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Opiate receptors in the periaqueductal gray mediate analgesic effect of nitrous oxide in rats

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Abstract

The site of action and the pathways which are activated by nitrous oxide (N_2O) to produce an analgesic effect are not well defined. Experiments were designed to determine whether N_2O produces analgesia by activating opiate receptors or α_2-adrenoceptors in periaqueductal gray. The analgesic effect of N_2O was determined using the tail flick response to noxious radiant heat in lightly anesthetized rats. Different antagonists were bilaterally microinjected into ventrolateral periaqueductal gray to determine whether the analgesic effect produced by N_2O was reversed. The increase in the tail flick latencies produced by N_2O was reversed by bilateral microinjection into the ventrolateral part of periaqueductal gray with the opiate receptor antagonist naloxone 2.5 $\mu g/0.5$ μl, but not with the α_2-adrenoceptors antagonist yohimbine 1.5 $\mu g/0.5$ μl. These results indicate that the N_2O analgesic effect is mediated by activation of opiate receptors, but not α_2-adrenoceptors, in the periaqueductal gray. Combined with the previous experiments that the N_2O analgesic effect is reversed by intrathecal injection of an α_2-adrenoceptor antagonist but not by an opiate receptor antagonist, it seems likely that N_2O causes activation of the opiate receptors in the periaqueductal gray, which in turn activate the noradrenergic descending pathways to the spinal cord to produce the analgesic effect. © 1997 Elsevier Science B.V.

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1. Introduction

Nitrous oxide (N_2O) is an inhalation agent that is widely used during general anesthesia. The inhalation of N_2O in concentrations below those required for anesthesia produce analgesia in humans and animals in a dose dependent manner (Dundee and Moore, 1960; Berkowitz et al., 1976, 1977). Inhalation of 20–25% N_2O in O_2 can produce an analgesic effect equivalent to that produced by 15 mg of morphine sulfate in humans (Chapman et al., 1943; Parbrook et al., 1964). N_2O analgesia, like morphine analgesia, is in part reversed by narcotic antagonists in humans and animals (Berkowitz et al., 1976, 1977; Chapman and Benedetti, 1979; Lawrence and Livingston, 1981; Yang et al., 1980). Furthermore, there is unilateral cross-tolerance between morphine and N_2O (Berkowitz et al., 1977, 1979). Based on these observations, a common mechanism of action for narcotics and N_2O was proposed in which N_2O activates endogenous opiate inhibitory systems to produce analgesia.

Recent and ongoing investigations provide more evidence for the involvement of the opioid mechanism in the N_2O analgesic effect. Neurochemical studies demonstrated that plasma β-endorphin concentrations and [Met]enkephalin concentrations in the cerebral spinal fluid increase after N_2O exposure (Quock et al., 1985). Later studies also showed that N_2O increase β-endorphin concentrations in the arcuate propriomelanocortin neuronal system in rats (Zuniga et al., 1987a) an effect that was reproduced in an in vitro system (Zuniga et al., 1987b). This same group also demonstrated that N_2O analgesic effect could be completely ablated after lesioning the periaqueductal gray (periaqueductal gray) in the rat (Zuniga et al., 1987c).

A large number of studies have established an important role for the periaqueductal gray in pain modulation (Mayer and Liebeskind, 1974; Akil et al., 1976; Sandkuhler and Gebhart, 1984; Hosobuchi et al., 1977; Richardson and

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Akil, 1977; Gebhart and Toleikis, 1978; Fardin et al., 1984a). Stimulation of periaqueductal gray inhibited nociception in rats (Mayer and Liebeskind, 1974; Akil et al., 1976; Sandkuhler and Gebhart, 1984), cats (Gebhart and Toleikis, 1978), and humans (Hosobuchi et al., 1977; Richardson and Akil, 1977), and microinjection of opioid agonists into the periaqueductal gray produces dose-dependent antinociception (Fardin et al., 1984b).

Subsequently, the ability of systemic naloxone to reverse N_2O analgesia was shown to be stereospecific suggesting that N_2O was acting through an opiate receptor (Quock and Graczak, 1988). Recently, in a study involving dogs, the levels of two derivatives of the proenkephalin system were found to be elevated in cerebral spinal fluid obtained from the third ventricle in chronically-cannulated animals administered N_2O (Finck et al., 1995). Therefore, it seems likely that N_2O provokes the release of endogenous opiate in the region of the periaqueductal gray.

In addition to the putative role of the opioidergic system, another neurotransmitter system, possibly noradrenergic, may also be involved. N_2O was shown to centrally activate the sympathetic nervous system and increase the plasma norepinephrine level (Ohara et al., 1993). Roizen et al. (1988) reported that a significant correlation was found between norepinephrine content in the medulla and N_2O MAC in mice selectively bred for resistance and susceptibility to N_2O analgesia. Furthermore, systemic injection of α_2-adrenoceptors antagonist yohimbine can decrease N_2O analgesic effect (Guo et al., 1996), implying that α_2-adrenoceptors play a key role in the N_2O antinociception.

Previous experiments in this laboratory demonstrated that the analgesic effect produced by 70% N_2O is also partially reversed by i.c.v. naloxone (Guo et al., 1996). The purpose of the present study was to determine the neuroanatomical site for the action of naloxone and yohimbine.

2. Materials and methods

2.1. Animal preparation

Male Sprague-Dawley rats (300–400 g, Bantin and Kingman, Fremont, CA, USA) were lightly anesthetized with pentobarbital (35 mg/kg, i.p.) for experiments using the tail flick test. Cholinergic muscarinic receptor antagonist atropine methyl nitrate (0.25 mg in 0.25 ml saline, i.p.) was administered 40 min before pentobarbital to control respiratory and salivary secretions. This quaternary ammonium derivative of atropine is a peripherally-acting cholinergic muscarinic receptor antagonist that does not enter the central nervous system (Weiner, 1985). Each animal was used once to obviate the possibility that tolerance had developed.

2.2. Nociceptive testing procedures

Nociceptive response was assessed by the tail flick response to a noxious thermal stimulus. A high intensity light beam focused on the tail and the time for the rat to move its tail out of the light was recorded as tail flick latency. This method has been described in the previous report (Guo et al., 1996). The latency from three sites on the tail were averaged. A cut-off time of 10 s was predetermined to prevent tissue damage. Baseline measurements consist of a set of three tail flick determination at 2 min intervals. Baseline tail flick latencies ranged between 3–4 s. Following baseline measurements, the receptor antagonists were bilaterally injected into the ventrolateral periaqueductal gray. Twenty min later, the rats were exposed to either air or 70% N_2O for 30 min, at which time nociceptive response to thermal stimuli was tested again.

2.3. Drug microinjection

Naloxone 2.5 μg (5.4 nmol, Sigma, St. Louis, MO, USA) or yohimbine 1.5 μg (3.8 nmol, Sigma) dissolved in physiological saline, were microinjected bilaterally into the ventrolateral periaqueductal gray at the level of trochlear nerve according to the coordinates: A = 0.3 mm, L = ±0.2 mm, V = 7.0 mm relative to the skull surface at lambda according to the atlas of Paxinos and Watson (1986). Naloxone, yohimbine or saline vehicle were microinjected in a volume of 0.5 μl over a 1-min period of time using an infusion pump. The selected doses of naloxone or yohimbine are capable of antagonizing the effect of their respective agonists in the brain (Jensen and Yaksh, 1984; Sagen and Proudfit, 1985).

To determine if the antagonists had an effect on nociception when injected alone, tail flick latencies were tested 20 min after drug injection, prior to gas exposure.

2.4. Gas exposure

The rats were placed into a plexiglass restrainer from which their tails extended. The internal volume was 800 ml with a rostral inlet and a caudal outlet to facilitate gas exchange. Oxygen concentration in the chamber was maintained at 30% while N_2O concentration chamber was maintained at 70%. Gas concentration were measured continuously by inserting a tubing in the restrainer and flow rates adjusted appropriately to maintain the desired concentrations.

Previous studies suggest that the analgesic effect is stable between 15 and 30 min of N_2O exposure. Therefore after 30 min exposure in the gas, rat tail flick latencies were determined.

2.5. Histological procedures

At the end of each experiment, the animal was killed with carbon dioxide inhalation, the brain was removed and

fixed in a solution of 10% formalin and 30% sucrose for 2 days. The brain was frozen, 40 μm transverse sections were cut using a cryostat microtome, and the sections were stained with cresyl violet. Injection sites were determined from serial sections through the cannula track and the section in which the injection cannula achieved its greatest depth of penetration was assumed to represent the tip of the cannula and the site of drug microinjection. Only those rats which had accurate bilateral injections in the ventrolateral periaqueductal gray were chosen for analysis.

2.6. Statistical analysis

Each treatment group consisted of 6–8 rats, and tail flick latencies are presented as the mean ± standard error of the mean. Statistical comparisons between treatment groups were made using two-way analysis of variance (two-way ANOVA) for repeated measures.

3. Results

3.1. Effect of microinjecting antagonists on nociception

Saline, naloxone or yohimbine was microinjected bilaterally in the ventrolateral periaqueductal gray at the level of the trochlear nucleus (Fig. 1). No spontaneous motor activity was observed when drug was injected in the periaqueductal gray. Injections of these compounds at the indicated doses do not change the tail flick latency for the more than 60 min period, during which they were tested (data not shown).

3.2. Effect of antagonists on N_2O analgesia

The elevations of the tail flick latencies produced by N_2O were significantly reduced by microinjecting naloxone bilaterally in the periaqueductal gray (two-way ANOVA, $p < 0.05$). The elevations of the tail flick latencies produced by N_2O were not significantly changed by microinjecting yohimbine bilaterally in the periaqueductal gray (two-way ANOVA, $p > 0.05$) (Fig. 2).

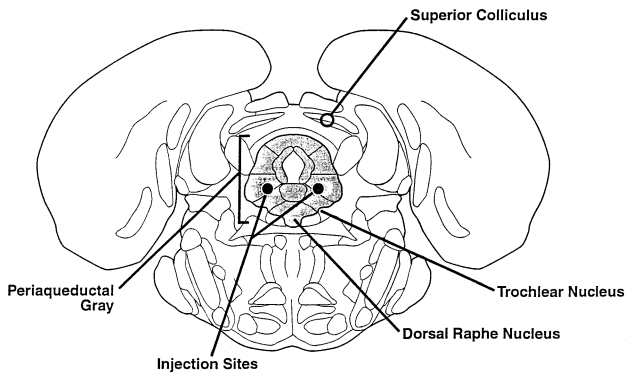

Fig. 1. Location of sites in the ventrolateral periaqueductal gray at which bilateral microinjection of drugs were made at the level of trochlear nerve according to the coordinates: A = 0.3 mm, L = ±0.2 mm, V = 7.0 mm relative to the skull surface at lambda.

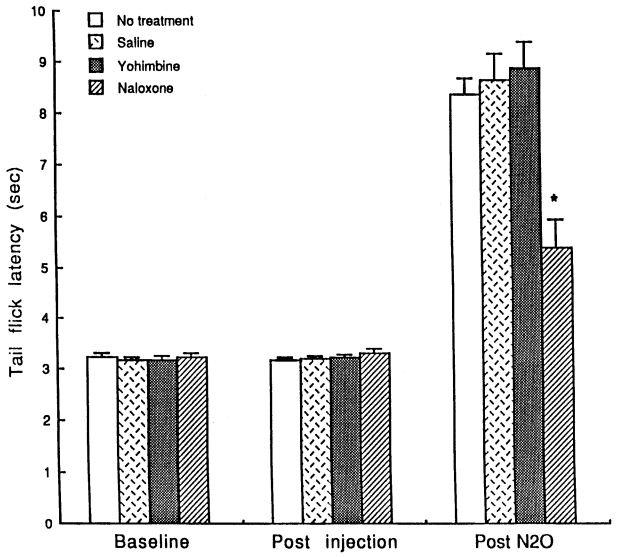

Fig. 2. Effects of naloxone and yohimbine on antinociceptive response. Four group of rats ($n = 6$/group) were microinjected either saline, naloxone or yohimbine bilaterally in the periaqueductal gray. Baseline tail-flick latencies were elicited before microinjection. 20 min after the microinjection, tail-flick latencies were elicited again. Then the rats were exposed to N_2O for 30 min before the final tail-flick latencies were elicited. Data are expressed as mean ± S.E.M. * Statistically significant different (ANOVA, $p < 0.05$).

4. Discussion

The main finding of this study indicates that opiate receptors, but not α_2-adrenoceptors, in the periaqueductal gray mediate the antinociceptive action of N_2O in rats. This interpretation is based on the fact that periaqueductal gray injection of naloxone but not yohimbine can reverse N_2O analgesic effect. N_2O may provoke the release of endogenous opiate ligands in the area of the periaqueductal gray by stimulating the hypothalamus, since the hypothalamus is the major source of afferent opioidergic projections to the periaqueductal gray (Beitz, 1982).

Stimulation of all regions of periaqueductal gray can generate analgesia, but stimulation of the ventrolateral part is most effective and produces 'pure' analgesia, free from motor side-effects or signs of aversion. In contrast, stimulation of dorsolateral part of periaqueductal gray produces analgesia and aversive motor reaction, making it difficult to separate the antinociception from the aversive behavior (Lovick, 1993). Therefore, in the present study, only those rats which had successful bilateral injections in the ventrolateral periaqueductal gray were chosen for data analysis. N_2O analgesia has also been studied by Quock's group using the hot plate test (Hodges et al., 1994). Their results

showed that N_2O analgesia was antagonized in dose-related fashion by intracerebral pretreatment with μ receptor antagonist, but not by δ- and κ-receptor antagonists. Furthermore, N_2O analgesia was antagonized by μ receptor antagonist directly administered in the periaqueductal gray in a dose-related fashion. Therefore, it is much likely that in our model using the tail flick test, opiate μ receptors in the periaqueductal gray mediate analgesia effect of N_2O.

Evidence has accumulated establishing that α_2-adrenoceptors can mediate an antinociceptive effect. A number of areas in the brain contain populations of α_2-adrenoceptors (Young and Kuhar, 1981), including the periaqueductal gray (Unnerstall et al., 1984). However, microinjection of an α_2-agonist in the periaqueductal gray did not produce an antinociceptive effect (Ossipov and Gebhart, 1983). Therefore, the α_2-adrenoceptors in the periaqueductal gray are not essential for mediating antinociception, which is consistent with the present finding.

Since α_2-adrenoceptors are involved in mediating the N_2O analgesic effect (Guo et al., 1996), but not at the periaqueductal gray site, the mechanism whereby stimulation of opiate receptors in supraspinal regions produces analgesia has been extensively investigated and appears, in part, to involve a descending noradrenergic pathway. Several lines of evidence indicate that spinally-projecting noradrenergic neurons mediate the antinociception produced by activating neurons in the periaqueductal gray. For example, intrathecal injection of a noradrenergic antagonist can reduce the antinociception produced by either electrical (Aimone et al., 1987) or chemical (Jensen and Yaksh, 1984) stimulation of periaqueductal gray neurons. Intrathecal injection of an α_2-adrenoceptors antagonist can also attenuate the antinociception produced by microinjection of morphine in the periaqueductal gray (Camarata and Yaksh, 1985). Additionally, discrete injection of morphine into the periaqueductal gray produces an increase in norepinephrine metabolites in the spinal cord and its analgesic effect is attenuated by prior depletion of norepinephrine stores in the spinal cord (Pang and Vasko, 1986). Electrophysiological studies also showed that α_2-adrenoceptors in the spinal cord mediate the periaqueductal gray-induced inhibition of dorsal horn cell activity (Peng et al., 1996).

Using the tract tracing methods and combinations of lesions with histochemical methods, numerous studies have demonstrated that noradrenergic neuronal projection to the spinal cord originate from the A5, A6 (locus coeruleus, subcoeruleus), and A7 cell (Westlund et al., 1984; Clark and Proudfit, 1993). The projections from the periaqueductal gray to the A5 (Byrum and Guyenet, 1987; Cameron et al., 1995), A6 (Beitz et al., 1988; Ennis et al., 1991), and A7 cell groups (Fang et al., 1995) have been demonstrated. Therefore, based on our findings, we speculate that N_2O provokes the release of endogenous endorphins and enkephalins which stimulate the opiate receptors in the periaqueductal gray region to cause release of norepinephrine in spinal cord. This in turn activates α_2-adrenoceptors in the dorsal horns to produce an antinociceptive effect (Kendig et al., 1991).

In conclusion, data from our study suggest that in the periaqueductal gray, opiate receptors, but not α_2-adrenoceptors mediate the N_2O antinociceptive effect.

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References

Aimone, L.D., Jones, S.L., Gebhart, G.F., 1987. Stimulation-produced descending inhibition from the periaqueductal gray and nucleus raphe magnus in the rat: Mediation by spinal monoamines but not opioids. Pain 31, 123.

Akil, H., Mayer, D.J., Liebeskind, J.C., 1976. Antagonism of stimulation produced analgesia by naloxone, a narcotic antagonist. Science 191, 961.

Beitz, A.J., 1982. The organization of afferent projections to the midbrain periaqueductal gray of the rat. Neuroscience 7, 133.

Beitz, A., Mullett, M.A., Brandt, N., 1988. The relationship of periaqueductal gray projections to bulbospinal neurons: A combined fluorogold-PHA-L analysis. Soc. Neurosci. Abstr. 14, 856.

Berkowitz, B.A., Ngai, S.H., Finck, D.A., 1976. Nitrous oxide 'analgesia': Resemblance to opiate analgesia. Science 194, 967.

Berkowitz, B.A., Finck, A.D., Ngai, S.H., 1977. Nitrous oxide analgesia: Reversal by naloxone and development of tolerance. J. Pharmacol. Exp. Ther. 203, 539.

Berkowitz, B.A., Finck, A.D., Hynes, M.D., Ngai, S.H., 1979. Tolerance to nitrous oxide analgesia in rats and mice. Anesthesiology 51, 309.

Byrum, C.E., Guyenet, P.G., 1987. Afferent and efferent connections of the A5 noradrenergic cell group in the rat. J. Comp. Neurol. 261, 529.

Camarata, P.J., Yaksh, T.L., 1985. Characterization of the spinal adrenergic receptors mediating the spinal effects produced by the microinjection of morphine into the periaqueductal gray. Brain Res. 336, 133.

Cameron, A.A., Khan, I.A., Westlund, K.N., Willis, W.D., 1995. The efferent projections of the periaqueductal gray in the rat: A *phaseoleus vulgaris*-leucoagglutin study. II. Descending projection. J. Comp. Neurol. 351, 585.

Chapman, C.R., Benedetti, C., 1979. Nitrous oxide effects on cerebral evoked potential to pain, partial reversal with a narcotic antagonist. Anesthesiology 51, 135.

Chapman, W.P., Arrowood, J.G., Beecher, H.K., 1943. The analgetic effects of low concentrations of nitrous oxide compared in man with morphine sulphate. J. Clin. Invest. 22, 871.

Clark, F.M., Proudfit, H.K., 1993. 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. 616, 200.

Dundee, J.W., Moore, J., 1960. Alterations in response to somatic pain associated with anesthesia. IV: The effects of sub-anesthetic concentrations of inhalation agents. Br. J. Anesth. 32, 453.

Ennis, M., Behbehani, M., Shipley, M.T., Van Bockstatele, E.J., Aston-Jones, G., 1991. Projections from the periaqueductal gray to the rostromedial pericoerulear region and nucleus locus coeruleus: Anatomical and physiological studies. J. Comp. Neurol. 306, 480.

Fang, F., Marczynski, T.J., Proudfit, H.K., 1995. Projections from neurons in the ventrolateral periaqueductal gray to the spinally-projecting noradrenergic neurons in the A7 catecholamine cell group. Soc. Neurosci. Abstr. 21, 386.

Fardin, V., Oliveras, J.L., Besson, J.M., 1984a. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat I. The production of behavioral side effects together with analgesia. Brain Res. 306, 105.

Fardin, V., Oliveras, J.L., Besson, J.M., 1984b. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat II. Different characteristics of the analgesia induced by ventral and dorsal PAG stimulation. Brain Res. 306, 125.

Finck, A.D., Samaniego, E., Ngai, S.H., 1995. Nitrous oxide selectively releases Met-enkephalin and Met-enkephalin–Arg–Phe into canine third ventricular cerebrospinal fluid. Anesth. Analg. 80, 664.

Gebhart, G.F., Toleikis, J.R., 1978. An evaluation of stimulation produced analgesia in cat. Exp. Neurol. 62, 570.

Guo, T.Z., Poree, L., Golden, W., Fujinaga, M., Maze, M., 1996. The antinociceptive response to nitrous oxide is mediated by supraspinal opiate alpha-2 adrenergic receptors in the rat. Anesthesiology 85, 846.

Hosobuchi, Y., Adams, J.E., Linchitz, R., 1977. Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science 197, 183.

Hodges, B.L., Gagnon, M.J., Gillespie, T.R., Breneisen, J.R., O'Leary, D.F., Hara, S., Quock, R.M., 1994. Antagonism of nitrous oxide antinociception in the rat hot plate test by site-specific μ and ε opioid receptor blockade. J. Pharmacol. Exp. Ther. 269 (2), 596–600.

Jensen, T.S., Yaksh, T.L., 1984. Spinal monoamine and opiate systems partially mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Res. 321, 287.

Kendig, J.J., Savola, M.K.T., Woodley, S.J., Maze, M., 1991. Alpha-2 adrenoceptors inhibit a nociceptive response in neonatal rat spinal cord. Eur. J. Pharmacol. 192, 293.

Lawrence, D., Livingston, A., 1981. Opiate-like analgesic activity in general anaesthetics. Br. J. Pharmacol. 73, 435.

Lovick, T.A., 1993. Integrated activity of cardiovascular and pain regulatory systems: Role in adaptive behavioural response. Prog. Neurobiol. 40, 631.

Mayer, D.J., Liebeskind, J.C., 1974. Pain produced by focal electrical stimulation of the brain: An anatomical and behavioral analysis. Brain Res. 68, 73.

Ohara, A., Zhang, P., Mashimo, T., Kamibayashi, T., Takada, K., Mammoto, T., Yamatodani, A., Yoshiya, I., 1993. Myocardinal effects of xenon and nitrous oxide during halothane anesthesia in dogs. Anesthesiology 79A, 613.

Ossipov, M., Gebhart, G.F., 1983. Absence of antinociceptive effect of alpha-2 agonists microinjected in the periaqueductal gray of the rat. Brain Res. 289, 354.

Parbrook, G.D., Ree, S., Robertson, G.S., 1964. Comparison of a 25% nitrous oxide and oxygen mixture with morphine. Br. Med. J. 2, 480.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, Sydney.

Pang, I.H., Vasko, M.R., 1986. Effect of depletion of spinal cord norepinephrine on morphine-induced antinociception. Brain Res. 371, 171.

Peng, Y.B., Lin, Q., Willis, W.D., 1996. Involvement of alpha-2 adrenoceptors in the periaqueductal gray-induced inhibition of dorsal horn cell activity in rats. J. Pharmacol. Exp. Ther. 278, 125.

Quock, R.M., Graczak, L.M., 1988. Influence of narcotic antagonistic drugs upon nitrous oxide analgesia in mice. Brain Res. 440, 35.

Quock, R.M., Kouchich, F.J., Tseng, L.F., 1985. Does nitrous oxide induce release of brain opioid peptides?. Pharmacology 30, 95.

Richardson, D.E., Akil, H., 1977. Pain reduction by electrical brain stimulation in man. II. Chronic self administration in the periaqueductal gray matter. J. Neurosurg. 47, 184.

Roizen, M.F., Koblin, D.D., Johnson, B.H., Eger, E.I. II, Bainton, C.R., 1988. Mechanism of age-related and nitrous oxide-associated anesthetic sensitivity: The role of brain catecholamines. Anesthesiology 69, 716.

Sagen, J., Proudfit, H.K., 1985. Evidence for pain modulation by pre- and postsynaptic noradrenergic receptors in the medulla oblongata. Brain Res. 331, 285.

Sandkuhler, J., Gebhart, G.F., 1984. Relative contribution of the nucleus raphe magnus and adjacent medullary reticular formation to the inhibition by stimulation in the periaqueductal gray of a spinal nociceptive reflex in the pentobarbital-anesthetized rat. Brain Res. 305, 77.

Unnerstall, J.R., Kopajtic, T.A., Kuhar, M.J., 1984. Distribution of alpha-2 agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res. 319, 69.

Weiner, N., 1985. Atropine, scopolamine and related antimuscarinic drugs. In: Gilman, A.G., Goodman, L.S., Rall T.W., Murad, F. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. Macmillan, New York, NY, p. 130.

Westlund, K.N., Bowker, R.M., Ziegler, M.G., Coulter, J.D., 1984. Origins and terminations of descending noradrenergic projections to the spinal cord of monkey. Brain Res. 292, 1.

Yang, J.C., Clark, W.C., Ngai, S.H., 1980. Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology 52, 414.

Young, W.S., Kuhar, M.J., 1981. Anatomical mapping of clonidine (alpha-2 noradrenergic) receptors in rat brain, relationship to function. In: Lal, H., Fielding, S. (Eds.), Psychopharmacology of Clonidine. Alan R. Liss, New York, NY, p. 41.

Zuniga, J.R., Joseph, S.A., Knigge, K.M., 1987a. The effects of nitrous oxide on the central endogenous pro-opiomelanocortin system in the rat. Brain Res. 420, 57.

Zuniga, J.R., Joseph, S.A., Knigge, K.M., 1987b. The effects of nitrous oxide on the secretory activity of pro-opiomelanocortin peptides from the basal hypothalamic cells attached to cytodex beads in a superfusion in vitro system. Brain Res. 420, 66.

Zuniga, J., Joseph, S., Knigge, K., 1987c. Nitrous oxide analgesia: Partial antagonism by naloxone and total reversal after periaqueductal gray lesions in the rat. Eur. J. Pharmacol. 142, 51.