On the Structure and Activity of Membrane Receptors: A Computational Simulation of Ligand-Triggered Activation in a Model 5-HT_{1A} Receptor

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ABSTRACT

The relation between receptor structure and the mechanism by which ligands with different pharmacological efficacy elicit a response is analyzed in a three-dimensional molecular model of the human 5-HT_{1A} receptor [Pardo et al., J. Biomed. Sci. **3**, 98 (1996)]. According to the model, the main interaction of the endogenous neurotransmitter serotonin (5-HT) to the human 5-HT_{1A} receptor consists of (*i*) the ionic interaction between the protonated side-chain amine of 5-HT and the conserved Asp-116, located in transmembrane helix (TMH) 3; (*ii*) the hydrogen bond between the 5-OH group of 5-HT and Thr-199, located in TMH 5; and (*iii*) the complex between the aromatic indole ring of 5-HT and His-192, located in TMH 5. Ab initio quantum chemical calculations were used to position ligands in molecular models of the binding pocket of the 5-HT_{1A} receptor consisting of these interacting residues. The consequences of the interactions between the ligands and the proposed recognition sites of the 5-HT_{1A} receptor, reflected in the electronic structure of the complexes, suggest a mechanism by which the receptor activation is triggered by ligand binding. Results from the computations show a more favorable interaction of the aromatic ring of 5-HT (or of the 5-HT_{1A} selective agonist

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8-OH DPAT) with the protonated form of His-192 than with the neutral form. The change in the reactivity of the imidazole ring then leads to the attraction of a proton from another site in the receptor: Arg-175 in TMH 4. This proton transfer to His-192 that is triggered by the interaction with ligand is facilitated by Asp-191 in TMH 5, as shown by energetic considerations. The position of the ligand recognition pocket in the transmembrane bundle of the 5-HT_{1A} receptor suggests that the ligand-induced proton transfer may cause a conformational change in the tertiary structure of the receptor that could be transmitted toward the intracellular end to facilitate the transmission of the signal.

Introduction

The process of signal transduction into the cell, meditated by G-protein-coupled transmembrane receptors (GPCRs), involves the formation of the ligand–receptor complex. A significant and spectacular advance in the study of the signal transduction process was the discovery of mutant receptors that are constitutively active [1–3], i.e., they are capable of transmitting a signal into the cell in the absence of the extracellular ligand. These findings suggested that the mutation allows the GPCRs to adopt a conformation corresponding to the activated state which is intrinsic to their molecular structure and is stabilized either by ligand binding or by mutation. Furthermore, it has been determined for Rhodopsin (RHO), one of the experimentally best characterized GPCR to date, that upon visible light absorption the *11-cis* retinal bound to RHO undergoes rearrangement to the intermediate states labeled bathorhodopsin, lumirhodopsin, metarhodopsin I, and metarhodopsin II (see [4] for a review). The end state of these molecular processes corresponds to an active state of RHO (the metarhodopsin II intermediate) which is able to couple with the G protein transducin to form the ternary complex. The structural similarity between RHO and other GPCRs suggests functional similarities, so that multiple equilibria may be a common feature in GPCRs. Thus, the available experimental data suggest that agonist–receptor complexes undergo rearrangement to one or several intermediates through a series of processes that are transmitted to the cytoplasmatic domains of the receptor, facilitating the binding of the agonist-bound receptor to the G protein.

To understand the mechanisms of receptor activation involving such rearrangement in a structural context, molecular models of GPCRs have been constructed by computer-aided model-building techniques (see [5] for a review). The nature of the mechanisms underlying ligand–receptor interaction and producing receptor activation can be explored from such models (e.g., [6, 7]). The precise molecular definition of properties commonly determined by pharmacological techniques including potency, intrinsic efficacy, and efficacy requires an understanding of the set of molecular processes that connect the ligand binding process to the activation of the receptor [8, 9].

We present here a study of the relation between receptor structure and the mechanism of response elicited with different efficacy by specific agonists, using a three-dimensional (3-D) model of the 5-HT_{1A} receptor (5-HT_{1A}R). The 5-HT_{1A}R model has been reported previously [10] and comprises seven transmembrane helices (TMH). We describe here the results of quantum mechanical and molecular mechanics calculations applied to study the recognition of ligands in this 5-HT_{1A}R model. The findings define a geometry for the consensus recognition pocket in the 3-D structure of the 5-HT_{1A}R and suggest mechanistic hypotheses regarding key structural features of both the agonists and the receptor that are responsible for the binding of the ligand to the receptor and for triggering the signal transduction mechanism.

Methods

A MODEL OF THE 5-HT_{1A} RECEPTOR

Computational methods for modeling and testing GPCR structure have been reviewed recently [5]. The specific 3-D model of the transmembrane domain of the 5-HT_{1A}R used here (see Fig. 3 in [10]) was constructed by computer-aided model building techniques as described elsewhere [10]. The main criteria used for modeling this specific GPCR included (*i*) the amino acid sequence [11–13]; (*ii*) topological criteria guided by inferences from sequence homologies [10, 14]; (*iii*) the

electron density projection map of bovine RHO obtained from electron microscopy [15]; (*iv*) predictions of helix–helix interactions in GPCRs [16, 17]; (*v*) the relevance of the extracellular disulfide-bonded cysteines in maintaining the tertiary structure of the receptor [18] and in forming the ligand binding site [19]; and (*vi*) directional constraints expressed in the various proposed hydrogen bonds between agonists and residues in TMHs 3 and 5 and between antagonists and residues in TMHs 3 and 7 constructed on the basis of structure–activity relationships [8, 10, 20, 21] and experimental results from site-directed mutagenesis on the 5-HT$_{1A}$R [22, 23] and other GPCRs that bind protonated amine neurotransmitters [24–29]. Other models of 5-HT$_{1A}$R have been proposed (e.g., see [30]).

A RECEPTOR MODEL FOR AGONIST RECOGNITION

The mode of recognition in the receptor of either 5-HT or the selective 5-HT$_{1A}$ ligand 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH DPAT) was determined by energy minimization of the ligands inside a model constructed from propanoate (representing Asp-116, SITE I, TMH 3), isopropanol (representing Thr-199, SITE II, TMH 5), 5-ethylimidazole (representing His-192, SITE III, TMH 5), and two ethane groups (representing, respectively, Ile-113 in TMH 3 and Ile-196 in TMH 5). These molecular fragments were positioned at the sites occupied by the C_α and the side chain of each of the putative residues of the ligand binding site. The Ile residues, with the isopropyl side chain replaced by a methyl, were included in the calculation to define the size of the recognition pocket (see [10]).

A MODEL OF RECEPTOR ACTIVATION

The model system to study the proton-transfer processes proposed as a trigger of receptor activation is composed of 5-ethylimidazole (His-192, SITE III, TMH 5), methylguanidinium (Arg-175, SITE IV, TMH 5), and propanoate (Asp-191, SITE VI[†], TMH 5), together with either the indole or phenyl ring of 5-HT or 8-OH DPAT. The C_α of Asp-191 and His-192, and the indole or phenyl ring, were kept fixed at the position previously obtained in the optimization of the recognition mode [see above]. A linear hydrogen bond between His-192 and Arg-175 at the internuclear N $\cdots$ N distance of 3.0 Å was also constrained. In the calculations of the ligand-aided proton-transfer mechanism, E_{react} represents the energy of reaction of the proton transfer from Arg-175 (SITE IV) to His-192 (SITE III) and is defined as the difference in energy between the initial guanidinium/imidazole ($GNDN^+/IM$) and the final guanidine/imidazolium ($GNDN/IM^+$) minima.

[†]Asp-191 is denoted SITE VI because SITE V is reserved to Asn-385 in TMH 7, a binding site of antagonists at the 5-HT$_{1A}$R (see [10]).

COMPUTATIONAL APPROACHES

Energy minimization of the macromolecular system was performed by molecular mechanics calculations with the CHARMM21 system of programs using default parameters [31]. The parametrization of 5-HT and 8-OH DPAT ligands was taken from the default parameters, and the charge distribution over atoms was calculated from a natural population analysis [32] with the 6-31G basis set. Quantum mechanical calculations were performed with the GAUSSIAN system of programs [33]. The structures of the isolated ligands (5-HT and 8-OH DPAT) were obtained from total geometry optimization with the semiempirical AM1 Hamiltonian [34]. Semiempirical structural optimizations of the complexes of 5-HT or 8-OH DPAT with the model binding site were carried out with the AM1 Hamiltonian [34]. The proton-transfer process proposed as a trigger of receptor activation was calculated ab initio with structural optimizations at the Hartree–Fock (HF) level with the standard 6-31G basis set. Electron correlation energy was calculated with both the 6-31G and the 6-31G* basis set at the MP2 level.

The calculated energies of interaction, E_{int}, for the complexes of the indole moiety of serotonin and the ionic and tautomeric forms of His-192, were decomposed with the method of Morokuma [35] into electrostatic (E^{ES}), polarization (E^{POL}), charge transfer (E^{CT}), exchange repulsion (E^{EX}), and residual energy (E^{MIX}) contributions. The Morokuma decomposition of E_{int} was carried out with the GAUSSIAN 80 UCSF program [36].

Results and Discussion

STRUCTURE OF THE LIGANDS

5-HT comprises an indole ring, a hydroxyl group, and a short ethylamine side chain (see **1** in

Table I). The protonated form, at the amine moiety of 5-HT, is predominant at physiological pH. The dihedral angles τ_1, τ_2, and τ_3 for the ethylammonium side chain and τ_4 for the hydroxyl group of 5-HT (see legend of Table I for notation) define the conformational parameters of the ligand. Variations in τ_3 correspond to the different conformations of the ammonium group. Structure–activity relationships centered on congeners of 5-HT [10, 21] revealed that the 5-OH hydroxyl group adopts the *cis* conformation relative to the C_5—C_6 bond ($\tau_4 = 0°$, see Table I for notation) in the interaction with the 5-HT_{1A}R sites. Thus, only conformational species in the proximity of $\tau_4 = 0°$ were calculated. The structure of 5-HT was obtained from total geometry optimization with the semiempirical AM1 Hamiltonian. Table I summarizes the dihedral angles τ_1 to τ_4 of the different 5-HT conformers. We were able to detect the *gauche,trans* ($\tau_1 = 73.4$, $\tau_2 = 187.1$) and *gauche,gauche* ($\tau_1 = 81.6$, $\tau_2 = -53.0$) conformations. There were no significant variations in τ_4 for these conformational species (see Table I). The *gauche,trans* conformation was found to be energetically less stable than was the *gauche,gauche* conformation by 3.8 kcal/mol.

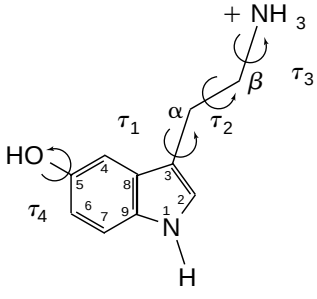
8-OH DPAT comprises a phenyl ring, a hydroxyl group, and a (di-*n*-propylamino)tetralin ring. The protonated form, at the amine moiety of 8-OH DPAT, is predominant at physiological pH. The binding of 8-OH DPAT to the 5-HT_{1A}R sites is stereoselective. The R form of 8-OH DPAT is a potent and full agonist [37, 38]. Table I shows the conformational parameters τ_0 to τ_4 (see Table I for nomenclature), with the semiempirical AM1

TABLE I

Dihedral angles (degrees) τ_0–τ_4 (see below for notation) of 5-HT and 8-OH DPAT in the receptor-binding conformation (see Fig. 2 for the drug-receptor complexes) as well as for the isolated ligand and optimized intermolecular hydrogen bonds distances (Å) between the protonated amine of the ligand and both O^{δ} (N — O^{δ}) of Asp-116 (SITE I; TMH 3), and the hydroxyl group of the ligand and O^{γ} (O — O^{γ}) of Thr-199 (SITE II; TMH 5).

Ligand	SITE III[a]	τ_0	τ_1	τ_2	τ_3	τ_4	O — O^{γ}	N — $O^{\delta 1}$	N — $O^{\delta 2}$
5-HT (*gauche,trans*)	[b]	—	67.1	188.9	179.7[c]	−2.8	—	—	—
5-HT (*gauche,gauche*)	[b]	—	81.6	−53.0	171.7[c]	−6.6	—	—	—
5-HT	HIS +	—	66.9	162.3	163.4[c]	14.0	3.29	2.63	2.65
5-HT	HIS–N^{δ}H	—	64.6	170.2	163.4[c]	2.8	3.19	2.66	2.66
5-HT	HIS–N^{ε}H	—	65.0	170.2	164.7[c]	−1.8	3.19	2.65	2.66
8-OH DPAT	[b]	167.8	44.7	167.0	−43.2	−0.1	—	—	—
8-OH DPAT	HIS +	167.7	44.7	167.0	−68.9	14.1	3.26	2.74	2.85
8-OH DPAT	HIS–N^{δ}H	167.8	44.7	167.1	−70.2	0.1	3.25	2.79	2.81

[a] HIS + : protonated His; HIS–N^{δ}H: tautomer N^{δ}H; and HIS–N^{ε}H: tautomer N^{ε}H.
[b] Dihedral angles of isolated ligand.
[c] Calculated relative to the closest *trans*-hydrogen.



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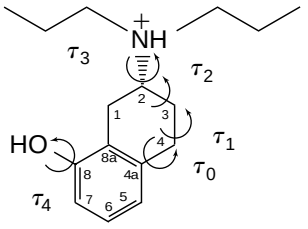

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1: τ_1 = C_9—C_3—C_α—C_β; τ_2: C_3—C_α—C_β—N; τ_3: C_α—C_β—N—H; τ_4: H—O—C_5—C_6
2: τ_0: C_5—C_{4a}—C_4—C_3; τ_1: C_{4a}—C_4—C_3—C_2; τ_2: C_4—C_3—C_2—N; τ_3: C_3—C_2—N—H; τ_4: H—O—C_8—C_7

Hamiltonian, of the R form of 8-OH DPAT (the propyl groups were replaced by methyls), starting from the low-energy conformation obtained by Arvidsson et al. [39]. The carbon atoms of the cyclohexene ring lie in the plane formed by the aromatic ring, with the exception of C_2 and C_3 which are puckered with dihedral angles $\tau_0 = 167.8°$ and $\tau_1 = 44.7°$. It is important to note that the protonated amine and the hydroxyl groups of 5-HT and 8-OH DPAT are equivalent in the 3-D space when the ethylammonium side chain of 5-HT adopts the *gauche,trans* conformation. Consequently, it is likely that 5-HT interacts with the 5-HT$_{1A}$R at a side-chain conformation close to the *gauche,trans*.

THE RECOGNITION OF SEROTONIN IN THE 5-HT$_{1A}$ RECEPTOR MODEL

The proposed recognition [10] of the endogenous ligand 5-HT in the 5-HT$_{1A}$R model (see Fig. 1) involves (*i*) the interaction between the protonated amine moiety and the conserved negative Asp-116, located in TMH 3; (*ii*) the hydrogen bond between the 5-OH group and Thr-199, located in TMH 5; and (*iii*) the interaction complex between the aromatic ring portion of the ligand and the neutral form of His-192, located in TMH 5. Moreover, two bulky residues Ile-113 and Ile-116, located in TMHs 3 and 5, are oriented toward the front and the back of the ligand and thus limit the size of the recognition cavity [10]. The nature of the interaction with His-192 and its consequences for triggering the activation of the receptor (see below) depend on the ionic and tautomeric forms of the imidazole side chain [8, 40]. To define the state of His, the structures of the complex of 5-HT and the receptor model were refined with quantum mechanical calculations. For practical reasons, given the size of the complex, the refinement was carried out with the semiempirical AM-1 algorithm. The 5-HT molecule was positioned in the

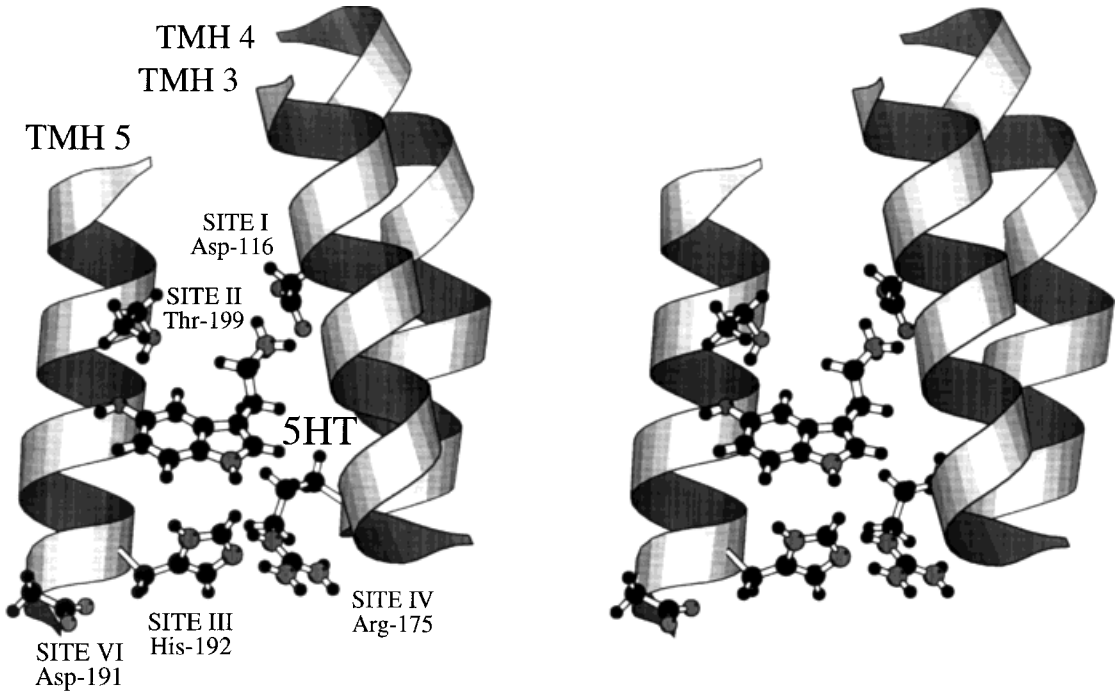

FIGURE 1. Molecular model of the agonist binding site of the 5-HT$_{1A}$R composed of residues in TMHs 3–5. Key residues of the receptor involved in ligand recognition and receptor activation are identified. The C_α traces are shown only for part of the TMHs pointing toward the extracellular side (bottom). As shown, the connectivity of the TMHs in the receptor model is clockwise when viewed from the extracellular side. The recognition pocket of the human 5-HT$_{1A}$R consists of (*i*) the ionic interaction between the ligand and Asp-116; (*ii*) the hydrogen bond between the hydroxyl group and Thr-199; and (*iii*) the complex between the aromatic ring of the ligand and the neutral form of His-192. The activation pocket of the receptor includes (*i*) a proton-donor group Arg-175; (*ii*) a proton-acceptor group His-192; and (*iii*) a negative group in the receptor Asp-191.

recognition pocket composed of the side-chain models of the residues in the receptor model (see Figs. 1 and 2 and Methods). During the energy optimization of the system, the C_α of the amino acids were kept fixed at the positions determined by the relative orientation of TMHs 3 and 5 where these residues are located. The internal coordinates optimized were (*i*) the relative orientation of 5-HT inside the receptor model; (*ii*) the dihedral angles χ_1 and χ_2 of Asp-116, His-192, and Thr-199; and (*iii*) the dihedral angles τ_1 to τ_4 of 5-HT (see Table I for nomenclature).

STRUCTURE OF THE SEROTONIN–RECEPTOR COMPLEXES

The optimized ligand/receptor complexes are depicted in Figure 2(a) for the tautomer N^ε—H of His, Figure 2(b) for the N^δ—H, and Figure 2(c) for protonated His. Table I shows the dihedral angles τ_1 to τ_4 of 5-HT in the receptor-binding conformation. From the present results, it appears that the interaction of 5-HT with the recognition pocket of the 5-HT_{1A}R occurs with the ethylammonium side chain close to the *gauche,trans* conformation, for any ionic and tautomeric species of His-192, as was predicted on the basis of structural comparisons between 5-HT and 8-OH DPAT (see above).

Table I shows, for the different ionic and tautomeric species of His-192, the optimized intermolecular distances between 5-HT and Asp-116 (SITE I) and Thr-199 (SITE II). The optimized distances between the nitrogen of the protonated ethylamine side chain of 5-HT and the oxygens of SITE I ($N—O^\delta$) are virtually identical for ionic and tautomeric forms of SITE III. Furthermore, the best alignment for the ionic interaction occurs when two N—H groups are pointing to both oxygens of Asp-116, as is reflected in Figure 2 and in the energy-optimized distances shown in Table I (compare $N—O^{\delta 1}$ with $N—O^{\delta 2}$). This type of interaction is in good agreement with the lower affinity measured for the 5-HT analog in which the side-chain primary amine is substituted by N-propyl groups [21]. On the other hand, the systems contain linear hydrogen bonds between the O atom of 5-HT (see **1** in Table I) and Thr-199, at the optimized distances of 3.3, 3.2, and 3.2 Å, respectively, for protonated His, the tautomer N^δ—H, and the tautomer N^ε—H. Thus, no major differences in the hydrogen-bond distances are observed for the optimized ligand/receptor complexes. Superposition

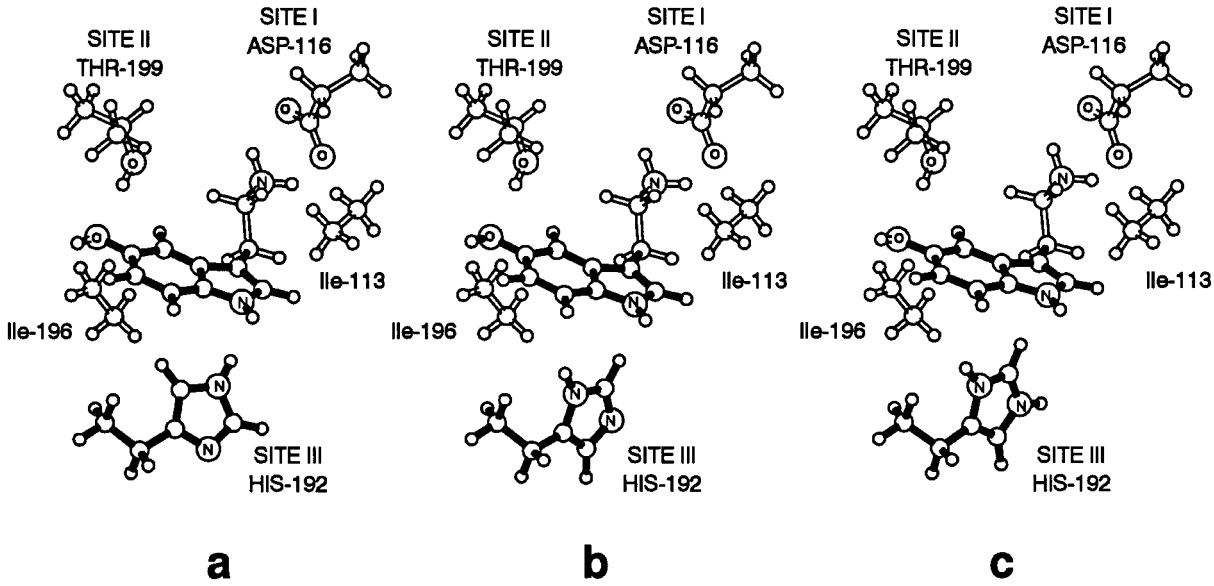

FIGURE 2. The 5-HT molecule positioned in the computational model of the recognition pocket composed of a propanoate residue (SITE I), isopropanol (SITE II), 5-ethylimidazole (SITE III), and two ethane groups, representing, respectively, the C_α and the side chains of Asp-116 (TMH 3), Thr-199 (TMH 5), His-192 (TMH 5), Ile-113 (TMH 3), and Ile-196 (TMH 5). Note the orientations of His relative to the indole ring when the imidazole is in the N^ε—H tautomeric form (a), the N^δ—H form (b), and in the protonated form (c). Fragments used in the ab initio quantum mechanical calculations are shown in black.

of the optimized structures of the complexes depicted in Figures 2(a)–(c) indicates that the orientation of 5-HT in the recognition pocket is analogous for any ionic or tautomeric form of the His-192 residue, as indicated by selected geometrical parameters (Table I).

Based on the optimized models shown in Figure 2, the properties of the interaction between 5-HT and the side chain of His-192 (SITE III) were further explored with ab initio calculations using the 6-31G basis set. During the optimization of the system, the indole group was kept fixed at the position determined in the previous optimization of 5-HT inside the defined receptor cavity. The results of the optimization are depicted in Figures 2(a)–(c), in solid lines, together with with the rest of the system. Table II shows θ, the dihedral angle between the planes of the aromatic rings of 5-HT (indole) and His-192 (either imidazole or imidazolium), obtained during the semiempirical and ab initio optimizations of the system. The semiempirical AM-1 calculation produces a situation in which the most stable orientation of the aromatic ring of His-192 strongly depends on its ionic and tautomeric form. Thus, the imidazolium ring of protonated His is near the perpendicular conformation ($\theta = 69.9°$), the imidazole ring of the N^{δ}—H tautomer is near the parallel stacked conformation ($\theta = 36.0°$), and the imidazole ring of the N^{ε}—H tautomer is found midway between previous orientations ($\theta = 51.1°$). The situation is significantly different when the ab initio HF/6-31G calculation is considered. Table II and Figure 2 show that the best orientation of His relative to the indole ring is nearly perpendicular for all the ionic and tautomeric forms (θ angles of 75.7°, 72.3°, and 52.3° for protonated His, N^{δ}—H, and N^{ε}—H tautomers, respectively). In such a conformation, the two aromatic rings achieve closer proximity than in the parallel stacked conformation. NMR and spectroscopic studies on oligopeptides containing both Trp and His show this type of interaction between the two aromatic rings [41], and this form of interaction is well known in the crystal structures of proteins (see [42, 43] and references therein).

ENERGETICS OF THE SEROTONIN–RECEPTOR COMPLEXES

The calculated energies of interaction, E_{int}, for the complexes of the indole moiety of serotonin and the ionic and tautomeric forms of His-192, at different levels of theory, are presented in Table II. It is clear that calculations with the semiempirical AM-1 algorithm yield poor results when compared with the results obtained at the HF level with the 6-31G basis set. For protonated His, E_{int} is underestimated by as much as 5.2 kcal/mol (−5.6 kcal/mol vs. −10.8 kcal/mol). In the neutral systems, E_{int} is also not predicted correctly (−1.3 kcal/mol vs. −2.8 kcal/mol for the N^{δ}—H tautomer; and −1.1 kcal/mol vs. −2.7 kcal/mol for

TABLE II

E_{int}, the energy of interaction (kcal / mol) between the indole ring of 5-HT and His-192 (TMH5; SITE III[a]) and θ, the dihedral angle (degrees) between the planes of the indole ring of 5-HT and either imidazole or imidazolium ring of His-192.

	SITE III			
Method		HIS +	HIS–N^{δ}H	HIS–N^{ε}H
AM-1 / / AM-1	θ	69.9	36.0	51.1
	E_{int}	−5.6	−1.3	−1.1
HF / 6-31G / / HF / 6-31G	θ	75.7	72.3	52.3
	E_{int}	−10.7	−2.8	−2.7
Morokuma decomposition	E^{ES}	−7.7	−2.5	−2.5
	E^{POL}	−2.9	−0.3	−0.3
	E^{CT}	−2.5	−0.9	−1.0
	E^{EX}	2.1	1.0	1.0
	E^{MIX}	0.3	0.0	0.0
MP2 / 6-31G / / HF / 6-31G	E_{int}	−13.0	−5.0	
HF / 6-31G* / / HF / 6-31G	E_{int}	−11.1	−3.0	
MP2 / 6-31G* / / HF / 6-31G	E_{int}	−14.4	−5.7	

[a]HIS + : protonated His; HIS–N^{δ}H: tautomer N^{δ}H; and HIS–N^{ε}H: tautomer N^{ε}H

the N^{ε}—H tautomer). E_{int} calculated with the semiempirical method is in all the systems half the value of E_{int} obtained with the ab initio method.

It is of interest to compare E_{int} of the indole ring of 5-HT with protonated and with neutral His. The calculations, at the HF/6-31G level of theory, show that E_{int} of indole with protonated His (−10.8 kcal/mol) is stronger than for the N^{δ}—H or N^{ε}—H tautomers of His (−2.8 and −2.7 kcal/mol, respectively). The Morokuma decomposition of E_{int} (see Table II and Methods) in protonated and neutral systems provides an explanation for the differences. The magnitudes of E^{ES} (−7.7 kcal/mol), E^{POL} (−2.9 kcal/mol), and E^{CT} (−2.5 kcal/mol) in the complex with protonated His are larger than in the complex with the N^{δ}—H tautomer (E^{ES} = −2.5 kcal/mol, E^{POL} = −0.3 kcal/mol, and E^{CT} = −0.9 kcal/mol) and with the N^{ε}—H tautomer (E^{ES} = −2.5 kcal/mol, E^{POL} = −0.3 kcal/mol, andE^{CT} = −1.0 kcal/mol). Note that the magnitude of E^{EX} is similar in the three complexes considered. However, the most important element in the interaction of indole with either protonated or neutral His corresponds to the electrostatic term (see E^{ES} in Table II) which prefers the protonated His. According to the present results, the recognition complex between 5-HT and the 5-HT_{1A}R occurs with the N^{δ}—H tautomer of His (see Fig. 1 and below). The molecular determinants in the recognition of 5-HT by His-192 (SITE III, TMH 5) of the 5-HT_{1A}R are basically electrostatic with a small component of charge transfer (see Morokuma decomposition in Table II).

The energetic driving force for triggering the activation of the receptor depends on the difference in E_{int} between protonated and the N^{δ}—H tautomer of His (see below). Therefore, it is desirable to examine this difference from calculations with larger basis sets and with the inclusion of correlation energy. The absolute values of E_{int} obtained with single-point calculations at the MP2 level with the 6-31G basis set (see Table II) are found to be larger than those at the HF level, by approximately −2.3 kcal/mol, reaching −13.0 and −5.0 kcal/mol for the protonated and N^{δ}—H forms of His, respectively. At the highest level of theory employed here (MP2/6-31G*), the value of E_{int} is −14.4 for protonated His and −5.7 for the N^{δ}—H tautomer. The significant magnitude of E_{int} obtained in this type of interaction between the indole ring of 5-HT and both forms of His clearly explains why aromatic–aromatic interactions have been suggested for mechanisms of protein structure stabilization [44].

A Trigger for Receptor Activation Resulting from Ligand Binding

THE ACTIVATION TRIGGER HYPOTHESIS

A computational exploration of the molecular properties of the recognition complex between 5-HT and the 5-HT_{1A}R model identifies the nature of the changes that can be produced by the presence of a ligand in the binding site. These changes can be interpreted in terms of rearrangements in the electronic structures and conformations of the molecules involved in the recognition complex. These rearrangements produced by the presence of the ligand in the binding site suggests a mechanism by which the receptor could be triggered for activation. The mechanism suggested here involves the same key His residue as an earlier proposal made solely on the basis of structure–activity considerations for 5-HT_{1A} ligands [8, 40]. From the present results, it appears that the interaction of the monocationic side chain of 5-HT with Asp-116 (TMH 3; SITE I), and of the hydroxyl group of 5-HT with Thr-199 (TMH 5; SITE II) positions the indole ring of 5-HT near the N^{δ}—H tautomer of His-192 (TMH 5; SITE III). Because the interaction of the indole ring of 5-HT with the protonated His is more favorable than with the neutral N^{δ}—H tautomer (−14.4 vs. −5.7 kcal/mol, see above), it will provide an energetic driving force that will induce the N^{ε} atom of the N^{δ}—H tautomer of His-192 to attract a proton from a proton donor site on the receptor (SITE IV). The hypothesis for the activation trigger is thus based on the proposal that a change in the reactivity properties of the His, produced by the presence of the ligand in the recognition pocket, will be satisfied by a proton transfer to N^{ε} from a neighboring proton donor residue.

In our model of the 5-HT_{1A}R, the lone pair of the N^{ε} of His-192, located in TMH 5, is pointing toward the helix bundle and more directly toward TMH 4. The protonated residue Arg-175 in TMH 4 could be the proton donor group in the proton-transfer process toward N^{ε} (see Fig. 1). The differences in the proton affinities of the two side chains in aqueous solution, expressed by their pK_a values, would not suggest such a direction for the proton transfer, but ligand binding and the recep-

tor environment could induce a change that would modify the relative proton affinities of the two participants in the H bonding to an extent appropriate for the transfer. Interestingly, the protonated Arg-175 is estimated to be in the membrane spanning region near the extracellular site, as reported in the original publications of the 5-HT_{1A} sequence [45]. The primary structure of the 5-HT_{1A}R does not suggest another putative proton donor (e.g., Lys, His) in the proximal sequence stretches. The calculations indicate that the proton transfer modeled here would occur under the influence of ligand (5-HT) binding and would change the properties of the region proximal to the ligand binding site. In fact, neighboring residues (e.g., Asp-191) have a significant role in this proposed proton transfer, as described below.

MOLECULAR BASIS FOR THE SPECIFICITY OF AGONISTS TOWARD THE 5-HT_{1A}R

Figure 3 summarizes the alignments of the TMHs 4 and 5, where Arg-175, Asp-191, and His-192 are located, for the known sequences of the 5-HT receptor family. Sequence alignments of GPCRs [46] superimpose the totally conserved Phe, Phe or Tyr, and Pro residues in TMH 5 (see shaded area in Fig. 3). Therefore, the homology proposed in Figure 3 makes the amino acids Asp-191 and His-192 of the 5-HT_{1A}R (see boxed area in Fig. 3) nonconserved among the 5-HT receptor sequences, if the segment actually adopts a helical structure. The lack of conservation at this locus is consistent with the proposed role of His-192 as a specific recognition element of the 5-HT_{1A}R.

A similar sequence analysis was carried out for

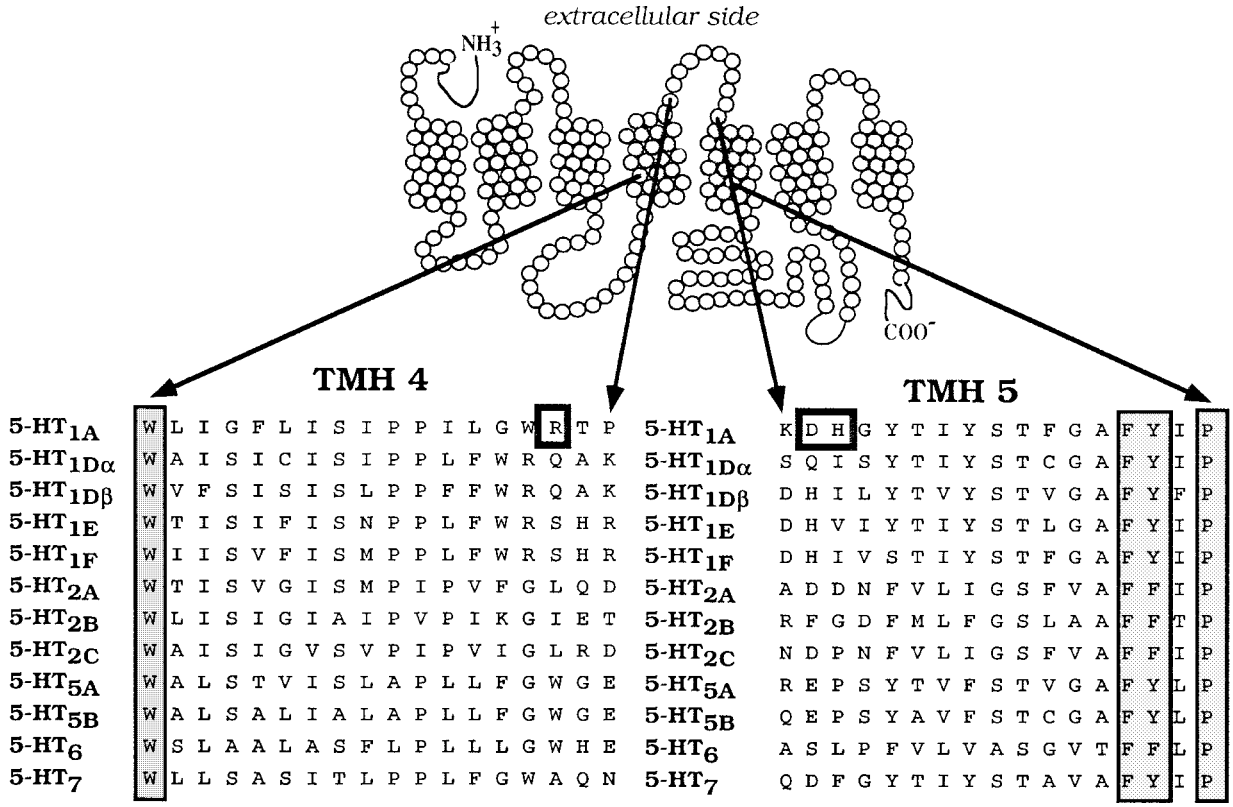

FIGURE 3. Sequence alignments of portions of TMHs 4 and 5 in members of the 5-HT receptor family (only selected portions of the sequences are shown). Optimal sequence alignments of GPCRs [46] superimpose the totally conserved Trp residue in TMH 4, and Phe, Phe, or Tyr, and Pro residues in TMH 5 (shaded area). Thus, residues Arg-175 in TMH 4 and Asp-191 and His-192 in TMH 5 (boxed area), which are proposed as key participants in the activation trigger mechanism of the 5-HT_{1A}R, are not conserved among the family of 5-HT receptor subtypes. The full sequence span of TMHs 4 and 5 used in [10] is similar to that in Scheme 1 of [30].

TMH 4 of GPCRs. The conserved residue Trp (see shaded area in Fig. 3) is normally superimposed in sequence alignments of GPCRs [46]. Therefore, the protonated residue Arg-175 in the sequence of the 5-HT_{1A}R (see boxed area in Fig. 3) is not conserved among the 5-HT receptor family members. Similarly to TMH 5, this nonconserved locus is considered to be part of the specific recognition pocket of the 5-HT_{1A}R.

We can conclude that residues Arg-175 (TMH 4), Asp-191 (TMH 5), and His-192 (TMH 5), which are proposed as key participants in the activation trigger mechanism of the 5-HT_{1A}R (see below), are not conserved among the family of 5-HT receptor subtypes. These residues are specific for the 5-HT_{1A}R sequence. Consequently, the 5-HT_{1A}R agonists may interact with these unique residues to trigger the specific activation of the 5-HT_{1A}R.

SIMULATION OF THE ACTIVATION TRIGGER BY 5-HT

To evaluate computationally the feasibility of the proposed proton transfer as a result of ligand binding, a molecular model system was constructed for the interaction of the indole ring of 5-HT with 5-ethylimidazole, representing His-192 (SITE III) and methylguanidinium, representing Arg-175 (SITE IV). Figure 4 shows, in solid lines, the systems optimized from ab initio calculations with the 6-31G basis set. The initial state of the proton-transfer reaction in which the proton is bound to Arg-175 is shown in Figure 4(a), and the end product of the reaction in which the proton is bound to His-192 is in Figure 4(b). As for the other energy optimizations described above, constraints were applied to keep the system compatible with the overall receptor model: The C_α of SITE III and the indole ring of 5-HT were kept fixed, and a linear hydrogen bond between His-192 and Arg-175 was constrained at the internuclear $N \cdots N$ distance of 3.0 Å. The proton transfer induced by 5-HT is characterized by a double-well potential, as observed earlier [40]. The calculated energies for the initial guanidinium/imidazole ($GNDN^+/IM$) and the final guanidine/imidazolium ($GNDN/IM^+$) forms of the complex, in the absence and presence of the ligand, are given in Table III. Note that in the absence of the 5-HT ligand the initial system $GNDN^+/IM$ is more stable than the final state $GNDN/IM^+$ by 11.6 kcal/mol. The favorable interaction between the aromatic rings of 5-HT and protonated His reduces this difference by 3.8 kcal/mol by stabilizing the proton of IM^+ more than that of $GNDN^+$ (11.6 kcal/mol vs. 7.8 kcal/mol; see Table III). However, this interaction alone is not strong enough to overcome the large difference in energy between

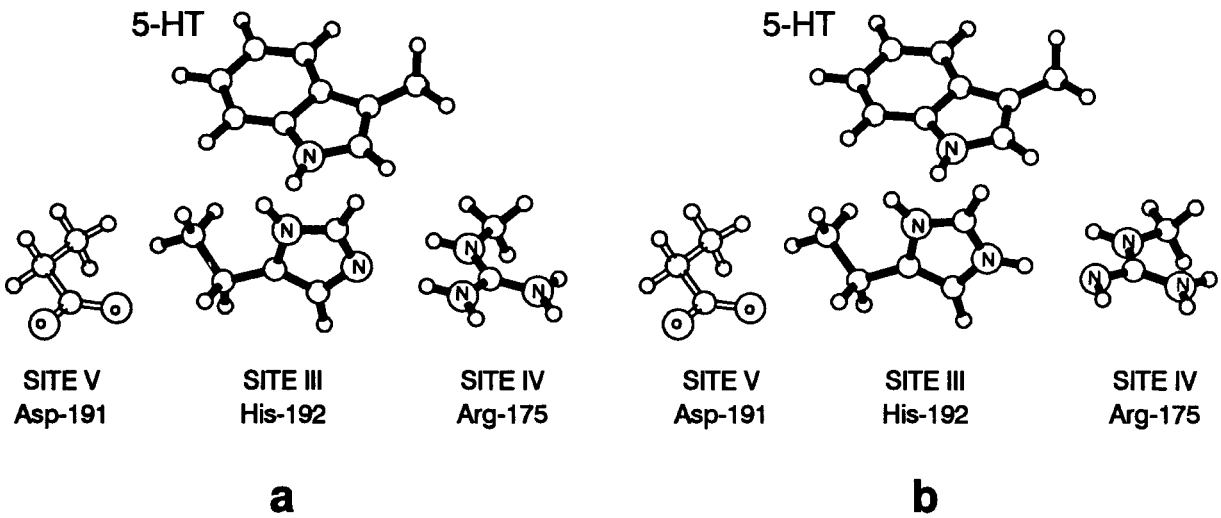

FIGURE 4. The system composed of the indole ring of 5-HT and models for Arg-175 (TMH 4), His-192 (TMH 5), and Asp-191 (TMH 5) used to explore the trigger for receptor activation resulting from ligand binding. The interaction of the ligand with His-192 generates an appreciable driving force for the proton to transfer from Arg-175 [in (a)] to His-192 [in (b)] as a trigger of receptor activation.

TABLE III

Total energies[a] (Hartrees) of the initial guanidinium / imidazole (GNDN$^+$/ IM) and final guanidine / imidazolium (GNDN / IM$^+$) states of the activation process at the 5-HT$_{1A}$ receptor, in the absence and presence of the 5-HT and 8-OH DPAT ligands and in the presence and absence of Asp-191 (TMH 5; SITE V). (E_{react} [kcal / mol] is the energy of reaction for the movement of the proton from Arg-175 (TMH 4; SITE IV) to His-192 [TMH 5; SITE III].)

Ligand	SITE V	Total energy		E_{react}[a]
		GNDN$^+$/ IM	GNDN / IM$^+$	
—	—	−546.25727	−546.23876	11.6
—	Asp-191[b]	−812.46643	−812.46380	1.6
5-HT[c]	—	−946.61558	−946.60308	7.8
5-HT[c]	Asp-191[b]	−1212.82262	−1212.82494	−1.5 (**−1.2**)
8-OH DPAT[d]	—	−815.90892	−815.89508	8.7
8-OH DPAT[d]	Asp-191[b]	−1082.11678	−1082.11822	−0.9 (**−0.7**)

[a] Calculated at the levels of HF / 6-31G / / HF / 6-31G, and MP2 / 6-31G* / / HF / 6-31G (in bold).
[b] The anionic site, Asp-191, is represented by FRM$^-$.
[c] The ligand, 5-HT, is represented by its indole moiety (see Fig. 4).
[d] The ligand, 8-OH DPAT, is represented by its benzene moiety (see Fig. 5).

the two states, and the proton transfer would not be induced by ligand binding if the recognition complex were isolated from the rest of the receptor environment.

It was shown before that the presence of the electrostatic field generated by the protein environment may have an important effect on the protonation preference of His residues [47]. Inspection of the receptor sequence reveals the presence of a negatively charged Asp-191 (TMH 5, SITE VI) in the vicinity of His-192 (see Figs. 1 and 3 for location of Asp-191 and His-192). Such negative charges have an important effect on the energy for proton transfer in hydrogen-bonded complexes. With this in mind, we added the C_α and the side chain of Asp-191 to the receptor model, as shown in Figure 4, to study the influence of the anion on the energetics of the proton transfer. The C_α of Asp-191 was kept fixed at the position determined by the location of TMHs 3 and 5 in the construction of the model above. The orientation of the side chain was determined by ab initio energy minimization (with the 6-31G basis set) on a reduced system containing Asp-191 and His-192. Clearly, inclusion of Asp-191 affects the calculated energy of reaction for the proton-transfer process. When results obtained in the presence and in the absence of Asp-191 are compared, E_{react} is found to change from 7.8 kcal/mol to −1.5 kcal/mol (see Table III). At the highest level of theory employed here (MP2/6-31G*), E_{react} changes somewhat to −1.2 kcal/mol.

Thus, the calculations show that the proton-transfer process from GNDN$^+$/IM to GNDN/IM$^+$ is energetically feasible in the presence of both the ligand and Asp-191. Notably, the effect of Asp-191 alone is not sufficient to promote the proton transfer (E_{react} is 1.6 kcal/mol, see Table III); ligand binding in the recognition pocket is necessary to trigger the process.

TRIGGERING OF 5-HT$_{1A}$R ACTIVATION BY 8-OH DPAT

The R enantiomer of 8-OH DPAT is one of the most potent and selective compounds for the 5-HT$_{1A}$R [39, 48]. The protonated amines and the OH substituents of 5-HT and 8-OH DPAT are sterically and chemically equivalent in terms of reactivity (see **1** and **2** in Table I). Therefore, the mode of recognition of 8-OH DPAT should follow the same trends obtained for 5-HT: The protonated site of the molecule anchors at Asp-116 (SITE I); the hydroxyl group forms a hydrogen bond to Thr-199 (SITE II); and the phenyl ring interacts with His-192 (SITE III). This proposed mode of binding makes the indole moiety of 5-HT equivalent to the phenyl moiety of 8-OH DPAT, in terms of chemical reactivity. Thus, the guiding assumption is that the phenyl ring of 8-OH DPAT interacts with His-192 (SITE III), providing the driving force for the proposed proton transfer from Arg to His as a trigger of receptor activation, in a similar fashion as the indole ring of 5-HT. Comparison of

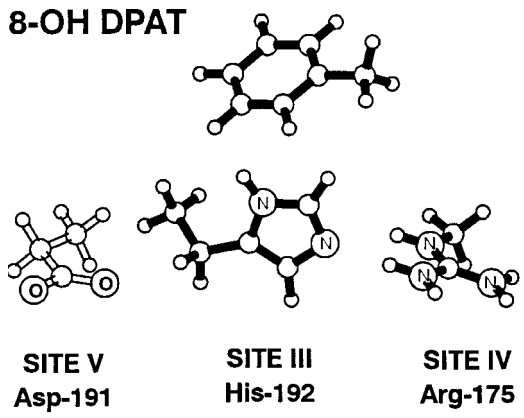

FIGURE 5. The model of the 5-HT_{1A}R recognition site used in the computational simulation of the activation trigger by 8-OH DPAT. See legend of Figure 4 for details.

the binding of 5-HT and 8-OH DPAT in our proposed model offers an opportunity to probe the model of the 5-HT_{1A}R recognition pocket and to test the mechanistic hypothesis for 5-HT_{1A}R activation.

The R enantiomer of the 8-OH DPAT ligand, in which the propyl groups were replaced by methyls, was docked into the binding site in the same manner as that used to position the 5-HT molecule (see above). The optimization inside the receptor model composed by the side chains of Asp-116 (SITE I), Thr-199 (SITE II), His-192 (SITE III), Ile-113, and Ile-196 was also carried out in the same way (see above). The characterization of the relative position of the benzene moiety of 8-OH DPAT in the receptor model was used to construct the proton-transfer system comprising the side chains of His-192 (SITE III), Arg-175 (SITE IV), Asp-191 (SITE V), and the benzene moiety of 8-OH DPAT (see Fig. 5). Table III shows the calculated energies for the initial $GNDN^+/IM$ form (Fig. 5) and the final $GNDN/IM^+$ form (not shown) of the proton-transfer process. Notably, a comparison of E_{react} obtained for 5-HT and 8-OH DPAT (-1.2 vs. -0.7 kcal/mol at the MP2/6-31G* level of theory) reveals that both ligands can provide the energetic driving force that induces His-192 to attract a proton from Arg-175 in the presence of Asp-191, as a trigger of receptor activation. Clearly, the aromatic rings of 5-HT and 8-OH DPAT serve the same function in receptor activation, with similar efficacy. *This finding is in good agreement with the known pharmacological properties of the ligands and provides a model for the manner in which new, structurally active, selective agonists of the 5-HT_{1A}R can be designed and tested computationally even before experimentation with the authentic receptor systems.*

Conclusions

Understanding the structural basis of ligand selectivity, as well as of the relation between receptor structure and the mechanism of response elicited with different efficacy by the specific agonists, requires a detailed 3-D structure of the receptor molecule. Use of such a model in computational simulations of the properties of agonist–receptor complexes for the 5-HT_{1A}R suggested a trigger for the receptor activation mechanism resulting from ligand binding. The interaction of agonists with the partially conserved recognition pocket of the 5-HT_{1A}R positions the ligand near the activation pocket of the receptor that includes (*i*) a proton donor group Arg-175, located in TMH 4; (*ii*) a proton-acceptor group His-192, located in TMH 5; and (*iii*) a negative group in the receptor Asp-191, located in TMH 5, that influences the energy for proton transfer in hydrogen-bonded complexes. The activation mechanism of the receptor resulting from ligand binding takes the form of a proton transfer from TMH 4 (Arg-175; SITE IV) to TMH 5 (His-192 and Asp-191; SITEs IV and VI, respectively). In the absence of the ligand, the process of proton transfer from Arg to His is energetically prohibited, keeping Arg-175 protonated and His-192 neutral. When the agonist approaches the proton-transfer system, the interaction between the aromatic rings of the ligand and His-192 generates an appreciable driving force for the proton to transfer from Arg-175 to His-192 as a trigger of receptor activation. The proton-transfer process explains the local changes induced by agonist in the receptor binding site. The structural consequences of these changes are likely to be involved in the propagation of the extracellular signal, encoded in the structure of the ligand, to the intracellular site. such a conformational change assisted by the conserved Pro residues in the middle of the TMHs has been identified from computational simulations as the molecular mechanism of signal transduction by a 5-HT_2 receptor [6, 7]. However, the present study has not identified the conformational changes in the tertiary structure of the receptor following agonist binding. Rather, it provides a direct relation between the interactions of the ligand in the recognition pocket and the triggering of

a significant change in the molecular properties of the receptor. The probability of producing this change by a given ligand through the triggering of the proton transfer constitutes in this model the expression of the pharmacological property of intrinsic efficacy ([49] and references therein). Agonist intrinsic efficacy is defined in explicit receptor models as the ability to produce the set of molecular processes that are induced by agonist binding to the receptor and lead to the active form of the receptor [8, 9]. A discrete molecular understanding of the classical pharmacological concepts of agonism and partial agonism emerges from the explicit description of this molecular process of receptor activation. As shown here, the computational analysis of the process relates elements of the structure to the trigger of the activation process. This insight can be used to guide the design of ligands with predetermined efficacies.

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References

1. M. A. Kjelsberg, S. Cotecchia, J. Ostrowski, M. G. Caron, and R. J. Lefkowitz, J. Biol. Chem. **267**, 1430 (1992).
2. R. J. Lefkowitz, S. Cotecchia, P. Samama, and T. Costa, Trends Pharmacol. Sci. **14**, 303 (1993).
3. Q. Ren, H. Kurose, R. J. Lefkowitz, and S. Cotecchia, J. Biol. Chem. **268**, 16483 (1993).
4. J. W. Lewis and D. S. Kliger, J. Bionerg. Biomembr. **24**, 201 (1992).
5. J. A. Ballesteros and H. Weinstein, Methods Neurosci. **25**, 366 (1995).
6. D. Zhang and H. Weinstein, J. Med. Chem. **36**, 934 (1993).
7. X. C. Luo, D. Q. Zhang, and H. Weinstein, Protein Eng. **7**, 1441 (1994).
8. H. Weinstein and R. Osman, Neuropsychopharmacology **3**, 397 (1990).
9. L. Pardo, M. Campillo, and J. Giraldo, submitted.
10. L. Pardo, M. Batlle, M. Duñach, and H. Weinstein, J. Biomed. Sci. **3**, 98 (1996).
11. B. K. Kobilka, T. Frielle, S. Collins, T. Yang-Feng, T. S. Kobilka, U. Francke, R. J. Lefkowitz, and M. G. Caron, Nature **329**, 1987 (1987).
12. A. Fargin, J. R. Raymond, M. J. Lohse, B. K. Kobilka, M. G. Caron, and R. J. Lefkowitz, Nature **335**, 358 (1988).
13. P. K. Chanda, M. C. Minchin, A. R. Davis, L. Greenberg, Y. Reilly, W. H. McGregor, R. Bhat, M. D. Lubeck, S. Mizutani, and P. P. Hung, Mol. Pharmacol. **43**, 516 (1993).
14. L. Pardo, J. A. Ballesteros, R. Osman, and H. Weinstein, Proc. Natl. Acad. Sci. U.S.A. **89**, 4009 (1992).
15. G. F. X. Schertler, C. Villa, and R. Henderson, Nature **362**, 770 (1993).
16. W. Zhou, C. Flanagan, J. A. Ballesteros, K. Konvicka, J. S. Davidson, H. Weinstein, R. P. Millar, and S. C. Sealfon, Mol. Pharmacol. **45**, 165 (1994).
17. S. C. Sealfon, L. Chi, B. J. Ebersole, V. Rodic, D. Zhang, J. A. Ballesteros, and H. Weinstein, J. Biol. Chem. **270**, 16683 (1995).
18. R. A. F. Dixon, I. S. Sigal, M. R. Candelore, R. B. Register, W. Scattergood, E. Rands, and C. D. Strader, EMBO J. **6**, 3269 (1987).
19. H. G. Dohlman, M. G. Caron, A. DeBlasi, T. Frielle, and R. J. Lefkowitz, Biochemistry **29**, 2335 (1990).
20. P. H. Reggio, H. Weinstein, R. Osman, and S. Topiol, Int. J. Quantum Chem., Quantum Biol. Symp. **8**, 373 (1981).
21. R. A. Glennon, R. B. Westkaemper, and P. Bartyzel, in *Serotonin Receptor Subtypes: Basic and Clinical Aspects*; S. J. Peroutka, Ed. (Wiley-Liss, New York, 1991), Vol. 15, pp. 19–64.
22. B. Y. Ho, A. Karschin, T. Branchek, N. Davidson, and H. A. Lester, FEBS Lett. **312**, 259 (1992).
23. X. Guan, S. J. Peroutka, and B. K. Kobilka, Mol. Pharmacol. **41**, 695 (1992).
24. C. D. Strader, I. S. Sigal, M. R. Candelore, M. R. Rands, W. S. Hill, and R. A. F. Dixon, J. Biol. Chem. **263**, 10267 (1988).
25. C. D. Strader, M. R. Candelore, W. S. Hill, I. S. Sigal, and R. A. F. Dixon, J. Biol. Chem. **264**, 13572 (1989).
26. C. Wang, M. A. Buck, and C. M. Fraser, Mol. Pharmacol. **40**, 168 (1991).
27. N. J. Pollock, A. M. Manelli, C. W. Hutchins, M. E. Steffey, R. G. MacKenzie, and D. E. Frail, J. Biol. Chem. **267**, 17780 (1992).
28. C. Wang, T. K. Gallaher, and J. C. Shih, Mol. Pharmacol. **43**, 931 (1993).
29. M. P. Johnson, R. J. Loncharich, M. Baez, and D. L. Nelson, Mol. Pharmacol. **45**, 277 (1994).
30. F. Fanelli, M. C. Menziani, M. Cocchi, and P. G. De Benedetti, J. Mol. Struct. (Theochem) **120**, 49 (1995).
31. B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus, J. Comput. Chem. **4**, 187 (1983).
32. A. E. Reed, R. B. Weinstock, and F. Weinhod, J. Chem. Phys. **83**, 735 (1985).

33. M. J. Frisch, M. Head-Gordon, G. W. Trucks, J. B. Foresman, H. B. Schlegel, K. Raghavachari, M. A. Robb, J. S. Binkley, C. Gonzalez, D. J. Defrees, D. J. Fox, R. A. Whiteside, R. Seeger, C. F. Melius, J. Baker, R. L. Martin, L. R. Kahn, J. J. P. Stewart, S. Topiol, and J. A. Pople, *Gaussian 90* (Gaussian, Inc., Pittsburgh, PA, 1990).
34. M. J. S. Dewar, E. G. Zoebish, E. F. Healy, and J. J. P. Stewart, J. Am. Chem. Soc. **107**, 3902 (1985).
35. K. Kitaura and K. Morokuma, Int. J. Quantum Chem. **10**, 325 (1976).
36. U. C. Singh and P. Kollman, *GAUSSIAN 80 UCSF* (1982).
37. L.-E. Arvidsson, U. Hacksell, A. M. Johansson, J. L. G. Nilsson, P. Lindberg, D. Sanchez, H. Wikstrom, K. Svensson, S. Hjorth, and A. Carlsson, J. Med. Chem. **27**, 45 (1984).
38. L. J. Cornfield, G. Lambert, L. Arvidsson, C. Mellin, J. Vallgårda, U. Hacksell, and D. L. Nelson, Mol. Pharmacol. **39**, 780 (1991).
39. L.-E. Arvidsson, A. Karlen, U. Norinder, L. Kenne, S. Sundell, and U. Hacksell, J. Med. Chem. **31**, 212 (1988).
40. R. Osman, S. Topiol, L. Rubenstein, and H. Weinstein, Mol. Pharmacol. **32**, 699 (1987).
41. H. Jäckle and P. L. Luisi, J. Phys. Chem. **88**, 1767 (1984).
42. S. K. Burley and G. A. Petsko, Adv. Protein Chem. **39**, 125 (1988).
43. J. Singh and J. M. Thornton, *Atlas of Protein Side-Chain Interactions* (IRL PRESS, Oxford, 1993).
44. S. K. Burley and G. A. Petsko, Science **229**, 23 (1985).
45. P. R. Albert, Q. Zhou, H. H. M. Van Tol, J. R. Bunzow, and O. Civelli, J. Biol. Chem. **265**, 5825 (1990).
46. W. C. Probst, L. A. Snyder, D. I. Schuster, J. Brosius, and S. C. Sealfon, DNA Cell Biol. **11**, 1 (1992).
47. G. A. Mercier, R. Osman, and H. Weinstein, Protein Eng. **2**, 261–270 (1988).
48. L.-E. Arvidsson, U. Hacksell, J. L. G. Nilsson, S. Hjorth, A. Carlsson, P. Lindberg, D. Sanchez, and H. Wikstrom, J. Med. Chem. **24**, 921 (1981).
49. T. Kenakin, *Pharmacologic Analysis of Drug-Receptor Interaction*, (Raven Press, New York, 1993).