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Behavioral Effects of the Putative Anxiolytic (±)-1-(2,5-Dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2) in Rats and Mice

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SCORZA, M. C., M. REYES-PARADA, R. SILVEIRA, H. VIOLA, J. H. MEDINA, M. B. VIANA, H. ZANGROSSI JR. AND F. G. GRAEFF. *Behavioral effects of the putative anxiolytic (±)-1-(2,5-Dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2) in rats and mice.* PHARMACOL BIOCHEM BEHAV **54**(2) 355–361, 1996.—Behavioral effects of the phenethylamine derivative (±)-1-(2,5-dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2) were studied in mice and rats. Murine locomotor activity, measured with a photocell actometer, was markedly depressed following IP injection of 2 and 6 mg/kg of the drug. The same doses of the drug also decreased frequency and duration of head dipping and the number of rearings in the hole board apparatus. In the murine elevated plus maze 2 and 6 mg/kg of ALEPH-2 increased the percentage of both open arm entries and time. The total number of entries into the enclosed arms was not significantly affected by the drug. In the rat, 2–12 mg/kg ALEPH-2, IP, decreased photobeam counts in the actometer in a dose-dependent fashion. Both 2 and 4 mg/kg of the drug increased the percentage of open arm entries, but only the highest dose significantly increased the percentage of time spent on the open arms. The dose of 4 mg/kg ALEPH-2 also significantly decreased the total number of enclosed arm entries. Finally, in a recently developed model of anxiety and memory, the elevated T-maze, the doses of 2 and 4 mg/kg ALEPH-2 did not change inhibitory avoidance of the open arms. Nevertheless, the highest dose had an amnestic effect on this task, repeated 72 h later in the absence of drug. In addition, this dose significantly increased the latency to escape from the open arms and had an amnestic effect measured 72 h later. Overall, these results indicate that ALEPH-2 possesses anxiolytic, amnestic as well as sedative and/or motor depressant actions.

ALEPH-2 Locomotor activity Hole board Elevated plus maze Elevated T-maze Rat Mouse Anxiolysis Amnesia Sedation

MOST of the benzodiazepines currently used in the treatment of anxiety have undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbances, and interaction with alcohol. Therefore, there exists great interest in the search for new anxiolytic agents with a mechanism of action other than that of the classical benzodiazepines Thus far, the most likely therapeutic alternatives seem to be drugs that act upon the serotonergic system (1). Thus, serotonin 5-HT_{1A} receptor agonists (4), 5-HT_2 (14), and 5-HT_3 (2) antagonists, have shown anxiolytic effects in several behavioral models of anxiety and, in some cases, in human clinical trials (5,9).

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It is well known that drugs with the phenethylamine skeleton affect monoaminergic neurotransmission in many ways (16). Concerning 5-HT, substituted amphetamines and phenethylamines are potent and selective 5-$HT_{2A/2C}$ ligands (10) or 5-HT uptake blockers (17). Some rigid analogs such as aminotetralins are potent and selective 5-HT_{1A} ligands (13), and several phenethylamine derivatives have monoamine oxidase inhibitory properties (8,20).

In the present study we report several behavioral effects of one phenethylamine derivative, (±)-1-(2,5-dimethoxy-4-ethylthiophenyl)-2-aminopropane hydrochloride (ALEPH-2), which is structurally related to serotonergic agents like 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). The behavioral tests used were: two photobeam apparatuses for measuring locomotor activity in rats and mice, respectively; a hole board for assessing directed exploration in mice; the elevated plus maze, a widely used animal model of anxiety (12, 15,18) in both rats and mice; and a newly developed model that allows measurement of two types of fear as well as memory in the same rat, named the elevated T-maze (11,22).

EXPERIMENT 1

This experiment was performed in mice and measured the effect of ALEPH-2 on locomotor activity in an actometer and on directed exploration in the hole board.

Method

Subjects and housing. Male CF1 mice weighing 25–35 g were used. Animals were housed in groups of 10–12 under a 12 L : 12 D cycle (lights on at 0800 and off at 2000 h) at 22 ± 1°C, 55% humidity, with free access to food and water. Only naive animals were used.

Apparatus. The apparatus used to measure locomotor activity consisted of a glass box of 36 × 15 × 20 cm (OPTO-VARIMEX, Sweden) and two lateral bars with 13 light beams (0.32 cm diameter; beam spacing 7.65 cm). The experimental device employed to carry out the test of the hole board in mice has been fully described recently (23). Briefly, the hole board consisted of a wooden box (60 × 60 × 30 cm) with four equidistant holes of 2-cm diameter in the floor.

Drug and injection. ALEPH-2 was synthesized following a previously reported procedure (21). The drug was dissolved in saline for IP injection, the solutions being prepared on the same day of the experiments. The volume injected was 5 ml/kg body weight.

Procedure. To measure locomotor activity, mice were injected with either ALEPH-2 (2 and 6 mg/kg) or saline, and 10 min later placed inside the actometer. Locomotor activity was measured during 5 min.

For the hole board experiment, another group of mice was injected with either ALEPH-2 (2 mg/kg) or saline. Twenty minutes later each animal was placed in a corner of the hole board and allowed to freely explore the apparatus for 5 min. The number of head dips, time spent head dipping, and the number of rearings were recorded.

Data analysis. For locomotor activity, the number of photobeam interruptions (counts) were submitted to analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison test. The measurements in the hole board test were analyzed by the unpaired *t*-test of Student. In all cases significance level was considered to be $p < 0.05$.

Results

Table 1 shows that ALEPH-2 markedly decreased murine locomotor activity. One-way ANOVA showed a significant effect of drug on the number of transitions through the beams, $F(2, 21) = 29.81$, $p < 0.001$. Post hoc comparisons performed by the Student–Newman–Keuls test showed that both doses of 2 and 6 mg/kg significantly decreased ($p < 0.001$) locomotor activity as compared to control mice.

As illustrated in Table 2, 2 mg/kg of ALEPH-2 significantly decreased the number of head dips, $t(17) = 7.47$, $p < 0.001$, and shortened head dipping time, $t(17) = 6.06$, $p < 0.001$. The number of rearings was also decreased, $t(17) = 5.22$, $p < 0.001$. These changes may reflect a sedative action of ALEPH-2.

EXPERIMENT 2

This experiment measured the effect of ALEPH-2 on the murine elevated plus maze.

Method

Subjects and housing. As in Experiment 1.

Apparatus. The experimental device employed to carry out the test of the elevated plus maze in mice has been fully described recently (23). Briefly, the elevated plus maze was made of wood and consisted of two opposed open arms (20 × 5 cm) perpendicular to two opposed arms of equal dimension that were surrounded by 35 cm high walls. The four arms were linked by a central square of 10 × 10 cm. The whole apparatus was elevated 50 cm from the floor.

Drug and injection. As in Experiment 1.

Procedure. Fifteen minutes after injection of either ALEPH-2 (2 and 6 mg/kg) or saline, each mouse was placed at the center of the elevated plus maze facing one of the enclosed arms and allowed to freely explore the maze for 5 min. Before the next mouse was introduced, the maze was cleaned with a solution of 20% ethanol and dried. The number of entries into open and enclosed arms and the time spent in each arm were evaluated by an observer inside the room.

Data analysis. For each animal, the total number of entries (open + enclosed arms), the percentage of open arm entries (100 × open/total), and the percentage of time spent on the open arms [100 × open/(open + enclosed)] were calculated by a specially designed computer program. The number of entries into the enclosed arms was used as an index of motor activity (3). One-way ANOVA, followed by the multiple comparison test of Newman–Keuls, were used for statistical analysis. The significance level was $p < 0.05$.

TABLE 1

DEPRESSANT EFFECT OF ALEPH-2 ON LOCOMOTOR ACTIVITY OF MICE IN A PHOTOBEAM ACTOMETER

Treatment	Beam Crosses	*n*
Control	747.0 ± 77.5	8
2 mg/kg	262.7 ± 62.2*	7
6 mg/kg	155.0 ± 31.9*	9

Data are expressed as means ± SEM. *Significantly different from control (Student–Newman–Keuls test, $p < 0.001$).

TABLE 2

DEPRESSANT EFFECT OF ALEPH-2 ON HOLEBOARD EXPLORATION OF MICE

Treatment	Head Dips	Time Head Dipping	Rearings	n
Control	10.10 ± 0.93	13.22 ± 1.62	9.54 ± 1.74	6
2 mg/kg	2.60 ± 0.38*	3.00 ± 0.52*	0.40 ± 0.20*	6

Data are expressed as means ± SEM. *Significantly different from control (Student's t-test, $p < 0.001$).

Results

The upper panel of Fig. 1 shows the effect of ALEPH-2 in mice tested in the elevated plus maze. One-way ANOVA showed a significant overall effect of drug on the percentage of open arm entries, $F(2, 23) = 8.80$, $p < 0.01$, and of time spent on the open arms, $F(2, 23) = 5.76$, $p < 0.05$. Post hoc comparisons made with the Newman-Keuls test indicated that the doses of 2 and 6 mg/kg were significantly different from control ($p < 0.01$) for the percentage of open arm entries. The same occurred for the percentage of time (2 mg/kg, $p < 0.01$; 6 mg/kg, $p < 0.05$). The number of enclosed arm entries was not significantly affected by ALEPH-2 (lower panel Fig. 1), $F(2, 23) = 2.44$, $p > 0.05$. Therefore, ALEPH-2 had a selective anxiolytic effect in this test.

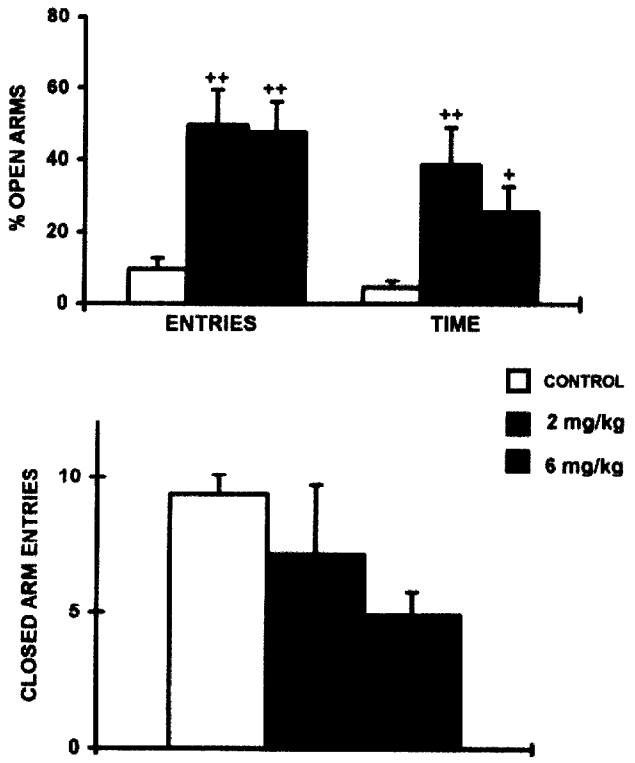

FIG. 1. Anxiolytic effect of ALEPH-2 on exploratory behavior of mice in the elevated plus-maze. Bars represent the mean and vertical lines the SEM. Mice were injected IP with either drug or saline 15 min before the experimental session. $+p < 0.05$, $++p < 0.01$ drug vs. control group. $n = 8$ for control, 7 for 2 mg/kg, and 9 for 6 mg/kg ALEPH-2.

EXPERIMENT 3

This experiment measured the effect of ALEPH-2 on locomotor activity in rats.

Method

Subjects and housing. Male wistar rats weighing 200–240 g were used. Animals were housed in groups of five to six under a 12 L : 12 D cycle (lights on at 0600 and off at 1800 h) at 23 ± 1°C, with free access to food and water. Only naive animals were used.

Drug and injection. As in Experiment 1, except that the volume injected was 1 ml/kg body weight.

Procedure. Immediately after injection of either different doses of ALEPH-2 or saline each animal was placed in an individual plastic cage (60 × 60 × 30 cm) equipped with photobeams. Spontaneous motor behavior was assessed by 20 photosensors placed 4 cm up from the floor of the cage. Sensors are mounted every 4 cm covering the entire area. Locomotor activity, defined as the number of beam crosses, was recorded for a period of 30 min, starting 10 min after injection.

Data analysis. Means ± SEM were calculated for each experimental group. The data were submitted to one-way analysis of variance (ANOVA) followed by the multiple comparison test of Student-Newman-Keuls. The significance level was $p < 0.05$.

Results

As illustrated in Fig. 2, ALEPH-2 caused a dose-dependent decrease in locomotor activity. One-way ANOVA showed a significant overall drug effect, $F(4, 26) = 15.45$, $p < 0.001$. Post hoc comparisons evidenced that the groups treated with 4, 6, and 12 mg/kg ALEPH-2 were significantly different from control ($p < 0.05$).

EXPERIMENT 4

In this experiment, the effect of ALEPH-2 on the rat elevated plus maze was measured.

Method

Subjects and housing. As in Experiment 3.

Apparatus. The elevated plus maze was made of wood, according to the specifications of Pellow et al. (18). The appa-

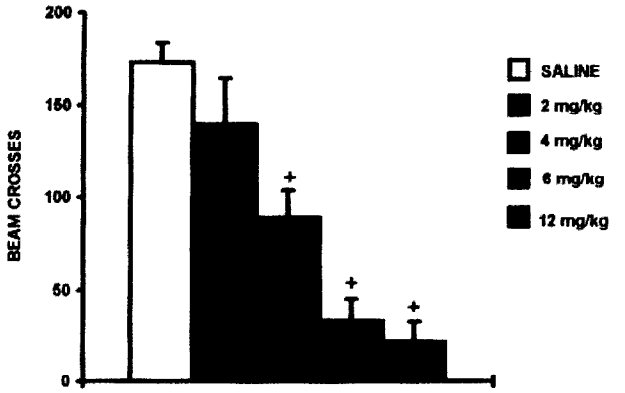

FIG. 2. Depressant effect of ALEPH-2 on locomotor activity of rats in the photobeam actometer. Bars represent the mean and vertical lines the SEM. Rats were injected IP with either drug or saline 10 min before the experimental session. $+p < 0.05$ drug vs. control group. $n = 7$ for control and 2 mg/kg, 6 for 6 mg/kg and 9 mg/kg, and 5 for 12 mg/kg ALEPH-2.

ratus consisted of two opposed open arms measuring 50 × 10 cm, crossed at right angle with two opposed arms of the same size. The latter were enclosed by walls 40 cm high, except for the entrance. The four arms delimited a square central area of 10 cm side. The whole apparatus was elevated 50 cm above the floor. To avoid rats falling down, a rim of Plexiglas 1 cm high was made to surround the open arms. The experimental sessions were recorded by a vertically mounted videocamera, linked to a monitor and VCR in an adjacent room.

Drug and injection. As in Experiment 3.

Procedure. Fifteen minutes after the injection of either ALEPH-2 (2 or 4 mg/kg) or saline, the rat was placed at the center of the elevated plus maze facing one of the enclosed arms and allowed to freely explore the elevated plus-maze for 5 min. Before the next rat was introduced, the maze was cleaned with a solution of 20% ethanol and dried. The number of entries into open and enclosed arms, and the time spent on open and enclosed arms were recorded by typing on a microcomputer keyboard.

Data analysis. As in Experiment 2.

Results

The upper panel of Fig. 3 shows the dose-dependent anxiolytic effect of ALEPH-2 in rats tested in the elevated plus maze. One-way ANOVA evidenced a significant overall effect of drug on the percentage of open arm entries, $F(2, 33) = 12.23$, $p < 0.001$, and on the percentage of time spent on the open arms, $F(2, 33) = 12.74$, $p < 0.001$. Post hoc comparisons made with the Student-Newman-Keuls test indicated that the doses of 2 mg/kg ($p < 0.05$) and 4 mg/kg ($p < 0.001$) were significantly different from control for the percentage of open arm entries. For the percentage of time, this difference was significant only at the dose of 4 mg/kg ($p < 0.001$).

As shown in the lower panel of Fig. 3, the number of enclosed arm entries was significantly affected by ALEPH-2, $F(2, 33) = 5.61$, $p = 0.008$. The Student-Newman-Keuls test showed that the dose of 4 mg/kg significantly ($p < 0.01$) decreased enclosed arm exploration, indicating that this dose already impaired locomotor activity.

EXPERIMENT 5

In this experiment, the effect of ALEPH-2 on the rat elevated T-maze was measured.

Method

Subjects and housing. As in Experiment 4.

Apparatus. The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by 40 cm high walls, was perpendicular to two opposed open arms. To avoid the rats falling down, the open arms were surrounded by a Plexiglas rim 1 cm high. The whole apparatus was elevated 50 cm above the floor. The experiments were recorded by an observer inside the room.

Drug and injection. Same as in Experiment 4.

Procedure. On the third and fourth days after their arrival in the laboratory animals were gently handled for 5 min. On

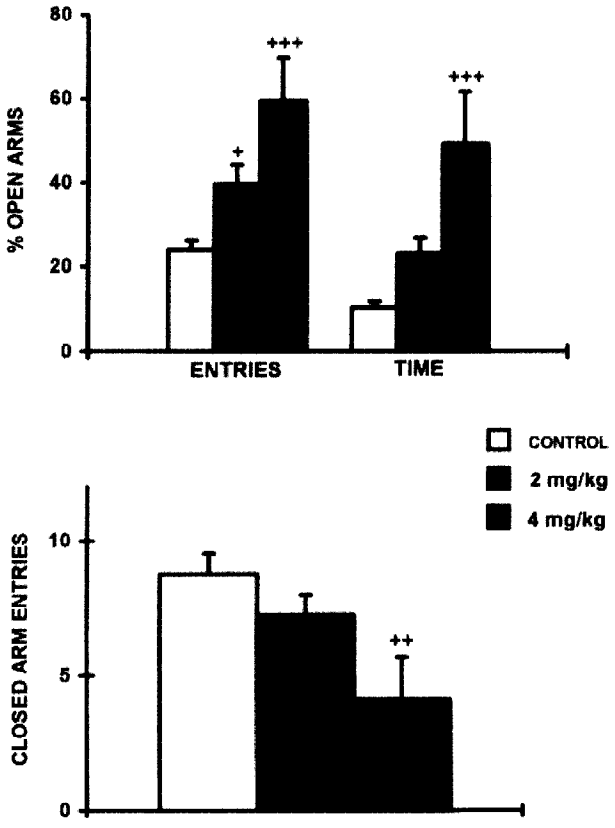

FIG. 3. Anxiolytic effect of ALEPH-2 on exploratory behavior of rats in the elevated plus maze. Bars represent the mean and vertical lines the SEM. Rats were injected IP with either drug or saline 15 min before the experimental session. $+p < 0.05$, $++p < 0.01$, $+++p < 0.001$ drug vs. control group. $n = 17$ for control, 9 for 2 mg/kg, and 8 for 4 mg/kg ALEPH-2.

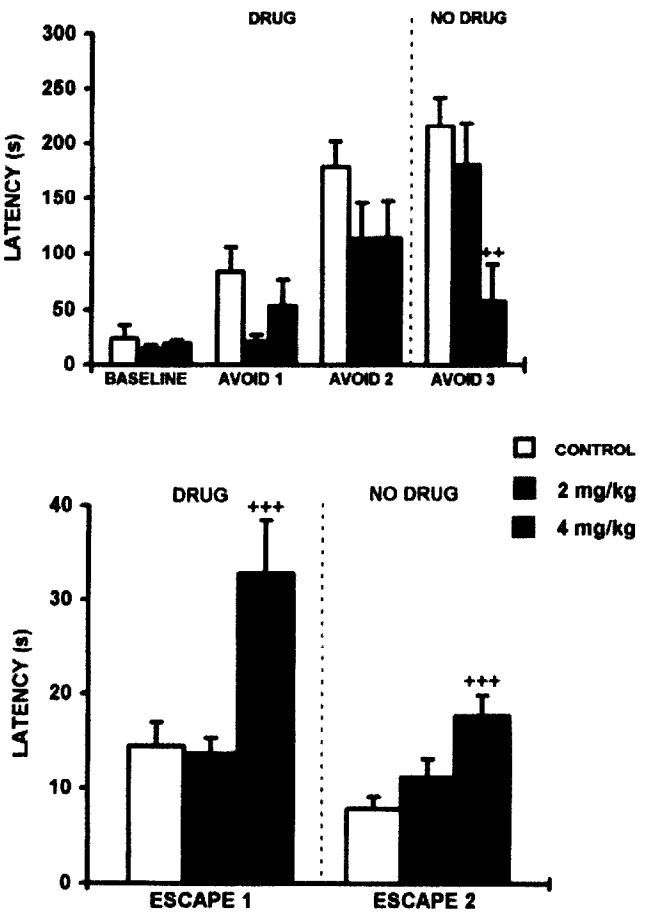

FIG. 4. Anxiolytic and amnestic effects of ALEPH-2 in rats tested in the elevated T-maze. Bars represent the mean and vertical lines the SEM. Rats were injected IP with either drug or saline 15 min before the experimental session. $++p < 0.01$, $+++p < 0.001$ drug vs. control group. $n = 25$ for control, 13 for 2 mg/kg, and 12 for 4 mg/kg ALEPH-2.

the fifth day, they were randomly assigned to treatment groups, and given either 2 or 4 mg/kg ALEPH-2 or control saline injection. After 15 min, each rat was placed at the end of the enclosed arm of the T-maze and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). Next, the same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2) at 30-s intervals. Following avoidance training (30 s), the rat was placed at the end of right open arm and the time taken to withdraw from the arm with the four paws to enter the enclosed arm was recorded (escape 1). Three days later, avoidance (avoidance 3) and escape (escape 2) latencies were measured again.

Data analysis. Avoidance and escape latencies were analyzed by a two-way between-within ANOVA. Because this ANOVA showed significant drug × trial interactions, one-way ANOVA, followed by the Student-Newman-Keuls test were performed. A p-value of 0.05 or less was required for significance.

Results

The upper panel of Fig. 4 illustrates the effect of ALEPH-2 on inhibitory avoidance in the elevated T-maze. Two-way ANOVA showed significant effects of trial, $F(3, 141) = 24.17, p < 0.001$, and drug, $F(2, 47) = 3.77, p = 0.030$. The drug × trial interaction was also significant, $F(6, 141) = 3.35, p = 0.005$. Further one-way ANOVAs evidenced significant between-group differences, $F(2, 47) = 6.39, p = 0.003$, at avoidance 3 only. Post hoc comparisons showed that the 4 mg/kg group differed significantly from both control ($p < 0.01$) and 2 mg/kg ($p < 0.05$) groups. Therefore, ALEPH-2 neither affected inhibitory avoidance acquisition nor had an anxiolytic effect on this type of (learned) fear. However, the dose of 4 mg/kg of the drug had an amnestic effect in this task.

The lower panel of Fig. 4 shows the effect of ALEPH-2 on one-way escape. A two-way ANOVA showed significant effect of both trial, $F(1, 47) = 22.20, p < 0.001$, and drug, $F(2, 47) = 13.94, p < 0.001$. A significant drug × trial interaction also occurred, $F(2, 47) = 3.22, p = 0.049$. Further one-way ANOVA across treatments at escape 1 showed an overall significance of drug, $F(2, 49) = 9.76, p < 0.001$. Post hoc comparisons evidenced that the group treated with 4 mg/kg significantly differed from both control an 2 mg/kg groups ($p < 0.001$). This may be interpreted as an anxiolytic effect of ALEPH-2 on innate fear.

One-way ANOVA at escape 2 showed a significant effect of treatment, $F(2, 49) = 8.44, p < 0.001$. Multiple comparisons evidenced that the group treated with 4 mg/kg ALEPH-2 differed significantly from control ($p < 0.001$) and 2 mg/kg ($p < 0.05$) groups.

Therefore, this dose has an amnestic effect.

DISCUSSION

The increase in open arm exploration presently caused by ALEPH-2 in the rat elevated plus maze indicates that this drug has anxiolytic properties. This conclusion is partially supported by the present results in the rat elevated T-maze, a recently developed test that is supposed to generate two types of fear in the same animal (11,22). Although inhibitory avoidance was not significantly changed by ALEPH-2, the drug impaired one-way escape in the elevated T-maze. This task is believed to represent innate fear and proved resistant to doses of either diazepam or ipsapirone that markedly impaired inhibitory avoidance (22). Therefore, the profile of ALEPH-2 in the elevated T-maze was opposite to that of diazepam and ipsapirone, suggesting a unique anxiolytic potential for ALEPH-2 that is worth further investigation.

However, the present results additionally show that the dose of ALEPH-2 that had a clear anxiolytic effect in the rat elevated plus maze and in the elevated T-maze (4 mg/kg) also had sedative and/or motor depressant effects. This is indicated by the significant decrease in closed arm entries in the elevated plus maze and by the significant reduction in photobeam crossings recorded in the actometer. In spite of this, a genuine anxiolytic effect of ALEPH-2 in the rat is likely to exist, because one of the indices of decreased anxiety in the elevated plus maze-entrances into the open arm-requires motor activity and, therefore, should be decreased by motor incapacitation. Also, in the elevated T-maze the baseline latency to withdraw from the enclosed arm was not affected by 4 mg/kg ALEPH-2 (upper panel of Fig. 4). Because the same

behavior walking along an alley is displayed in withdrawing from either the enclosed (baseline latency) or the open (escape) arm, this result indicates that motor ability for this task is preserved.

The present results additionally show that 4 mg/kg ALEPH-2 impaired memory of both inhibitory avoidance and one-way escape tasks in the elevated T-maze. In the same test, anxiolytic doses of diazepam had an amnestic effect on inhibitory avoidance, but not on escape (11,22). Therefore, the amnestic action of ALEPH-2 seems to be more pronounced than that of benzodiazepines.

In the murine elevated plus-maze, the doses of 2 and 6 mg/kg of ALEPH-2 significantly increased the percentage of open arm entries and of time spent on this arm, without significantly affecting the number of closed arm entries. This suggests a selective anxiolytic effect of the drug. However, the same doses of ALEPH-2 markedly reduced locomotor activity in the actometer. The discrepancy between the last result and the absence of drug effect on closed arm entries in the murine elevated plus maze is intriguing, because factor analysis studies performed in rats evidenced that number of enclosed arm entries loads heavily and exclusively on a factor related to motor activity (3,8). Furthermore, the present results show that ALEPH-2 decreased head dipping and rearing in the mouse hole board test, an effect that has been interpreted as indicative of sedation (6,19).

In summary, although this study indicates that ALEPH-2 has anxiolytic action, it also reveals potent sedative and amnestic properties of the drug that are manifested at the same range of doses that causes anxiolytic-like effects in animal models of anxiety. In addition, hallucinogenic effects of ALEPH-2 have been reported in healthy volunteers (21). In spite of these limitations, the finding that this type of molecule has anxiolytic effects may be a starting point for the development of a new family of anxiolytics. This can be achieved through systematic modification of the molecular structure of ALEPH-2 aimed at reducing the untoward effects while preserving or intensifying the anxiolytic action. On the other hand, the sedative effects of ALEPH-2 shown by the present results may be of interest regarding the development of new hypnotics.

Concerning the mechanisms underlying the behavioral effects of ALEPH-2, little can be said at present. The close structural similarity of ALEPH-2 with DOI and DOM suggests that changes in 5-HT neurotransmission may be involved. In this regard, doses of ALEPH-2 higher than those used in the present experiments induced behavioral changes characteristic of the serotonergic syndrome, and in binding assays the same compound displayed high affinity for 5-HT_2 receptors (Reyes-Parada and Nichols, unpublished results). However, the participation of other neurotransmitters, such as noradrenaline, GABA, glutamate, and several neuropeptides, cannot be excluded. Further studies are necessary to determine the exact mechanism of each of the behavioral effects anxiolytic, sedative, amnestic, and hallucinogenic caused by ALEPH-2.

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