Molecular Modeling of the Interaction of LSD and Other Hallucinogens With 5-HT_2 Receptors

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INTRODUCTION

Multiple populations of serotonin (5-hydroxytryptamine [5-HT]) receptors have been identified, including 5-HT_{1A}, 5-HT_{lB}, 5-HT_{1c}, 5-HT_{1D}, 5-HT_2, 5-HT_3, and 5-HT_4 subtypes. The 5-HT_2 and possibly the 5-HT_{1C} receptors have been implicated as the probable sites of action of classical hallucinogenic agents (Glennon 1990). The goal of this chapter is to identify potential modes of interaction of hallucinogens with 5-HT_2 receptors,

In general terms, there are two ways to embark on a program designed to elucidate drug-receptor interactions. These have been referred to as the ligand-ligand and ligand-receptor approaches (Westkaemper and Glennon 1991). In the first approach, indirect information about specific receptor structural features and their locations in three dimensions is inferred indirectly from the structures of ligands. Typically, common features of structurally related ligands are graphically superimposed to illustrate common and disparate features that aid formulation of hypotheses related to specific drug-receptor interaction.

For example, (+)lysergic acid diethylamide (LSD) contains many structural features in a rigid polycyclic template that are important for 5-HT_2 receptor binding. Figure 1 shows one way in which the structures of LSD and the more flexible hallucinogenic agent 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) can be related. For additional examples of the application of this approach to serotonergic agents, see Glennon and colleagues (199 1).

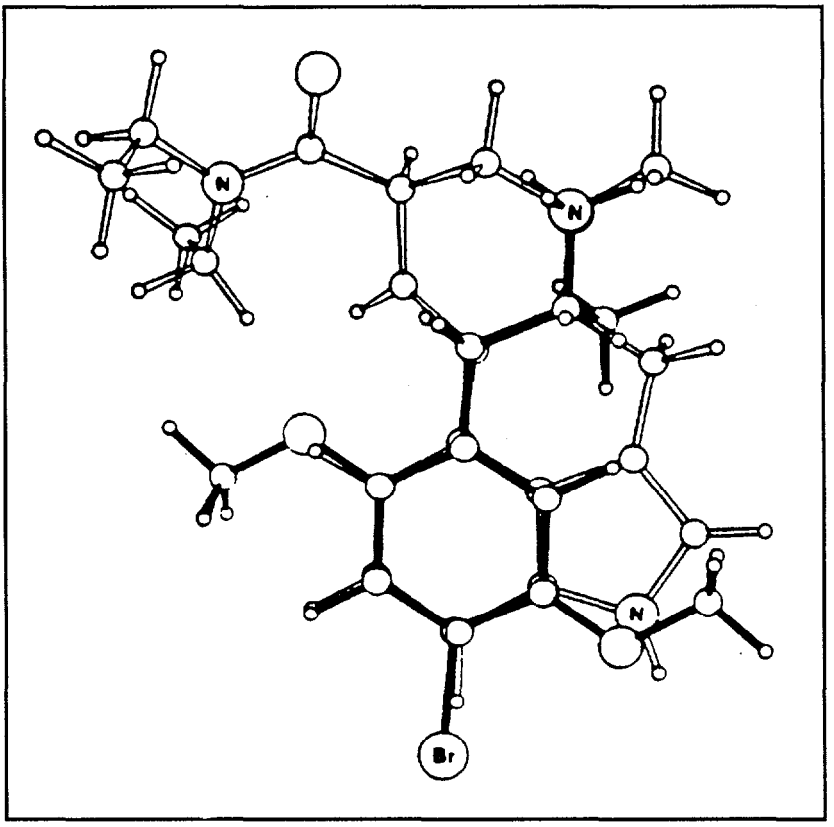

FIGURE 1. *DOB (solid bonds) superimposed on the structure of (+)LSD.*

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The ligand-receptor approach has been used extensively for biological macromolecules for which high resolution x ray structures are available. Until recently, this approach has not been feasible for neurotransmitter receptors because the three-dimensional (3-D) structures of neurotransmitter receptors have yet to be determined. Some of the earliest approaches to modeling ligand-receptor interactions utilized

simulations employing fragments that might be present at the ligand-binding site of a receptor.

Kier and Aldrich (1974) investigated ligand-receptor interactions using semiempirical molecular orbital methods to calculate the interaction energies between a drug and a small molecule thought to mimic a portion of the receptor binding site in a process known as receptor mapping using model interaction energy calculations. Later, this paradigm was applied to hallucinogenic phenethylamines (Di Paolo et al. 1978). In these studies, interaction energies between ring-substituted phenethylamines and methylindole, a presumed tryptophan surrogate, were found to correlate with hallucinogenic potency.

Weinstein explored a similar approach by calculating interaction energies between an imidazolium ion as a receptor model and 5-HT (Weinstein and Osman 1989). Although the implications of such studies are intriguing, the receptor models do not incorporate what is known about the molecular architecture of proteins. Later, more elaborate models, based on known protein structures not related to neurotransmitters, were used to provide a framework for the incorporation of ligand and receptor-like features (Weinstein and Osman 1990).

The construction of more complete receptor models had not been possible because information pertinent to 3-D structure, such as deduced amino acid sequence, was not available, Although several 5-HT receptors have now been cloned and their sequences reported (Fargin et al. 1988; Hamblin and Metcalf 1991; Hartig 1989; Hartig et al. 1990*a*,*b*; Huang and Julius 1991; Julius et al. 1988, 1990; Pritchett et al. 1988; Shih and Chen 1990; Voigt et al. 1991), their 3-D structures remain unknown.

On the other hand, the 3-D structure of the light-sensitive proton pump from bacteria, bacteriorhodopsin (BRH), has been determined to near atomic resolution (Henderson et al. 1990; Henderson and Schertler 1990). Although BRH is not a guanosine triphosphate binding protein (G-protein) receptor, it may be possible to deduce the structures of neurotransmitter receptors from it. BRH consists of seven membrane-spanning α-helices linked by extracellular and intracellular loops. The N-terminus is extracellular, and the C-terminus is located within the cell. The amphiphilic helices are found to form a pore in the membrane, with lipophilic sides oriented toward the membrane and with the more hydrophilic residues located in the central cavity.

The mammalian visual pigment rhodopsin, which is G-protein coupled, is functionally different from BRH, but both are light- and retinal-dependent (Henderson and Schertler 1990). There is little sequence homology between the mammalian visual pigments and BRH (Dohlman et al. 1987). However, evidence suggests that the overall topology for the mammalian protein is similar to that of BRH: both consist of seven membrane-spanning α-helices (see Westkaemper and Glennon 1991).

There is a significant homology between rhodopsin and the G-protein-coupled neurotransmitter receptors and an even greater homology between the neurotransmitters. Studies utilizing secondary structure-predicting methods, the analysis of the distribution of hydrophobic spans of amino acids, and the fact that the G-protein receptors have the greatest degree of sequence homology in the putative helical segments suggest that the architecture of G-protein neurotransmitter receptors is similar to that observed for BRH and to that presumed for rhodopsin (Dohlman et al. 1987).

If sequence homology between BRH and serotonin receptors were good, construction of 3-D receptor models could begin by graphically changing the amino acid side chains of the experimentally determined structure to those of the receptor of interest. Because there is no unambiguous alignment of the transmembrane portions of the 5-HT receptors and BRH sequences, model building is much more tenuous. Given that the helical segments of the serotonin receptors can be identified, placement of these in three dimensions requires the establishment of helix connectivity, vertical placement of each helix (perpendicular to the membrane), rotational alignment (in the plane of the membrane), and side chain geometry, none of which can be known with absolute certainty.

Given these ambiguities, abbreviated receptor models in which only a single helix is represented were chosen for investigation (Westkaemper and Glennon 1991). Docking studies performed with 5-HT ligands and models of helix 3 and helix 2 of 5-HT_2 (and other) receptors indicated that helix 3 is the more probable primary ligand-binding site; this has been recently verified by site-directed mutagenesis (Shih et al., this volume). Calculated binding energies for 5-HT ligands and the helix 3 model of 5-HT_2 receptors correlated qualitatively with experimentally determined affinities for a wide range of structural types, indicating that the features of helix 3 can account for ligand binding.

Despite this success, more complete receptor models are necessary for detailed understanding of ligand-receptor interaction and, in particular, linkage between agonist binding and second messenger coupling.

Hibert and coworkers (1991) recently described the first seven-helix model of a serotonin receptor. A potential sequence alignment between the helical segments of multiple G-protein receptors and BRH was established. The helix connectivity (both in the Hibert model and in the model presented below) of BRH was preserved; that is, helix 1 of the 5-HT receptor corresponds with helix 1 of BRH and so on. Alternative connectivity schemes have been proposed (Weinstein et al., this volume). Although not described, it appears that the final receptor configuration was arrived at by rotational adjustment of helices guided by the principles that highly conserved and charged amino acids should be oriented toward the receptor pore (central to the aggregate of helices). Consideration also was given to site-directed mutagenesis data for ß-adrenergic receptors (ß-AR).

The authors have taken a somewhat different approach and have attempted to identify sequence alignments between the 5-HT receptors and BRH that could be used to construct serotonin receptor models by mutating amino acid side chains without manual modification of helix disposition. White and Jacobs (1990) recently have shown that lengths and centers of helical spans in the photosynthetic reaction center and BRH can be accurately predicted (helical centers within 0.2 amino acids and helix ends within 1 to 3 residues of the experimentally determined values) using a modified hydrophobicity index, and that the helix turn potential has a minimum value at helix centers. Figure 2 shows the results of such an analysis for the 5-HT_2 receptor sequence.

The authors have chosen to evaluate the hypothesis that helical centers of serotonin receptors and BRH (see figure 3 for amino acid sequence of BRH) are structurally coincident. Once such a coincidence and sequence alignment are established, 3-D models of serotonin receptors can be constructed, subjected to molecular mechanics geometry optimization, and evaluated for reasonableness with respect to important criteria: helix packing, disposition of polar and hydrophobic residues, orientation of highly conserved residues, and accessibility of side chains known to interact with ligands.

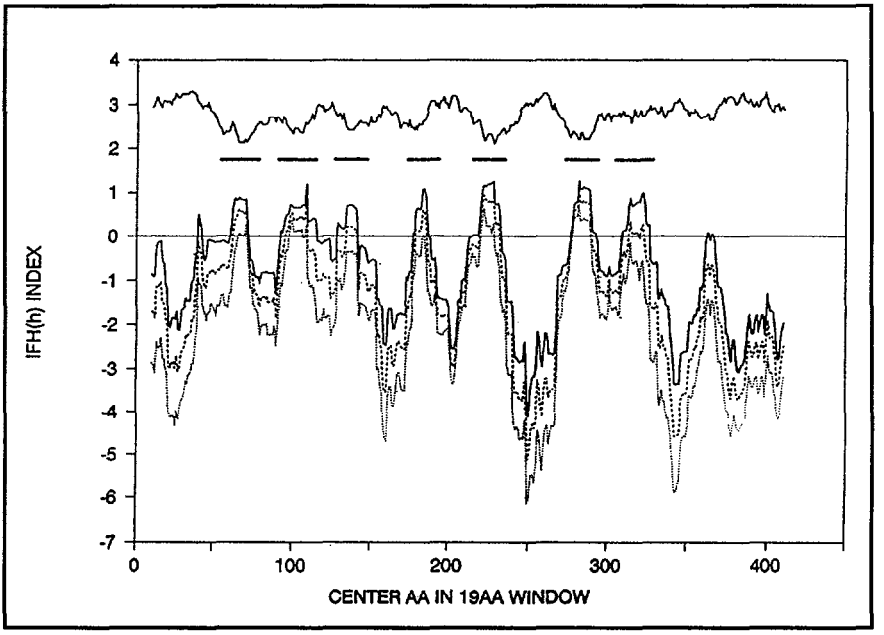

FIGURE 2. *Hydrophobiciy and turn potential analysis of the 5-HT_2 receptor sequence. Horizontal bars indicate helical segments. The upper trace represents turn potential. The lower traces are the hydrophobicity indexes for interfacial hydrophobicity scale where h=0, 0.5, 1.0.*

RECEPTOR MODEL FEATURES

As determined by alignment, the disposition of the ammoniumion-binding aspartate of helix 3 with respect to the rest of the aggregate is perhaps the most important single issue in the model-building process. Unfortunately, identification of the helix center is the least certain in the case of helix 3, perhaps because of the presence of several highly hydrophilic residues. Needleman and Wunsch (1970), using either physical properties or identity as the similarity criteria, produced an alignment shown in figure 4, panel A (identity of 23 percent, alignment score 0.6+0.08; 0.46 for jumbled alignments) which is within the range of possibilities identified using the method of White and Jacobs (1990). The second and third alignments (figure 4, panels B and C, respectively) are also consistent with helix center identification but result only in 4 percent identity. The first alignment places the helix 3 aspartate near the interface

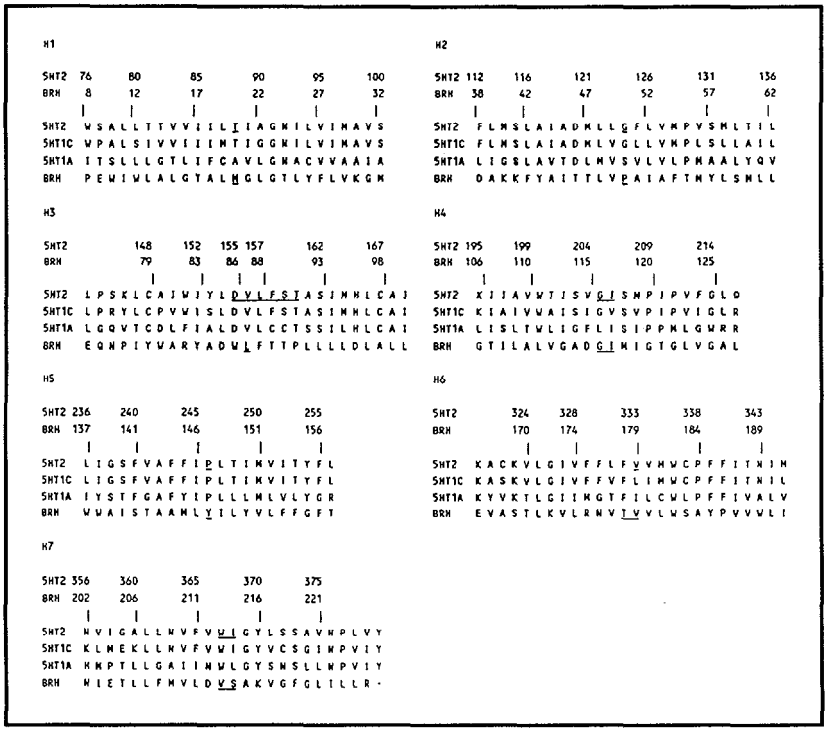

FIGURE 3. *Amino acid sequences for the* seven *transmembrane helices of 5-HT$_2$, 5-HT$_{1C}$, and 5-HT$_{1A}$ receptors and BRH. Underlined residues indicate the most probable locations of helix centers.*

between helix 3 and helix 2. The third alignment places the helix 3 aspartate inaccessible to ligands at the interface between helix 3 and helix 4 and closer to the membrane than to the pore of the receptor. Although identity is poorer than that for the first alignment, the second alignment has been evaluated most extensively because it places the aspartate of the helix in the central pore.

The 5-HT$_2$ receptor was generated by mutation of the BRH side chains according to the alignment shown in figure 3, retaining as much of the original side chain geometry as possible. The entire structure was subjected to molecular mechanics geometry optimization (Weiner et al. 1984) after removing obviously bad side chain contacts. The model of the 5-HT$_2$ receptor constructed in this manner (figures 5 and 6) is only one of several possibilities. Because of limited sequence homology, this

and all models should be considered provisional; nevertheless, several interesting points emerge from the model.

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A   BRH          PSKLCAIWIYLDVLFSTASIMHLCAIS
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL

B   BRH          LPSKLCAIWIYLDVLFSTASIMHLCAI
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL

C   BRH          PLPSKLCAIWIYLDVLFSTASIMHLCA
    5-HT2        EQNPIYWARYADWLFTTPLLLLDLALL
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FIGURE 4. *Several potential alignments of the helix 3 portions of the 5-HT_2 receptor and BRH.*

- The overall packing geometry of BRH is retained in the energy-minimized 5-HT_2 receptor model. The total calculated energy of the receptor model is comparable to the calculated energy of BRH. Helix-helix interactions observed in the model are qualitatively consistent with the experimental structures of adjacent proteins with nearly parallel α-helices.

- Conserved residues generally are located in the central cavity, as are most of the polar amino acid side chains. Exceptions occur at helix termini.

- The ammonium ion-binding aspartate of helix 3 is located in the central cavity nearer to the extracellular side than to the interior of the cell. It is central to two potential ligand binding cavities, one formed from helices 4, 5, and 6 (site I) and a second formed by helices 1, 2, and 7 (site II) (figure 7, panel A).

- Phenylalanine (Phe)-89 and tryptophan (Trp)-82 flank aspartic acid (Asp)-86 of helix 3 (see figure 8) and are in positions that would be accessible for interaction with a bound ligand.

- The hydroxy group of tyrosine (Tyr)-216 of helix 7 forms a hydrogen bond with Asp-86 (figure 8). This structural finding is particularly interesting given the observation that it is conserved only in G-protein receptors that have ammonium ion ligands (Hulme et al. 1990).

- Valine (Val)-212 of helix 7 is near the helix 3 aspartate. An asparagine at the analogous position in the 5-HT_{1A} receptor may modulate ligand selectivity (Surryanarayana et al. 1991).

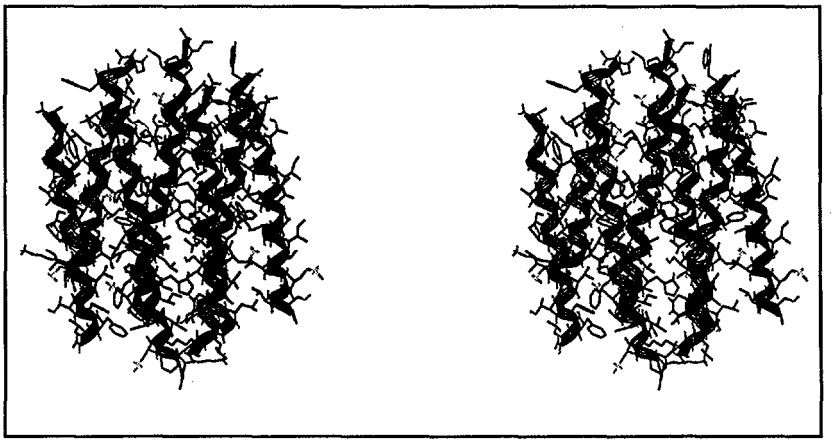

FIGURE 5. *Stereoscopic view of a 5-HT_2 receptor model as viewed from the plane of the cell membrane.*

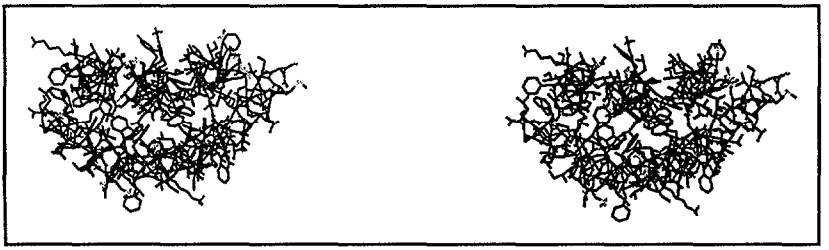

FIGURE 6. *Stereoscopic view of a 5-HT_2 receptor model shown in figure 5 as viewed from the extracellular (N-terminal) side.*

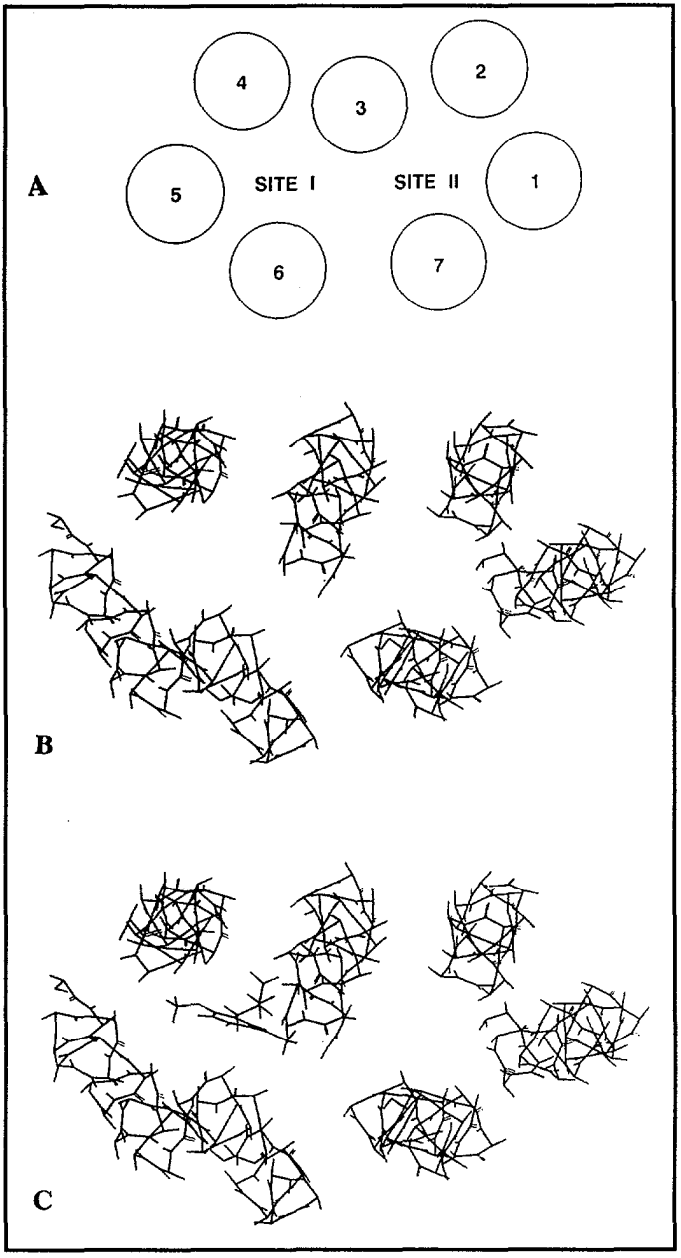

FIGURE 7. *A schematic view of sites I and II formed by helices 4, 5, and 6 and by helices 2, 1, and 7, respectively (A). A view of sites I and II showing only the helix backbone of the $5HT_2$ receptor model (B). A representation of DOB bound in site I of the $5HT_2$ receptor model (C).*

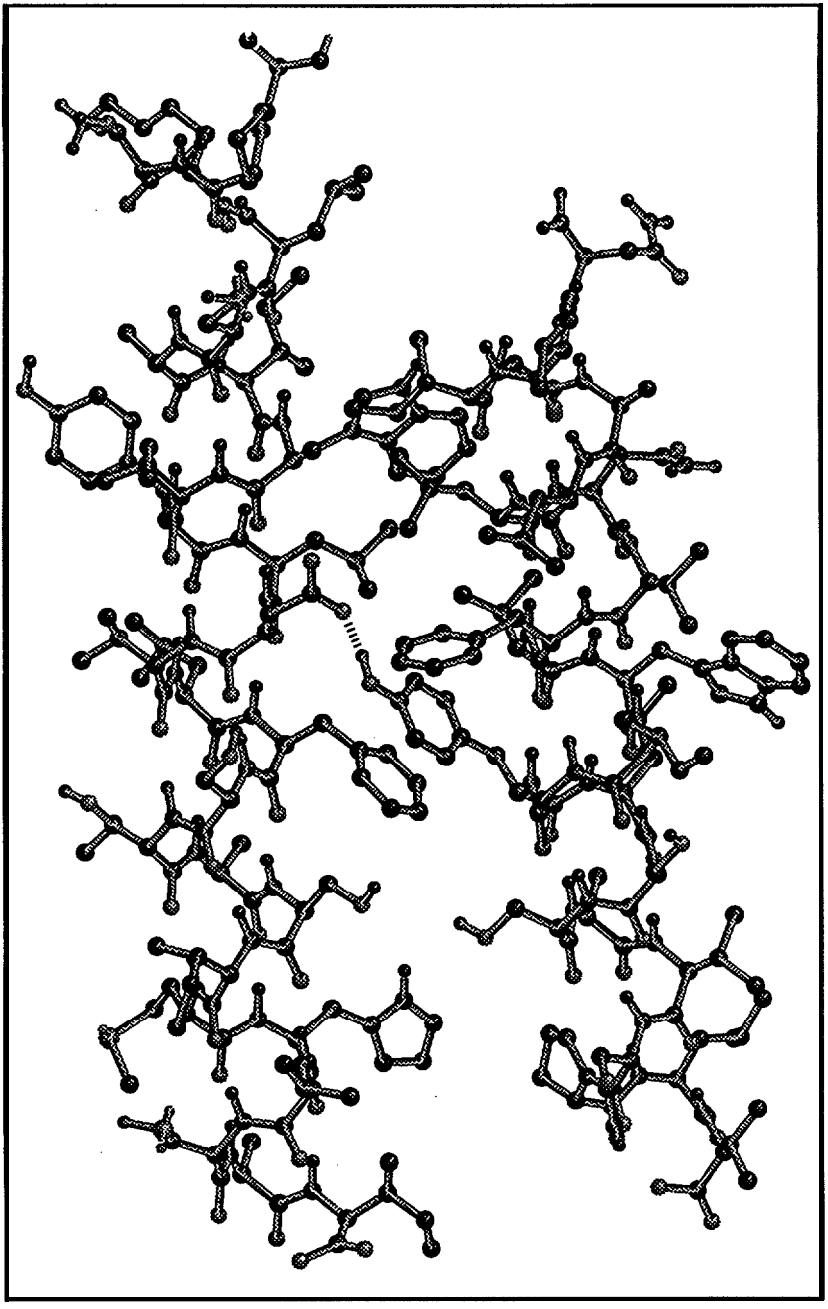

FIGURE *8.* *Closeup of the region surrounding Asp-86 (helix 3) and Tyr-216 (helix 7) of the 5-HT_2 receptor model. Note the hydrogen bond between Asp-86 and Tyr-216.*

One major difference between the model reported here and that reported by Hibert and colleagues (1991) is the rotational orientation of helix 5. In the present model, one of the same two serine (Ser) residues that may be involved in ligand-receptor interactions for the ß-AR (Strader et al. 1989) appears at the interface between helix 4 and helix 5.

LIGAND-RECEPTOR DOCKING

Several structurally similar 5-HT2 receptor models have been developed. One way to evaluate potential receptor models is to investigate modes of ligand-receptor interactions. This process requires selection of an appropriate ligand. Ideally, the ligand should be conformationally rigid and sterically demanding. Table 1 depicts the relative merits of dihydroergotamine (DHE), LSD, and DOB as ligands. LSD and DOB, both of which are hallucinogenic, were selected for initial docking studies. Preliminary studies using the program DGEOM (Blaney et al. 1990) for the automated docking of LSD and a 5-HT_2 receptor model indicated that, for steric reasons, the only reasonable orientation of the rigid polycyclic structure was in sites I or II (figure 7, panel A), with the plane of the aromatic nucleus of LSD more or less parallel to the helical axes. The program GRID (Goodford 1985) was used to identify receptor features that could provide energetically favorable noncovalent interactions. Probe species included an aromatic carbon, water, a methoxy group, an ammonium ion, a carbonyl moiety, and a bromine The information from structure-activity relationship (SAR) studies of ring-substituted phenylisopropylamines such as DOB also was used to guide docking studies (Seggel et al. 1990). The ligand binding site of the receptor must account for the fact that the 2-methoxy group of DOB is critical for high affinity. The 5-methoxy substituent is also important for 5-HT_{1C} and 5-HT_2 affinity, and may play a recognition role analogous to the indole nitrogen of serotonin and LSD. The substituent at the 4-position (e.g., the Br of DOB) modulates affinity over a 50,000-fold range. Typically, nonpolar substituents enhance affinity, the most effective being the halogens (H<F, Cl, Br, I) and alkyl groups (e.g., methyl, ethyl, propyl, butyl, t-butyl, amyl, hexyl, octyl). The receptor recognition site must accommodate alkyl groups as large as 4-octyl and 4-(3-phenylpropyl). In addition, primary amine alkylation decreases affinity, whereas stereochemistry and the presence of an a-methyl group have little effect on receptor affinity.atom. Manual docking was performed using GRID contours as a guide.

TABLE 1. *Potential ligands for receptor model docking.*

	DHE	LSD	DOB
Steric demand	+	+	-
Conformationally restricted	+	+	-
Subtype selectivity			+
SAR data			+
Hallucinogenic		+	+

KEY: DHE = dihydroergotamine; DOB = 1-(4-bromo-2,5-dimethoxyphenyl)-2aminopropane; SAR = structure-activity relationships

Both sites I and II can accommodate the steric bulk of DOB. Both sites have numerous polar, hydrogen-bond-donating side chains, but the side chains are somewhat more numerous in site II. Both sites have aliphatic amino acid side chains in the receptor pore, but site I is slightly richer in aromatic residues at the level of the helix 3 aspartate. Several potential modes of DOB-receptor binding have been evaluated, but only one is discussed in this chapter. There is some evidence that the analogous site I receptor features may be the important ligand recognition region for G-protein receptors other than serotonin receptors (Strader et al. 1989).

The first fact that becomes evident (partially from DGEOM docking experiments) is that the helix 3 aspartate must move away from its hydrogen-bonded, unligated conformation (figure 8) with rotation about the Cα–CTJ bond and move toward site I to allow the bound ligand access to functionally significant side chains. This process is shown schematically in figure 9. Disruption of the resting receptor conformation, in which the helix 3 aspartate and helix 7 Tyr hydrogen bond is intact, may prove to be the event initiating a conformational change that may be responsible for receptor activation.

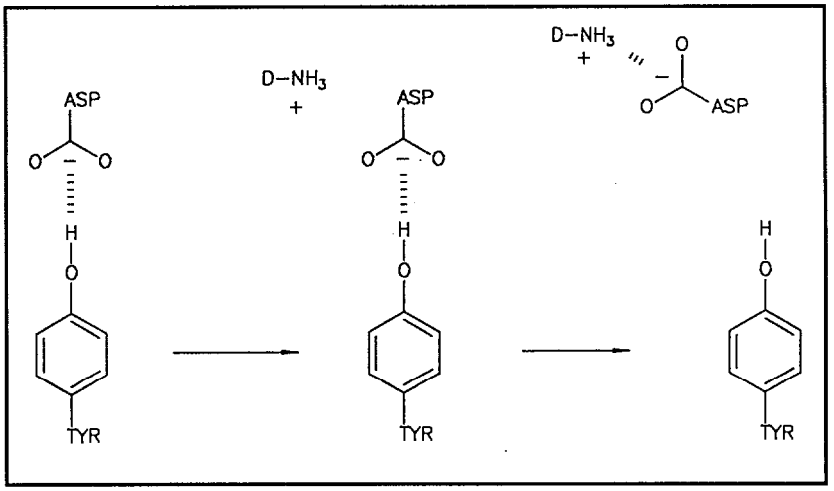

FIGURE 9. *Schematic representation of the possible sequence of events that occurs on the interaction of a ligand (D-NH_3+) with the helix 3 aspartate of a 5-HT_2 receptor model.*

DOB was found to bind favorably in site I (figure 7, panel C), with the aromatic ring approximately parallel to the helical axis, with the primary amine pointed toward the extracellular side and the 4-Br group downward toward the intracellular side. The helix 3 Asp-86 forms an ionic bond with the primary ammonium ion (figure 10). Ser-118 of helix 4 donates a hydrogen bond to the 2-metboxy group, and Ser-90 of helix 3 donates a hydrogen bond to the 5-methoxy substituent. Two aromatic residues (Phe-145 and Phe-185) form an edge-to-face hydrophobic interaction with the phenyl ring of DOB. Aliphatic side chains complete the remainder of the phenyl ring binding site. Trp-182 of helix 6 appears near the bottom of the DOB phenyl ring near the 4-Br group (figure 10), suggesting that this residue may form part of the hydrophobic region that can accommodate a variety of hydrophobic substituents. Further in the intracellular direction, the 4-position of DOB is surrounded by aliphatic and aromatic residues in a way that results in the formation of a short hydrophobic tunnel that can be expected to accommodate several substituents, even long chain aliphatic ones. A serine analogous to Ser-118 (figure 10) is uniformly conserved in the serotonin receptors as well as in the receptors for catecholamines.

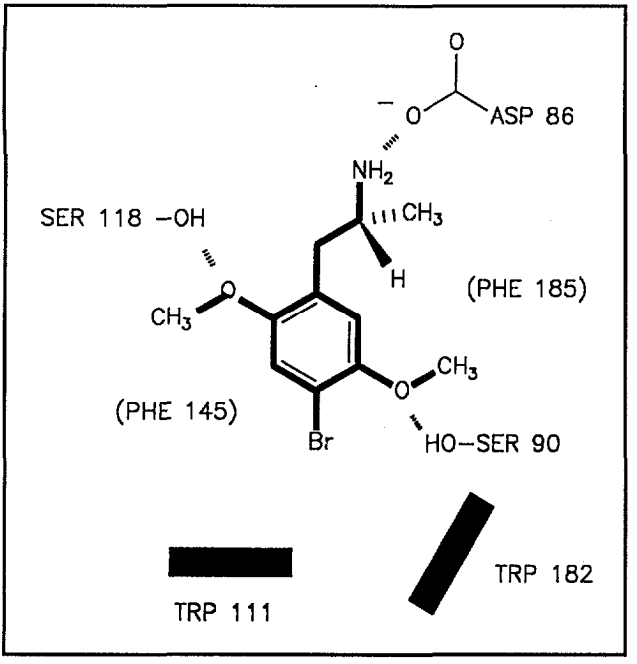

FIGURE 10. *Schematic of possible interactions between DOB and features of a 5-HT$_2$ receptor model as derived from figure 6, panel C*

The neurotransmitters for these receptors all have a phenolic hydroxy group in the position analogous to the 2-methoxy group of DOB (e.g., the 5-hydroxy group of 5-HT). Receptors for acetylcholine, which do not have an aromatic hydroxyl group, have a tryptophan residue at this position. Among the serotonin receptors, a Ser-90 at helix 3 occurs only for the 5-HT$_2$ and 5-HT$_{1C}$ receptors. This position bears a cysteine (Cys) in all other serotonin subtypes. Although a Cys can donate a hydrogen bond, the bond is considerably less energetically favorable than that formed by the Ser hydroxy group. This finding is consistent with the observation that groups analogous to 5-methoxy of DOB are not necessary for 5 HT$_{1A}$ binding. Taken together, these observations indicate that the mode of interaction described is feasible but is not proven.

LSD can bind to the receptor in a fashion analogous to DOB. Ser-118 of helix 4 may form a hydrogen bond with the amide carbonyl or with the C10-C11 double bond of LSD, and Ser-90 interacts with the indole

nitrogen (figure 11). Aromatic residues Phe-185, Phe-145, and Trp-182 can interact with the aromatic ring system of LSD as shown in figure 11. Other closely situated residues may participate in ligand-receptor interaction.

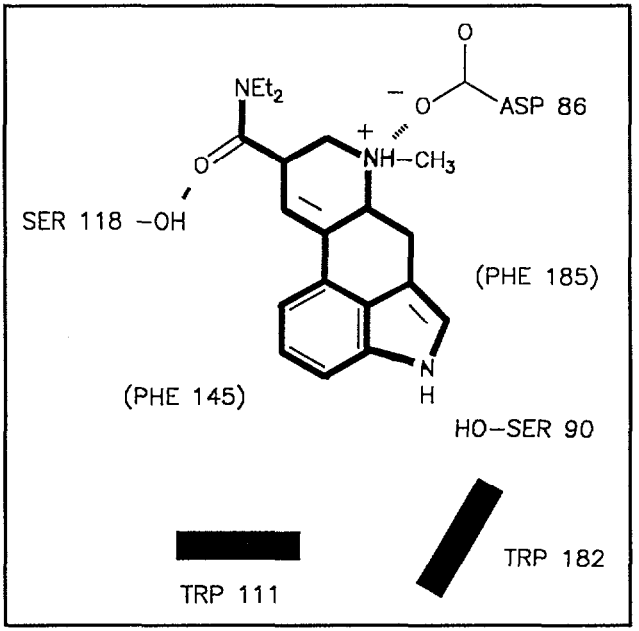

FIGURE 11. Schematic *of possible interactions between (+)LSD and features of a 5-HT_2 receptor model*

SUMMARY

Current evidence suggests that classical hallucinogens act via a 5-HT_2 (possibly 5-HT_{1C}) agonist mechanism. To gain further insight into the mechanism of action of these agents, the authors have attempted to model this drug-receptor interaction. Because of the unavailability of the 3-D structure of neurotransmitter receptors, it was necessary first to develop a hypothetical 5-HT_2 receptor model. By analogy to the structure of BRH and by utilizing pertinent information from other neurotransmitters, several 5-HT_2 receptor models were developed; one of these is shown in figures 5 and 6.

Using this model, the authors attempted to dock the structures of DOB and (+)LSD using DGEOM and GRID to aid this process. Docking also was guided by known SAR. (That is, the validity of any model rests with its ability to account for what is known about the binding of hallucinogens at 5-HT_2 receptors.) Two potential binding domains were identified (i.e., site I and site II, see figure 7). Several different modes of binding are possible within each of these sites. Figure 7, panel C shows one way in which DOB can bind at site I of the 5-HT_2 receptor model. A closeup view of the specific amino acid residues that may be involved in binding is shown as a schematic (figure 10). The proposed mode of interaction also accounts for the binding of (+)LSD and is consistent with established SAR. The proposed mode of interaction between drug and receptor is neither inflexible nor static. (That is, ligands with slightly different aromatic substitution patterns may still interact in an analogous manner with the receptor features that have been identified.) Furthermore, it must be emphasized that figures 10 and 11 represent one potential mode of binding of DOB and (+)LSD at site I of a single multiple-helix 5-HT_2 receptor model. Other modes of binding-indeed, other 5-HT_2 receptor models-are possible. Although the present drug-receptor model is certainly reasonable, not all other possible drug-receptor models have been fully evaluated yet.

The objective is to identify the most likely drug-receptor models using site-directed mutagenesis. For example, all models utilize the helix 3 aspartate as the ammonium ion binding site; thus, this aspartate must play a critical role in binding, and its elimination or replacement should result in reduced affinity. In collaboration with Dr. Shih, the authors now have demonstrated that replacement of this aspartate (but not the aspartate of helix 2) by asparagine (Asn) results in a dramatic decrease in affinity for various 5-HT_2 ligands. Similar studies are required to test other potential binding features consistent with the different models.

The classical hallucinogens bind at 5-HT_{1C} receptors with affinities comparable with the affinities for 5-HT_2 receptors. Indeed, there is less than a tenfold difference in 5-HT_{1C} versus 5-HT_2 affinities for a large series of DOB-related analogs (Glennon et al. 1992). Thus, any drug-receptor model developed for 5-HT_2 receptors should possess those (or structurally analogous) features found in 5-HT_{1C} receptors. In contrast, because classical hallucinogens (with the exception of (+)LSD) typically bind with low affinity at 5-HT_{1A} receptors, structural features common to 5-HT_2 and 5-HT_{1A} receptors would likely be unable to account for selectivity. One of several possible drug-receptor 5-HT_2 models is

described here in detail. The model accounts for, and is consistent with, much that is known about classical hallucinogens. Studies are under way to further validate this (and other) models. These studies, in addition to being relevant to the investigation of hallucinogenic agents, go well beyond the present limited scope of study and can have a significant influence on the understanding of drug-receptor interactions in general.

NOTE

All sequence numbers refer to the BRH numbering system for purposes of comparison and uniformity. The actual numbering system for the amino acid sequence of 5-HT_2 receptors is shown in figure 3.

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