Hallucinogens Acting at 5-HT Receptors: Toward a Mechanistic Understanding at Atomic Resolution

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INTRODUCTION

The cumulative scientific evidence, including new data discussed at the National Institute on Drug Abuse (NIDA) technical review meeting and presented elsewhere in this chapter, suggests that entire classes of compounds which elicit the complex array of psychotomimetic responses classifying them as hallucinogens shark specific actions on 5-hydroxy-tryptamine (5-HT) receptors and bind to 5-HT binding sites (for some additional recent reviews see Glennon 1990; Glennon et al. 1991; Peroutka 1991; Weinstein and Osman '1990*a* ; Weinstein et al. 1987). The correlation between affinity for 5-HT_2-type binding sites and receptors and the doses required to elicit hallucinogenic responses in humans has been demonstrated repeatedly (Glennon 1984, 1990). Of special interest in this context are similarities and differences in the pharmacological profiles of compounds belonging to the various families of chemical structures that yield hallucinogenic agents, that is, tryptamine (TRYP) derivatives (including the hallucinogenic N,N,-dimethyltryptamine), ergolines (including d-lysergic acid diethylamide [d-LSD]), and derivatives of phenylethylamine and phenylisopropylamine (including mescaline, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane [DOM], and methylenedioxymethamphetamine [MDMA]). These families of compounds are active both on the 5-HT_{1A} and 5-HT_2 receptor systems (Weinstein and Osman 1990*a*), but hallucinogenic action has been connected with partial agonism on 5-HT_2 receptors (Sanders-Bush et al. 1988) and more recently on 5-HT_{1C} receptors (Burris et al. 1991).

If one can understand what in the structures of the ligands is most directly involved in receptor activation and what receptor activation involves in terms of specific changes in molecular structure resulting from ligand binding, then one can design drugs to target such receptors and activate or inactivate them, as required. The actions of any other receptor ligands, the hallucinogens among #em, then could be understood and

countermanded by such specifically targeted and designed drugs. In addition to novel understanding of cellular signaling processes at the molecular level, the practical significance of this insight is therefore inherent to the potential it offers for targeted molecular design. Such designed compounds should provide new tools for investigation of the mechanisms of actions of hallucinogenic compounds and a promise of new therapeutic and preventive modalities.

The design of effective therapeutic agents with predetermined actions at specific receptors requires solid information on the pharmacology of the target compounds. A detailed understanding of the processes of drug-receptor interaction and the mechanisms involved in the evolution of pharmacological responses elicited by ligand-receptor interactions is also required (Jensen et al. 1990). All of these requirements are essential elements in rational design (Weinstein and Osman 1989*a*, 1989*b*, 1990*b*; Weinstein et al. 1987) and are subjects of the authors' studies (Weinstein and Osman 1990*a*; Weinstein et al. 1988). The resulting insights are summarized elsewhere; recent developments are reviewed briefly in this chapter. These insights and developments constitute the first steps in a novel understanding of the relationships between ligand structure, receptor structure, and receptor response.

This novel understanding is achieved at an atomic level of resolution from the analysis, modeling, and simulation of molecular mechanisms of drug-receptor interaction. Such computational simulations have become possible because of new information available on the molecular and structural biology of 5-HT receptors (Adham et al. 1992; Demchyshyn et al. 1992; Guan et al. 1992; Hen 1992; Julius et al. 1988, 1989; Shih et al. 1991) combined with insights from the molecular pharmacology of hallucinogens and other 5-HT receptor ligands (Mylecharane et al. 1989; Peroutka 1991).

An initial focus of the authors' work in the molecular and structural biology of the 5-HT receptors has been the computational modeling of the transmembrane helix bundle of the serotonin receptors of the 5-HT_2 subtype. This chapter describes success in the first steps of constructing macromolecular models of the membranal portions of the receptors because of a redefinition and refinement of structural criteria and functional considerations. These models are used to simulate computationally the effects of some receptor ligands binding inside the structure of the model receptor proteins under various conditions in order to explore the mechanisms of receptor activation.

The first aim of these computational simulations is to investigate the structural correlates of properties that affect the receptor proteins function. The current literature contains ample examples for simulations of this kind, usually performed on cytosolic proteins and water-soluble systems and leading to targeted experimental probing (e.g., through mutation studies) (Aqvist and Warshel 1992; Karplus and Petsko 1990; Levitt and Sharon 1988; Pascual-Ahuir et al. 1991; van Gunsteren et al. 1992; Weinstein 1986; Weinstein and Mehler 1992; Weinstein and Osman 1989*b*, 1990*b*).

The computational exploration of membrane protein systems is more complex because of methodological difficulties and uncertainties related to the representation of the protein in the membrane environment and the nature of the embedding of the entire system in a surrounding aqueous solvent (Ahlstrom et al. 1989; Milik et al. 1992; Stigter et al. 1992; Wendoloski et al. 1989; Xing and Scott 1992). Nevertheless, cautious computational studies performed on the transmembrane bundle components of the receptor structures, combined with the experimental probing, can be used as described below to define molecular correlates of pharmacological efficacy. In the context of computational simulations of receptor function, these insights can be evaluated further with known ligands exhibiting a range of efficacies at the 5-HT receptor subtypes.

Difficulties in the accurate description of the biophysical mechanisms further increase the importance of experimental validation of the inferences emerging from the computational simulations. Such validation depends on a concomitant probing of all the resulting pharmacological models and predictions in collaborative efforts, including experimental determination of the pharmacological profiles of compounds acting at the 5-HT receptor subtypes. As illustrated by examples from recent publications, the pharmacological assays used in the authors' collaborative studies have included measurements of responses mediated by the 5-HT_1A, receptor coupledto adenylate cyclase in membrane preparations from rat hippocampus (DeVivo and Maayani 1990), the contraction of the isolated rabbit aorta mediated by 5-HT_2 receptors (Ben-Harari et al. 1991; Christ et al. 1990; Mahle et al. 1990), and the response to 5-HT_1C receptors coupled to the hydrolysis of phosphatidyl inositol in selected areas of the brain (Burris et al. 1991). In addition, the collaborative experimental studies probe the modulation of agonist binding in various brain sections by receptor-guanosine triphosphate binding protein (G-protein) interactions to learn about the relations between receptor selectivity in vitro and the likelihood that the agonists

will elicit responses on multiple receptors in the brain (Yocca et al. 1990). The results are analyzed for clues on the chemical determinants required for recognition (binding) and activation (efficacy) for each of the two receptors.

The combination of computational simulation studies and experimental verifications is expected to yield a quantitative relationship between the molecular structures of such ligands and their efficacy in activating the receptor. Such a mechanistic understanding of structure-function relationships will lead to new molecular structures that will be selective for 5-HT receptor subtypes and have a predicted degree of activity on these receptors. The following sections provide a brief overview of recent results from such studies.

CONSTRUCTION AND EXPLORATION OF MOLECULAR MODELS FOR THE TRANSMEMBRANE PORTION OF 5-HT RECEPTORS

Identification of an Appropriate Template

The construction of molecular models for the transmembrane region of the 5-HT receptor subtypes by homology modeling and energy refinement requires specific structural templates. The molecular architecture of bacteriorhodopsin (BR) and the photosynthetic reaction centers (PRCs) is commonly regarded as a model for membrane proteins (Rees et al. 1989). In particular, BR has been offered as a structural template for the three-dimensional (3-D) structure of membrane receptors that are functionally coupled to regulatory guanine nucleotide-binding proteins (G-proteins) (Findlay and Eliopoulos 1990).

More recently, specific molecular models of such G-protein coupled receptors (GPCRs) were constructed on the basis of the functional and structural relation of rhodopsin to BR and the sequence homology between rhodopsin and the GPCR (Dahl et al. 1991; Hibert et al. 1991). Such models of GPCRs suffer from the apparent lack of any significant degree of sequence homology between the seven transmembranal helices (TMHs) of BR and the portions in the sequence of the various GPCRs that are considered their transmembrane domain (TMDs). However, strong arguments have been proposed in favor of the structural relation between BR and the opsins and, hence: the GPCR (Lefkowitz 1991).

These arguments prompted the authors' investigation of the possibility that the true sequence homology, including any existing similarity in the distribution of kink-inducing proline (Pro) among the helices, might have been obscured by the assumption that the TMHs maintained their sequential order from BR in the evolution of the mammalian proteins. With a definition of the TMHs in the neurotransmitter GPCRs guided by hydropathicity predictions and additional criteria used to define the span of each helix, an optimal alignment is achieved for each pair of sequences calculated with no gaps allowed in the matching (Pardo et al. 1992). This proposed alignment reveals considerable homology between the TMHs in BR and those in GPCRs, if the sequential order of the helices is ignored. The findings point to the possibilities of modifying the BR template to account for the correct packing of the helices in the tertiary structures of GPCRs.

This strategy was followed in the construction of a 3-D model of the neurotransmitter GPCR of 5-HT_2 on the basis of specific interhelical interactions such as those observed in BR and in PRC as well as on additional criteria. The main advantage of the resulting modified template for the modeling of GPCRs based on the nonsequential homology to BR is that it is possible to incorporate several additional criteria in the construction of the helix bundle at the level of protein sequence comparisons as well as at the level of helix-helix and helix-membrane interactions. Such considerations (described in the next section) should improve the quality of 3-D models of GPCRs and facilitate inferences on the mechanisms by which these proteins perform their biological functions.

ANALYSIS AND REFINEMENT OF THE CRITERIA FOR PREDICTING THE STRUCTURE AND RELATIVE ORIENTATION OF TRANSMEMBRANE HELICES IN MODELS OF THE NEUROTRANSMITTER RECEPTOR

The membrane-spanning domain of the serotonin GPCRs is considered to share at least the general topology of a seven-transmembrane helix bundle observed in BR. As a result of the significant homologies that were found (as described above, by allowing for a rearrangement of the helix-bundle template), the strategy for building the model for the 5-HT_2 receptor has been to construct hypotheses concerning helix-helix interactions, their orientations, and their arrangement in bundles surrounded by lipid based on inferences and observations from the

3.5 angstrom (Å) resolution structure of BR (Henderson et al. 1990). Criteria resulting from such considerations are being tested against the 2.3 Å resolution Structure of the PRC from *Rhodobacter viridis* (Deisenhofer and Michael 1989).

These comparisons led to a reevaluation of some of the methods available for the prediction and relative orientations of membrane-embedded α-helices (Ballesteros and Weinstein 1992*a*). The authors found that methods used in the construction of helical transmembrane domains could be misleading; e.g., by assuming that the arginine (Arg) and lysine (Lys) residues that appear preferentially at the cytoplasmic end of the transmembrane helices of BR and PRC must be inside the cell because of their polar nature. More careful structural considerations would place only the ends of these residues close to the phospholipid headgroups of the membrane, with the rest of the residues inside the lipid portion. Thus, the intramembrane helix "gains" 1 to 1.5 turns, and the span is redefined by 3 to 7 residues (Ballesteros and Weinstein 1992*a*). Because the orientation of the p-carbon atom positions the Arg-Lys side chains in a direction pointing from the C-terminus toward the N-terminus of the helix, the inaccuracy in determining the helix span caused by the Arg-Lys residues affects the helices traversing the membrane from inside to outside (i.e., N-terminal to C-terminal) more than it affects those oriented inward.

Consequently, the inaccuracy can lead to significant errors in packing the helices against each other in the transmembrane bundle and thus induce errors in the construction of models for these structures. Therefore, these considerations will have a significant effect on the results of methods commonly used in constructing such models, including the criteria based on hydrophobicity profiles, hydrophobic moments, helix amphiphilicity, and charge neutralization.

Another consideration centers on the structural role of the kinks induced by the presence of Pro residues in the a-helix. (For some recent reviews of these structural properties of Pro-containing helices and their possible consequences for protein function, see MacArthur and Thornton 1991 and Williams and Deber 1991.) Because the region of the Pro-induced kink does not have a 3.6 amino acids/turn periodicity, one would expect a twisting of the faces before/after the Pro-kink compared with the case of a straight helix. Such an effect would vitiate the profiles for amphiphilicity and hydrophobic moments of Pro-containing helices because the

algorithms for the calculation of these properties assume that the helices are regular and straight.

Yet, such profiles are commonly used for the prediction/modeling of transmembrane helices without special attention to the effects of Pro-induced kinks. Consequently, the modeling approaches will require modification according to the authors' findings. The proposed refinements from the considerations presented above overcome some of the shortcomings inherent in the use of the above-mentioned methods; they constitute an additional guide for identifying the cytoplasmic end of a transmembrane helix. Importantly, such criteria can be explored quantitatively from computational simulations after a specific mechanistic model has been proposed.

The authors have recently described such a model for a dynamic activation mechanism in the function of a transmembrane protein based on the structural properties induced by the presence of Pro residues in key transmembrane helices (Ballesteros and Weinstein 1992*a*,*b*). The model takes advantage of the following properties of the kink region: A Pro residue within a helix disrupts two 1-4 backbone H-bonds: the $C{=}O_{i\text{-}4}...CN_i$ due to the imide functional group replacing the amine and the $C{=}O_{i\text{-}3}....HN_{i+1}$ due to the axial kink. This can generate up to three putatively H-bond-free reactive sites around the kink region, the C=Os being unusually exposed (Woolfson and Williams 1990). Most charged and polar side chains in transmembrane domains are likely to be involved in extensive H-bonding because of the low dielectric constant of the transmembrane domain. Consequently, the three types of reactive sites in the backbone should be expected to make up a significant percentage of the total number of free H-bonding donors or acceptors within the transmembrane region.

The involvement of such available polar sites in the function of the proteins has been illustrated recently in the structure of the bacterial aspartate receptor where the crystal structure of the binding domain shows the ligand binding site to include the -C=O groups exposed by a Pro-induced kink (Milburn et al. 1991) and by the structure of a bacterial Ca^{2+}-channel crystallized in nonpolar solvents (Karle et al. 1991).

The putative gating mechanism for a channel formed by the transmembrane arrangement of a bundle of helical leucine (Leu)-zervamicin peptides that the authors proposed recently (Ballesteros and Weinstein 1992*b*) is based on the likely dynamic properties of the

structure containing Pro-induced kinks in a transmembrane helix. These dynamic properties are induced by the Pro-kink which, as shown below from a molecular dynamics simulation of the 5-HT_2 receptor model, is likely to be involved in the propagation of the structural response of the receptor protein to ligand binding. Thus, the dynamic mechanism supported by the special structure around a Pro site in a helix is likely to be involved in the relay of information about ligand binding into the transmembrane structure connected to the effector (i.e., a G-nucleotide binding regulatory protein). This mechanism requires a low activation energy as illustrated by the structure and dynamics of the Pro-containing helix of melittin (Bazzo et al. 1988; Pastore et al. 1989) and thus provides a reasonable mode for the transduction of a signal produced by ligand binding. Such mechanistic hypotheses for receptor activation, anchored in the structural and dynamic properties of a 3-D model of the receptor protein, are amenable to exploration by the methods of theoretical biophysics used in the computational simulations described briefly below.

A Three-Dimensional Structural Model of the 5-HT_2 Receptor

The authors have constructed a complete 3-D model of the TMD of the 5-HT_2 receptor at atomic resolution. Preliminary molecular dynamics simulations also have been carried out on the receptor model and its complexes with ligands, including full agonists, partial agonists, and antagonists. The results suggest an activation mechanism of the receptor based on the structural response of the receptor to the binding of ligands. Both the local effects at the recognition site, which produce the activation trigger, and the distal effects, which are propagated from the recognition site into the receptor region responsible for coupling to the effector, are identifiable from the analysis of the dynamic behavior of the ligand-receptor complex.

The 5-HT_2 receptor model was constructed based on specific criteria from the following considerations: ligand, structure-affinity relationships developed for pharmacologically well-characterized ligands, structural features of membrane proteins for which structures are available at atomic resolution (BR and PRC-see above), and results of studies on the molecular biology of GPCRs.

The construction of the 5-HT_2 receptor model and the first computational simulation of the dynamic behavior of the transmembrane helix bundle in the presence and absence of ligand bound at the putative recognition site are described in detail elsewhere (Zhang and Weinstein 1993). The main

considerations in the construction of the model and the qualitative features of the results from the computational simulations are outlined briefly here.

Ligand Binding Site. As discussed in detail elsewhere (Weinstein and Osman 1990*a*), the nature of the interaction between ligands and recognition sites in the various subtypes of 5-HT receptors is expected to be *different*, consonant with the differences in ligand selectivity of the receptor subtypes. The main molecular property related to recognition at 5-HT_{1A} receptors was shown to be the directional character of the electrostatic potential generated by the ligands in the molecular region corresponding to the indole in 5-HT. The corresponding recognition site in the receptor was predicted to have properties of a positively charged (imidazolium) form of the side chain of a histidine residue (Mercier et al. 1988, 1989; Weinstein and Osman 1989*b*, 1990*a,b*).

From studies of model proteins incorporating the type of residues proposed for the recognition site in the structural arrangement required to be selective for 5-HT_{1A} ligands, the mechanism of recognition at the 5-HT_{1A} receptor was shown to be electrostatic and conducive to a triggering of the receptor response through the change in the electronic structure of the imidazolium recognition site when it interacts with an activating ligand (agonist) (Weinstein and Osman 1990*u*). This effect was shown to induce a proton transfer from the ring to a neighboring residue to which it can be hydrogen bonded in the resting state (Mercier et al. 1988, 1989; Weinstein and Osman 1990*b*).

However, a different model was proposed for selective recognition at the 5-HT_2receptors based on structural details of 5-HT-binding peptides (Weinstein and Osman 1990*a*). The recognition site in the 54-HT_2 receptor was thus proposed to consist of two aromatic residues separated by a hydrophilic residue.

In contrast to the model for 5-HT_{1A}, the recognition was considered to be based on the interaction of neutral molecules with the stabilization provided by dispersion forces (Weinstein and Osman 1990*a* and references therein). These inferences from structure-activity relationships (SAR) and specific calculations of molecular complexes (see Weinstein and Osman 1990*a* and references therein) were incorporated in the construction of the receptor model.

Putative residues of the ligand binding site in the 5-HT_2 receptor sequence were determined according to the requirements for matching recognition sites in the ligand binding pocket based on specific models proposed from SAR studies (Glennon et al. 1991; Weinstein and Osman 1990*a*); the specific sequence of amino acids in the receptor subtype and its relation to BR (Pardo et al. 1992) and the other 5-HT receptors (Adham et al. 1992; Hartig 1989; Hen 1992), and published results of GPCR structures modification using methods of molecular biology, including point mutations and chimeric receptors of various types (Guan et al. 1992; Shih et al. 1991; Strader et al. 1989). The positions of the putative residues of the ligand binding site were used as constraints in positioning the TMHs relative to one another.

The ligand binding sites are assumed to be aspartic acid (Asp) 133 (TMH3) to interact with the protonated amine group of the ligand; side chains of phenylalanine (Phe)-218 and Phe-222 (TMH 5) to interact with the aromatic rings of the ligand; methionine (Met)-313 (TMH 6) to match the region of the ligand corresponding to N1, C7 of the indole ring in 5-HT; and serine (Ser)-350 (TMH 7) to interact with the region corresponding to the 5-OH of 5-HT.

Assembly of the Helix Bundle. With 5-HT as the template of ligands, TMH 3,5, 6, and 7 were positioned so that the residues in the putative ligand binding site could interact with the key groups of 5-HT as mentioned above. These initial positions of the TMHs were refined by the guideline of maximizing interactions between conserved residues. The position of TMH 2 is determined further by possible interaction between conserved residues, specifically Asp-98 (TMH 2), Ser-140 (TMH 3), and asparagine (Asn)-354 (TMH 7). The position of TMH 1 is determined by allowing for an interaction between Asn-79 (TMH 1) and histidine (His)-143 (TMH 3), which also interacts with Asp-98 (TMH 2). Such a positioning of TMH 1 relative to TMH 2 is based on the assumption that conserved residues in a receptor sequence play an important role in determining the structure and function of the receptor and that their interaction is essential for accomplishing this function. Consequently, the conserved residues, like the polar residues, were placed in the interior of the assembled helix bundle to the fullest extent possible.

Molecular Dynamics Simulations. After the assembly of the seven TMHs, energy minimization and dynamics simulation were run to optimize the structure of the model. Average structures were obtained

from the simulations, energy was minimized, and then the average structures were used as the starting structures for studies on the interaction between the receptor model and ligands of the receptor. Ligands were roughly docked into the putative ligand binding site with nuclear overhauser effect (NOE)-type constraints to the ligand recognition sites. Molecular dynamics simulations were applied again to obtain the structure of model/ligand complexes and to study the interactions between ligands and the model.

All the molecular dynamics simulations on the model were performed with the CHARMm program, following protocols similar to those described in other publications (Mehler and Weinstein 1990; Pascual-Ahuir et al. 1991) but with a fixed dielectric permittivity constant of $\epsilon = 4$. The energy-minimized average (more than 20 picoseconds [ps]) of a final equilibrated structure was taken as a starting structure for studies of the interaction between the receptor model and various ligands.

The detailed analysis of the ligand-receptor complexes is expected to yield information on the mode of ligand recognition expressed as the physicochemical nature of the complex between the ligand and the recognition sites as well as on the mechanism by which the response of the receptor to ligand binding is triggered and then propagated toward the effector. The resulting activation mechanism from such a mode of ligand recognition and binding is based on a structural rearrangement propagated from the recognition site into the receptor protein structure connected to the portion that interacts with the effector protein. These inferences were explored with the computational simulations of the dynamics of the modeled transmembranal region of the 5-HT_2 receptor.

Dynamics of the Receptor Response to Ligand Binding. The conformational changes of the receptor model induced by the interaction with ligands were analyzed from the root mean square (rms) deviation of the alpha-carbon (C-α) atoms of the complexes with respect to the starting structures to obtain information on the structural response of the receptor to the binding of the ligand. The preliminary results indicate that the patterns of the distribution of rms are different for the three complexes of full agonists, partial agonists, and antagonists, suggesting that different types of the ligands induce different types or degrees of conformational changes of the receptor model (Zhang and Weinstein 1993). In the results from initial simulations, these conformational changes were found to be consistent with experimental results, most

significantly by their concentration in the region connecting TMHs 5 and 6.

Mutation experiments and the pharmacology of chimera from different' receptor subtypes have indicated that the beginning and ending regions of the third intracellular loop are important for GPCRs to interact with G-proteins and that the regions that affect the binding of the activating ligands are the extensions of TMH 5 and TMH 6 in the intracellular side (Strader et al. 1989; Kjelsberg et al. 1992).

Therefore, it is reasonable to expect that the binding of an agonist would induce the most significant conformational changes in these two helices and that the binding of a partial agonist also would induce conformational changes similar to, but less significant than, those induced by an agonist. The binding of an antagonist should not induce conformational changes of this kind. All these expectations were met by the first results from the computational simulations (Zhang and Weinstein 1993).

Thus, figure 1 illustrates a comparison between the averaged structures obtained from a simulation of the receptor model and a simulation of the receptor with a 5-HT ligand in the recognition site described above. The initial position of the 5-HT molecule is indicated in the same shade as the structure of the receptor without the ligand (light grey), but the molecular dynamics run had been performed in the absence of ligand. The structures of the helices and the ligand outlined in solid black represent the result of the simulation of the receptor-ligand complex. It is evident that the position of 5-HT in the recognition pocket changes significantly during the simulation (compare the position of the initial [gray] structure of 5-HT with that of the final black one).

Although the interaction with Asp-133 is maintained at the same distance, there is considerable rearrangement around the flexible side chain with a resulting strengthening of the interactions indicated in the description of the recognition site. However, the overall orientation of the indole ring remains similar to the starting point, and the planes are nearly parallel. This finding is in striking contrast to the results obtained with TRYP as a ligand (figure 2), where a "flip" of the indole ring positions the bond dipole of the N-H group in the indole in roughly the same position relative to the recognition site that the -OH group occupies in the complex with 5-HT (Zhang and Weinstein 1993).

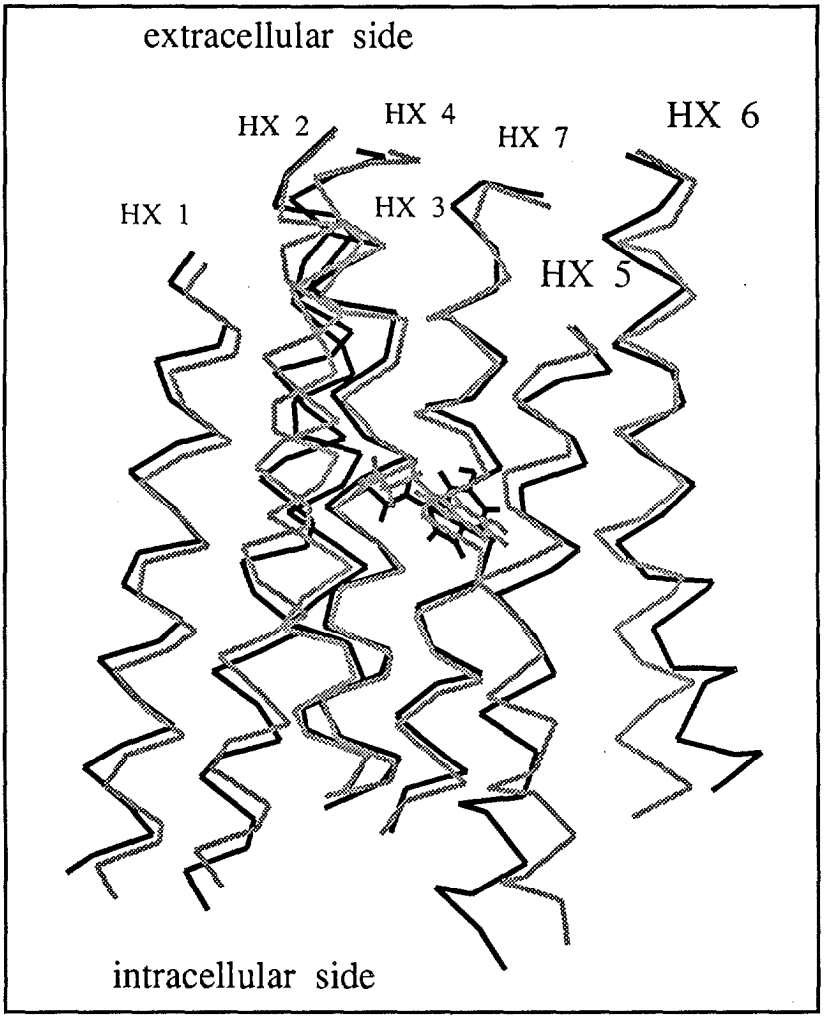

FIGURE 1. *Comparison of the structures of the molecular model of the transmembrane helix bundle of the 5-HT$_2$ receptor in the absence and presence of 5-HT. The C-a ribbon structures of the helices are shown in a view parallel to the membrane bilayer. The structures represent the results from molecular dynamics simulations (see text) of the receptor model without ligand (40 picosecond average structure representing 215 picosecond run, in shaded gray) and in the presence of 5-HT bound in the recognition site (snapshot at 65 picoseconds, in solid black). The initial position of 5-HT in the receptor is indicated in the gray shade; the position after dynamics simulation is shown in solid black.*

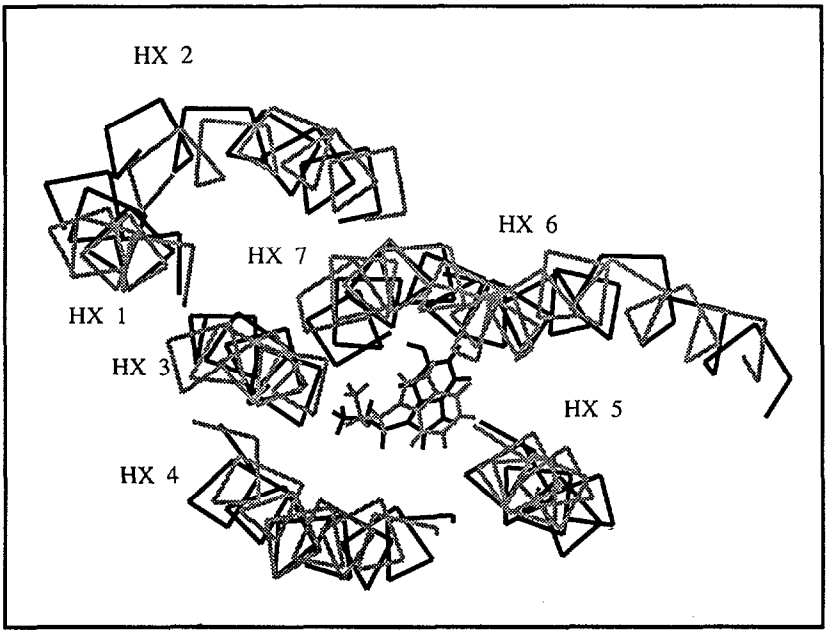

FIGURE 2. *Comparison of the structure of the 5-HT$_2$ receptor model in the presence of different ligands bound in the recognition site: 5-HT (snapshot at 65 picoseconds, in solid black) and TRYP (snapshot at 115 picoseconds, shaded gray). The view is perpendicular to the plane of the membrane, from outside the cell. See text and figure I legend for other details.*

The structural rearrangement in the helix bundle also is identifiable qualitatively from the comparison in figure 1. The Cα ribbons of helices 1, 2, 3, 4, and 7 are similarly arranged, whereas those of helices 5 and 6 show clear differences in the lower half pointing toward the cell interior. It is noteworthy that the major rearrangements in the structures of the two helices occur after the Pro-kink that exists in both. All the helices move during the dynamics simulation, but the major changes occur only in helices 5 and 6.

The differences in the structures of these helices in the presence and absence of the ligand are recognized in the dynamic averages from the results of the simulation, showing that the binding of the ligand has induced a force that causes the distortion in the positions of these two helices more than for the others in the bundle. The involvement of only

helices 5 and 6 at this level of comparative analysis (for more extensive details and analyses see Zhang and Weinstein 1993) is significant because these helices are the intramembrane ends of the cytoplasmic Loop III that constitutes the major structural element in the interaction of the receptor with the effector system (Kjelsberg et al. 1992; Strader et al. 1989).

As a probe of the validity of this receptor model, it is most gratifying that the main structural response to ligand binding seems to be in the region required for transmission of the signal to the effector system. The strong correlation between the structure of this region and ligand binding has been demonstrated from mutation studies in the general area of the loop (Strader et al. 1987) and has focused more specifically on one residue in that region (Kjelsberg et al. 1992).

Further support for the relation between the structural rearrangement caused by the ligand binding in the recognition site and the mechanism of receptor activation toward interaction with the effector is provided by the correlation the authors observed between the extent of the rearrangement and the known pharmacological efficacy of the ligand (Zhang and Weinstein 1993). This is illustrated in figure 2, which shows the comparison between the receptor helix bundle in the presence of the full agonist 5-HT and the partial agonist TRYP. The view of the helix bundles in figure 2 is perpendicular to the plane of the membrane. Careful inspection reveals that the bundles representing complexes with 5-HT (solid black) and TRYP (gray) differ little with the exception of helix 6. In fact, helix 6 in the TRYP-bound receptor is not significantly distorted from its position in the free receptor. Because 5-HT distorts helix 6, the superposition shown in figure 2 identifies a difference in that region. On the other hand, helix 5 superimposes well from the dynamics of the receptors with 5-HT and with TRYP, showing that the distortion produced by 5-HT (see above) is similar to that induced by the binding of TRYP. Overall, TRYP induces less distortion in the structure of the Loop III region than 5-HT does, consonant with its lower efficacy indicated by the pharmacological property of partial agonism.

A computational simulation of the receptor complex with the structurally related 5-HT_2-receptor antagonist gramine (not shown here) is remarkable in that ***no distortion*** in the average structure of the receptor bundle is observed in the key regions of helices 5 and 6 (Zhang and Weinstein 1993). Similar results are being obtained in preliminary studies with the 5-HT_2 antagonist ketanserin and with the hallucinogenic DOM, which behaves in this simulation in accordance with its pharmacological profile

as a partial agonist. Therefore, it is clear that a coherent outline of the molecular mechanism of ligand recognition and receptor response to ligand binding is emerging from the computational simulations with the present model of the transmembrane helix bundle of the 5-HT_2 receptor. The qualitative features of this mechanism of receptor function at atomic resolution are in good agreement with the molecular pharmacology of the receptor.

CONCLUSION

The major work of probing against experimental data the inferences obtained here for the first time from the simulation of the dynamic response of a receptor model to the binding of structurally related ligands is still ahead. To validate the model and the inferences to produce a reliable description of the molecular pharmacology of hallucinogens, it is necessary to extend these exciting preliminary findings to agonists, partial agonists, and antagonists in different chemical classes and to relate the structural effects of ligand binding to a reliable mechanistic hypothesis at atomic resolution. Such developments would permit specific physicochemical testing of the mechanisms and examination in detail of the behavior of hallucinogenic compounds in models of this type for other relevant receptor subtypes (e.g., 5-HT_{1C}).

These aims constitute only some of the exciting tasks that can be addressed by computational simulations with the 3-D receptor model. Such projects offer a comprehensive analysis of fundamental aspects of mechanisms of drug-receptor interactions and receptor function described at atomic resolution and also offer arguably the best hope of interfering with and/or exploiting the properties of hallucinogenic substances based on rational mechanistic means and specifically designed medications.

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