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SHORT COMMUNICATION

Involvement of the κ-opioid receptor in nitrous oxide-induced analgesia in mice

Tomohiro Koyama · Kazuhiko Fukuda

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Abstract Nitrous oxide (N_2O)-induced analgesia is thought to be mediated by endogenous opioids. We previously showed that the μ-opioid receptor is not required for the analgesic action of N_2O in mice using a gene knockout approach. In this study, we examined the effect of κ- (KOP)- or δ-opioid receptor (DOP)-selective antagonists on N_2O-induced analgesia. The analgesic effect of N_2O was evaluated using a writhing test. Male C57BL/6 mice aged 7–8 weeks were assigned to control, N_2O, KOP agonist, and DOP agonist groups. According to the group assignment, mice were pretreated with a KOP antagonist, nor-binaltorphimine (nor-BNI), a DOP antagonist, naltrindole hydrochloride (NTI), a KOP agonist U50488, and a DOP agonist SNC80. Mice in the control, KOP agonist, and DOP agonist groups were exposed to 25% oxygen/75% nitrogen for 30 min, and mice in the N_2O group were exposed to 25% oxygen/75% N_2O for 30 min. Nor-BNI [10 mg kg^{-1}, subcutaneously (s.c.)] significantly suppressed the analgesic effect of N_2O and U50488. In contrast, NTI (10 mg kg^{-1} s.c.) did not significantly affect the analgesic action of N_2O, but almost completely inhibited the analgesic effect of SNC80. These results suggest that KOP plays an important role in the analgesic effect of N_2O in mice.

Keywords Nitrous oxide · Analgesia · Opioid receptor

The greater potency of the analgesic effect of nitrous oxide (N_2O) compared to other inhaled anesthetics is the most conspicuous advantage of N_2O. The underlying mechanisms of the analgesic action of N_2O have been extensively investigated, but many aspects of the mechanism remain unclear. Since 1976 [1], there have been several reports describing the opioidergic mechanism of N_2O-induced analgesia [2–4], but the opioid receptors and endogenous opioid peptides that are involved have not been clarified. We have previously shown that an absence of μ-opioid receptors (MOP) in mice does not affect the analgesic effect of N_2O or the antagonistic effect of naloxone on N_2O-induced analgesia [5]. Thus, we hypothesized that κ- (KOP) and/or δ-opioid receptors (DOP) are involved in N_2O-induced analgesia in mice. To examine this hypothesis, we determined whether nor-binaltorphimine (nor-BNI), a highly selective KOP antagonist [6, 7], and naltrindole hydrochloride (NTI), a highly selective DOP antagonist [8], antagonize the analgesic effect of N_2O in the writhing test.

This study was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. Male C57BL/6 mice (7–8 weeks old; Japan SLC, Shizuoka, Japan) were allowed access to food and water ad libitum, and were housed in an air-conditioned room (24 ± 2°C room temperature, 50% relative humidity) with lights on from 7 a.m. to 9 p.m. All chemicals were obtained from Sigma Chemical (St. Louis, MO, USA). Mice were exposed to the mixed gas for 30 min in a polypropylene chamber (12 cm in height, 22 cm in diameter) and then injected intraperitoneally with 10 ml kg^{-1} 0.7% glacial acetic acid. Five minutes after the injection, the number of writhing responses (lengthwise stretches of the torso with concave arching of the back) in each mouse was counted for 10 min. The mixed gas was continuously delivered into the chamber until the end of the observation. Mice were used only once in the writhing test. To test the

T. Koyama (✉) · K. Fukuda
Department of Anesthesia, Kyoto University Hospital,
54 Kawahara-cho, Shogoin, Sakyo-ku,
Kyoto 606-8507, Japan
e-mail: yama8899@gmail.com

effect of a KOP antagonist, mice were assigned to a control, N_2O, or KOP agonist group. Mice in the control and KOP agonist groups were exposed to 25% oxygen (O_2)/75% nitrogen (N_2) for 30 min, and mice in the N_2O group were exposed to 25% O_2/75% N_2O for 30 min. A selective KOP antagonist, nor-binaltorphimine (nor-BNI) (3 or 10 mg kg^{-1}), or vehicle (normal saline) was injected subcutaneously (s.c.) 4 h before the writhing test. Mice in the KOP agonist group received the KOP agonist U50488 [*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidiny)-cyclohexyl)-benzeneacetamide methanesulfonate] [9] (5 mg kg^{-1} s.c.) 15 min before the writhing test. To test the effect of a DOP antagonist, mice were assigned to a control, N_2O, or DOP agonist group. Mice in the control and DOP agonist groups were exposed to 25% O_2/75% N_2 for 30 min, and mice in the N_2O group were exposed to 25% O_2/75% N_2O for 30 min. A selective DOP antagonist, NTI (10 mg kg^{-1}), or vehicle (distilled water) was injected s.c. 30 min before the writhing test. Mice in the DOP agonist group received the DOP agonist SNC80 [10] (10 mg kg^{-1} s.c.) 10 min before the writhing test. Data are shown as the mean ± SD. The results were analyzed by one-way analysis of variance followed by a Bonferroni post hoc test or unpaired *t* test. A P value < 0.05 was considered significant.

In the absence of opioid antagonists, the number of writhes was significantly decreased by N_2O and the KOP agonist compared with the control group (N_2O, 4.8 ± 3.2, $n = 16$; KOP agonist, 2.0 ± 2.6, $n = 8$; control, 10.0 ± 6.0, $n = 13$; $P < 0.01$) (Fig. 1). Pretreatment with the KOP antagonist nor-BNI (10 mg kg^{-1}) significantly increased the number of writhes in the N_2O group (4.8 ± 3.2 vs. 9.1 ± 6.9, $n = 16$, $P < 0.05$) and the KOP agonist group (2.0 ± 2.6 vs. 5.9 ± 2.6, $n = 8$, $P < 0.05$), whereas nor-BNI had no effect on the number of writhes in the control group. Figure 2 shows that the number of writhes in mice of the DOP agonist group and the N_2O group was significantly smaller than in the control group (N_2O, 3.8 ± 4.6, $n = 8$; DOP agonist, 5.4 ± 4.0, $n = 8$; control, 9.6 ± 2.9, $n = 8$; $P < 0.05$) in the absence of opioid antagonists. The DOP antagonist NTI significantly increased the number of writhes in the DOP agonist group (5.4 ± 4.0 vs. 9.6 ± 3.5, $n = 8$, $P < 0.05$) but showed no effect on the number of writhes in the control and N_2O groups.

In the present study, pretreatment with nor-BNI almost completely antagonized the analgesic effect of N_2O in mice. On the other hand, a selective DOP antagonist NTI at a dose that fully blocked the analgesic effect of SNC80 had no effect on N_2O-induced analgesia. We have previously shown that the analgesic effect of N_2O and the antagonistic effect of naloxone on N_2O-induced analgesia are not significantly affected by knockout of the MOP gene [5]. Collectively, these findings suggest that KOP, but not DOP

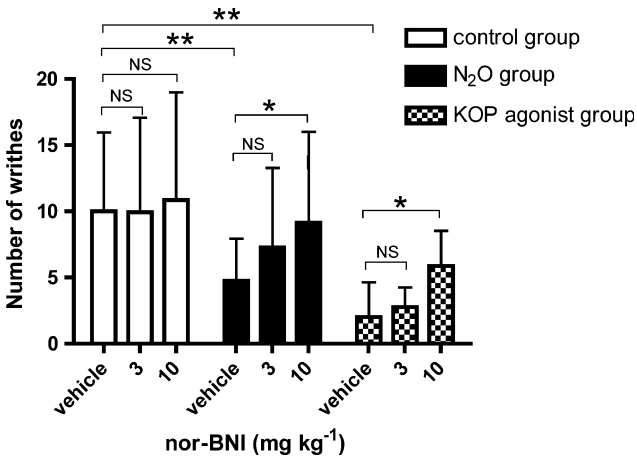

Fig. 1 Effect of nor-binaltorphimine (*nor-BNI*) on the analgesic action of nitrous oxide in the writhing test. Number of writhes (mean ± SD) induced by intraperitoneal injection of acetic acid plotted against the dose of nor-BNI. *N_2O* nitrous oxide, *KOP* κ-opioid receptor. *$P < 0.05$; **$P < 0.01$; *NS* not significantly different

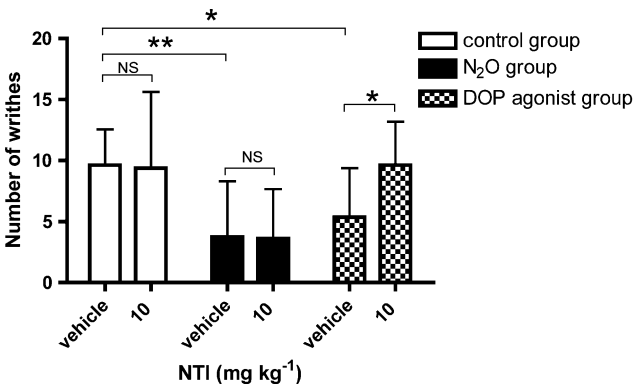

Fig. 2 Effect of naltrindole hydrochloride (*NTI*) on the analgesic action of nitrous oxide in the writhing test. Number of writhes (mean ± SD) induced by intraperitoneal injection of acetic acid plotted against the dose of NTI. *N_2O* nitrous oxide, *DOP* δ-opioid receptor. *$P < 0.05$; **$P < 0.01$; *NS* not significantly different

or MOP, is mainly involved in the analgesic action of N_2O in mice, in agreement with the findings of Quock and colleagues [11, 12] and Kingery et al. [13]. This result is not contradictory to previous reports showing antagonism of N_2O-induced analgesia by naloxone, because naloxone can inhibit cellular responses mediated by MOP, KOP, or DOP, although it shows higher affinity for MOP than for KOP and DOP. To further clarify the involvement of KOP in N_2O-induced analgesia, it will be necessary to investigate whether KOP is activated by N_2O inhalation in the central nervous system and whether N_2O induces release of dynorphin, an endogenous KOP agonist.

There have been several reports suggesting possible mechanisms other than the opioid system in N_2O-induced analgesia. It has been reported that N_2O directly inhibits

glutamatergic transmission in the spinal cord [14]. The serotonin system also has been suggested to be involved in the analgesic effect of N_2O [15, 16]. Furthermore, it has been reported that N_2O antagonizes nicotinic acetylcholine receptors [17] and potentiates two pore-domain potassium channels [18]. In future, further studies are needed to completely clarify involvement of these mechanisms in the analgesic effect of N_2O.

N_2O-induced KOP activation may suggest a negative interaction between N_2O and opioid agonists in clinical settings, because an opposing interaction between KOP and MOP has been reported [19, 20]. It was demonstrated that morphine, a MOP agonist, reduces the minimum alveolar concentration of isoflurane, but that this effect of morphine is suppressed by N_2O [21], which may reflect suppression of morphine-induced MOP activation by N_2O-induced KOP activation. Because it may be possible that KOP activation by N_2O attenuates MOP-mediated analgesia by MOP agonists such as fentanyl, morphine, and remifentanil, it will be interesting to compare the dose of opioids necessary to suppress nociceptive responses with or without simultaneous administration of N_2O.

A potential limitation of this study is that we cannot completely exclude the possibility that nor-BNI and NTI act on other opioid receptors, although these antagonists show high selectivity for KOP (>150-fold over DOP and MOP [7]) and DOP (>100-fold over KOP and MOP [8]), respectively, and were administered according to previously described protocols [22, 23]. To overcome this pharmacological limitation, further experiments using mice deficient for KOP and DOP will be required.

In summary, we have shown that N_2O-induced analgesia is almost fully reversed by a KOP antagonist in mice. Taken together with our previous report [5], this finding indicates that the KOP system plays an essential role in the analgesic action of N_2O.

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