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Nitrous oxide increases serotonin release in the rat spinal cord

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Abstract
The mechanism of the antinociceptive action of nitrous oxide (N_2O) is not fully understood. It was reported that N_2O induces opioid peptide release in the rat midbrain, which can activate the descending inhibitory system in the spinal cord. Although effects of N_2O on the noradrenergic descending inhibitory system have been established, effects of N_2O on the serotonergic descending inhibitory system have not been extensively investigated. We measured the extracellular level of serotonin by using in vivo microdialysis in the dorsal horn of the spinal cord in rats. The serotonin release increased to 213.01 ± 24.87% (mean ± SEM) of the baseline level from 20 to 40 min after applying N_2O, which was followed by a gradual decrease. It is suggested that the serotonergic descending pathway is activated by N_2O.

Key words Nitrous oxide · Serotonin · Microdialysis · Descending inhibitory system

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 years ago, its mechanism of action has not been completely understood. It has been reported that N_2O induces opioid peptide release in the rat midbrain [1], which can activate the descending inhibitory system in the spinal cord. The descending inhibitory system involves noradrenergic, serotonergic, and opioidergic neurons [2]. Although several reports on the effect of N_2O on noradrenergic neurons have been published already [3,4], the effects of N_2O on the serotonergic descending inhibitory system have not been extensively investigated.

In the present investigation, to examine the effect of N_2O on serotonergic neurons, we measured the extracellular level of serotonin by using in vivo microdialysis in the dorsal horn of the spinal cord in rats.

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This study was approved by the Animal Research Committee of Kyoto University Faculty of Medicine. Sixteen male Wistar rats, weighing 250–310 g, were used. They were housed, 1 per cage, under a 12-h light-dark cycle, with free access to food and water. The 16 animals were allocated randomly to either a control or an N_2O group, each consisting of 8 rats.

The dialysis probe was constructed from a 9-mm-long piece of dialysis fiber (200 μm in diameter; molecular weight cutoff, 50000; EICOM, Kyoto, Japan). The preparation of the spinal cord dialysis was similar to that described previously [5]. The rats were anesthetized with pentobarbital (40 $mg \cdot kg^{-1}$, i.p.) and the dialysis fiber was positioned in the dorsal spinal cord, including the bilateral dorsal horns at the Th13 level. The animals were allowed to recover for at least 1 day. Any animals displaying any sign of limb paralysis were excluded from further study. The dialysis probe was perfused continuously with artificial cerebrospinal fluid (CSF) solution (147 mM Na^+, 2.3 mM Ca^{2+}, 4 mM K^+, 156 mM Cl^-) at the rate of 2.0 $\mu l \cdot min^{-1}$. Perfusate samples were collected every 20 min.

Neurologically intact animals were placed in clear plastic boxes (20 × 20 × 15 cm), which were gassed continuously with a mixture of 25% oxygen (1 $l \cdot min^{-1}$) and 75% nitrogen (3 $l \cdot min^{-1}$) from the port. The animals were exposed to either 75% nitrogen/25% O_2 (control group; $n = 6$) or 75% N_2O/25% O_2 (N_2O group; $n = 5$) for 2 h after the three initial dialysate collections. After discontinuation of the N_2O, samples were collected for 1 h.

N_2O, oxygen, and carbon dioxide concentrations in the box were monitored with an anesthetic gas monitor (Type 1304; Brüel and Kjær, Copenhagen, Denmark). The behavior of the animals was observed throughout the experiments.

To verify that the fiber had transversed the dorsal horn, histological verification of the dialysis probe sites was performed at the end of each experiment. Only rats

in which the dialysis probe was located dorsal to the central canal were included for data analysis.

Serotonin levels in the dialysates were measured using high-performance liquid chromatography. In brief, an Eicompack PP-ODS column (particle size 2 μm; ϕ4.6 × 30 mm; EICOM) was used for separation. The mobile phase contained sodium phosphate buffer 0.1 M (pH 6.0), sodium 1-decansulfonate 450 mg·l^{-1}, and ethylenediamine tetraacetic acid (EDTA) 50 mg·l^{-1}, and was delivered with a pump (model 203; EICOM) at 0.5 ml·min^{-1}. Compounds in the dialysate were quantified by electrochemical detection, using a glassy carbon working electrode set at 400 mV against a silver-silver chloride reference electrode (WE-3G; EICOM).

Values for all results, expressed as percentages of the mean of three baseline values, are shown as means ± SEM. The results were assessed for statistical significance by analysis of variance, and when significant F values were encountered, Fisher's protected least significant differences test was used to confirm significant differences between treatment means. A probability level of $P < 0.05$ was considered statistically significant.

Because of limb paralysis and inappropriate position of the probe, data from three and two rats, respectively, were discarded.

The time course of the serotonin release in rats in the control group and the N_2O group is shown in Fig. 1. Mean serotonin release at the baseline condition was 5.86 ± 0.73 fmol·20 min^{-1} ($n = 11$). In the control rats, there were no significant changes in serotonin content in the samples obtained during the 4-h observation. Inhalation of N_2O significantly increased serotonin release

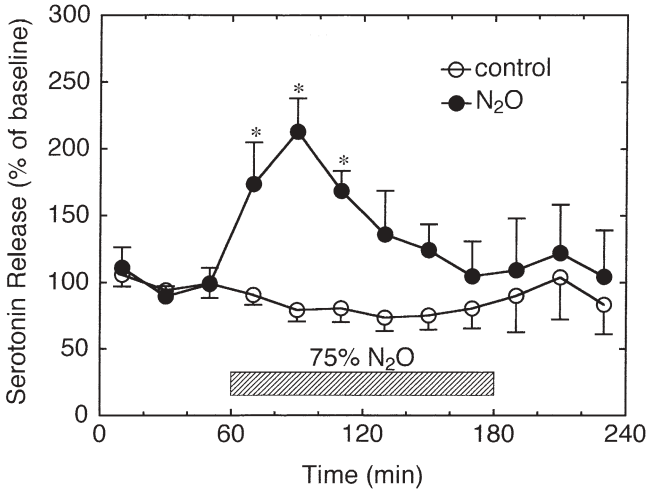

Fig. 1. Effects of 75% N_2O on dialysate serotonin content. Serotonin in each fraction is shown as a percentage of the mean of three initial collections. Data values are expressed as means ± SEM ($n = 5$ and 6, for N_2O and control groups, respectively). *$P < 0.05$ vs control values

in the dorsal horn of the spinal cord. The maximal serotonin release was 213.01 ± 24.87% ($n = 5$) of the baseline level at 20–40 min of exposure to N_2O ($P < 0.01$), which was followed by a gradual decrease. Serotonin release was restored to the control level after 80-min exposure to N_2O. Rats were calm before the application of N_2O. They became alert and moved around in response to N_2O for approximately 20 min, and then gradually became quiet. Within 5 min after the stopping of N_2O administration, they became alert.

The mechanism of N_2O-induced antinociceptive effects has been extensively studied, mainly by the use of pharmacological methods. It has been demonstrated that N_2O exerts its analgesic action by activating the descending inhibitory neurons in the spinal cord [6]. Further studies have indicated that N_2O induces opioid peptide release in the periaqueductal gray area of the midbrain, leading to the activation of the descending inhibitory pathways, which results in the modulation of nociceptive processing in the spinal cord [7].

Three major descending inhibitory pathways—noradrenergic, serotonergic, and opioidergic inhibitory pathways—project to the spinal cord from the brainstem [2]. Zhang et al. [4] have demonstrated that N_2O provokes norepinephrine release in the spinal cord in rats, and that N_2O is no longer able to produce its antinociceptive effect when norepinephrine is depleted or the spinal cord is transected. Thus, it appears that spinal cord norepinephrine is necessary for the analgesic action of N_2O. With respect to the involvement of the serotonergic system in the analgesic action of N_2O, it was shown that a serotonin receptor antagonist did not inhibit the antinociceptive effect of N_2O or N_2O-induced c-Fos expression in the spinal cord [3]. These results suggest that the descending serotonergic inhibitory pathways are unlikely to be involved in the N_2O-induced antinociceptive effect [7]. In contrast, a 5-hydroxytryptamine 3 receptor antagonist suppressed the antinociceptive effect of N_2O in an acetic-acid abdominal constriction test in rats [8]. This discrepancy between the two studies [7,8] may have been due to the differences in the nociceptive stimuli used, radiant heat on the plantar surface of the hind paw, or the abdominal injection of acetic acid. The enhancement of serotonin release by N_2O in our study suggests that the antinociceptive effect of N_2O may be mediated by the serotonergic pathway, as well as the noradrenergic system, although it is also possible that N_2O directly activates the spinal neurons, resulting in the local release of serotonin. Thus, to completely clarify the involvement of the serotonergic descending inhibitory system in the antinociceptive effect of N_2O, further study is necessary.

Acute tolerance has been observed in various pharmacological actions of N_2O, including changes in

electroencephalography [9], anticonvulsive actions [10], activation of the dopaminergic system [11], and the enhancement of acetylcholine release [12]. The maximum effects of N_2O in these studies were observed during the first 30 min, subsiding gradually within 2 h, and then reaching a steady state. This time course resembles the time-dependent increase in serotonin release in the rat spinal cord observed in the present study. It is possible that a common mechanism is involved in these apparently diverse N_2O actions. The continuous antinociceptive effect of N_2O may be based on other mechanisms, including noradrenergic and opioidergic pathways.

In conclusion, N_2O increased serotonin release in the dorsal horn of the rat spinal cord, suggesting that the serotonergic descending pathway is activated by N_2O. This pathway may be involved in the antinociceptive effect of N_2O.

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