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ALEPH-2, a suspected anxiolytic and putative hallucinogenic phenylisopropylamine derivative, is a 5-HT_{2a} and 5-HT_{2c} receptor agonist

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Abstract

To assess the pharmacodynamic profile of ALEPH-2, a phenylisopropylamine derivative with alleged anxiolytic and hallucinogenic properties, *Xenopus laevis* oocytes were microinjected with either of the rat cRNA for the 5-HT_{2A} or the 5-HT_{2C} receptor. Concentration-response curves were obtained following the exposure of the oocytes to varying concentrations of either ALEPH-2 or 5-hydroxytryptamine (5-HT) for 10 s. ALEPH-2 is a partial agonist on the 5-HT_{2A} receptor with a similar potency to 5-HT. In contrast, ALEPH-2 is a full 5-HT_{2C} receptor agonist and is about 15-fold less potent than 5-HT. Pre-application of 1 μM ritanserin antagonized the responses induced by 5-HT and ALEPH-2 to the same extent; however, the 5-HT_{2A} receptor is more sensitive to ritanserin blockade than the 5-HT_{2C} receptor.

Keywords: ALEPH-2; 5-hydroxytryptamine; 5-HT_{2A} and 5-HT_{2C} receptors; Phenylisopropylamine derivatives

Introduction

ALEPH-2, [(±)-1-(2,5-Dimethoxy-4-ethylthiophenyl)-2-aminopropane] is a ring-substituted amphetamine derivative that is said to have psychedelic effects in humans at low oral doses

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[1] and also exhibits an anxiolytic-like profile in rodents in different behavioral models [2]. ALEPH-2 displaces the binding of [^{3}H]-ketanserin to rat brain membranes [3] suggesting that, like phenethylamine hallucinogens such as DOM [1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane] or its 4-bromo (DOB) and 4-iodo (DOI) analogs, ALEPH-2 has affinity for the 5-$HT_{2A/2C}$ receptor subtypes. In addition, it does not have significant affinity for either the 5-HT_{1A}, $GABA_A$ or benzodiazepine receptors [3].

When evaluated in different behavioral models of anxiety, 5-HT_2 receptor agonists, including hallucinogenic amphetamine derivatives, generally elicit anxiogenic-like responses [4-6]. On the other hand, it has been suggested that the blockade of 5-HT_{2B} and/or 5-HT_{2C} receptors may mediate the anxiolytic-like effects of certain serotonergic drugs [7,8].

In the light of emerging concepts in the psychopharmacology of substituted phenethylamines, and to gain further details regarding the molecular mechanism of the action of these drugs, we were prompted to assess the efficacy of ALEPH-2 in 5-HT_2 receptors. We reasoned that such information is relevant to the understanding of the behavioral properties of this compound and would allow a better interpretation of the mechanisms involved. An attractive model system to study the efficacy of drug action is the expression of receptors in *Xenopus* oocytes. We therefore examined the pharmacodynamic profile of ALEPH-2 in *Xenopus laevis* oocytes microinjected with cRNA of the 5-HT_{2A} and the 5-HT_{2C} receptors, and compared its properties to those of 5-HT.

Methods

Xenopus laevis ovary lobes were surgically removed; stage V and VI oocytes were manually defolliculated and further treated with collagenase as previously described [9]. Oocytes were microinjected intracytoplasmically with 10–20 ng of cRNA for either the rat 5-HT_{2A} or 5-HT_{2C} receptor clones [9]. After 24-48 h of incubation at 15°C in modified Barth's solution, the oocyte membrane potential was held at −70 mV. Recordings were performed with the two-electrode technique using an OC-725C oocyte clamp (Warner Instrument Corp.). The composition of the buffer solution used to record the drug-evoked currents was detailed by Huidobro-Toro and harris [9]. Since these are metabotropic receptors, 5-Ht and related agonists evoke an indirect, Ca-activated chloride current. Control, non-injected oocytes did not respond to applications of either 5-HT nor ALEPH-2, allowing us to rule out the interaction of this drug with other oocyte cell membrane proteins, among which the chloride channels might be relevant.

To ascertain the expression of the receptors transfected, the oocytes were challenged for 10 s with various concentrations of 5-HT or ALEPH-2 every 20–30 min. The drugs were applied by superfusion at a constant flow rate of 2 ml/min. The currents generated by 5-HT or ALEPH-2 were plotted against concentration using the Graph-Pad Software (Graph-Pad Inc., San Diego, CA) to obtain the maximal current (I_{max}), the 50% effective concentration (EC_{50}) and the Hill coefficient [9]. Curves were normalized against the 5-HT concentration that produced the maximal current (300 nM for the 5-HT_{2A} and 20 nM for the 5-HT_{2C} receptor, respectively). Independent protocols evaluated the currents generated by ALEPH-2 or 5-Ht following the pre-application of 1μM ritanserin for 1 min. At least two batches of oocytes were used to study the pharmacodynamics of 5-HT and ALEPH-2; each protocol was repeated in 5–9 separate oocytes.

Kruskal-Wallis and Friedman and Quade tests were used for statistical analysis. In all cases, significance was set at a P value < 0.05. Results are shown as mean $\pm$ S.E.M. ALEPH-2 hydrochloride was synthesized by B.K. Cassels. Ritanserin and ketanserin tartrate were purchased from RBI (Natick, Mass. USA), and 5-HT oxalate was purchased from Sigma Chemicals.

Results

Activity at the 5-HT$_{2A}$ receptor

In agreement with the known pharmacology of 5-HT$_2$ receptor subtypes, the 5-HT EC_{50} was 56.2 $\pm$ 11.7 nM (n=11). 300 nM 5-HT consistently yielded the I_{max} (Fig. 1 A-B). ALEPH-2 evoked concentration-dependent currents; its EC_{50} was 62.1 $\pm$ 18.0 nM (n=7) and the I_{max} was 58.6 $\pm$ 9.1% of that attained with 5-HT (n=7, $P < 0.001$, Fig. 1B). The Hill coefficient of 5-HT and ALEPH-2 was not different and was around 2 for the fitting model used. The responses evoked by 100 nM ALEPH-2 (n=4) or 100 nM 5-HT (n=5) were significantly reduced by pre-application of 1 μM ritanserin (88.8 $\pm$ 11.2% and 81.4 $\pm$ 12.5%, respectively, $P < 0.001$, Fig. 1C).

Activity at the 5-HT$_{2C}$ receptor

5-HT evoked concentration-dependent currents; its EC_{50} was 4.6 $\pm$ 0.6 nM (n=6). 5-HT concentration-response curves were normalized with 20 nM 5-HT, a concentration that reliably attained I_{max} (Fig. 2 B). Compared to the 5-HT$_{2A}$ receptor, 5-HT is about 10-fold more potent in this clone, in agreement with Leonhardt et al. (1992, [10]). ALEPH-2 elicited a parallel concentration-response curve displaced to the right about 15-fold; its EC_{50} was 85.7 $\pm$ 16.5 nM ($P < 0.001$ as compared to 5-HT, n=6). Both ALEPH-2 and 5-HT produced a similar I_{max} (Fig. 2B); the Hill coefficient did not differ significantly and was around 2. The responses evoked by 100 nM ALEPH-2 or 10 nM 5-HT were significantly reduced by 1 μM ritanserin pre-treatment (33.5 $\pm$ 5.8 and 44.5 $\pm$ 11.4%, respectively, $P < 0.01$, n=5, Fig. 2 C).

Discussion

Even though correlations between pharmacology and behavioral data must be taken with caution, the present findings describing the efficacy of ALEPH-2 on 5-HT$_2$ receptors shed light on the putative mechanisms underlying the psychedelic and anxiolytic effects of ALEPH-2. The EC_{50} of ALEPH-2 on the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors indicates that the compound binds with high affinity to both sites, which is consistent with its ability to displace [^{3}H]-ketanserin binding at nanomolar concentrations [3]. While ALEPH-2 is a full agonist of the 5-HT$_{2C}$ receptor, it is a partial 5-HT$_{2A}$ receptor agonist.

Structurally related 2,5-dimethoxy-4-substituted amphetamine analogs, such as the extremely potent hallucinogen DOI and its 4-trifluoromethyl analogue DOTFM, have high binding affinity for 5-HT$_{2A/2C}$ receptors and behave as full agonists in cell lines expressing both receptor subtypes [12]. Selective 5-HT$_{2A}$ antagonists block the discriminative cues of DOM or DOI [13,14]; however, a selective 5-HT$_{2C/2B}$ antagonist did not show a similar effect

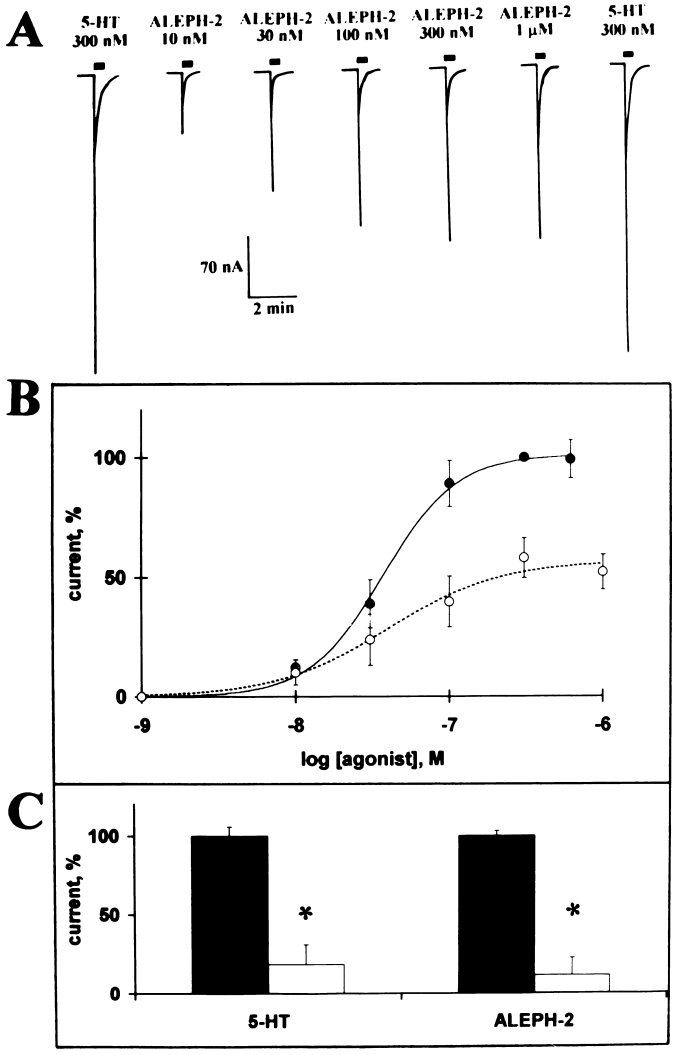

Fig. 1. ALEPH-2 is a 5-HT_{2A} receptor partial agonist **A:** representative tracings from a single oocyte expressing the rat 5-HT_{2A} receptor clone demonstrate that applications of ALEPH-2 do not produce the same maximal current attained with 100 nM 5-HT. **B:** ALEPH-2 (open circles-dashed line) and 5-HT (filled circles) concentration-response curves. **C:** Blockade of the responses following a 1-min pre-incubation with 1 μM ritanserin (*, $P < 0.001$). The control columns show the S.E.M. of the intra-oocyte variation.

[15]. These and other results prompted the notion that 5-HT_{2A} receptors are a key to the hallucinogenic action of phenethylamine and tryptamine psychedelics [16]. As Glennon has pointed out [17], these drugs are either full or partial 5-HT_{2A} receptor agonists, but not antagonists. It should be noted however, that all psychedelic phenethylamines tested so far also bind to the 5-HT_{2C} receptor, displaying affinities in the same range as required for the 5-HT_{2A} receptor subtype [18,19]. The present results are entirely consistent with the hallucinogenic

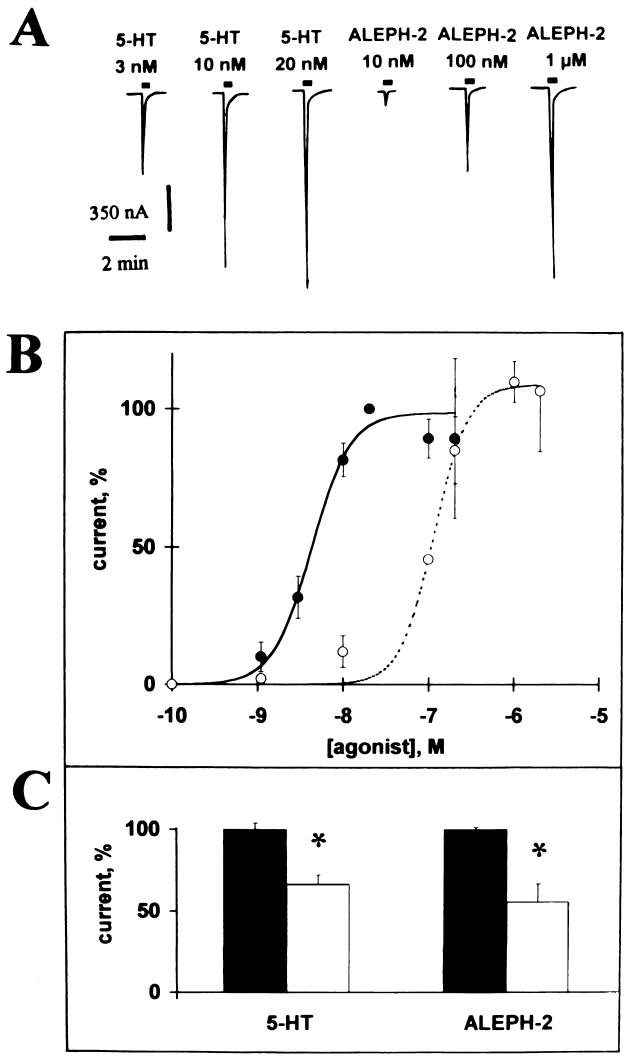

Fig. 2. ALEPH-2 is a 5-HT_{2C} receptor full agonist **A**: representative tracings show the full agonist activity of ALEPH-2. Recordings were obtained from the same oocyte, tested with both ALEPH-2 and 5-HT. **B:** concentration-response currents elicited by ALEPH-2 (open circles) and 5-HT (filled circles) in oocytes expressing the 5-HT_{2C} receptor clone. **C:** Blockade of the ALEPH-2 and 5-HT responses following a 1-min pre-incubation with 1 μM ritanserin (*, $P < 0.01$). The control column shows the S.E.M., including the intra-oocyte variation.

effect of ALEPH-2 in humans, strengthening the notion that phenethylamine psychedelics are 5-HT_2 receptor agonists.

The responses elicited by ALEPH-2 and 5-HT were reduced by 1 μM ritanserin, an accepted 5-HT_2-receptor antagonist. In agreement with its relative selectivity for the 5-HT_{2A} receptor subtype, ritanserin reduced the currents elicited by 5-HT acting on 5-HT_{2A} significantly more than when acting on the 5-HT_{2C} receptor. This result is compatible with current

5-HT receptor pharmacology [11]. In parallel experiments we also tested 1 μM ketanserin, a structurally related 5-HT antagonist, which was devoid of receptor subtype selectivity in this model. 1-3 μM methysergide evidenced partial agonism in 5-HT_{2C} injected oocytes as previously described [11], forcing us to discontinue the methysergide protocol.

ALEPH-2 showed anxiolytic effects in the elevated T- and plus-maze models. On the basis of results obtained in the T-maze, a model that discriminates between conditioned fear (related to generalized anxiety disorder) and unconditioned fear (associated with panic disorder, [20]), it has been suggested that activation of the 5-HT_{2C} receptor may inhibit unconditioned fear responses [21]. In this model, ALEPH-2 significantly inhibited the one-way escape response, suggesting a reduction of unconditioned fear [2,20]. The observation that ALEPH-2 is a full 5-HT_{2C} agonist is in agreement with its anxiolytic effect in the T-maze.

The anxiolytic effect of ALEPH-2 in the plus-maze test is not as easily interpreted. Usually, the blockade of 5-HT_{2C} (and more recently also 5-HT_{2B}) receptors is associated with anxiolytic-like profiles in the plus-maze [7]. Therefore, it is relevant and surprising that ALEPH-2, which is a full 5-HT_{2C} receptor agonist, induces in rodents a significant anxiolytic response in the plus-maze [2]. The activity of ALEPH-2 in the plus-maze, the anxiolytic effect of DOI in the rat ultrasonic vocalization test, and the possibility that 5-HT_2 receptor activation might play a role in the anxiolytic effect of antidepressant drugs [22], suggest that the function of 5-HT_2 receptors in anxiety might be more complex than originally envisaged. We cannot disregard the possibility that non-serotonergic mechanisms may also be involved in the anxiolytic effect of ALEPH-2.

Finally, experiments using *Xenopus* oocytes as a model to express receptors and study their functional properties highlight the significance of combining behavioral with molecular studies to understand the neurochemical basis of behavior and the underlying mechanisms of psychotropic drug action. In this context, a family of ring-substituted phenethylamine derivatives and their beta-methoxy analogues [23,24] are our next candidates for functional studies.

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