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Nociceptin receptor antagonist JTC-801 inhibits nitrous oxide-induced analgesia in mice

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Abstract
The mechanism of the analgesic effect of nitrous oxide (N_2O) has not been completely clarified. Although we have reported that the analgesic effect of N_2O was significantly decreased in nociceptin-orphanin FQ (N/OFQ) receptor (NOP)-deficient mice, the effect of nociceptin receptor antagonists on N_2O-induced analgesia has not been reported. In this investigation, we examined the effect of the NOP antagonist JTC-801 on N_2O-induced analgesia in 129Sv mice by the writhing test and tail flick test, and demonstrated that the analgesic effect of N_2O was suppressed by the intraperitoneal administration of JTC-801.

Key words Nitrous oxide · Analgesia · Nociceptin

Nitrous oxide (N_2O) has been used clinically for more than 150 years. The major advantage of N_2O is its analgesic effect, which is more potent than that of other inhaled anesthetics. Findings to date suggest that N_2O induces the release of endogenous opioid peptides in the periaqueductal gray area of the midbrain and exerts its analgesic effect via activation of the descending inhibitory pathway [1–4].

Nociceptin-orphanin FQ (N/OFQ) has been identified as an endogenous agonist of the opioid receptor-like 1 receptor (NOP) that is highly homologous to classical opioid receptors [5,6]. N/OFQ and NOP are widely expressed in the central nervous system [7,8] and have been shown to be involved in the modulation of nociceptive signals. Although we previously reported that the analgesic effect of N_2O was markedly smaller in NOP-deficient mice than in wild-type mice [9], it is not known whether NOP antagonists affect N_2O-induced analgesia. The aim of the present work was to investigate the effect of a NOP antagonist, JTC-801, on N_2O-induced analgesia, to gain further insight into the mechanism of N_2O action.

This study was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. We used male 129Sv mice (7–8 weeks old; Japan SLC, Shizuoka, Japan). They were allowed ad libitum access to food and water, and were housed in an air-conditioned room (room temperature, 24 ± 2°C; 50% relative humidity) with lights on from 7 a.m. to 9 p.m. Gas exposure was performed in a polypropylene chamber (12 cm in height, 22 cm in diameter). Mice were exposed to either 75% N_2O/25% O_2 (N_2O group) or 75% N_2/25% O_2 (control group) continuously delivered from an anesthetic machine (Aika; Ichikawa Shiseido, Tokyo, Japan) into the chamber through an inflow port; the gas was exhausted through an outflow port (total gas flow rate, 5 $l \cdot min^{-1}$). The chamber was temporarily opened during intraperitoneal (i.p.) administration of saline or JTC-801, or injection of acetic acid. The analgesic effect of N_2O was measured using the writhing test and the tail flick test. For the writhing test, mice ($n = 8$ for each group) were exposed to the mixed gas for 30 min and then injected i.p. with 0.7% glacial acetic acid (0.1 ml per 10 g body weight). Five minutes after the injection of acetic acid, the number of writhing responses (lengthwise stretches of the torso with concave arching of the back) in each mouse was counted for 10 min. Until the end of the observation, the mixed gas was continuously delivered into the chamber. JTC-801 (Tocris Bioscience, Bristol, UK) was dissolved in normal saline. JTC-801 (0.05, 0.1, 0.5, 1, or 5 $mg \cdot kg^{-1}$) or normal saline was i.p. injected 10 min before the i.p. injection of acetic acid. For the tail flick test ($n = 10$ for each group), we determined tail-flick latencies (TFLs) from the mean of two consecutive latencies, using a tail-flick apparatus (MK-330B; Muromachi Kikai, Tokyo, Japan). The level of heat intensity was preset so that the baseline TFL ranged between 2.7 and 3.3 s. To prevent tissue damage, the cutoff time was 6 s. After the measurement

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of baseline TFL, each mouse was exposed to the mixed gas for 30 min, and then transferred outside the chamber to determine the post-treatment TFL. JTC-801 (0.05, 0.1, 0.5, 1, or 5 mg·kg^{-1}) or vehicle was i.p. injected 15 min before determination of the post-treatment TFL. The maximum possible effect (%MPE) was calculated as follows: %MPE = (post-treatment TFL–baseline TFL)/(cutoff time (6 s)–baseline TFL) × 100. Data values are presented as means ± SEM. The results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test or unpaired *t*-test for pairwise comparisons. A *P* value of less than 0.05 was considered significant.

The number of writhing responses induced by the i.p. injection of acetic acid is shown in Fig. 1A. In vehicle-treated mice, N_2O significantly reduced the number of writhing responses. In the control group, the NOP antagonist JTC-801 had no effect on the number of writhing responses. On the other hand, JTC-801, at 0.1 and 0.5 mg·kg^{-1}, increased the number of writhing responses in the N_2O group. JTC-801 alone, at doses of up to 5 mg·kg^{-1}, did not induce writhing responses (data not shown). Figure 1B demonstrates %MPE values for the tail flick test. There were no significant differences in baseline TFL values between the treatment groups. In vehicle-treated mice, N_2O showed a significant increase in %MPE. In the control group, JTC-801 did not significantly change %MPE, whereas, in the N_2O group, JTC-801 in the range of 0.05–5 mg·kg^{-1} decreased %MPE.

The present results are compatible with our previous report that N_2O exerted significantly less analgesic effect in NOP-deficient mice than in wild-type mice, and the results suggest that the N/OFQ system in the brain and/or the spinal cord is activated by N_2O, resulting in analgesic effect.

Although N/OFQ and NOP are highly homologous to dynorphin and the classical opioid receptors, respectively, the influence of the N/OFQ system on nociception is apparently different from that of the classical opioid peptide system [10]. There has been controversy as to the role of N/OFQ in pain modulation. It was reported that the intracerebroventricular administration of N/OFQ induced hyperalgesia [5,6], but spinal administration produced an antinociceptive effect [11]. Morphine analgesia was antagonized by supraspinal N/OFQ, but potentiated by spinal N/OFQ [12]. Thus, N/OFQ modulates nociceptive responses differently in the brain and the spinal cord [13]. Therefore, it remains to be examined how the N/OFQ system is activated by N_2O and how activation of the N/OFQ system leads to antinociception.

There are limitations of the present investigation. First, although JTC-801 has been demonstrated to possess at least eightfold selectivity for NOP over the classical opioid receptors, we cannot completely exclude the possibility that the suppressive effect of JTC-801 on the analgesic action of N_2O is due to its antagonistic effect on the classical opioid receptor. To exclude this possibility in future, we should also test other NOP antagonists, including J-113397 and SB-612111 [14,15].

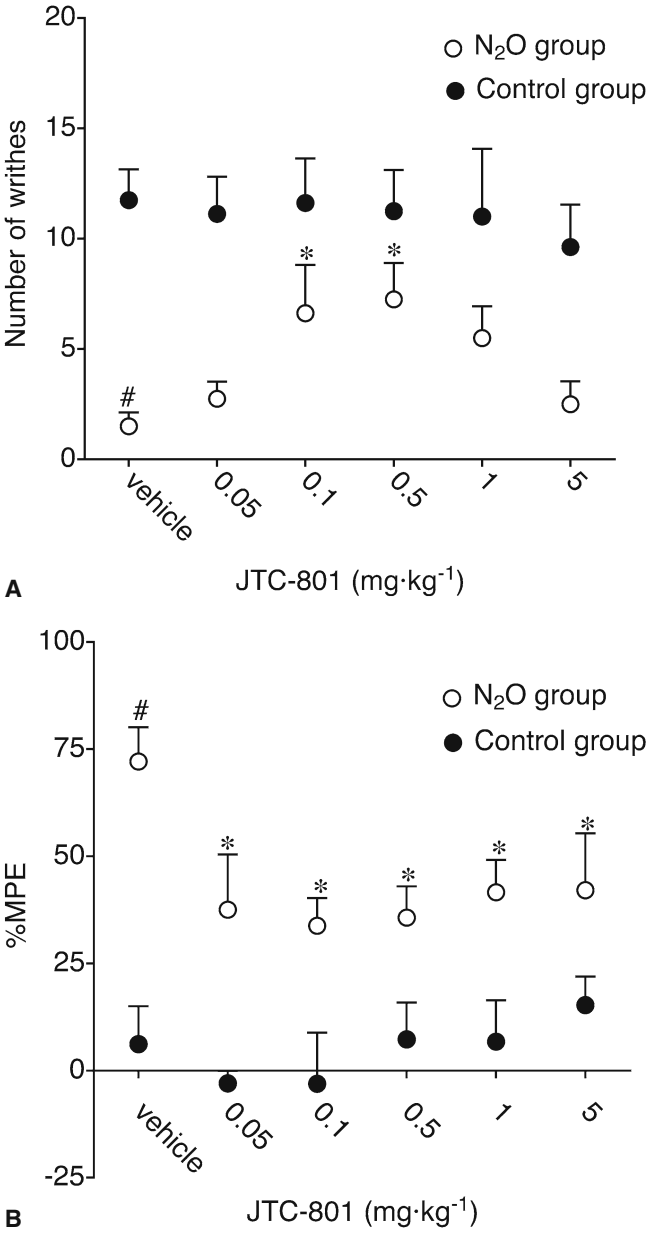

Fig. 1A,B. **A** Effect of JTC-801 (Tocris Bioscience) on nitrous oxide (N_2O)-induced analgesia in the writhing test. The number of writhing responses (*writhes*; mean ± SEM; $n = 8$) induced by the i.p. injection of acetic acid is plotted against the dose of JTC-801. **B** Effect of JTC-801 on nitrous oxide (N_2O)-induced analgesia in the tail flick test. The maximum possible effect *(%MPE*; mean ± SEM; $n = 10$) is plotted against the dose of JTC-801. $^{\#}P < 0.001$ versus control-group mice treated with vehicle; $^{*}P < 0.05$ versus N_2O-exposed mice treated with vehicle

Second, it remains to be clarified why a high dose of JTC-801 significantly suppressed the analgesic effect of N_2O in the tail flick test but not in the writhing test. This observation suggests that the analgesic effect of JTC-801 [16,17] on visceral pain may be stronger than the effect on somatic pain, but it is not clear why no obvious analgesic effect of JTC-801 was observed when it was administered without N_2O inhalation in the present study. It is possible that the analgesic efficacy of JTC-801 may depend on the type of noxious stimulus and the activation state of the N/OFQ system.

In conclusion, the present study demonstrated that the NOP antagonist JTC-801 attenuated the analgesic effect of N_2O. Further studies using other NOP antagonists will enable us to clarify the involvement of the N/OFQ system in N_2O-induced analgesia.

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