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Yohimbine and flumazenil: effect on nitrous oxide-induced suppression of dorsal horn neurons in cats

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Abstract
Purpose. The purpose of this study was to determine the mechanisms of nitrous oxide (N_2O) antinociception at the spinal level with yohimbine (an α_2-adrenergic antagonist) and flumazenil (a specific benzodiazepine antagonist) using chemonociceptive stimuli in spinal dorsal horn neurons in the cat.
Methods. A lumbar laminectomy extending from L4 to L6 was performed to allow insertion of a extracellular recording device via a microelectrode. Additional laminectomy was performed at the T12 level to transect the spinal cord. As a noxious stimulus, bradykinin (BK) was injected via the cannula inserted into the femoral artery. Animals were divided into four treatment groups for subsequent experiments: N_2O + flumazenil, N_2O + yohimbine, flumazenil (alone), and yohimbine (alone).
Results. N_2O suppressed BK-induced nociceptive responses in transected feline spinal cords. The BK-induced neuronal firing rates were significantly suppressed: to 69.2%, 61.8%, and 52.2% of the baseline firing rate at 10, 20, and 30 min, respectively, after N_2O administration. The 47.8% suppression on BK-induced neuronal responses at 30 min after N_2O administration was reversed 5 min after administration of yohimbine (25.2% suppression). Similarly, N_2O suppression (42.5%) on chemically induced neuronal responses was reversed by flumazenil (24.9% suppression) at identical post-administration intervals.
Conclusion. These data imply that N_2O suppresses the nociceptive responses, in part probably through its agonistic binding activity to the α_2-adrenergic, γ-aminobutyric acid (GABA)-benzodiazepine, or both receptor systems in dorsal horn neurons of the feline spinal cord.

Key words: N_2O, Spinal dorsal horn neuron, Nociceptive response, Yohimbine, Flumazenil

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Introduction

Nitrous oxide (N_2O) is an excellent analgesic agent in humans and animals if used at concentrations well below those required for inducing anesthesia. The precise mechanism underlying the antinociceptive effect of N_2O has been unclear. Various studies have investigated the interaction between N_2O and the opioid systems [1–5]. The opioid receptor systems are possible target sites of influence by N_2O. N_2O, however, may possess nonopioid mechanisms for eliciting its antinociceptive effects. The interactions of N_2O with the adrenergic and benzodiazepine systems are currently of interest. The adrenergic and benzodiazepine systems have been documented to partially mediate the action of N_2O [6–8], but it remains to be elucidated whether the effects of N_2O are mediated at the spinal level by the adrenergic receptor, the benzodiazepine receptor, or both. The dorsal horn of the spinal cord is an important site for central-acting drugs to mediate the transmission of noxious inputs. The suppressive effects of N_2O on the bradykinin (BK)-induced nociceptive stimuli may be, at least in part, due to direct action on the spinal dorsal horn [5].

The purpose of this study was to determine the mechanism(s) of N_2O antinociception at the spinal level, using yohimbine (an α_2-adrenergic antagonist) and flumazenil (a specific benzodiazepine antagonist), with chemonociceptive stimuli in spinal dorsal horn neurons.

Methods

The protocol was approved by our institutional animal care committee. A total of 21 cats (2.4–3.2 kg) were used in the experiments. Details of the experimental method have been previously described [9]. Briefly, surgical procedures were carried out under enflurane/N_2O and oxygen anesthesia. Following tracheostomy the internal

jugular vein was cannulated for drug administration and the carotid artery for continuous blood pressure monitoring. Nociceptive responses of spinal dorsal horn neurons were induced by intraarterial BK injections. A cannula was retrogradely introduced into the bifurcation site of bilateral femoral arteries for the intraarterial BK injection.

Anesthetized animals, physically immobilized with their heads fixed in a stereotaxic apparatus in a prone position, were decerebrated at the level of the midbrain reticular formation. A lumbar laminectomy extending from L4 to L6 was performed to enable insertion of a recording microelectrode. Additional laminectomy was performed at the T12 level to transect the spinal cord with a scalpel. After these surgical procedures, anesthesia was discontinued.

Animals were immobilized by pancuronium bromide and artificially ventilated. The $PaCO_2$ and systolic blood pressure were maintained at 35–40mmHg and more than 100mmHg by controlling the tidal volume and intravenous fluid volume, respectively. The body temperature was maintained using a rectal probe connected to a servo-controlled heating pad adjusted to 37°–38°C.

The noxious stimulus, BK, was injected via the cannula inserted into the femoral artery. BK was dissolved in physiological saline (100µg/ml), and 0.1ml of solution was injected as a single bolus administration over 1–2s at 10- to 12-min intervals. Only dorsal horn neurons that responded to repeated BK injections were selected for this study. Extracellular recordings from lamina V-type cells (1500–2500µm deep from the dorsum of spinal dorsal horn) were traced on a ratemeter via a tungsten microelectrode (tip diameter 3–4µm). Unitary activities from the recording tungsten microelectrode were amplified and monitored with a dual-beam oscilloscope; the action potentials were counted with a discriminator. The number of unitary spikes per minute before and after each BK injection was counted. The pre-BK recording was referred to as spontaneous neuronal activity. The BK-induced nociceptive response was determined by subtracting the spontaneous reading from the number of unit discharges immediately after BK injection. This corrected BK-induced neuronal count was taken as the control for the nociceptive response before drug treatment.

After two or three recordings of the alternating spontaneous and BK-induced neuronal activity animals were allowed to inhale 75% N_2O for 40min, with their unitary neuronal responses monitored. An N_2O concentration of 75% was considered to be far higher than that required to suppress nociceptive stimuli in the spinal cord [5].

Cats were divided into four treatment groups for subsequent experiments: N_2O + flumazenil, N_2O + yohimbine, flumazenil alone, and yohimbine alone. Neuronal firing was recorded before and at 10, 20, and 30min after N_2O inhalation. Yohimbine ($0.2\,mg{\cdot}kg^{-1}$) and flumazenil ($0.2\,mg{\cdot}kg^{-1}$) were separately administered intravenously 35min after N_2O inhalation to study the pharmacological aspects of N_2O-induced suppression on spinal dorsal horn neurons. The effects of intravenously administered flumazenil or yohimbine on the neuronal activities were investigated in different animals. To evaluate the effects of N_2O inhalation we compared the neuronal responses of spontaneous and BK-induced neuronal activity before and after inhalation of N_2O. The reversal effects of yohimbine and flumazenil on N_2O-induced suppression were evaluated. Based on the spontaneous and BK-induced neuronal responses obtained 30min after inhalation of N_2O. N_2O was discontinued immediately after the assessment at 40min (5min after administration of flumazenil or yohimbine).

Results were expressed as the mean ± SEM. The statistical significance was verified with the Wilcoxon signed rank test. Differences were considered significant at $P < 0.05$.

Results

Treatment with N_2O plus yohimbine

Seven neurons were studied to determine the effect of yohimbine on the suppressive effects of N_2O. The mean BK-induced neuronal firing rate was 900.5 ± 206.2 (con-

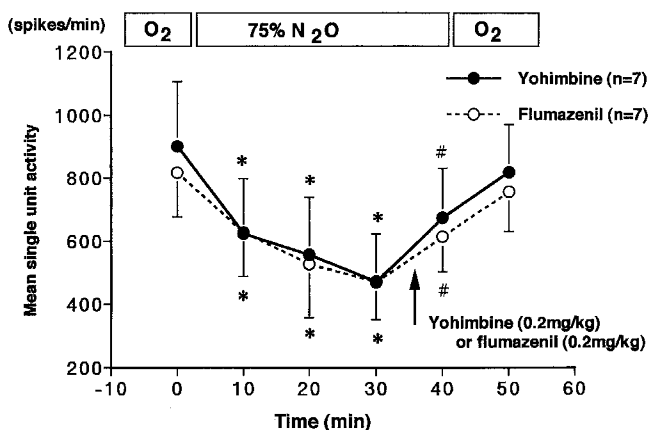

Fig. 1. Effects of yohimbine and flumazenil on N_2O-elicited suppression of bradykinin (BK)-induced activities in feline spinal dorsal horn neurons. The suppressive effect on BK-induced neuronal activities of spinal lamina V-type neurons in the dorsal horn at 10, 20, and 30min after N_2O inhalation was significant (*$P < 0.05$). The suppressive effects were significantly (#$P < 0.05$) reversed 5min after administration of either yohimbine or flumazenil ($0.2\,mg{\cdot}kg^{-1}$, IV)

trol) spikes/min. The BK-induced responses were significantly suppressed to 69.2%, 61.8%, and 52.2% of the baseline firing rate at 10, 20, and 30 min, respectively, after N_2O inhalation. The chemonociceptive responses of N_2O inhibition (47.8% suppression 30 min after N_2O inhalation) were reversed with yohimbine ($0.2 mg \cdot kg^{-1}$, IV) 5 min after administration (25.2% suppression) (Fig. 1).

Treatment with N_2O plus flumazenil

Seven neurons were studied to determine the effects of flumazenil on N_2O-induced suppression. The mean BK-induced neuronal firing rate was 817.3 ± 140.8 spikes/min (control). The BK responses were significantly suppressed to 76.4%, 64.5%, and 57.5% of the baseline firing rate at 10, 20, and 30 min, respectively, after N_2O inhalation. The N_2O-inhibited chemonociceptive neuronal response (42.5% suppression at 30 min after N_2O inhalation) was reversed by flumazenil ($0.2 mg \cdot kg^{-1}$, IV) 5 min after administration (24.9% suppression) (Fig. 1).

Treatment with yohimbine or flumazenil alone

In another group of cats, flumazenil or yohimbine administered alone did not elicit any changes in the BK-induced neuronal activities in five spinal neurons of spinalized cats.

Discussion

In this electrophysiological study, both yohimbine and flumazenil antagonized the suppressive effects of N_2O on cat spinal dorsal horn neurons. Our results indicate that the antinociceptive effect of N_2O is in part mediated through both the α_2-adrenergic and γ-aminobutyric acid (GABA)–benzodiazepine complex systems.

Various studies have investigated the interaction between N_2O and the opioid systems [1–5]. Because the antinociceptive effects of N_2O are partially attributed to activation of the endogenous opioid systems, N_2O probably stimulates neuronal release of endogenous opioid peptides, which subsequently bind to opioid receptors, producing analgesia by triggering the central nervous system (CNS) inhibitory systems. N_2O has been described as a specific agonist against μ opioid receptors without binding the κ receptors [3,4]. The opioid receptor systems, necessary for the production of antinociceptive effects are a possible target site influenced by N_2O.

Particular attention has been focused on the interaction between anesthetics and GABA–benzodiazepine receptors in recent years. Benzodiazepines enhance not only the probability of GABA channel openings but also GABAergic synaptic transmission because benzodiazepine receptors are linked to GABA receptors via a chloride coupling unit. Many anesthetics, such as volatile anesthetics, barbiturates, and benzodiazepines are known to enhance endogenous GABA-mediated inhibition in the mammalian CNS [10]. These drugs effectively increase the GABA concentration within synaptic clefts by inhibiting metabolic breakdown of GABA, leading to an accumulation of this inhibitory neurotransmitter at the synaptic site, thereby inducing anesthesia [10]. If antinociceptive effects of N_2O were antagonized by flumazenil, such GABA–benzodiazepine receptor complex systems may, in part, play a role in N_2O antinociception.

In the tail flick model, interaction of N_2O antinociception with drugs that act on the benzodiazepine receptors implicates a commonality of action between N_2O and benzodiazepine receptors [6]. Furthermore, as flumazenil antagonizes the reduction in rearing activity by N_2O in mice, involvement of benzodiazepine receptors in mediating certain behavioral effects of N_2O cannot be discounted [7]. These findings provide evidence that the GABA–benzodiazepine receptor complex system produces a modulating effect on N_2O antinociception. Moreover, drugs with no activity against the benzodiazepine receptors do not influence N_2O antinociception [6], which further suggests the presence of specific benzodiazepine receptors that could modulate the antinociceptive effects of N_2O along the nociceptive inhibitory pathways. In addition, it is possible that N_2O activates brain benzodiazepine receptors, either directly by binding to the benzodiazepine receptors or via indirect mechanisms such as altering the binding or release of an endogenous benzodiazepine-like substance.

A close association of benzodoazepine with opiate receptors has been evidenced by a direct effect of midazolam on the binding of opioid ligands to spinal opioid receptors [10]. As such, the mechanism of interaction between benzodiazepines and N_2O may depend on the allosteric interaction between benzodiazepine receptors and opioid receptors.

Other than the antinociceptive mechanisms of opiates and benzodiazepines, the sympathetic nervous system appears to play an important role in N_2O antinociception. N_2O administration results in activation of the efferent nerve activity directed to the vasculature that supplies skeletal muscles and an increase in plasma catecholamine levels [11]. It is possible that the antinociceptive effects of N_2O are exerted partially through mediation of α_2-adrenergic receptors. Intrathecal administration of α_2-adrenergic agonists have produced antinociception at the spinal level in various animal models [12,13] and humans [14].

These antinociceptive effects are mediated predominantly via the presynaptic α_2-adrenergic receptors located in the dorsal horn of the spinal cord. Furthermore, GABAergic nerve terminals in the rat hippocampus have been documented to possess α_2-adrenergic receptors whose activation causes enhancement of GABA release [15]. Moreover, α_2-adrenergic agents increase GABAergic inhibition by presynaptically facilitating endogenous GABA release [16]. N_2O might cause activation of both the α_2-adrenergic and GABAergic systems to elicit antinociception.

It is believed that volatile anesthetics, including N_2O, act nonspecifically on lipid membranes in the CNS independent of the direct anesthetic effects on specific receptors. Because some of the N_2O effects are antagonized by both yohimbine and flumazenil, direct binding of N_2O to the specific receptors or via other indirect mechanisms such as alteration of the binding sites or neurotransmitter release by the drug may also be possible.

In conclusion, both yohimbine and flumazenil reversed N_2O-elicited suppression of BK-induced nociceptive neuronal responses in the feline spinal cord. It is possible that an α_2-adrenergic/GABA–benzoziazepine receptor complex produces a modulating effect on N_2O antinociception.

References

1. Chen DC, Quock RM (1990) A study of central opioid receptor involvement in nitrous oxide analgesia in mice. Anesth Prog 37:181–185
2. Quock RM, Walczak CK, Henry RJ, Chen DC (1990) Effect of subtype-selective opioid receptor blockers on nitrous oxide antinociception in rats. Pharmacol Res 22:351–357
3. Quock RM, Best JA, Chen DC, Vaughn LK, Portoghese PS, Takemori AE (1990) Mediation of nitrous oxide analgesia in mice by spinal and supraspinal $\varkappa$-opioid receptors. Eur J Pharmacol 175:97–100
4. Ori C, Ford-Rice F, London ED (1989) Effects of nitrous oxide and halothane on μ and $\varkappa$ opioid receptors in guinea-pig brain. Anesthesiology 70:541–544
5. Miyamoto K (1985) The effects of nitrous oxide on the lamina V type neuron of the spinal dorsal horn of the rabbit. Med J Kinki Univ 10:347–355
6. Emmanpuil DE, Quockm RW (1989) Modification of nitrous oxide analgesia by benzodiazepine receptors. Anesth Prog 36:5–8
7. Quock RM, Emmanouil DE, Vaughn LK, Pruhs RJ (1992) Benzodiazepine receptor mediation of behavioral effects of nitrous oxide in mice. Psychopharmacology 107:310–314
8. Rattan AK, McDonald JS, Tejwani GA (1991) Differential effects of intrathecal midazolam on morphine-induced antinociception in the rat. Role of spinal opioid receptors. Anesth Analg 73:124–131
9. Miyamoto K, Wakita K, Okuda T, Suekane K (1992) Suppressant effects of midazolam on responses of spinal dorsal horn neurons in rabbits. Neuropharmacology 31:49–53
10. Tanelian DL, Kosek P, Mody I, Maclver MB (1993) The role of the GABA receptor/chloride channel complex in anesthesia. Anesthesiology 78:757–776
11. Ebert TJ, Kampine JP (1989) Nitrous oxide augments sympathetic outflow. Direct evidence from human peroneal nerve recordings. Anesth Analg 69:444–449
12. Yaksh TL, Reddy SVR (1981) Studies in the primate on the analgesic effects associated with intrathecal actions of opiates, α-adrenergic agonists, and baclofen. Anesthesiology 54:451–467
13. Wakita K (1992) The antinociceptive effects of fentanyl, midazolam and clonidine and their interactions in the spinal dorsal horn. Jpn J Anesth 41:1881–1888
14. Eisenach JC, Detweiler D, Hood D (1993) Hemodynamic and analgesic actions of epidurally administered clonidine. Anesthesiology 78:277–287
15. Maura G, Pittaluga A, Ulivi M, Raiteri M (1988) Enhancement of endogenous GABA release from rat synaptosomal preparations is mediated by α_2-adrenoceptors pharmacologically different from α_2-autoreceptors. Eur J Pharmacol 157:23–29
16. Doze VA, Cohen GA, Madison DV (1991) Synaptic localization of adrenergic disinhibition in the rat hippocampus. Neuron 6:889–900