Anesth Prog 37:181–185 1990

A Study of Central Opioid Receptor Involvement in Nitrous Oxide Analgesia in Mice*

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This study was undertaken to assess the sensitivity of nitrous oxide (N_2O) analgesia to antagonism by intrathecally (IT) and intracerebroventricularly (ICV) administered antagonists selective for kappa- and mu-opioid receptors. Male ICR mice were pretreated IT or ICV with the kappa antagonist nor-binaltorphimine (nor-BNI), 1 or 50 nmol, respectively, or distilled water (control), then exposed to N_2O (50% or 75% in oxygen). Compared with IT control mice, IT nor-BNI-pretreated mice responded with significantly less analgesia. Compared with ICV control mice, ICV nor-BNI-pretreated mice also showed markedly reduced analgesic response. Other mice were pretreated IT or ICV with either the selective and irreversible mu antagonist β-funaltrexamine (β-FNA, 5.0 μg) or distilled water (control). When exposed to N_2O 24 h later, β-FNA-pretreated and control mice exhibited comparable analgesic responses. These preliminary results suggest that N_2O analagesia in mice may involve spinal and supraspinal kappa-opioid receptors but not mu-opioid receptors.

Nitrous oxide (N_2O) is commonly used as an adjunct for behavior modification in clinical dentistry.[1,2] However, the effectiveness of N_2O analgesia has not received as much attention as its use for managing anxiety of dental patients. N_2O possesses analgesic activity that is comparable in many respects to that of morphine, the classical opioid analgesic standard. One old report suggests that exposure to 20% N_2O produces as much pain relief as does treatment with 15 mg morphine sulfate.[4]

The first direct evidence linking the analgesic effect of N_2O to endogenous opioid systems was the demonstration by Berkowitz and associates that opioid receptor blockers can attenuate N_2O analgesia in mice;[5,6] in addition, the analgesic effect of N_2O was reduced by morphine tolerance.[5–7] Berkowitz and co-workers suggested that N_2O might release endogenous opioid peptides in the brain to stimulate opioid receptors and cause analgesia.[7] Quock and others showed that N_2O could increase the amount of methionine-enkephalin in perfusate of centrally perfused rats[8] and increase the methionine-enkephalin content of selected brain regions as well.[9] Zuniga and others have shown that N_2O can increase the amount of β-endorphin in medial basal hypothalami of rats[10] and also increase release of β-endorphin from rat hypothalamic cells in vitro.[11] These indications of N_2O release of opioid peptide imply that activation of opioid receptors by peptides might be responsible for analgesia.

Quock and Graczak[12] demonstrated that N_2O analgesia in mice might involve kappa-opioid receptors because the drug effect was antagonized by MR-2266, a putative kappa-opioid antagonist. But since MR-2266 blocks both kappa- and mu-opioid receptors,[13] the present study was undertaken to assess the sensitivity of N_2O analgesia to antagonism by separate and more selective mu- and kappa-opioid antagonists administered directly into the central nervous system.

MATERIALS AND METHODS

Animals and N_2O Exposure

Male ICR mice (Sasco/King Animal Laboratories, Oregon, WI) weighing 20–25 g were randomly allocated to different pretreatment groups used in this study. Groups

Received June 14, 1989; accepted for publication April 30, 1990.

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*1989 Astra Anesthesia Research Award recipient.

ISSN 0003-3006/90/$3.50

of five mice each were housed in a plastic cage (30 cm long × 19 cm tall × 16 cm wide) inside a sealed, medium-sized inflatable polyethylene glovebag (Aldrich, Milwaukee, WI). N_2O and oxygen (O_2) were delivered into the sealed glovebag with a final inflow rate of 10 L/min (50% N_2O : 5.0 L/min N_2O plus 5.0 L/min O_2 or 75% N_2O : 7.5 plus 2.5 L/min, respectively), using a standard N_2O/O_2 anesthesia machine (Adec, Newburg, OR). The N_2O concentration in the glovebag was periodically checked by drawing gas samples into evacuated flasks and determining the contents with a Hewlett Packard 1100 medical gas analyzer. Exhausted gas was vented from the glovebag to a nearby fumehood via a second length of polyethylene tubing. A beaker of soda lime was placed within the glovebag for absorption of carbon dioxide. Control experiments were conducted with the unsealed glovebag open to room air. The temperature within the glovebag was kept constant at 24°C. All exposures and experiments were conducted between 0900 and 1230 h. Values were determined from 20 animals per pretreatment group.

Analgesia Assessment

N_2O analgesia was measured by assessing the ability of N_2O (50% or 75%) to suppress acetic acid-induced abdominal constrictions.[14] A 0.6% solution of acetic acid was administered by intraperitoneal (IP) injection, and immediately afterwards the mice were placed into the glovebag for exposure to N_2O/O_2. Exactly 5 min following the acetic acid challenge, the number of abdominal constrictions in each animal was counted for a 6-min period. The experiment was then terminated, and the animals were removed from the glovebag and killed.

Analgesia was also measured by the heat-irradiant tail-flick assay.[15] A high-intensity light was directed at the middle third of the animal's tail simultaneously with the start of a photoelectric timer. The number of seconds required for the animal to flick its tail out of the light path was recorded. The machine and animals were placed inside the glovebag which was then filled with N_2O/O_2 when testing for N_2O analgesia. A cut-off time of 10 s was used to prevent tissue damage.

Intrathecal and Intracerebroventricular Injections

Two methods of central drug pretreatment were used. Intrathecal (IT) injections were made by direct lumbar puncture of nonanesthetized mice at the $C_{5,6}$ level, using a Hamilton microsyringe, according to the method of Hylden and Wilcox.[16] To account for possible effects of stress, control animals received a similarly administered IT injection of vehicle (distilled water). Intracerebroventricular (ICV) injections were made, also using a Hamilton microsyringe, in lightly halothane-anesthetized mice, following exposure of the calvarium, according to a modification of the method of Haley and McCormick.[17] ICV control mice received injections of vehicle. The volume of IT and ICV injections was 4–5 μl.

Drugs

The following were used in this study: nitrous oxide, U.S.P. and oxygen, U.S.P. (Bentley Welding Supply, Milwaukee, WI): β-funaltrexamine (β-FNA) and nor-binaltorphimine (nor-BNI) (Research Biochemicals, Wayland, MA). Except for N_2O and O_2, all drugs were prepared in distilled water. Doses reflect weights or moles of the respective salts of the drugs. β-FNA pretreatment was administered 24 h before analgesia testing; β-FNA (5 μg/4 μl) was injected either ICV or IT. The 24-h pretreatment time for β-FNA reportedly allows for dissipation of a kappa-opioid agonist effect of β-FNA while maintaining irreversible blockade at mu-opioid receptors.[18] Nor-BNI (50 nmol/5 μl) ICV pretreatment was administered 60 min before the experiment whereas nor-BNI (1 nmol/5μl) IT pretreatment was administered 15 min before the test. The different doses and times for ICV and IT nor-BNI pretreatments are reportedly effective in blocking kappa-opioid receptors in brain and spinal cord.[19]

Statistical Analysis of Data

The degree of analgesia (% analgesia) evoked by nitrous oxide in the abdominal constriction test was determined as

$$100 \times \frac{\begin{array}{c}\#\text{ of abdominal constrictions} \\ \text{in control mice}\end{array} - \begin{array}{c}\#\text{ of abdominal constrictions} \\ \text{in test mice}\end{array}}{\#\text{ of abdominal constrictions in control mice}},$$

and in the tail flick test, as

$$100 \times \frac{\begin{array}{c}\text{experimental tail} \\ \text{flick time}\end{array} - \begin{array}{c}\text{control tail} \\ \text{flick time}\end{array}}{10 - \text{control tail-flick time}}.$$

The percentage of analgesia of control and drug pretreated groups were compared, using a two-tailed Student's *t*-test.

RESULTS

Preliminary experiments have shown that exposure of mice to N_2O results in suppression of acetic acid-induced abdominal constrictions and also in prolongation of tail-flick latencies. However, because of the different experimental paradigms, the identical concentration N_2O may result in different degrees of analgesia in the two para-

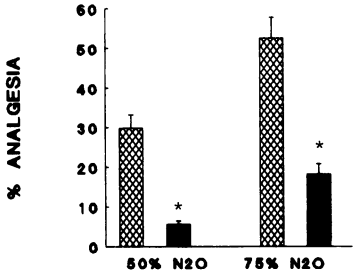


Figure 1. Influence of IT nor-BNI on 50% and 75% N_2O analgesia in the abdominal constriction test. Crosshatched bars represent the mean analgesic responses of vehicle control mice 15 min following IT injection of 5 μl distilled water and the solid bars represent the mean analgesic responses of mice 15 min following IT injection of nor-BNI (1 nmol/5 μl). Vertical lines represent the SEM. Significance of difference: *, $P < 0.05$, compared to control group.

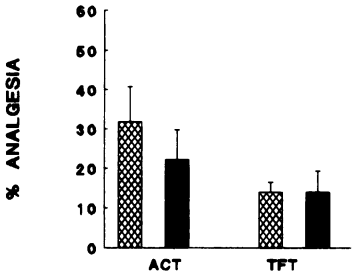


Figure 3. Influence of IT β-FNA on 75% N_2O analgesia in the abdominal constriction test (ACT) and tail-flick test (TFT). Crosshatched bars represent the mean analgesic responses of vehicle control mice 24 h following IT injection of 4 μl distilled water and the solid bars represent the mean analgesic responses of mice 24 h following IT injection of β-FNA (5 μg/4 μl). Vertical lines represent the SEM. Responses were not significantly different in either test.

digms. Preliminary data also indicates that treatment with β-FNA or nor-BNI alone was without significant influence upon pain thresholds in the experimental paradigms.

IT nor-BNI-pretreated mice responded with significantly less analgesia in the abdominal constriction test than IT control mice at 50% and 75% N_2O (Figure 1). Compared with ICV control mice, ICV nor-BNI-pretreated groups also showed markedly reduced analgesic responses at 50% and 75% N_1O (Figure 2).

When exposed to N_2O 24 h later, β-FNA-pretreated and control groups exhibited comparable analgesic responses. In IT β-FNA-pretreated groups, abdominal constriction and tail-flick tests did not reveal any significant differences with the IT control group (Figure 3). Similar responses were observed in ICV β-FNA-pretreated and control groups (Figure 4), although in other experiments, similar pretreatment with β-FNA effectively antagonized analgesic effects of morphine, sufentanil, and phosphoramidon (results not shown here).

It also appeared that IT vehicle control mice exhibited less analgesia in response to N_2O than ICV vehicle control mice. This might be due to the process of the IT injection into spinal cord and influence upon spinal mechanisms underlying the tail-flick response.

DISCUSSION

It has been demonstrated by pharmacological and neurochemical means that there are multiple opioid receptors and that there are at least three subtypes of opioid receptors that can mediate analgesia, notably mu, kappa, and delta receptors.[13,20,21] Recent studies have also shown that there are multiple sites of action for opioid analgesic drugs within the central nervous system, namely in the brain and the spinal cord.[22–24] Our study was designed to obtain preliminary data as to whether N_2O analgesia is mediated through mu- and/or kappa-opioid receptors and whether

Figure 2. Influence of ICV nor-BNI on 50% and 75% N_2O analgesia in the abdominal constriction test. Crosshatched bars represent the mean analgesic responses of vehicle control mice 60 min following ICV injection of 5 μl distilled water and the solid bars represent the mean analgesic responses of mice 15 min following ICV injection of nor-BNI (50 nmol/5 μl). Vertical lines represent the SEM. Significance of difference: *, $P < 0.05$, compared to control group.

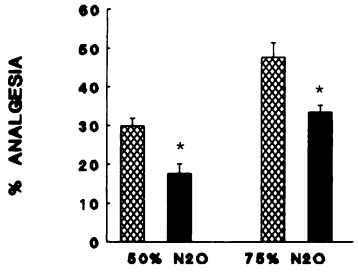
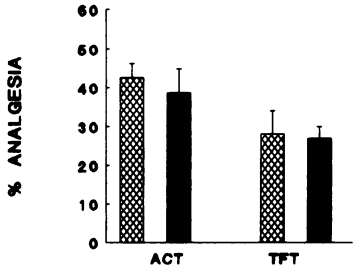


Figure 4. Influence of ICV β-FNA on 75% N_2O analgesia in the abdominal constriction test (ACT) and tail-flick test (TFT). Crosshatched bars represent the mean analgesic responses of vehicle control mice 24 h following ICV injection of 4 μl distilled water and the solid bars represent the mean analgesic responses of mice 24 h following ICV injection of β-FNA (5 μg/4 μl). Vertical lines represent the SEM. Responses were not significantly different in either test.



these target sites are located in the brain or in the spinal cord.

Our results show that nor-BNI, a selective kappa-opioid antagonist, significantly blocked N_2O analgesia following either IT or ICV pretreatment. On the other hand, at the doses used in this study, β-FNA, a selective mu-opioid antagonist, was without appreciable effect on N_2O analgesia. Although our findings implicate kappa-opioid receptors while mitigating against mu-opioid receptors in N_2O analgesia, the findings are preliminary and need to be interpreted in light of relative drug specificity and optimal doses and times for selective receptor blockade. Confirmation by other experimental approaches is required. One interesting tact might be to use animals in which selected opioid receptor subtypes are altered by means other than receptor blockade. For instance, CXBK/ByJ mice that have been reported to be deficient in brain mu-opioid receptors[25] still exhibit an analgesic response to N_2O similar to that of the C57BL/6ByJ parent strain.[26] This observation supports the contention that N_2O analgesia might be independent of mu-opioid receptors.

CONCLUSION

These results suggest that N_2O analgesia may involve kappa-opioid receptors but not mu-opioid receptors, and that these participating opioid receptors may be located both in the brain and the spinal cord.

ACKNOWLEDGEMENTS

This research was supported by NIH grant DE-06894 and the Marquette University School of Dentistry.

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