxide in humans: A possible acute drug tolerance. Anesth Analg 1990; 70:369-74

41. Rupreht J, Dworacek B, Bonke B, Dzoljic MR, van Eijndhoven JH, de Vlieger M: Tolerance to nitrous oxide in volunteers. Acta Anaesthesiol Scand 1985; 29:635-8

42. Ngai SH, Finck AD: Prolonged exposure to nitrous oxide decreased opiate receptor density in rat brainstem. ANESTHESIOLOGY 1982; 57:26-30