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Involvement of Nitric Oxide in Nitrous Oxide Anxiolysis in the Elevated Plus-Maze[1]

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CATON, P. W, S. A. TOUSMAN AND R. M. QUOCK. *Involvement of nitric oxide in nitrous oxide anxiolysis in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **48**(3) 689–692, 1994.—We recently reported that inhibition of nitric oxide (NO) production by the NO synthase (NOS) inhibitor L-N^G-nitro arginine (L-NOARG) antagonized the behavioral effects of a benzodiazepine (BZ) in a mouse paradigm for screening anxiolytic drug activity. Because other research has found that the anesthetic gas nitrous oxide (N_2O) also produces BZ-like behavioral effects, the present research was conducted to ascertain whether NO might also be involved in N_2O anxiolysis. Male Swiss–Webster mice were tested in an elevated plus-maze inside an inflatable glovebag. Exposure to N_2O significantly increased exploratory activity on the open arms of the plus-maze, as measured by the number of entries into the open arms and the time spent on the open arms. Pretreatment with L-NOARG significantly reduced the N_2O-induced elevation in open arm activity. This antagonism of the N_2O effect was reversed by ICV treatment of L-NOARG-pretreated mice with L-arginine but not D-arginine. These findings indicate that NO possibly mediates behavioral effects of N_2O in an animal model for anxiety.

Nitrous oxide Nitric oxide Elevated plus-maze Nitric oxide synthase inhibition Mouse

NITRIC oxide is a naturally occurring vasodilating gas that is synthesized from L-arginine in the vascular endothelium through the action of the catalytic enzyme NO synthease (NOS) (9,15). More recently, NOS has been localized in central nervous tissue (1) and NO has been postulated to act as a neurotransmitter in the central nervous system (2,6,26). There is growing evidence of NO involvement in various centrally mediated physiological and pharmacological effects. Recently, we demonstrated that pretreatment with the NOS-inhibitor L-N^G-nitro arginine (L-NOARG) antagonized the anxiolytic effect of the benzodiazepine (BZ) chlordiazepoxide in mice (23). That this antagonism was related to inhibition of NO production was demonstrated by stereoselective reversal of the antagonism by L-arginine, which is the natural substrate for NOS, but not by D-arginine, which is not. We have also previously shown that nitrous oxide produced BZ-like behavioral effects in a number of animal models for anxiety and that these effects of nitrous oxide can be antagonized by flumazenil, a BZ receptor blocker (4,5,11,21,24). The present investigation was carried out to determine whether NO might also be involved in the anxiolytic effect of nitrous oxide in the mouse elevated plus-maze (13).

METHOD

Animals

Male Swiss–Webster mice weighing 20–30 g were obtained from Sasco Inc. (Omaha, NE) and used in this research. Animals were used only once.

Behavioral Testing

The elevated plus-maze was constructed from Plexiglas and consisted of two open arms (30 cm L × 5.5 cm W) and two enclosed arms (30 cm L × 5.5 cm W × 16 cm H) mounted at 90° to one another. The arms were made of black Plexiglas but the walls were made of clear Plexiglas. The plus-maze was mounted on Plexiglas legs at a height of 24 cm. In this test,

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animals were individually placed in the central area of the elevated plus-maze then observed for a 5-min period. The number of entries into either open or enclosed arms of the maze was recorded, as was the duration of exploratory activity on open and enclosed arms of the plus-maze.

Exposure to Nitrous Oxide

The elevated plus-maze was inserted inside a large-size AtmosBag® glovebag (Aldrich, Milwaukee, WI) along with a cage of five mice, a pair of long forceps for transferring animals between the cage and maze, and a moist sponge for cleaning the maze and reducing residual odors. The sealed AtmosBag® was filled with equal portions of nitrous oxide and oxygen or with compressed air at a total inflow rate of 10 l/min using a dental sedation machine (Porter, Hatfield, PA). The AtmosBag® was inflated for 30 min, a time predetermined for achieving and maintaining the desired atmosphere. A POET II anesthetic monitoring system (Criticare Systems, Milwaukee, WI) was used continuously to measure the nitrous oxide and oxygen levels within the glovebag. Exhausted gases were vented to a nearby fumehood. Consequently, mice were in a nitrous oxide atmosphere for 35–60 min (30-min acclimation during filling of the glovebag and 5 min each during testing). Analysis of individual responses indicated no difference in response between the first and last mouse tested in each group. Following removal from the nitrous oxide-filled glovebag, the behavioral effects of nitrous oxide quickly dissipated.

Drugs

Drugs used in this research included the following: nitrous oxide, U.S.P., oxygen, U.S.P., and compressed air, U.S.P. (Rockford Industrial Welding, Rockford, IL); and L-NOARG, L-arginine, and D-arginine (all purchased from the Sigma Chemical Company, St. Louis, MO). Nitrous oxide and oxygen were administered by inhalation as described above. All other drugs were freshly prepared in 0.9% physiological saline. L-NOARG was administered SC 60 min prior to testing. The 10-mg/kg dose of L-NOARG was delivered in an injection volume of 0.1 ml per 10 g b.wt. This dose of L-NOARG was previously determined to be the optimal dose for inhibition of NO production in mice (14,27). Thirty minutes prior to testing, L-arginine and D-arginine were administered by central microinjection in mice during a brief anesthesia with halothane by the method of Haley and McCormick (7). The ICV dose of both L-arginine and D-arginine was 20 μg and was delivered in a volume of 4 μl. These doses were based on earlier whole-animal studies in our and other laboratories (14,23,27). Control animals received no prior drug treatment before being tested on the plus-maze.

Statistical Analysis of the Data

The following results were tabulated for each treatment group: the mean number of entries into open arms of the plus-maze; the mean number of entries into enclosed arms of the plus-maze; the mean duration of exploratory activity on open arms of the plus-maze; and the mean duration of exploratory activity on enclosed arms of the plus-maze. To demonstrate that the increased open arm activity reflected a specific anxiolytic drug effect, the open arm data were converted into percent total entries into open arms of the plus-maze and into percent of the total test time (300 s) spent on the open arms of the plus-maze. Following an arc-sin conversion, the data for each treatment group of mice were compared by one-way ANOVA followed by a post hoc Tukey's test (18).

RESULTS

As shown in Fig. 1, mice exposed to nitrous oxide exhibited significant increases in both the percent of entries into open arms and the percent time spent on open arms of the elevated plus-maze. Nitrous oxide produced an overall net increase in the mean number of overall (open plus enclosed) arm entries (from 6.0 ± 1.1 to 24.6 ± 2.9), indicating an increase in locomotor activity. Mice were pretreated with 10 mg/kg L-NOARG SC 60 min before exposure to nitrous oxide; these

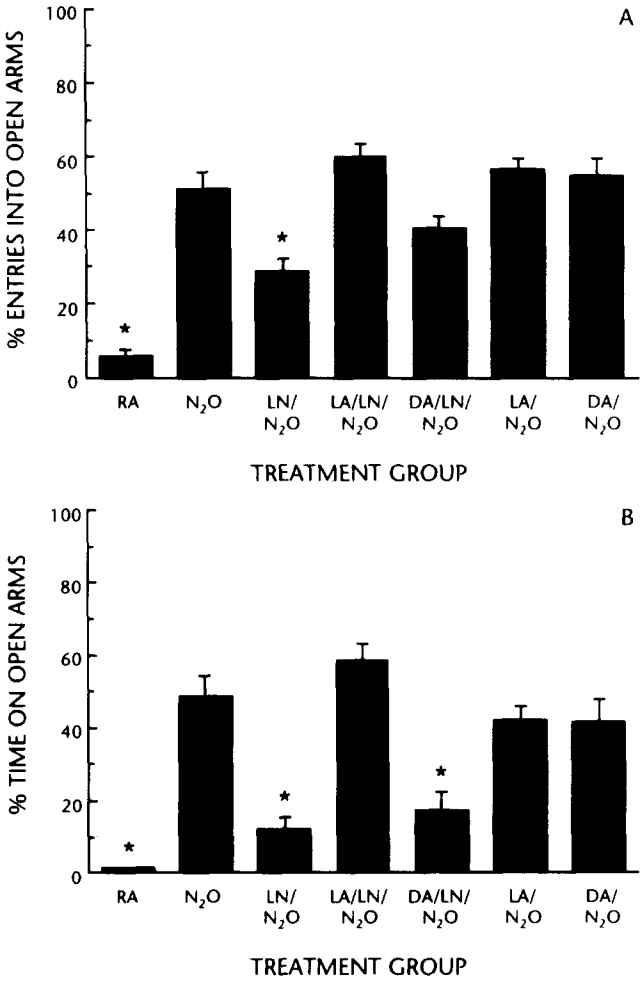

FIG. 1. The effects of nitrous oxide on the percent total entries into the open arm (A) and the percentage of time spent on the open arms (B) during a 5-min test on an elevated plus-maze. Abbreviations of different treatment groups: RA = room air, N_2O = nitrous oxide (50% by inhalation), LN/N_2O = L-NOARG (10 mg/kg, SC) plus nitrous oxide (50%), LA/LN/N_2O = L-arginine (20 μg, ICV) and L-NOARG (10 mg/kg, SC) plus nitrous oxide (50%), DA/LN/N_2O = D-arginine (ICV) and L-NOARG (10 mg/kg, SC) plus nitrous oxide (50%), LA/N_2O = L-arginine (20 μg, ICV) plus nitrous oxide (50%), DA/N_2O = D-arginine (20 μg, ICV) plus nitrous oxide (50%). The height of each bar represents the mean and each vertical bar represents the SEM of 15–20 mice per group. Significance of difference, $p < 0.05$ one-way ANOVA and post hoc Tukey's test).

animals exhibited a significant decrease in the amount of total entries into and of the total time spent on the open arm of the plus-maze. This dose of L-NOARG alone had no effect on baseline activity in the plus-maze (data not shown). When L-NOARG-pretreated mice were given an additional ICV injection of 20 μg L-arginine 30 min prior to the nitrous oxide exposure, open arm activity was restored to the same level as observed in the nitrous oxide control group. When L-NOARG-pretreated mice were treated ICV with 20 μg D-arginine 30 min prior to nitrous oxide exposure, open arm activity was not significantly increased from the L-NOARG group. Mice receiving ICV pretreatment with only L-arginine or D-arginine prior to nitrous oxide exposure displayed open arm activity comparable to the nitrous oxide control group.

DISCUSSION

There is growing evidence of an important intracellular and interneuronal messenger role for NO in the central nervous system (2,6,26). Earlier research in our laboratory has shown that subanesthetic concentrations of nitrous oxide produce behavioral effects that are reminiscent of drug effects of the BZs (5,11,21,24). Moreover, these effects of nitrous oxide are sensitive to antagonism by the BZ receptor blocker flumazenil, and there appears to be cross-tolerance to these behavioral effects of nitrous oxide in chlordiazepoxide-tolerant animals (5,11,21). Recently, we implicated NO in mediation of the anxiolytic effect of chlordiazepoxide in mice in the elevated plus-maze (23). The present study was designed to ascertain whether NO might also be involved in the anxiolytic effect of nitrous oxide by pretreating mice with L-NOARG, which is reportedly the most selective inhibitor of brain NOS (12).

A popular, pharmacologically valid test for screening both anxiolytic and anxiogenic compounds is the elevated plus-maze (13). According to this model, mice generally spend only a minute portion of time on the open arms of the elevated plus-maze because of their anxiety in wide, open spaces (i.e., the open arm of the plus-maze). When challenged with BZs and other anxiolytic drugs, the mice exhibit more and more activity on the open arms as their anxiety level is reduced. Comparable to administration of BZs, exposure to nitrous oxide increased exploratory activity on the open arms of the plus-maze; the number of entries into open arms of the plus-maze and the duration of exploratory activity on the open arms of the plus-maze were both significantly increased. The fact that there is approximately a fourfold increase in overall (open plus enclosed) arm entries is consistent with earlier reports that nitrous oxide lacks sedative and anesthetic properties in mice and, in fact, produces an opioid receptor-mediated locomotor stimulatory effect (8,25).

The increase in open arm activity on the elevated plus-maze was reduced by pretreatment with L-NOARG, which competitively inhibits NOS and prevents conversion of L-arginine to NO. When L-NOARG-pretreated mice were treated with L-arginine, the open arm activity was restored to the levels originally seen in the nitrous oxide group. When L-NOARG-pretreated mice were treated with D-arginine, which is not a substrate for NOS, the nitrous oxide effect remained reduced. This stereoselective reversal of L-NOARG antagonism by L-arginine is a clear indication of an involvement of NO in the mechanism of nitrous oxide action. Further experiments showed that when mice were pretreated with L-arginine alone, the open arm response to nitrous oxide was not potentiated in comparison to the nitrous control condition. Open arm activity was also unaffected by pretreatment with D-arginine.

Besides its anxiolytic effect, nitrous oxide also possesses prominent analgesic properties. In earlier work, we have demonstrated that nitrous oxide-induced analgesia is mediated by central opioid mechanisms (19,20,22) whereas the anxiolytic effect of nitrous oxide appears to be mediated by BZ mechanisms (4,5,11,21,24). In the mouse staircase test, an opioid receptor-mediated locomotor stimulatory effect (8) appeared to mask the anxiolytic effect of nitrous oxide, making it necessary to neutralize the opioid influence by naloxone (21,25). However, such pretreatment was not necessary in the elevated plus-maze to demonstrate an anxiolytic response to nitrous oxide. Nonetheless, there appears to be some involvement of opioid receptors, as evidenced by a recent report that low doses of morphine in rats induced a naloxone-sensitive increase in the number of open arm entries and time spent in the open arms of an elevated plus-maze (17). The role of opioid receptors in anxiolysis remains to be elucidated.

In summary, we found that the anxiolytic effect of nitrous oxide in the mouse elevated plus-maze was significantly attenuated by inhibition of brain NO production. The stereospecific reversal of this inhibition by L-arginine is consistent with the fact that L-NOARG competes with L-arginine to inhibit NOS (10,16). These results, therefore, support the hypothesis that NO plays an important role in the mechanism by which nitrous oxide produces its anxiolytic effect in mice in the elevated plus-maze.

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