Pharmacology Biochemistry and Behavior, Vol. 46, pp. 161–165, 1993
Printed in the U S.A.

0091-3057/93 $6.00 + .00

Benzodiazepine Receptor-Mediated Behavioral Effects of Nitrous Oxide in the Rat Social Interaction Test

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QUOCK, R. M., P. J. WETZEL, R. H. MAILLEFER, B. L. HODGES, B. A. CURTIS AND D. A. CZECH. *Benzodiazepine receptor-mediated behavioral effects of nitrous oxide in the rat social interaction test.* PHARMACOL BIOCHEM BEHAV **46**(1) 161–165, 1993.—The present study was conducted to ascertain whether an anxiolytic effect of nitrous oxide was demonstrable in rats using the social interaction test and whether this drug effect might be mediated by benzodiazepine receptors. Compared to behavior of vehicle-pretreated, room air-exposed rats, rat pairs exposed to nitrous oxide showed a generally inverted U-shaped dose-response curve with the maximum increase in social interaction encounters occurring at 25% and significant increase in time of active social interaction at 15–35%; higher concentrations produced a sedative effect that reduced social interaction. Treatment with 5.0 mg/kg of the anxiolytic benzodiazepine chlordiazepoxide also increased social interaction. Pretreatment with 10 mg/kg of the benzodiazepine receptor blocker flumazenil, which alone had no effect, significantly antagonized the social interaction-increasing effects of both nitrous oxide and chlordiazepoxide. In summary, these findings suggest that nitrous oxide produces a flumazenil-sensitive effect comparable to that of chlordiazepoxide and implicate central benzodiazepine mechanisms in mediation of the anxiolytic effect of nitrous oxide.

Nitrous oxide Behavioral effects Flumazenil Rats Benzodiazepine receptors Social interaction

NITROUS oxide is an inorganic gas with a variety of pharmacological effects, including anesthesia, analgesia, and anxiolysis. It is widely used as an anesthetic in combination with more potent inhalation agents for achieving balanced anesthesia for surgical procedures. Nitrous oxide is also used in clinical dentistry for reduction of patient pain and anxiety (1,2,16). While the anesthetic and analgesic effects of nitrous oxide have been widely studied, the mechanism of its anxiolytic effect has remained unknown. Earlier research in our laboratories has demonstrated that nitrous oxide exerted an apparent anxiolytic effect in mice in various models of experimental anxiety (6–8,15,17,18). The present study was conducted to assess the behavioral effects of nitrous oxide in rats, and then determine the sensitivity of such effects to antagonism by a benzodiazepine receptor blocker in the social interaction test, which is based on the uncertainty generated by placing unacquainted rats in an unfamiliar and brightly illuminated environment (10).

METHOD

Animals

Male Long–Evans hooded rats weighing 200–300 g were purchased from Sasco Inc. (Omaha, NE) for this research. They were individually housed in the animal quarters under controlled temperature and humidity.

Social Interaction Test

The social interaction of rat pairs under nitrous oxide or compressed air was studied in the enclosed, air-tight apparatus shown in Fig. 1. The apparatus consisted of a large test chamber (A) (60 cm L × 60 cm W × 65 cm H) connected to a pair of black Plexiglas air-tight acclimating cylinders (B) (10.5 cm diameter × 32 cm L) via flexible accordion-like dryer tubing (C) (the right one shown in a compressed position and the left one in the extended position). These two lengths of flexible tubing were attached to openings (D) in a heavy, removable, circular Plexiglas lid atop the test chamber (E). Thus, test gases flowed into the bottom of each cylinder, through connecting tubes, and into the test chamber. Exhausted gases flowed out through a pair of outflow ports (F) on top of the test chamber to a nearby fumehood via lengths of polyethylene tubing (not shown). The test chamber was set on the floor in a dimly lit room. The ambient room temperature was 24 ± 1°C.

On experiment days, pairs of rats matched for body weight were placed inside individual acclimating cylinders for a 30-

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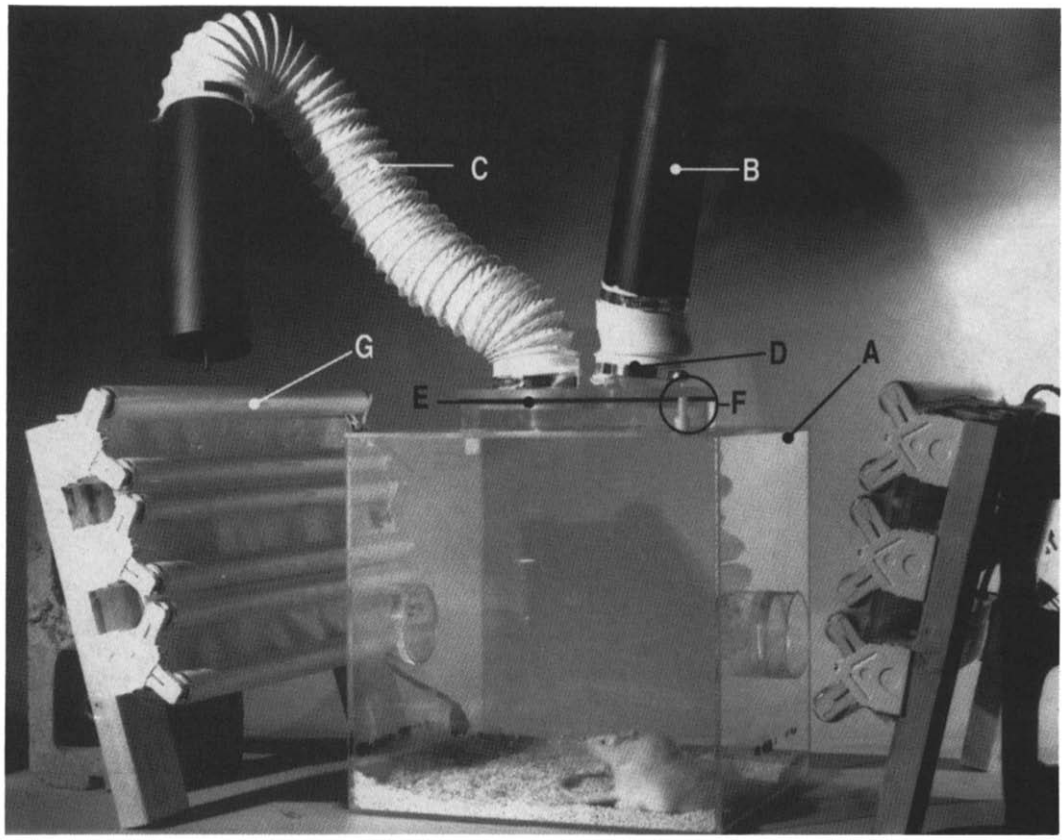

FIG. 1. Test apparatus

min exposure to either 50% nitrous oxide or compressed air. At the end of this exposure period, the acclimating cylinders were rotated upside down over the test chamber, the connecting tubing was compressed, and the rats were deposited into the test chamber. Two banks of six fluorescent tubes (each 20 W) each (G) were positioned at opposite sides of the test chamber to provide illumination. A videocamera was strategically mounted in front of the test chamber at a distance of 1.5 m to record the social interaction between rats inside the test chamber. Under these conditions, the temperature inside the test chamber generally increased 1.5°C during the 10-min test period and never exceeded 26.5°C.

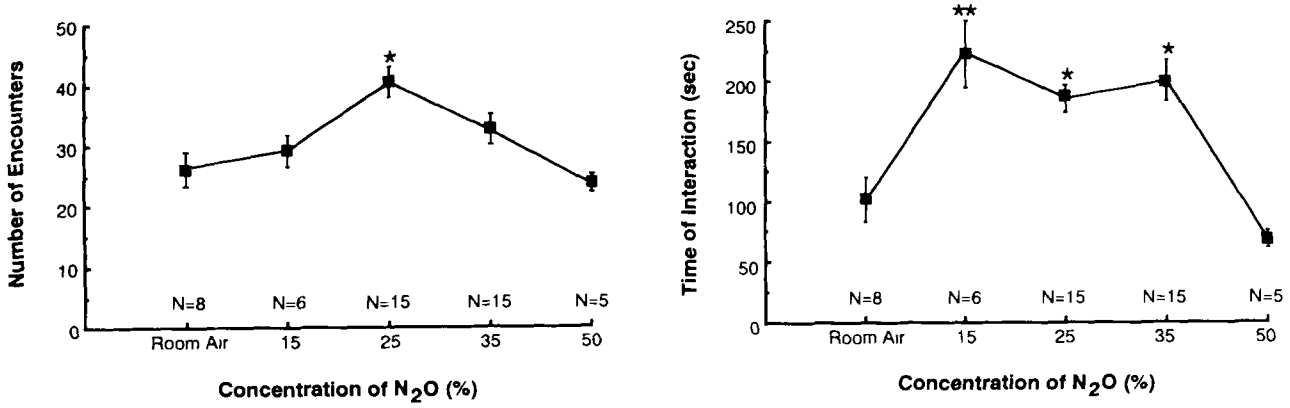

FIG. 2. The influence of increasing concentrations of nitrous oxide on the number of social interaction encounters (left) and the time of active social interaction (right). Each symbol represents the mean and each vertical line the SEM. The N of each group is shown above the x-axis. Significance of difference. $*p < 0.05$, compared to room air control (ANOVA and Dunnett's t-test).

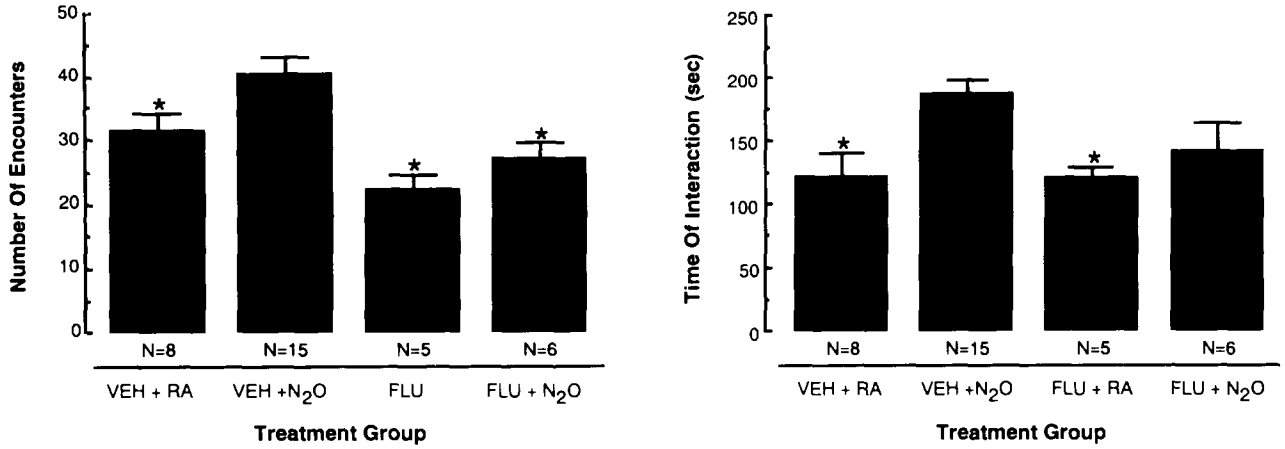

FIG. 3. The interaction between flumazenil (10 mg/kg) and nitrous oxide (25%) on the number of social interaction encounters (left) and the time of active social interaction (right). Each symbol represents the mean and each vertical line the SEM. The *N* of each group is shown above the x-axis. Abbreviations: VEH, vehicle; RA, room air; FLU, flumazenil; N_2O, nitrous oxide. Significance of difference: $*p < 0.05$, $**p < 0.01$, compared to Tween-80 plus 25% nitrous oxide group (ANOVA and Duncan's multiple-range test).

The social interaction of rat pairs following treatment with chlordiazepoxide or vehicle was studied in the same apparatus but with the lid left off and open to room air. Under these conditions, the temperature inside the test chamber generally remained the same as ambient room temperature (24 ± 1°C).

Thus, each rat was paired with an unacquainted rat in an unfamiliar and highly illuminated environment. These conditions are maximally anxiogenic and produce lower levels of active social interactive behavior. The activities of each rat pair were videotaped for 10 min, then the apparatus was flushed with compressed air, the lid to the test chamber was removed, and the rats were returned to their cages. The videotapes were later replayed and the number of social interaction encounters and the time of active social interaction (duration in s) were recorded by an observer (who was unaware of the drug treatment) using a hand-held tally counter and a stopwatch, respectively. Multiple observers were employed and their findings were averaged to provide mean numbers of social interaction encounters and times of active social interaction.

Exposure to Nitrous Oxide

Test gases were delivered at an inflow rate of 10 l/min into the acclimating cylinders containing the rats using a portable dental sedation system (Porter, Hatfield, PA). Control rat pairs in the nitrous oxide experiments were exposed to compressed air delivered into the acclimating cylinders at an identical inflow rate of 10 l/min. Appropriate probes were

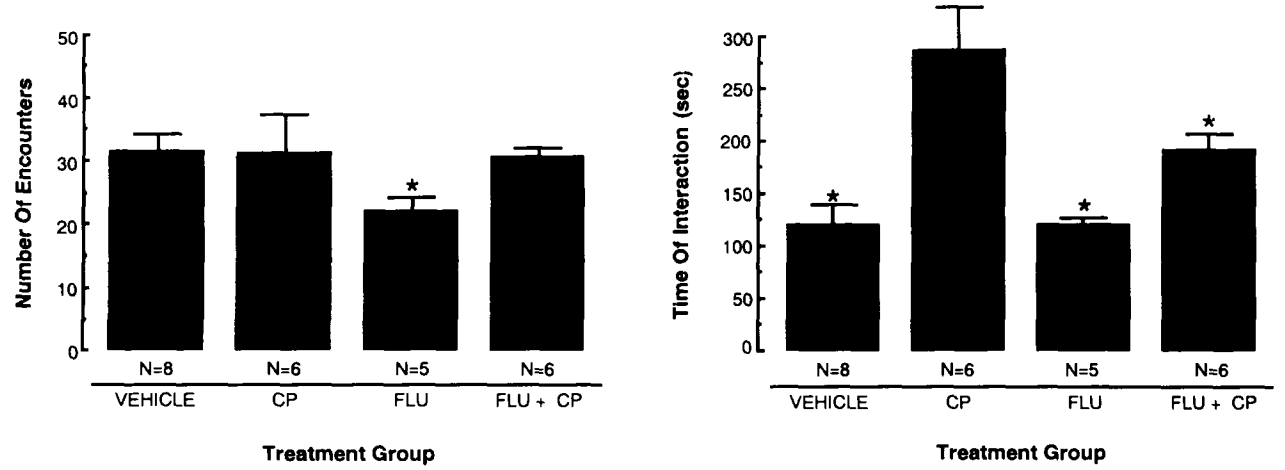

FIG. 4. The interaction of flumazenil (10 mg/kg) and chlordiazepoxide (5.0 mg/kg) on the number of social interaction encounters (left) and the time of active social interaction (right). Each symbol represents the mean and each vertical line the SEM. The *N* of each group is shown above the x-axis. Abbreviations: CP, chlordiazepoxide; FLU, flumazenil. Significance of difference: $*p < 0.05$, $**p < 0.01$, compared to 5.0 mg/kg chlordiazepoxide group (ANOVA and Duncan's multiple-range test).

threaded through the outflow tubing into the test chamber for monitoring of the test gas atmosphere and temperature inside the test chamber using a POET II anesthetic monitoring system (Criticare Systems, Milwaukee, WI) and a telethermometer (Yellow Springs Instruments, Yellow Springs, OH), respectively.

Drugs

Drugs used in this research included Nitrous Oxide, U.S.P., Oxygen, U.S.P. and Compressed Air, U.S.P. (Rockford Industrial Welding Supply, Rockford, IL), chlordiazepoxide hydrochloride (Sigma, St. Louis, MO) and flumazenil (Roche, Nutley, NJ). Gases were delivered into the test chamber as described above. Chlordiazepoxide was prepared in 0.9% physiological saline and administered IP 30 min prior to testing. Flumazenil was suspended in 0.3% Tween 80 solution and administered SC 30 min prior to testing. Doses of chlordiazepoxide and flumazenil were determined in preliminary experiments and were consistent with doses reported for rats in the literature. Control rat pairs in the chlordiazepoxide experiments were treated SC with vehicle. All drugs were delivered in an injection volume of 0.1 ml per 100 g body weight.

In the chlordiazepoxide and attendant control experiments, the lid to the test chamber was left off and the test was conducted in room air, as opposed to compressed air delivered into the closed chamber in the controls for the nitrous oxide experiments.

Statistical Analysis of Data

Findings were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test or Dunnett's *t*-test, two-tailed.

RESULTS

Increasing concentrations of nitrous oxide initially increased then decreased the amount of social interaction between rats (Fig. 2). Compared to behavior of vehicle-pretreated, room air-exposed rats, rat pairs exposed to nitrous oxide showed basically an inverted U-shaped dose-response curve with the maximum increase in social interaction encounters occurring at 25% and significantly increased times of social interaction at 15–35%. Fifty percent nitrous oxide produced significant sedation, and 70% caused profound sedation (not shown) that reduced both the number of social interaction encounters and the time of active social interaction.

Based on the concentration-response relationship, a standard challenge of 25% nitrous oxide was adopted in the social interaction test. Exposure to nitrous oxide significantly increased both the number of social interaction encounters and the time of active social interaction. Pretreatment with 10 mg/kg flumazenil, which alone had no effect on social interaction, resulted in antagonism of the nitrous oxide effect. The number of social interaction encounters produced by nitrous oxide was returned to baseline, while the time of active social interaction was reduced, though this decrease was not statistically significant (Fig. 3).

Treatment with 5.0 mg/kg chlordiazepoxide significantly increased the time of active social interaction, though not the number of social interaction encounters. Chlordiazepoxide at 10 mg/kg produced sedation, which lowered both the number of social interaction encounters and the time of active social interaction (data not shown). Pretreatment with 10 mg/kg flumazenil antagonized the increase in time of active social interaction seen in rat pairs treated with 5.0 mg/kg chlordiazepoxide (Fig. 4).

DISCUSSION

Nitrous oxide has long been known to produce significant effects on behavior (3,5,19–21), and this behavioral effect has been clinically efficacious in alleviation of patient anxiety (1,2,4,16,22). Though the anesthetic and analgesic effects of nitrous oxide have been the subject of extensive study, the mechanism of its anxiolytic effect remains uncertain. To our knowledge, our laboratories were the first to demonstrate benzodiazepine-like behavioral effects of nitrous oxide in mice and antagonism of nitrous oxide effects by a benzodiazepine receptor blocker (6,8,15,17).

The rat social interaction test was used to study nitrous oxide in the present investigation. Active social interaction between rats is greatest if the rats are familiar to each other and the environment is likewise familiar and dimly illuminated. On the other hand, if the rats are unacquainted with one another, if the animals are unfamiliar with their surroundings, and if the level of illumination is high, the amount of social interaction between the rats is greatly diminished as a result of uncertainty, which is a natural stimulus for anxiety. The ability of benzodiazepines to increase social interaction in an unfavorable environment has been accepted as a screen for anxiolytic drug activity (10). The social interaction test has been validated physiologically and pharmacologically, as well as behaviorally, and is widely used (9,11).

Results of the present study show that exposure to 25% nitrous oxide significantly increased both the number of social interaction encounters and the time of active social interaction. This is comparable to the pattern of behavioral response expected of benzodiazepines in this paradigm. Unlike the effects of nitrous oxide in the staircase test (17,18), this effect did not require naloxone pretreatment for expression. It is speculated that the anxiolytic effect of nitrous oxide in the mouse staircase paradigm may be masked by its opioid receptor-mediated locomotor stimulatory effect (12–14). Since rats become sedated rather than stimulated when exposed to higher concentrations of nitrous oxide, there was possibly no locomotor hyperactivity to mask the anxiolytic activity of the drug, thus eliminating the need for naloxone. The effects of nitrous oxide and chlordiazepoxide in the social interaction test were both reduced by flumazenil pretreatment. These results, therefore, support the hypothesis that nitrous oxide produces an anxiolytic effect that is mediated by benzodiazepine receptors.

It is also of interest to note that the social interaction behavior of compressed air-exposed animals (from the nitrous oxide experiment) and vehicle (saline)-treated, room air-exposed animals (from the chlordiazepoxide experiment) was comparable. Since the former group was exposed to the same 1.5°C increase within the test chamber as were nitrous oxide-exposed animals and the latter group was tested at room temperature, it can be conjectured that the elevated environmental temperature failed to influence social interaction behavior and that the behavioral effects of nitrous oxide were independent of the increased temperature within the test chamber.

ACKNOWLEDGEMENTS

This research was supported by NIH grant DE-09378. Flumazenil was a generous gift of Roche Laboratory (Nutley, NJ). We are grateful to Betsy Ellenberger, Janet Mueller, David O'Leary, and Beverly Reynolds for acting as observers in reviewing tapes.

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