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Anxiolytic-like action in mice treated with nitrous oxide and oral triazolam or diazepam

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Abstract

Few animal studies have explored the interaction of nitrous oxide (N_2O) with a benzodiazepine (BNZ) administered by the oral route, as used in clinical procedures involving "conscious sedation". The purpose of this study was to evaluate the relative "anxiolytic-like" and sedative effectiveness of N_2O, oral triazolam (TRIAZ; Halcion®) or oral diazepam (DIAZ; Valium®), either alone or in various combinations of drugs and doses. One hundred and twelve Swiss Webster male mice, 35–45 days old, were assigned to 28 groups, each of which contained four mice. The mouse staircase test was used for the assessment of anxiety (number of rearings) and sedation (number of steps ascended). Three doses of oral TRIAZ (0.1, 0.3, 1.0 mg/kg) or DIAZ (2.0, 3.5, 5.0 mg/kg) were given in combination with room air, or N_2O/O_2 at a N_2O concentration of 25, 50 or 75%. Each mouse was tested once. N_2O alone did not reduce NR in any concentration, but caused a significant increase in locomotion. DIAZ without N_2O reduced NR only with the middle and high doses, but the addition of N_2O significantly enhanced the anxiolytic-like effect of all DIAZ doses. TRIAZ, alone, reduced NR only in the highest dose, but added N_2O resulted in anxiolytic-like behavior with all three TRIAZ doses. The sedative effects of the BNZs were extremely variable. Only the middle dose of DIAZ plus 25% N_2O unequivocally reduced the number of steps ascended, i.e., caused sedation. TRIAZ lacked the inverted U-shaped dose-response relationship with NR usually seen with DIAZ. TRIAZ, therefore, provides better dose

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control. This behavioral animal model indicates that the optimal combinations for reduction of anxiety-like behavior with minimal effects on sedation are 0.1 mg/kg oral TRIAZ with 25% N_2O or 2.0 mg/kg oral DIAZ with 25% N_2O.

Keywords: Nitrous oxide; Diazepam; Triazolam; Benzodiazepines; Conscious sedation; Sedation; Behavioral; Anxiolytic; Anxiolytic-like; Mice

Introduction

Conscious sedation with combinations of nitrous oxide (N_2O) and a benzodiazepine (BNZ) is a common clinical procedure, especially in pediatric medicine and dentistry. Many pediatric dentists use some form of conscious sedation for young children in their office practice (Houpt, 2002). The main clinical objective of conscious sedation is to reduce anxiety while maintaining vital reflexes in order accomplish a medical or dental treatment (Amer. Acad. Ped., 1992). An animal behavioral model referred to as the "staircase" paradigm has been validated for screening potential BNZs for conscious sedation (Simiand et al., 1984; Steru et al., 1987; Quock et al., 1992). Triazolam (TRIAZ) has not been evaluated for its anxiolytic-like effect in laboratory experiments, either alone, or in combination with N_2O. The present study was performed in mice using the staircase test to establish whether N_2O plus oral TRIAZ, would be an effective combination for reducing anxiety-related behavior.

Methods and materials

Animals

Swiss Webster male mice (Charles River Laboratory) approximately 4–5 weeks old and weighing 16–18 g were housed at the UMDNJ Animal Laboratory Facility in groups of 6 under standard conditions of temperature, lighting, and ventilation, with food and water freely available. The day before testing, groups of mice were put into separate cages in the testing laboratory for acclimatization. Three hours before testing they were denied food and water.

The mice were 35–45 days old when tested, approximately at puberty, a time associated with increased anxiety in novel situations. One hundred and twelve animals were randomly assigned to one of 28 experimental groups, each of which contained four mice. Each mouse was tested only once. The 24 groups were used for 3 doses of TRIAZ and 3 doses of DIAZ each of which was administered together with room air or 1 of 3 doses of nitrous oxide. Four groups that received only room air or nitrous oxide were used as controls.

Equipment

A clear plexiglass chamber (100L vol.) was fitted with inflow (top) and outflow (bottom) tubes to accept N_2O and O_2 from a McKesson analgesia machine. The outflow tube was exhausted into a gas

hood. Investigator access to the chamber was through a gas-tight neoprene accordion sleeve and glove. The chamber contained the staircase, individual holding cages for the mice, and a thermistor probe. The temperature was maintained at 27 ± 0.5 C. Fifteen minutes prior to introducing the mice into the chamber, it was filled with the appropriate gas mixture at a flow rate of 10L/min (see also Drugs in Methods).

An opaque, white plastic staircase was fabricated to the specifications of Simiand (Simiand et al., 1984), which were modifications of the original developed by Thiebot (Thiebot et al., 1973). A mouse was placed singly on the floor of the chamber with its back to the staircase. The number of steps climbed or ascended (NSA) and the number of rears (NR) the mouse made over a three-minute period was recorded with a dual blood cell counter. A step was counted only if the mouse placed all four paws on the step. Steps descended were ignored. A rearing occurred when the mouse rose on its hind legs with its nose in the air for exploration. After each animal was tested, the staircase was cleaned with distilled water to eliminate any olfactory cues.

Drugs

TRIAZ and DIAZ (Sigma, St. Louis, Mo.) were suspended in a 2% aqueous suspension of methyl cellulose. They were delivered directly to the stomach through a 1 in, 22 gauge straight epigastric feeding needle with a 1.25 mm balled tip. A standard 0.l ml/kg volume was delivered using a 1.0 ml disposable syringe. Drug dosages were: DIAZ- 2.0, 3.5, 5.0 mg/kg; TRIAZ- 0.1, 0.3, 1.0 mg/kg; N_2O- 25, 50 and 75%, with O_2 as the remainder. Room air was used as the control, since 100% O_2 would have been toxic. Mice received the BNZ doses 30 minutes before being placed in a holding box embedded in the experimental chamber. The chamber was equilibrated with N_2O or room air for 15 minutes before the treated mice were placed in the chamber. The mice remained in the gas phases for 15 minutes before they were tested on the staircase. Therefore, the tests were made 45 minutes after a BNZ dose and 15 minutes after breathing the gas phases. The gas mixture had flowed into the chamber for 30 minutes before the mice were tested.

Experimental design

Vials containing the drugs were coded. The design was a randomized design in which the administrator of the coded treatments, as well as the observer of treatment effects, was one person who was blinded as to the BNZ treatments. To avoid any systematic bias between testing sessions, one treatment from each of the 7 major treatment groups was assigned at random for each session utilizing 7 × 7 Latin squares (Fisher and Yates, 1949). Twenty-eight treatments were each replicated 4 times resulting in 112 observations. The protocol was approved by the Institutional Animal Care and Use Committee of the UMDNJ-New Jersey Medical School.

Statistical analysis

The NR and NSA were counted for a standard three-minute period and subjected to a oneway ANOVA with Bartlett's test for variance homogeneity (STATA computer program); and Dunnett's two-tailed, post-hoc test contrasting the single control (air plus placebo) with the 27 remaining treatments (Dunnett, 1964). For comparisons within any drug group, oneway ANOVA was used with

Bonferroni's two-tailed post-hoc test. The 95% level of significance ($\alpha = 0.05$) was used for all statistical decisions.

Results

Table 1 presents a statistical summary of the NR and NSA data. A reduction in anxiety-linked behavior was indicated by a reduction in NR from the appropriate control. Sedation was evaluated by NSA, which reflects locomotion capacity. A oneway ANOVA of NR or NSA showed significant differences between treatment groups ($F = 10.84$, $P < 0.0001$, and $F = 3.46$, $P < 0.0001$, respectively. Bartlett's test was significant for heterogeneous variances for NR and NSA, (X^2 (27) = 52.10, $P = 0.003$ and 56.90, $P = 0.001$, respectively). No standard data transformation procedure could correct the heteroscedasticity, precluding the use of standard post-hoc contrasts among all treatments. Since a

Table 1

Drug	% N_2O	Number of Rears (NR) (*Anxiety*)*		Number of Steps Ascended (NSA) (*Sedation*)**	
		Mean ± SEM		Mean ± SEM	
Control	0	27.0	1.8	24.0	1.1
	25	30.8	0.9	39.0	1.2
	50	28.3	2.0	39.5	2.1
	75	35.8	2.7	55.0	4.2
Diazepam 2.0 mg/kg	0	21.0	1.6	40.5	5.5
	25	10.5	0.5	28.5	2.9
	50	7.8	1.3	22.8	1.3
	75	16.5	2.2	57.3	12.1
Diazepam 3.5 mg/kg	0	11.3	1.7	41.3	7.2
	25	0.5	0.3	7.0	4.4
	50	6.8	2.5	20.3	9.1
	75	9.8	2.5	68.3	14.0
Diazepam 5.0 mg/kg	0	19.8	1.5	46.0	7.4
	25	6.8	5.4	18.0	11.5
	50	2.5	2.2	16.0	11.2
	75	6.8	2.5	44.3	18.2
Triazolam 0.1 mg/kg	0	24.3	2.4	34.8	3.6
	25	5.3	2.2	17.0	6.5
	50	17.8	4.7	28.0	3.1
	75	6.3	1.0	50.8	5.2
Triazolam 0.3 mg/kg	0	13.0	6.4	23.5	5.3
	25	2.3	1.7	9.3	5.7
	50	13.3	5.6	51.5	11.0
	75	9.0	3.5	44.0	12.4
Triazolam 1.0 mg/kg	0	7.0	1.3	19.8	8.5
	25	8.0	2.8	26.8	9.8
	50	4.0	2.3	22.0	10.3
	75	7.8	2.8	29.0	5.2

* Greater number of rears (NR) indicates increased anxiety-like behavior.

** Lesser number of steps ascended (NSA) indicates increased sedation.

principal objective of the study was to determine if any of the treatments were superior to the control treatment (air plus vehicle), Dunnett's two-tailed t test with expanded number of groups was used. Graphic results are in Figs. 1 and 2.

Anxiolytic-like effect; nitrous oxide

N_2O/O_2 did not reduce NR at any of the three concentrations when compared to air alone (Fig. 1). The highest concentration, however, actually had a significant anxiogenic-like effect, with a 33% increase in NR over air control (oneway Anova with Bonferroni's test; $p < 0.05$).

Anxiolytic-like effect; diazepam

In the absence of N_2O, i.e., in air, DIAZ reduced NR at the middle and high doses (Fig. 1). The addition of the 3 concentrations of N_2O to the low DIAZ dose unmasked an anxiolytic-like effect of the combinations (one-way Anova with Bonferroni's test; $p < 0.05$). It is noteworthy that the low dose of DIAZ overcame the increase in NR seen with 75% N_2O given alone. The two higher doses of DIAZ, which reduced NR by themselves, exhibited greater reduction in NR when combined with any of the three concentrations of N_2O (oneway Anova with Bonferroni's test; $p < 0.05$ with both doses). The greatest anxiolytic-like effect of DIAZ, numerically, occurred with the middle dose (3.5 mg/kg) plus 25% N_2O. However, this level of NR reduction is not significantly different from that obtained with the high dose of DIAZ plus 50% N_2O (oneway Anova with Bonferroni's test; $p > 0.05$). Thus, maximum anxiolytic-like activity from DIAZ was obtained with 3.5 mg/kg DIAZ plus 25% nitrous oxide, or 5.0

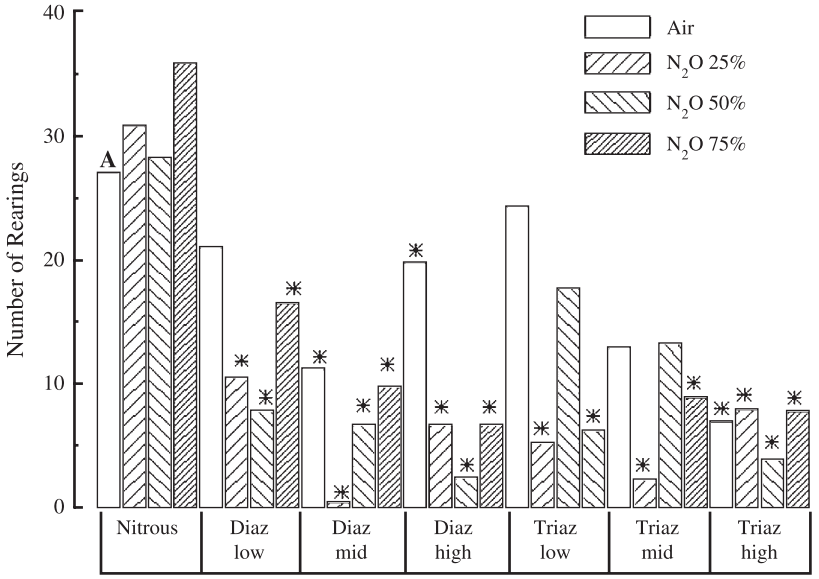

Fig. 1. The bars represent the mean rearings for each treatment (n = 4 per treatment). (Detailed summary statistics are given in Table 1). Diaz low, Triaz mid, etc., represent treatment groups receiving the various doses of diazepam and triazolam, respectively. * Indicates treatments that were statistically different when compared to AIR-VEHICLE control (A), ($P<0.05$, Dunnett's test; two-tailed).

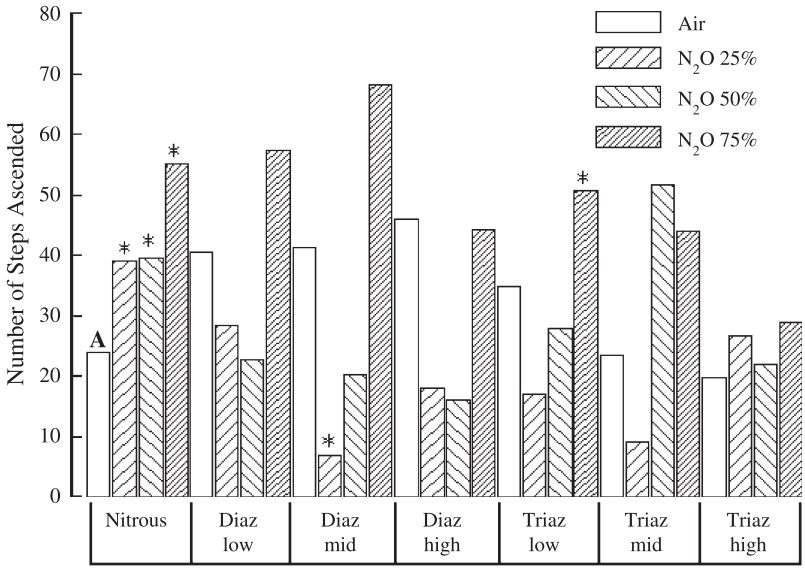

Fig. 2. The bars represent the mean number of steps ascended for each treatment (n = 4 per treatment). (Detailed summary statistics are given in Table 1). Diaz low, Triaz mid, etc., represent treatment groups receiving the various doses of diazepam and triazolam, respectively. * Indicates treatments that were statistically different when compared to AIR-VEHICLE control (A), ($P<0.05$, Dunnett's test; two-tailed).

mg/kg DIAZ plus 50% N_2O. From the perspective of safety alone, i.e., minimum effective doses, the 3.5 mg doses of DIAZ with 25% N_2O would be preferable between the two treatments. But as will be seen in the sedation results, this choice will be modified.

Anxiolytic-like effect; triazolam

TRIAZ, without N_2O, reduced NR only at the highest dose, causing a 74% reduction in NR (Fig. 1). N_2O did not further enhance this effect (oneway Anova with Bonferroni's test; $p > 0.05$), most likely because the high dose of TRIAZ alone was already causing close to a ceiling effect. The addition of 25% N_2O to the low and middle doses evoked a significant decrease in NR, but paradoxically, these two doses did not lower the NR when combined with 50% N_2O. The numerically greatest NR reduction occurred with 25% N_2O plus the middle dose of TRIAZ.

Sedation; nitrous oxide

N2O/ O_2, alone in all concentrations, did not reduce NSA. But the NSA increased by 63 to 129% above the control (Fig. 2). This phenomenon of increased motor activity has been previously reported (Hynes and Berkowitz, 1979; Pruhs and Quock, 1989).

Sedation; diazepam

DIAZ had no consistent effect on locomotion by itself (Fig. 2). The only significant reduction in NSA occurred when the middle dose was combined with 25% N_2O. This sedative effect detracts from the

excellent anxiolytic-like effect of that combination reported above. Therefore, the lowest dose of DIAZ with 25% N_2O provides the optimum combination.

Sedation; triazolam

TRIAZ had no effect on locomotion when given alone in any dose, nor did N_2O produce any statistically significant change in locomotion except at a 75% concentration with the low dose of TRIAZ, where N_2O increased motor activity similar to 75% N_2O alone (Fig. 2).

Discussion

The present investigation successfully detected the anxiolytic-like effects of DIAZ and TRIAZ in mice when given orally with specific concentrations of N_2O. There are two unique features in this investigation using this type of animal model: (1) this was the first study of TRIAZ in this paradigm; and (2) the first examination of the effectiveness of these two BNZs when given by mouth. Clinically, the oral route would be more acceptable to patients, especially in pediatrics. A primary objective was to establish a minimum dose combination of N_2O and oral BNZ which produces an anxiolytic-like effect without causing motor impairment. This was accomplished optimally with either 0.1 mg/kg TRIAZ and 25% N_2O, or 2.0 mg/kg DIAZ with 25% N_2O.

N_2O, by itself, had no anxiolytic-like effect at any concentration. Conversely, it had an anxiogenic-like action at the 75% concentration, i.e., increased NR. This finding differs from that of Quock et al., 1992, who used a different strain of mouse and found no effect of 75% N_2O on NR. This anxiogenic-like action is most likely caused by the emergence of an excitement stage of "anesthesia", since it is accompanied by and large increase in locomotion. A significant increase in NSA (increased locomotor activity) is seen at three N_2O concentrations. It is noteworthy that all doses of DIAZ or TRIAZ overcame the anxiogenic-like effect of 75% N_2O. There is intriguing evidence that N_2O has a potential anxiolytic-like effect mediated through the BNZ receptors. But the effect is masked if opiate receptors are open. When the opiate receptors are blocked, a substantial N_2O anxiolytic-like behavior is seen (Quock et al., 1987, 1992).

TRIAZ given orally without N_2O reduces NR only at the highest dose. But N_2O generally enhanced its anxiolytic-like profile. DIAZ, given alone, reduces NR only at the upper two doses. N_2O enhanced all DIAZ doses, even the lowest dose.

Comments

The administration of two or more drugs for the purpose of achieving an enhanced effect poses a number of important pharmacological problems. The drugs must possess pharmacodynamic actions that are capable of cooperative actions, and must have pharmacokinetic properties that allow the expression of this cooperation. Experimental behavioral evidence, however, indicates that some BNZs, such as DIAZ, have an inverted U-shaped dose-response curve which complicates the dose-response pharmacology (Steru et al., 1987). In the present study, TRIAZ alone does not appear to have this unusual dose-response relationship. The generation of active and long-lasting metabolites of DIAZ adds a significant pharmacokinetic element to its use, whereas TRIAZ has no active metabolite. The

interrelationships between the BNZ receptor and opiate receptor further complicates the picture, as does the fact that N_2O also has more than one dose-dependent action, including analgesia. The behavioral outcome of the joint administration of N_2O and a BNZ, both having narrow operational windows, is, therefore, not easily predictable.

A clinical evaluation in dental patients of orally administered TRIAZ, given without N_2O, was found to be superior to DIAZ in reducing anxiety, but caused a greater impairment of cognitive function (Ehrich et al., 1997). An earlier clinical study in adults subjected to surgical removal of impacted third molars, oral TRIAZ, given alone, was anxiolytic. The addition of 40% N_2O, however, did not improve the anxiolysis, probably because the TRIAZ, itself, was giving ceiling effects as in our animal study (Kaufman et al., 1993).

Conclusions

The mouse staircase model for detection of anxiolytic-like effects of pharmacological agents provides evidence for the increased effectiveness of conscious sedation with certain combinations of N_2O and orally delivered BNZs. The doses of the BNZs and the concentrations of N_2O are critical. The dosage windows for both drug classes are narrow.

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