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Spiro[9,10-dihydroanthracene]-9,3′-pyrrolidine—a structurally unique tetracyclic 5-HT_{2A} receptor antagonist

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Abstract

Structural elaboration of phenylethylamine to spiro[9,10-dihydroanthracene]-9,3′-pyrrolidine (SpAMDA) produces an agent with unexpectedly high affinity (K_i = 4 nM) at 5-HT_{2A} receptors. It was shown that SpAMDA acts as a 5-HT_{2A} receptor antagonist. The structure and molecular geometry of SpAMDA are not consistent with existing pharmacophore features, and a novel 5-HT_{2A} antagonist pharmacophore model is proposed for the binding of aminomethyl-9,10-dihydroanthracene analogs. Thus, SpAMDA may be a structurally novel parent of a new class of 5-HT_{2A} receptor antagonists that binds to the receptor in a unique fashion that is distinct from the binding topology of existing 5-HT_{2A} receptor antagonists.

Keywords: 5-HT receptor; 5-HT_{2A} receptor antagonist; SpAMDA

Serotonin (5-HT, 5-hydroxytryptamine) has been implicated in numerous functions ranging from hematological, cardiovascular, respiratory, and endocrine activities to regulation of mood states. Numerous studies have shown that aberrant central 5-HT neurotransmission is associated with several psychiatric disorders, including depression and anxiety. The diverse pharmacological actions of 5-HT are due to the existence of at least 14 different 5-HT receptors which have been distinguished on the basis of operational (function, antagonism, location), transductional (G protein, ion channel), and structural (gene sequence, chromosomal location) criteria. (Roth, 1994, Glennon et al., 1999). 5-HT_{2A} receptors have been implicated as the site of action of hallucinogens, atypical antipsychotic drugs and certain atypical antidepressants. The fact that many psychotherapeutic agents have high 5-HT_{2A} receptor affinity has stimulated a search for structurally unique 5-HT_{2A} receptor antagonists (Westkaemper and Glennon, 2002).

The contemporary process of drug discovery can make use of protein sequence data, information from site-directed mutagenesis, and evaluation of hypothetical 3-dimensional graphics models coupled with classical, intuition-driven structural modification of active, lead compounds. The 5-HT G-protein-coupled receptors (GPCRs) are predicted to be heptahelical structures, with an extracellular amino terminus, seven relatively hydrophobic transmembrane helices connected by three extracellular and three intracellular loops of varying lengths, and an intracellular carboxyl terminus. Recently, an X-ray crystal structure of nearly the entire molecule (including most of the intra- and extracellular loops) of the G-protein-coupled visual pigment bovine rhodopsin was reported (Palczewski et al., 2000). Molecular models based on the 5-HT_{2A} receptor sequences and the crystal structure of bovine rhodopsin have been used to visualize ways in which existing and designed compounds may interact with multiple aromatic residues within the ligand binding site (Westkaemper and Glennon, 2002).

Typically, simple unsubstituted phenylethylamines show very low affinity for 5-HT_{2A} receptors (e.g., phenylethylamine, PEA; 5-HT_{2A}; K_i>10,000 nM). Some time ago, examination of receptor models suggested that the affinity of structures containing a phenylethylamine skeleton could be enhanced by introducing a second aromatic moiety, perhaps by participating in additional aromatic–

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aromatic interactions between ligand and receptor (Westkaemper et al., 1992). This prompted us to prepare and evaluate 9-aminomethyl-9,10-dihydroanthracene (Fig. 1A, AMDA) which proved to have high affinity (K_i = 21 nM; Westkaemper et al., 1999) at ketanserin-labeled 5-HT_{2A} sites compared to phenylethylamine. We have now found that presentation of the ethylamino functionality of AMDA within a relatively uncommon spiropyrrolidine ring unexpectedly enhances affinity for 5-HT_{2A} sites even further. Incorporation of a spiropyrrolidine moiety at the 9-position of 9,10-dihydroanthracene generates SpAMDA (Fig. 1B, SpAMDA, K_i = 4 nM) which binds to 5-HT_2 receptors with at least 2500- and 5-fold higher affinity than PEA and AMDA, respectively. This structural modification induces small changes in the geometric relationship between the two aromatic rings and constrains the amino group to four energy equivalent ($\Delta E < 1$ kcal/mol) conformations differing in the geometry of pucker of the pyrrolidine ring. All four SpAMDA conformations resemble the exo form as opposed to the endo form of AMDA (Runyon et al., 2002). Evaluation of SpAMDA, with its multiple but geometrically restricted conformations, was conducted to further refine a recently proposed alternative pharmacophore model based on AMDA and AMDA derivatives (Westkaemper and Glennon 2002). We now report the characterization of SpAMDA as a high-affinity 5-HT_{2A} receptor antagonist.

SpAMDA was synthesized through a series of reactions starting from 9,10-dihydroanthracene-9-carboxamide, which was dehydrated to give 9-cyano-9,10-dihydroanthracene. Alkylation with ethyl bromoacetate in the presence of C_2H_5ONa followed by reductive cyclization using H_2-Pd/C gave spiro[9,10-dihydroanthracene]-9,4′-pyrrolidine-2′-one which was reduced with borane-tetrahydrofuran to provide SpAMDA (hemifumarate, mp 190.5–191.5 °C). Ligand binding assays using [^{3}H]ketanserin and phosphoinositide (PI) hydrolysis assays were performed as previously described. (Roth et al., 1997) using cloned receptor preparations. K_i values are reported as means of two to three separate determinations.

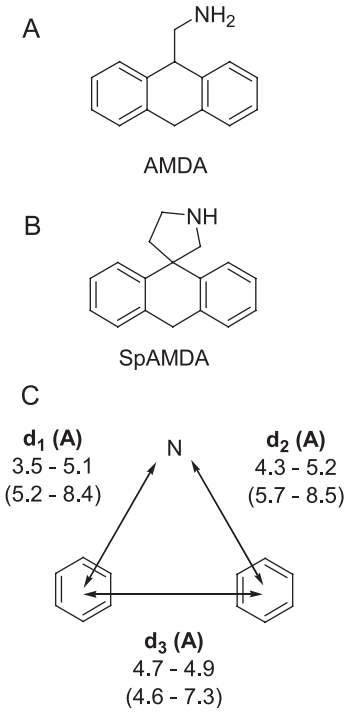

Fig. 1. Chemical structures of AMDA (A) and SpAMDA (B). Comparison of the Andersen/Mokrosz pharmacophore model geometries (in parentheses) with those of the current proposed pharmacophore (C).

SpAMDA binds with significantly enhanced affinity at ketanserin-labeled ($K_i = 4 \pm 2.1$ nM) 5-HT_{2A} sites compared to PEA (K_i>10,000 nM) and AMDA (K_i = 21 nM). The enhanced affinity for SpAMDA at ketanserin-labeled 5-HT_{2A} receptors is most likely due to conformational restriction of the side chain to a few (four) energy-equivalent forms all of which resemble one of two geometrically distinct, low energy conformations of AMDA.

To determine whether SpAMDA is an agonist or antagonist, dose–response studies for PI hydrolysis were performed using stably transfected 3T3 cells expressing the 5-HT_{2A} receptor. Dose–response studies show that SpAMDA is devoid of agonist activity up to a concentration of 10,000 nM while 5-HT behaved as an agonist with a K_{act} of 41 nM. The K_{act} value for 5-HT is similar to that previously reported (Roth et al., 1997). Inhibition studies showed that SpAMDA inhibited the ability of 10 μM 5-HT to activate PI hydrolysis in a concentration-dependent fashion. At a concentration of 10 μM SpAMDA inhibition was complete. Further studies suggest that SpAMDA is a competitive antagonist at 5-HT_{2A}receptors (pA_2 = 7.1).

Several pharmacophore models for 5-HT_{2A} receptor binding have been proposed based on the structure–affinity relationships of known antagonists. Typically, the essential geometric characteristics are described by the distances between two aromatic functionalities (d_3 = 4.6–7.3 Å) and the distances between each aromatic ring and a basic amine nitrogen atom (d_1 = 5.2–8.4 Å and d_2 = 5.7–8.5 Å, Fig. 1C.) (Andersen and co-workers and Mokrosz and co-workers, reviewed in Westkaemper and Glennon, 2002). The corresponding dimensions for the minimum energy conformation of AMDA are similar to existing agents with respect to aromatic interring distance (d_3 = 4.9 Å) but deviate substantially in that AMDA is not symmetrical with respect to the two amine-ring distances (d_1 = 3.5 Å and d_2 = 5.2 Å), d_1 being much shorter than is considered optimal. The ranges for these three geometric parameters for the four conformers of SpAMDA are d_1 = 3.9–5.1 Å, d_2 = 4.3–5.2 Å, and d_3 = 4.7–4.8 Å. A composite pharmacophore model consisting of the distance ranges presented by AMDA and SpAMDA is shown in Fig. 1C (d_1 = 3.5–5.1 Å, d_2 = 4.3–5.2 Å, and d_3 = 4.7–4.9 Å). Including conformationally constrained SpAMDA along with the AMDA series analogs allows a more

complete elaboration of a new pharmacophore model for 5-HT_{2A} antagonists as summarized in Fig. 1C. While inclusion of SpAMDA produces a pharmacophore model that is somewhat more symmetrical with respect to d_1 and d_2 than that based on AMDA alone, the new model is still clearly different from the Andersen/Mokrosz model in that all of the geometric parameters are more narrowly defined with generally shorter distances between pharmacophore features.

SpAMDA is a high-affinity, 5-HT_2-selective antagonist that possesses a geometry inconsistent with previously reported 5-HT_2 antagonist pharmacophore models. With the exception of its two aromatic rings and basic nitrogen atom, SpAMDA is remarkably devoid of the pharmacophore features usually associated with high-affinity receptor ligands such as the heteroatom hydrogen bonding features of the endogenous ligand serotonin. This is remarkable because SpAMDA was conceptually derived by modification of substructures with little or no measurable affinity for the receptor. Comparison of the geometric parameters of SpAMDA with structure–affinity relationships formulated from typical antagonists suggest that SpAMDA may bind at the receptor in a fashion that is different from known antagonists but may bind in a manner similar to AMDA and AMDA derivatives. This, coupled with the fact that there is little structural resemblance between SpAMDA and known serotonergic antagonists, suggests that SpAMDA may be a member of a structurally novel class of compounds that seems to bind to the receptor in a manner distinct from classical 5-HT_{2A} receptor antagonists. It is anticipated that structural elaboration of the SpAMDA may generate 5-HT_{2A} receptor antagonists with novel pharmacological properties.

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