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# (±)-1-(2,5-Dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2), a novel putative anxiolytic agent lacking affinity for benzodiazepine sites and serotonin-1A receptors

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**Abstract** Serotonergic behavioral responses, effects on motor activity and core temperature, and binding properties of the novel putative anxiolytic amphetamine derivative (±)1-(2,5-dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2), were examined in rodents in order to elucidate the mechanism underlying its anxiolytic-like effect. After peripheral administration in rats, ALEPH-2 induced some symptoms of the serotonergic syndrome, e.g. forepaw treading and flat body posture. Additionally, a decrease in motor activity was observed. No significant effects on the number of head shakes were observed after injection, although high inter-subject variability was noted. Higher doses of ALEPH-2, in the range exhibiting anxiolytic properties (4mg/kg), elicited significant hypothermia in mice. The affinity of the drug for 5-$HT_{2A/2C}$ receptors ([$^3$H]ketanserin sites) was in the nanomolar range ($K_i$ = 173 nM), whereas for 5-$HT_{1A}$, benzodiazepine sites, and $GABA_A$ receptors, the affinity was micromolar or lower. Based on these results the mechanism of action and the anxiolytic-like properties of ALEPH-2 are discussed.

**Key words** ALEPH-2 · Serotonin receptor binding · Anxiolytics · Serotonin syndrome · Phenylisopropylamines · Rat · Hypothermia · Psychedelics

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## Introduction

The incidence of undesirable side effects concomitant with benzodiazepine treatments has generated a great deal of interest in the search for new anxiolytic agents with mechanisms of action other than those of the classical benzodiazepines. Thus far, the most likely therapeutic alternatives seem to be drugs that act upon the serotonergic system (Barrett and Vanover 1993). Thus, serotonin 5-$HT_{1A}$ receptor agonists (full and partial) (De Vry et al. 1991), 5-$HT_2$ antagonists (usually non selective 2A/2C agents) (Kock et al. 1992) and 5-$HT_3$ antagonists (Costall and Naylor 1991) have shown anxiolytic effects in several behavioral models of anxiety and, in some cases, in human clinical trials (Feighner et al. 1982; Gammans et al. 1992).

It is known that methoxylated and alkylthio phenethylamine derivatives affect serotonergic neurotransmission in many ways (Nichols 1994). Hallucinogenic 2,5-dimethoxy amphetamine derivatives, for instance, are potent and selective 5-$HT_{2A/2C}$ ligands (Glennon et al. 1984). *Para*-substituted analogues such as *p*-methylthio- and *p*-methoxyamphetamine (PMA) are potent 5-HT releasing agents (Huang et al. 1992; Nichols et al. 1993; Nichols 1994). Additionally, PMA is a selective monoamine oxidase-A inhibitor (Green and El Hait 1980). However, anxiolytic effects have not been reported for any members of these classes of drugs.

We have recently shown that (±)1-(2,5-dimethoxy-4-ethylthiophenyl)-2-aminopropane (ALEPH-2), a phenethylamine derivative which has been reported to have psychedelic effects in humans (Shulgin and Shulgin 1991), exhibits an anxiolytic-like profile in mice and rats in the elevated plus maze test as well as in the elevated T-maze test (Scorza et al. 1996).

In the present study we report the behavioral (serotonergic syndrome), biochemical (binding properties) and physiological (effects on body temperature) profile of ALEPH-2, in comparison with the actions

of some structurally related phenethylamine derivatives without anxiolytic properties, in an effort to elucidate the mechanism underlying its putative anxiolytic effect.

## Methods

*Drugs.* ALEPH-2, (±)1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) and (±)1-(2,4,5-trimethoxyphenyl)-2-aminopropane (TMA-2), all as hydrochloride salts, were synthesized following published procedures (Shulgin and Shulgin 1991). Amiflamine was generously donated by ASTRA Arcus A.B. (Sweden). Cinanserin was a gift from the Squibb Institute for Medical Research. 8-Hydroxy-N,N-dipropylamino tetralin (8-OH-DPAT) was from RBI USA, and 5-HT was from SIGMA. [$^3$H]ketanserin, [$^3$H] 8-OH-DPAT, [$^3$H] flunitrazepam ([$^3$H]FNZ) and [$^3$H]muscimol were purchased from New England Nuclear (Boston) at specific activities of 61.9, 132.8, 87 and 20 Ci/mmol, respectively.

All drugs were dissolved in the corresponding vehicle (saline or water), on the same day the assays were performed. In the behavioral studies, volumes injected were 1 ml/kg and 5 ml/kg body weight in rats and mice, respectively.

### Behavioral studies

*Animals.* Male Wistar rats (Instituto de Investigaciones Biológicas Clemente Estable animal stock) weighing 200–240 g were used. Animals were housed in groups of 5–6, under a 12-h light/dark cycle (lights on at 08:00 and off at 20:00) at 22 ± 1 °C, with free access to food and water.

*5-HT syndrome.* The rats were placed separately in cages 5 min before the i.p. injection of equimolar doses of the drugs (30 μmol/kg). Control animals received saline under the same conditions. Immediately after injection, each animal was placed in an individual plastic cage (60 × 60 × 36 cm) equipped with photobeams, and its motor activity, defined as the number of beam crosses, was scored during 30 min (Scorza et al. 1996). During the same period, observation sessions lasting 45 s each, beginning 3 min after the injection of the drug, were repeated every 3 min. Reciprocal forepaw treading, hindlimb abduction and flat body posture were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, 3 = intense (the maximum score, summed over the 10 observation periods, amounted to 30 for each symptom/animal) (Tricklebank et al. 1985).

*Head shakes.* In the first series of experiments, rats were injected i.p. with ALEPH-2 at the same dose used in the 5-HT syndrome studies (8.7 mg free base/kg, 30 μmol/kg) and with a reportedly anxiolytic dose (4 mg free base/kg, 13 μmol/kg). Control animals received saline and a group of rats injected i.p. with the 5-HT$_{2A/2C}$ agonist DOB (0.5 mg free base/kg, 1.6 μmol/kg) was included as a positive control. Immediately after injection rats were placed in the observation cage and the number of head shakes, defined as rapid side to side rotations of the head and ears (Bedard and Pycock 1977), was quantified for 30 min.

In the second series of experiments, the ability of ALEPH-2 to prevent DOB-induced head shakes was evaluated. Rats were pretreated with either saline or ALEPH-2 (4 mg/kg i.p) and 30 min later the animals were injected with DOB (0.5 mg/kg). Immediately after injection of DOB, head shakes were quantified as described above.

In all behavioral studies, the experiments were performed between 09:00 and 12:30 h. The observers (two for each experiment) were always blind to the drug treatment condition of the animals.

### Radioligand binding studies

*5-HT receptor binding.* A previously described procedure was employed (Johnson et al. 1990). Briefly, the frontal cortex or hippocampal brain regions from 20–40 male Sprague-Dawley rats (175–200 g, Harlan Laboratories, Indianapolis) were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in four or eight volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenate was centrifuged at 36000 × g for 10 min, and the resulting pellets were resuspended in the same volume of sucrose solution. Separate aliquots of tissue were frozen at − 70 °C until assay.

For each separate experiment, a tissue aliquot was thawed slowly and diluted 1:25 with 50 mM Tris HCl (pH = 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500 × g for 10 min, with an intermittent wash with buffer. The resulting pellet was resuspended in 50 mM Tris HCl containing 0.5 mM $Na_2EDTA$, 0.1% Na ascorbate, and 10 mM pargyline HCl. In experiments with [$^3$H]ketanserin, 5.7 mM $CaCl_2$ was included. A second preincubation for 10 min at 37 °C was conducted, and the homogenate suspensions were than cooled in an ice bath. All experiments were performed with triplicate determinations using the appropriate buffer, to which 200–400 μg of protein was added, in a final volume of 1 ml. The contents of the tubes were allowed to equilibrate for 15 min at 37 °C before filtering through Whatman GF/C filters using a cell harvester (Brandel, Gaithersburg Md., USA) followed by two 5 ml washes using ice-cold Tris buffer. Specific binding was defined as that displaceable with 10 μM cinanserin in the [$^3$H] ketanserin and with 10 μM 5-HT in the [$^3$H] 8-OH-DPAT binding studies, respectively. Filters were air-dried, placed in scintillation vials with 10 ml of Ecolite scintillation cocktail, and allowed to sit overnight before counting at an efficiency of 37%.

Five to six concentrations of radioligands were used in both [$^3$H]ketanserin and [$^3$H]8-OH-DPAT saturation experiments. [$^3$H]ketanserin bound to a single site (Hill coefficient 1.08 ± 0.06) with a $B_{max}$ of 180 ± 19 fmol/mg protein and a $K_D$ of 0.83 ± 0.08 nM. [$^3$H]8-OH-DPAT bound to a single site (Hill coefficient 1.00 + 0.01) with a $B_{max}$ of 110 + 10 fmol/mg protein and a $K_D$ of 0.67 ± 0.10 nM. The ability of 8–9 concentrations of test drug to displace 0.75 nM [$^3$H] ketanserin or [$^3$H]-8-OH-DPAT was determined in drug displacement studies.

*BDZ and GABA$_A$ receptor binding studies.* The affinity of ALEPH-2 for the central type BDZ receptor was measured using [$^3$H]FNZ. Briefly, for each assay, triplicate or quadruplicate membrane samples containing 200–400 μg protein, determined by the Lowry method, were suspended in 1 ml Tris-HCl buffer, pH 7.3 and incubated at 4 °C for 60 min with 0.7 nM [$^3$H]FNZ. Non-specific binding was determined by parallel incubation in the presence of 3 μM FNZ or clonazepam and amounted to 5–15% of the total. Membranes were harvested by rapid filtration through GF/B filters with 3 washes using 5 ml of the incubation buffer each time. Then, filters were dried and transferred to vials with scintillation cocktail (2.5 ml diphenoxyloxazole-xylene) and the radioactivity was measured with 40% efficiency.

GABA$_A$ receptors were assayed using [$^3$H]muscimol as the radioligand. Each assay run in triplicate used 100–150 μg protein incubated in 1 ml Tris-HCl buffer, pH 7.4, with 13 nM [$^3$H]muscimol for 30 min at 4 °C. Non-specific binding was measured by performing the incubation in the presence of 100 μM GABA and represented 10–20% of the total. The membranes were harvested after addition of 5 ml ice-cold buffer by rapid filtration through Whatman GF/C filters followed by two washes with 5 ml of buffer.

Effects on body temperature

*Animals.* Male CF1 mice (Instituto de Investigaciones Biológicas Clemente Estable animal stock) weighing 25–35 g were used. Animals were housed in groups of 10–12, under a 12-h light/dark cycle (lights on at 08 : 00 and off at 20 : 00) at 22 ± 1 °C, with free access to food and water.

*Hypothermia.* Mouse core temperature was measured by inserting the probe of a digital thermometer approximately 2.5 cm into the rectum while lightly restraining the animal immediately before ($t_0$) and 10, 20, 30, 60 and 120 min after an i.p. injection of saline or drugs. The results are expressed as the change in body temperature ($\Delta t$), with respect to the basal temperature, measured at the beginning of the experiment ($t_0$) (Martin et al. 1992).

*Statistical analysis.* Means ± SEM were calculated and are presented for each experimental group. In the 5-HT syndrome, head shakes, motor activity and hypothermia studies, statistical significance was assessed by analysis of variance (ANOVA) followed, when appropriate, by Mann-Whitney *U*-test and Student's *t*-test. In the receptor binding studies, data were analyzed using the computer programs EBDA and Ligand as described by McPherson (McPherson 1985). In all cases the significance level was found to be $P < 0.05$.

## Results

### Behavioral studies

Table 1 shows the results of the effects of equimolar doses (30 μmol/kg) of phenethylamine derivatives on components of the serotonin syndrome. Amiflamine, a selective monoamine oxidase-A inhibitor and TMA-2, a psychoactive phenethylamine derivative (Shulgin and Shulgin 1991) with relatively weak affinity for 5-$HT_{2A/2C}$ receptors (Glennon et al. 1984) were included in this study. All the drugs tested induced responses suggesting activation of 5-HT receptors, and were very potent inducers of the flat body posture response. The most interesting difference was the significant ability of ALEPH-2 to elicit forepaw treading, a behavior that has been associated with the selective activation of 5-$HT_{1A}$ receptors (Tricklebank 1985). It is worth pointing out that all behavioral responses began almost immediately after injection.

Figure 2 compares the motor activity elicited by injection of the different phenethylamine derivatives.

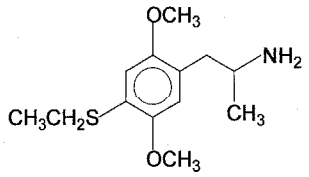


**Fig. 1** Chemical structure of ALEPH-2

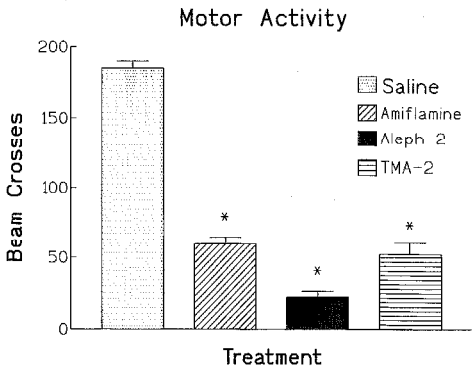


**Fig. 2** Effects on locomotor activity induced by equimolar doses of phenethylamine derivatives. The measurements were made immediately after i.p. injection of the drugs. Each bar represents the mean ± SEM of beam crosses of 10 rats per condition. *significant difference ($P < 0.05$ ANOVA-Student's *t*-test.)

All drugs tested significantly decreased the number of beam crosses as compared with controls.

The effects of ALEPH-2 on head shake behavior are summarized in Tables 2 and 3. As is shown in Table 2, the drug did not induce the response to a significant extent at either of the two assayed doses. However, we noted that many rats (7 out of 10) did not display the behavioral response, while a large number of head shakes (mean ± SEM = 22 ± 2) were observed in the remainder of the group. Almost the same behavior was observed at both dose levels. Additionally, ALEPH-2 was very potent in preventing the head shakes induced by DOB, Table 3.

### Effects on body temperature

When mice were injected with increasing doses of ALEPH-2 (1, 2, 4, 8 mg/kg), a dose-dependent decrease

**Table 1** Behavioral scores for individual 5-HT syndrome components induced by three phenethylamine derivatives

| | ALEPH-2 | TMA-2 | Amiflamine | Saline |
|---|---|---|---|---|
| Forepaw treading | 25 ± 0.7* | 4 ± 0.5 | 0.0 | 0.0 |
| Hindlimb Abduction | 0.0 | 6 ± 1.3 | 0.0 | 0.0 |
| Flat body posture | 26 ± 0.5* | 26 ± 0.7* | 25 ± 0.8* | 0.0 |

Each sign was scored at 3 min intervals during 30 min on a ranked intensity scale (0–3). The measurements started 3 min after i.p. injection of equimolar doses (30 μmol/kg) of the drugs, and lasted 45 s each. Values given are the mean scores ± SEM of 10 rats per condition. *significant difference ($P < 0.05$ ANOVA-Mann-Whitney *U*-test.)

**Table 2** Head shakes induced by different doses of ALEPH-2

| | ALEPH-2 (4 mg/kg) | ALEPH-2 (8.7 mg/kg) | Saline | DOB (0.5 mg/kg) |
|---|---|---|---|---|
| Head shakes | 6.6 ± 10.3 | 6.2 ± 8.2 | 2.7 ± 0.9 | 28 ± 4.5* |

The number of head shakes was counted during 30 min. The measurements were made immediately after i.p. injection of each drug. Values given are the mean scores ± SEM of 10 rats per condition, except in the case of DOB ($n = 6$). * significant difference ($P < 0.05$ ANOVA-Mann-Whitney $U$-test.)

**Table 3**

| | Saline + DOB | ALEPH-2 + DOB |
|---|---|---|
| Head shakes | 31 ± 4.2 | 3.4 ± 4.2* |

Rats were injected with either saline or ALEPH-2 30 min before DOB. The number of head shakes was counted during 30 min. The measurments were made immediately after i.p. injection of DOB. Values given are the mean scores ± SEM of 8 rats per condition. * significant difference ($P < 0.05$ ANOVA-Mann-Whitney $U$-test.)

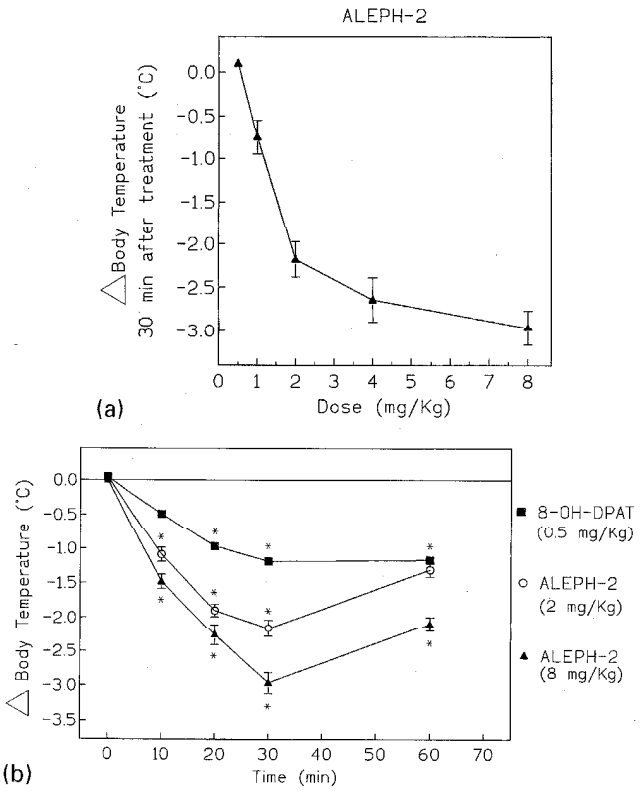


**Fig. 3** Dose-response (**A**) and time course (**B**) curves for the effect of ALEPH-2 on the body temperature in mice. *Each point* represents the mean ± SEM of 7–8 mice per condition. *significant difference ($P < 0.05$ ANOVA-Student's $t$-test.)

in the rectal temperature was observed (Fig. 3A). Time-course studies showed that significant decreases, for each dose, could be detected starting 10 min after injection, with the maximum hypothermic effect observed 30 min after ALEPH-2 (Fig. 3B). The temperature returned to baseline within 120 min after injection of the drug (data not shown). 8-OH-DPAT at a dose of 0.5 mg/kg i.p., was injected as a positive control, and produced basically the same time-course as ALEPH-2 (Fig. 3B).

## Radioligand studies

Table 4 lists the $K_i$ values determined for ALEPH-2 using the four radioligand binding assays utilized in this study (see Materials and methods). At [$^3$H]ketanserin binding sites, ALEPH-2 has an affinity in the nanomolar range, which is about three times lower and about seven times higher than the structurally related drugs DOB and TMA-2, respectively.

At the [$^3$H]8-OH-DPAT site, ALEPH-2 has an affinity in the micromolar range. The $K_i$ of ALEPH-2 for the [$^3$H]8-OH-DPAT site is similar to that of DOB and about five times lower than that of TMA-2.

ALEPH-2 does not recognize central BDZ binding sites or $GABA_A$ receptors. The $K_i$ values for [$^3$H]FNZ and [$^3$H]muscimol binding sites are higher than 100 μM.

## Discussion

A few reports exist describing the subjective effects of ALEPH-2 in humans. Like a number of other 4-substituted-2,5-dimethoxyamphetamine derivatives, ALEPH-2 is regarded as a psychedelic drug (Shulgin and Shulgin 1991). However, like other 4-alkylthio derivatives, this drug seems to differ from classical psychedelics, tending to produce a state of emotional detachment and anhedonia (Shulgin and Shulgin 1991; Nichols 1994).

The hypothermic action in mice and the behavioral responses elicited in rodents by ALEPH-2, i.e., the ability to induce forepaw treading, the small number of head shakes induced after its i.p. injection and the previously reported anxiolytic-like profile (Scorza et al. 1996) are in agreement with the idea that this drug could have a pharmacological profile that differs from that of some other psychotropic phenethylamines. In this respect, most psychedelic drugs, including hallucinogenic amphetamine derivatives, induce hyperthermia in rodents (Gudelsky et al. 1986; Nash et al. 1989; Glennon 1990) and their effects in anxiety tests are either nonsignificant or suggest some induction of anxiogenesis (Critchley and Handley 1987; Tomkins et al. 1990; Handley et al. 1993). Additionally, most of the evidence indicates that drugs such as 1-(2,5-dimethoxy-4-iodo)amphetamine (DOI) or its brominated congener DOB, do not induce serotonergic syndrome responses, but do induce head shakes, a property which

**Table 4** Displacement of [$^3$H]8-OH-DPAT, [$^3$H]ketanserin, [$^3$H]flunitrazepam and [$^3$H]muscimol binding by ALEPH-2 and some congeners

| | [$^3$H]8-OH-DPAT | | [$^3$H] Ketanserin | | [$^3$H] FNZ | | [$^3$H] Muscimol | |
|---|---|---|---|---|---|---|---|---|
| | $K_i$(nM) | $n$ | $K_i$(nM) | $n$ | $K_i$(nM) | $n$ | $K_i$(nM) | $n$ |
| ALEPH-2 | 5980 ± 450 | 6 | 173 ± 25 | 6 | > 100000 | 5 | > 100000 | 5 |
| DOB | 4450 ± 350 | 4 | 60 ± 5 | 4 | ND | | ND | |
| TMA-2 | 26800 ± 2300 | 5 | 1112 ± 97 | 4 | ND | | ND | |
| Amiflamine | ND | | 11380 ± 700 | 4 | ND | | ND | |

ND, not determined

has been associated with their affinity for 5-HT$_2$ subtype receptors (Yap and Taylor 1983; Glennon and Lucki 1988; Glennon 1990).

The findings put forth above suggested to us the possibility that ALEPH-2 might be a 5-HT$_{1A}$ ligand. Nevertheless, the radioligand displacement studies showed that the drug has a very weak affinity for these receptors. Furthermore, these studies revealed that ALEPH-2 has a remarkably high affinity for 5-HT$_{2A/2C}$ receptors ([$^3$H]ketanserin sites) which, like that of other 2,5-dimethoxy amphetamine derivatives, correlates well with its reported hallucinogenic potency in humans (Glennon et al. 1984; Sadzot et al. 1989; Shulgin and Shulgin 1991). As expected, the drug has no affinity for benzodiazepine sites or GABA$_A$ receptors, ruling out the possibility of its anxiolytic-like activity being mediated by in these systems.

A rather intriguing result was the observation that ALEPH-2 also behaved in a different way from its hallucinogenic congeners regarding its effects on the head shake response. Head shakes are a normal behavior in rats, but the administration of selective 5-HT$_{2A/2C}$ agonists clearly increases their number (Yap and Taylor 1983; Glennon and Lucki 1988; and see Table 2, DOB effects). As was observed, ALEPH-2 not only did not significantly elicit this response, but was even able to prevent the behavior induced by DOB injected 30 min later. It is worth pointing out that in the case of the head shakes induced by ALEPH-2 a large inter-subject variability was observed. This last finding could be a correlate of the highly variable effects observed in humans (Shulgin and Shulgin 1991).

In agreement with previous observations (Scorza et al. 1996), ALEPH-2 induced a decrease in motor activity, an effect which was also produced by the other phenethylamine derivatives evaluated.

Even though the sum of the results reported here does not completely clarify the mechanism by which ALEPH-2 behaves as an anxiolytic in rodents and further studies are needed, for example using diverse 5-HT antagonists, we feel that several new hypotheses must be considered. Thus, the simplest possibility is that ALEPH-2 acts as an antagonist or a weak partial agonist of 5-HT$_{2A/2C}$ receptors, since the drug displayed a relatively high affinity for these sites and blocks a behavioral response induced by an agonist. There exists well documented evidence for non-selective 5-HT$_2$ antagonists showing anxiolytic profiles in behavioral tests (Barrett and Vanover 1993; Koek et al. 1992). However, the psychedelic effects of ALEPH-2 in humans and its ability to induce some serotonergic syndrome responses in a way similar to other serotonergic transmission enhancers suggest that ALEPH-2 is an agonist rather than an antagonist at 5-HT$_2$ receptors.

However, 5-HT$_2$ agonists, including psychotomimetic amphetamine derivatives, elicit no consistent anxiogenic-like responses in different behavioral models. Also, the anxiolytic effects of 5-HT$_2$ antagonists can be overcome by agonists of these receptors. In addition, it has been suggested that 5-HT$_2$ receptors may play an inhibitory role on plus-maze behavior and that a down-regulation of these receptors may be responsible for the anxiolytic profile observed 48 h after mianserin administration (Benjamin et al. 1992). Thus, a different explanation for the mechanism of action could be offered. It has recently been reported that the selective blockade of 5-HT$_{2C}$ receptors seems to be responsible for the anxiolytic activity of non selective 5-HT$_{2A/2C}$ antagonists (Kennett 1992; Kennett et al. 1994). Furthermore, the relatively selective 5-HT$_{2C}$ agonist *m*-chlorophenylpiperazine (*m*-CPP), which is anxiogenic in humans and rodents (Kahn et al. 1988; Kennett et al. 1989; Murphy et al. 1991), induces hyperthermia in rats (Wozniak et al. 1989; Murphy et al. 1991; Klodzinska and Chojnacka-Wójcik 1992).

Considering the unusual combination of effects exerted by ALEPH-2 in rats, e.g. hypothermic and anxiolytic-like activity and the inability to consistently induce head shakes, it may be possible that this drug could act as an antagonist at 5-HT$_{2C}$ receptors, and still retain its 5-HT$_{2A}$ agonist action. In this sense, it is interesting to speculate about the role of 5-HT$_{2C}$ receptors in the effects of psychedelics in humans. Basically, all hallucinogenic drugs are non-selective with regard to 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Glennon et al. 1992), but the relative contribution of each receptor to the overall effect of these substances has not been elucidated. Nevertheless, Sanders-Bush and colleagues have demonstrated that several structurally diverse hallucinogens are agonists at 5-HT$_{2C}$ receptors (Burris et al. 1991; Sanders-Bush and Breeding 1991). Thus, considering the differences between classical psychedelics and ALEPH-2, this compound, and perhaps other

sulfur-containing phenethylamine derivatives, might be useful tools to dissect the different components of the psychedelic experience.

Additionally, 5-$HT_3$, cholecystokinin or glutamate receptor-mediated effects cannot be discarded as elicitors of the anxiolytic response, but the lack of structural similarity between ALEPH-2 and drugs that affect these receptors acts against this explanation for the mechanism of action of this phenethylamine derivative.

Another possible explanation for the anxiolytic-like activity of ALEPH-2, is the formation of some active metabolite, possibly a sulfoxide or a sulfone, which could be responsible for the in vivo actions. Obviously, this metabolite would not be detected in the in vitro binding studies. Experiments to determine the effects of a possible ALEPH-2 metabolite are currently being performed.

An additional tempting possibility is the existence of a new mechanism for alleviating anxiety. This is based on the fact that ALEPH-2 does not show affinity for 5-$HT_{1A}$ or benzodiazepine receptors, thus ruling out a mode of action similar to those of the most widely used anxiolytic drugs. In this sense, ALEPH-2 might be a useful tool to investigate such a hypothetical new mechanism.

Finally, although this possibility seems even less likely, ALEPH-2 might have some action of 5-$HT_{1A}$ autoreceptors. There exists increasing evidence that 5-$HT_{1A}$ autoreceptors (located in the raphe nuclei) are functionally different from the 5-$HT_{1A}$ receptors located postsynaptically (See for example Radja et al. 1992). Because the radioligand experiments for 5-$HT_{1A}$ receptors were only done using hippocampal membranes, i.e., postsynaptic receptors, we cannot discard a possible contribution of 5-$HT_{1A}$ autoreceptors to the anxiolytic-like effects of ALEPH-2.

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