0026-895X/87/050699-07$02.00/0

MOLECULAR PHARMACOLOGY, 32:699-705

A Molecular Model for Activation of a 5-Hydroxytryptamine Receptor

ROMAN OSMAN, SID TOPIOL, LESTER RUBENSTEIN, AND HAREL WEINSTEIN

Departments of Physiology and Biophysics (R.O., H.W.) and of Pharmacology (R.O., S.T., L.R., H.W.), Mount Sinai School of Medicine of the City University of New York, New York, New York 10029; and the Department of Medicinal Chemistry (S.T.), Berlex Laboratories, Inc., Cedar Knolls, New Jersey 07927

Received May 5, 1987; Accepted August 12, 1987

SUMMARY

The extension of a model proposed previously for molecular *recognition* at a serotonin (5-hydroxytryptamine (5-HT)) receptor makes possible the formulation of a molecular mechanism of receptor *activation*. The activation mechanism proposed here is based on the changes induced in the drug and in a model receptor by the interaction mimicking the formation of a drug-receptor complex. This mechanism was simulated by quantum mechanical calculations of molecular interactions between 5-HT and a model for a receptor represented by an imidazolium-ammonia complex that serves as a proton transfer model (PTM). The movement of the proton in the PTM is promoted by the interaction with 5-HT, suggesting a process by which 5-HT can trigger the activation of the receptor. The elements of the activation mechanism revealed by the results of the simulation are: (a) the electrostatic alignment between the PTM and 5-HT, which guides the recognition of 5-HT by the PTM; (b) the contraction of the distance between the hydrogen bonded components of the PTM, induced by the interaction of 5-HT with the PTM, which leads to a decrease in the barrier to proton transfer in the PTM; (c) an additional decrease of the barrier to proton transfer produced by the negative electrostatic potential of 5-HT, which stabilizes the transition state; and (d) the increased preference for product over reactant in the interaction complex between 5-HT and the PTM, which constitutes a driving force for the proton transfer process. According to this model, compounds that activate the 5-HT receptor should bind in a mode that induces the changes described above in the PTM and thus triggers the proton transfer.

Any formulation of a molecular mechanism of drug action must address two questions. The first refers to the high selectivity of the *recognition* by the receptor of a specific class of molecules, including both agonists and antagonists, which is expressed in their affinity for the receptor. The second question is related to the changes induced by the drug-receptor interaction to precipitate an activation process that leads to a measurable *response*. A complete separation of the drug-receptor interaction into a recognition stage and an activation stage may not be achieved in reality, but it nevertheless serves as a useful conceptual framework that relates to the separate pharmacological measurements of affinity and response. The practical advantage of such a conceptual separation is that it enables the definition of elements of recognition, which are the molecular properties required for high affinity interaction with the receptor, as well as the construction of models for receptor activation. These activation models represent molecular processes that result from the specific interaction between a ligand known to activate the receptor and a receptor model.

We have used this approach to model drug action at a class of receptors with high affinity for 5-HT, which also recognize lysergic acid diethylamide (LSD) (termed "the 5-HT/LSD receptor" even before the current classification of subtypes was completed) (1–6). Based on experimental evidence and theoretical studies, two major elements of recognition at receptors shared by 5-HT and LSD were identified from the molecular properties common only to the ligands that have high affinity for such receptors. The first is the protonated side chain amine, which can interact with a negative region in the binding site. This interaction is likely to be the primary event of the recognition process; it anchors the compound and triggers the second recognition element (5, 6). The second recognition element relates to the electrostatic properties of the molecules and is expressed by the directional character of the molecular electrostatic potential (MEP) generated by 5-HT (and LSD) over the indole portion (or its equivalent in other compounds). In this model for recognition, the anchoring of the side chain causes a neutralization of the positive charge on the side chain amine

This work was supported in part by the National Institute on Drug Abuse (NIDA) under grant DA-01875 and a Research Scientist Development Award DA-00060 (to H. W.), and Irma T. Hirschal Career Scientist Award (to S. Topiol while at Mount Sinai).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide; PTM, proton transfer model; MEP, molecular electrostatic potential; IM^+, imidazolium; IM, imidazole; AM, ammonia; AM^+, ammonium; TS, transition state; SCF, quantum mechanical self-consistent field (calculation).

and an enhancement of the negative values of the MEP generated by the molecule (1). The MEP was shown (1–5) to have a characteristic directionality that aligns itself with respect to an implied complementary directional character of the charge distribution in the receptor in order to produce an optimal electrostatic interaction (6). For congeners of 5-HT in which the directionality of the MEP differs from that of 5-HT itself, the simultaneous anchoring at the negative site of the receptor and the electrostatic alignment of the MEP can only be achieved by means of a conformational change in the side chain (5).

Because nothing was known about the molecular structure of the receptor that recognizes 5-HT with high affinity, the first hypothesis requiring testing was that the molecular properties chosen as molecular determinants for receptor recognition are predictive of the mutual orientations of molecules with which tryptamine congeners were known to form intermolecular complexes. For this test, imidazolium cation was chosen as a probe of the interaction directionality of the substituted tryptamines. The choice of imidazolium was guided by experimental studies (7) in which imidazolium was found to form stacking complexes with indole containing compounds. Calculations showed that the charge distribution of imidazolium cation exhibits a directionality that makes it a useful probe of the alignment hypothesis (2, 3).

Initial studies of the supermolecular complexes of 5-HT and 6-HT with imidazolium (2–4) indicated that, indeed, the orientation of the substituted tryptamines with respect to the imidazolium shows a preference for an alignment in agreement with the directionality predicted on the basis of the analysis of the MEP. Furthermore, an analysis of the decomposition of the energy of interaction using the scheme proposed by Morokuma (8, 9), and of the charge redistribution that took place between the interacting molecules (2, 3), led to the conclusion that the interaction was electrostatic, augmented by mutual polarization (2, 3, 10).

Insight into the development of a molecular model for activation of a 5-HT receptor was obtained from an analysis of the charge redistribution in the interacting molecules (3). As a consequence of the interaction with 5-HT, a major change in the charge distribution of imidazolium occurred in the vicinity of the nitrogen atoms and approximately along the direction of the N-H bond. This indicated a change in the proton affinity of the imidazolium and the possibility that imidazolium can act as a proton donor. A model capable of a proton transfer was constructed on this basis (11). In this model, the imidazolium, acting as a proton donor, was coupled to an ammonia molecule, acting as a proton acceptor. We report here the investigation of the interaction of 5-HT with this PTM.

The mechanism by which the interaction between 5-HT and the PTM triggers the transfer of a proton from the imidazolium to the ammonia was investigated by computational simulation of the process. We report here the results of the simulation and discuss this process as a plausible primary event in the activation of a 5-HT receptor. Based on the description of the possible molecular events in receptor activation provided by this model, a mechanistic explanation for the pharmacological property of antagonism at this 5-HT receptor emerges naturally from the construct in which a molecule can be recognized in the same way as an agonist but will not be able to trigger the primary molecular event in the activation of the receptor.

Methods

Construction of the PTM

The PTM was constructed as an extension of the imidazolium ion, which was previously used as a discriminant probe to study the molecular determinants of recognition at 5-HT/LSD sites (2–6). These studies showed that the positive charge distributed in the imidazolium ion was responsible for the electrostatic interaction with 5-HT and for the proper alignment of the ion with the directionality expressed in the electrostatic potential. The PTM had to preserve such a charge distribution as far as possible in order to conserve the recognition properties. Furthermore, the design of the PTM had to produce a system that had the potential to respond to the interaction with 5-HT; i.e., the interaction had to affect the properties of the system so as to increase its tendency to undergo a transition from one well-defined state to another. To satisfy these requirements, an ammonia molecule was positioned to act as a putative acceptor for a proton released by imidazolium. The proton transfer process from imidazolium to ammonia constitutes the response of the system to an interaction with 5-HT. The initial position of the ammonia nitrogen at a distance of 3.0 Å from the nitrogen of the imidazolium ion was chosen arbitrarily to model a neighboring residue in a hydrogen bonded system within a receptor protein. In the PTM, it constitutes an externally imposed geometric constraint that was subsequently found, from searches of the Protein Data Bank, to correspond to the range of distances observed between the imidazole nitrogen in histidine residues and putative hydrogen bonding groups in proteins (G. Mercier *et al.*, unpublished results). The resulting PTM is illustrated in Fig. 1.

In the course of the proton transfer from imidazolium to ammonia, the complex undergoes structural changes: imidazolium (IM^+) becomes imidazole (IM), and ammonia (AM) becomes an ammonium ion (AM^+). Using a scheme developed for the construction of a proton transfer curve from the separately optimized components of a hydrogen bonded system (12), the potential energy curve that would have been obtained from a complete optimization of the PTM for each position of a proton along the proton transfer coordinate was reproduced. The curve

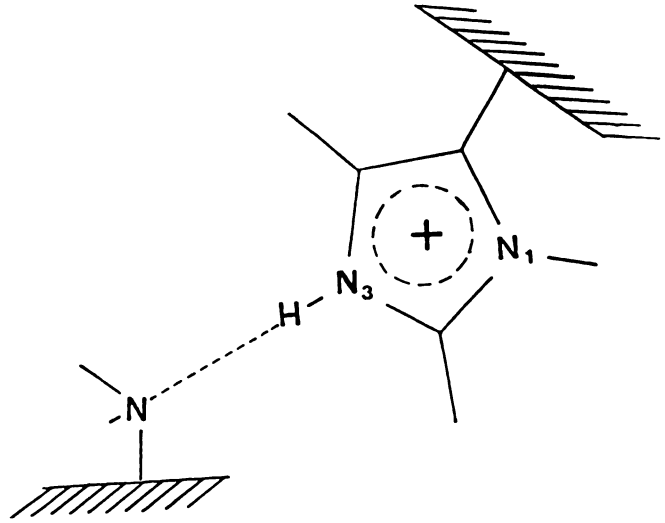

Fig. 1. The PTM constructed from IM^+ hydrogen bonded to AM at a distance of 3.0Å. The shaded areas represent the protein structure of which the PTM is a part.

was constructed from the two half-curves that correspond to the IM^+/AM complex (i.e., the proton is close to the imidazole) and the IM/AM^+ complex (i.e., the proton is close to the ammonia). The crossover point from one half-curve to the other was obtained by performing calculations with both complexes and then selecting the one with the lower energy. The positions of the proton at the extrema of the potential energy curve (i.e., the two minima of the reactants and products and the maximum at the transition state (TS)) were obtained from a quartic polynomial that was fitted to the calculated points. The quantum mechanical (SCF) calculations were performed with the GAUSSIAN 80 package of programs.[1] The STO-3G optimized structures of the neutral and protonated forms of IM and AM were used throughout.

Electrostatic Scans

Because the interaction between 5-HT and IM^+ was shown to be electrostatic in nature (2, 3, 10, 13), and because the charge distribution of the PTM was very similar to that of IM^+ alone, the complete scan of the potential surface of the interaction energy between 5-HT and the PTM was limited to the calculation of the electrostatic component of the interactions. The scan was performed by moving the PTM systematically on a grid of 20 × 20 bohr in steps of 0.5 bohr while keeping the 5-HT fixed in the geometry described earlier (1). The scan was performed in a plane parallel to the indole portion of 5-HT at a distance of 6.238 bohr on the side that is farthest from the nitrogen of the side chain. This intermolecular distance was chosen as described before (2, 3) on the basis of X-ray crystallography studies on tryptamine complexes, and it is shown to correspond to an optimal interplanar distance calculated for the interaction of 5-HT and IM^+ (10). At each position of the scanning molecule, a complete rotational scan (360°) around the hydrogen-bonded nitrogen of the IM^+ was performed with a step size of 10 degrees, out of which only the lowest energy orientation was saved for subsequent display in the isoenergy contour maps (see Figs. 3–5). Following an approximation analyzed in detail for this system (6), the electrostatic interaction energy was calculated as a sum of monopole-monopole interactions between point charges positioned on the interacting molecules. The monopoles were Mulliken net charges obtained from wave functions calculated with the LP-3G basis set (14).

SCF Calculations

To test the approximations inherent to limiting the calculation of intermolecular interaction energies to the electrostatic component evaluated from monopole-monopole interactions, complete quantum mechanical calculations of the supermolecular complex at selected check points were performed for test cases with the GAUSSIAN 80 system of programs.[1] Effective core potentials (15) were used with the LP-3G basis set (14) in order to exploit the documented advantages of this method in the representation of stacking complexes (13), especially with regard to the basis set superposition error.

[1] Binkley, J. S., R. A. Whiteside, R. A. Krishnan, R. Seeger, D. J. DeFrees, H. B. Schlegel, S. Topiol, L. R. Kahn, and J. A. Pople. GAUSSIAN 80 IBM version. Unpublished report.

Results and Discussion

The PTM

The isolated PTM system was first characterized to enable a quantitation of the changes it undergoes upon interaction with 5-HT. The energy for the movement of the proton from a position 0.8 Å away from the IM nitrogen to a position 0.8 Å away from the AM in steps of 0.2 Å was calculated as described in Methods, according to a scheme developed previously (12). At each point calculated along the path of the proton, the appropriate geometries of either IM^+/AM or IM/AM^+ was used to construct the entire potential energy curve. In order to evaluate the effect of the distance between IM and AM on the characteristic properties of the PTM, the entire procedure was carried out with the distance between the nitrogens of IM and AM set at 3.0 Å and at 2.8 Å.

Because the point charges obtained from calculations with the LP-3G basis set are of reasonably good quality (14), and such point charges are required for the electrostatic scans (see below), all the calculations of the potential energy curves for proton transfer have been performed with this basis set. The potential energy curves at the two distances are shown in Fig. 2. The transfer of the proton from IM^+ to AM is characterized by a double-well potential. At the N-N distance of 3.0 Å, the preference of the IM/AM^+ state over the IM^+/AM is only 2.1 Kcal/mole, indicating that the calculated gas-phase proton affinities of IM and AM are nearly the same. However, the transfer of the proton from IM^+ to AM has to overcome a barrier of 11.2 Kcal/mole, so that at the N-N distance of 3.0 Å, the proton shows a small preference for the AM site, but there is a significant barrier for the proton transfer. These results are summarized in Table 1. The barrier for the proton transfer originates from the increase in the energy of the system caused by removal of the proton from the stabilizing field of the lone pair of the IM. Because of the large distance between the nitrogens of IM and AM, this increase in energy is not compensated by a stabilizing interaction of the lone pair of the AM.

The changes in the energy upon transfer of the proton are paralleled by the changes in charge distribution throughout the process of proton transfer. The Mulliken net charges of the three structures at the extrema of the potential energy curve are shown in Table 2. The movement of the proton from IM^+ to AM decreases the values of the charges on the IM portion and increases the charges on the AM portion. For example, the total charge of the IM changes from 0.4670 to 0.2126, and, finally, to 0.0588 in the course of the proton transfer. The total charge on the AM changes in the opposite direction, from 0.0500 to 0.2765, and, finally, to 0.4552. The Mulliken charge of the proton increases as it reaches the TS and returns to almost its original value as it comes to a bonding distance on the other side of the barrier. These changes not only provide an explanation for the origin of the barrier but also play an important role in the electrostatic interaction between the PTM and 5-HT.

The characteristics of the PTM at the shorter distance of 2.8 Å between the nitrogens on IM and AM are consistent with the known dependence of the barrier to proton transfer on the distance between the hydrogen bonded molecules (16, 17). It also provides support for the interpretation given above for the origin of the barrier. At the shorter N-N distance, the potential energy curve shows a significantly lower barrier. The IM/AM^+ form is still preferred by 1.9 Kcal/mole over the IM^+/AM form,

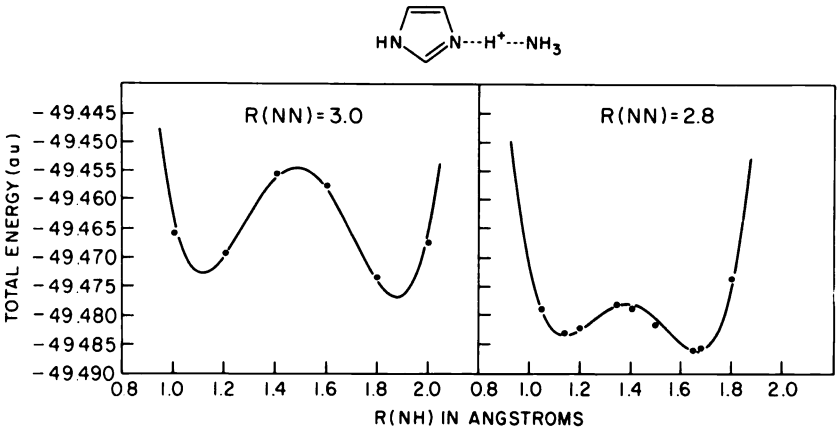

Fig. 2. Potential energy curves for the process of proton transfer in the PTM at two distances. Energies calculated with the LP-3G basis set using the coreless Hartree Fock pseudopotentials. The curves are quartic polynomials fitted to the calculated values.

TABLE 1

Characterization of the PTM composed of a complex between IM^+ and AM at an N . . . N distance of 3.0Å[a]

Form of Complex	STO-3G Optimized[b]		LP-3G Optimized[c]	
	R(N-H)Å	E (in hartrees)	R(N-H)Å	E (in hartrees)
IM^+/AM	1.079	−49.472911 (0.0)[d]	1.116	−49.473114 (0.0)[d]
TS	1.519	−49.455065 (11.2)[d]	1.483	−49.454323 (11.8)[d]
IM/AM^+	1.859	−49.476344 (−2.1)[d]	1.879	−49.476689 (−2.2)[d]

[a] All the calculations were done using the STO-3G optimized structures of the neutral and protonated forms of the IM and AM molecules according to the schemes in (12), as described in the text.

[b] The energies are the results of calculations with the LP-3G basis set using the Coreless Hartree-Fock pseudopotentials (15) performed on STO-3G optimized structures at the critical points of the curve.

[c] The energies are the results of calculations with the LP-3G basis set using the Coreless Hartree-Fock pseudopotentials (15) performed on LP-3G optimized structures at the critical points of the curve.

[d] Numbers in parentheses are relative energies in Kcal/mole, calculated with respect to the initial state with the proton on the IM.

TABLE 2

Net charges from Mulliken population analysis of the proton transfer model and its isolated components as a function of the distance of the proton from the IM nitrogen [R(N-H)]

	R(N-H) = 1.079 Å	R(N-H) = 1.519 Å	R(N-H) = 1.859 Å	Isolated ($IMID^+$)
IM				
N1	−0.3873	−0.4091	−0.4182	−0.3743
C2	0.0517	−0.0152	−0.0582	0.0602
N3	−0.3838	−0.3831	−0.3900	−0.3740
C4	−0.1579	−0.2116	−0.2376	−0.1564
C5	−0.1553	−0.1701	−0.1809	−0.1551
H1	0.4383	0.4179	0.4048	0.4596
H2	0.3669	0.3315	0.3130	0.3686
H4	0.3471	0.3180	0.3026	0.3537
H5	0.3473	0.3343	0.3233	0.3551
	0.4670	0.2126	0.0588	0.5374
AM				
N	−0.8602	−0.8188	−0.7971	
HA	0.3020	0.3636	0.4160	
HB	0.3039	0.3655	0.4177	
HC	0.3043	0.3662	0.4186	
	0.0500	0.2765	0.4552	
The proton				
H	0.4830	0.5109	0.4860	0.4626

but the barrier is now reduced from 11.2 to 2.9 Kcal/mole. This lowering of the barrier is caused by the stabilizing effects of the closer lone pairs of the IM and the AM on the proton at the transition state.

Because previous work showed that the LP-3G basis set is not reliable for structure optimizations (14), the potential energy curve for the proton transfer constructed from the calculations with the LP-3G basis set was tested against calculations with the STO-3G basis set (18), which is known to give reasonable structures. The structures of the IM^+/AM and IM/AM^+ systems were kept in the STO-3G optimized forms for both the LP-3G and the STO-3G calculation of the proton transfer. Subsequently, at the critical points from these calculations (i.e., the two minima and the maximum, see below), the energies of the system were recalculated with the LP-3G basis set. The results are presented in Table 1. Comparison of the results obtained with the LP-3G basis set with those obtained with the STO-3G basis set (and subsequently recalculated with the LP-3G basis set for comparison) shows that the potential energy curves for the proton transfer are very similar. For example, the calculated values of the N-H distances at the two minima and at the maximum on the potential energy curve agree well between the two basis sets, and the differences between them do not exceed 0.037Å. Similarly, the relative energies of the three extrema calculated with the two basis sets agree very well. In both curves, the IM/AM^+ form is preferred by approximately 2 Kcal/mole over the IM^+/AM form. The difference between the barriers obtained with the two basis sets is only 0.6 Kcal/mole, and the TS point occurs in both basis sets very close to the halfway point between the two minima. Thus, the particular process of proton transfer from IM^+ to AM does not exhibit the problems that were encountered in structure optimization using minimal valence basis sets (14).

The Electrostatic Scans

Separate scans of the electrostatic interaction energy between 5-HT and PTM were performed with each of the three states of the PTM: IM^+/AM in which the proton is in the minimal energy position next to IM^+; TS, in which the proton is in the TS position between IM and AM; and IM/AM^+, in which the proton is in the minimal energy position next to the AM (Table 1). LP-3G charges were used because they provide a good representation of the monopole-monopole approximation (6). The charges of the three forms of the PTM are listed in Table 2. The results of the scans are represented as isoenergy

contours in Figs. 3–5. The oriented structures of the PTM are sketched on the map in local minima obtained from the scan.

Fig. 3 shows the electrostatic scan of the PTM in the IM^+/AM form with 5-HT. The electrostatic interaction energy is attractive over the entire scan and shows three local minima. Local minimum #1 is positioned near the hydroxyl of 5-HT and has an interaction energy of −7.8 Kcal/mole. The other two local minima, #2 and #3, are clustered together and are only slightly different in their interaction energies, −9.9 and −10.4 Kcal/mole, respectively. They show, however, a distinct difference in their orientation with respect to the 5-HT molecule; although the PTM in local minimum #3 has a slightly lower energy, it is oriented away from the indole portion of 5-HT, whereas the PTM in local minimum #2 is oriented toward the indole ring, with the AM positioned directly over the indole. Also, the positions of these two local minima are near the position found in the electrostatic scan of 5-HT with IM^+ alone (6), but only the PTM in local minimum #2 has the same orientation as the isolated IM^+ (6). Results in Table 2 show

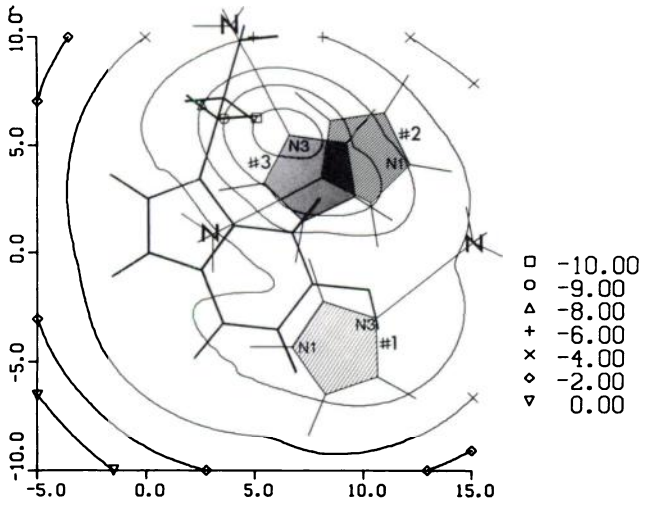

Fig. 3. Electrostatic interaction map (Kcal/mole) calculated within the approximation of monopole-monopole interactions between 5-HT and the PTM in the IM^+/AM form. The isoenergetic contours are for the best orientation of the PTM in each point obtained from a 360° rotation scan. The interplanar distance between 5-HT and the PTM is 6.238 bohr.

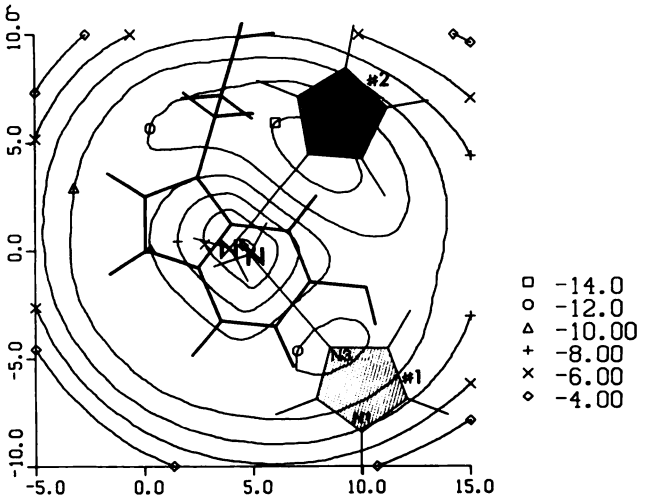

Fig. 4. Electrostatic interaction map between 5-HT and the PTM in the TS for the proton transfer. Other details as in legend to Fig. 3.

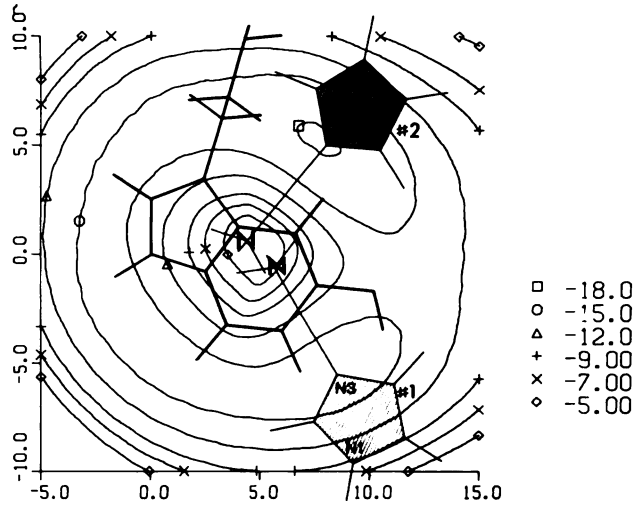

Fig. 5. Electrostatic interaction map between 5-HT and the PTM in the IM/AM^+ form. Other details as in legend to Fig. 3.

TABLE 3

Comparison of electrostatic and SCF interaction energies of 5-HT with the proton transfer model obtained from the electrostatic scan and from SCF calculations. The intermolecular distance is fixed at 6.238 bohr

Local Minimum	Geometry		Stabilization Energy	
	Location (x, y)[a]	Angle (degrees)	Electrostatic (Kcal/mole)	SCF (Kcal/mole)
LM #1	(−3.0, 10.5)	0.0	−7.8	−6.7
		110.0	−6.7	−5.0
LM #2	(3.5, 8.0)	170.0	−9.9	−8.5
		70.0	−9.2	−8.6
		290.0	−7.9	−5.7
LM #3	(5.5, 6.5)	80.0	−10.4	−9.9
		200.0	−8.8	−7.3

[a] Position in bohr units on electrostatic scan map shown in Fig. 3.

that the net charge distribution in IM^+ of the PTM complex is virtually the same as in the isolated IM^+, indicating why the directionality of the PTM is similar to that of the IM^+. It follows that this orientation of the PTM in local minimum #2 is aligned with the electrostatic directionality defined by the MEP of 5-HT in the indole portion of the molecule (2, 10).

Because the electrostatic scans serve in this work to guide the selection of geometries for more detailed calculations of the proton transfer process, it was of interest to determine whether the energies of the relative position and their orientations obtained from these scans agree with those obtained from SCF calculations. Results from previous studies show that the selectivity with respect to orientation of an isolated IM^+ obtained from electrostatic scans with 5-HT agrees well with that obtained from SCF calculations (6, 10). A similar test was performed here to establish whether the extension of the recognition probe (IM^+) by the addition of the AM portion changes this property. An additional rotational scan was therefore carried out at each local minimum to identify possible rotational minima. This scan revealed that upon rotation, the energy passes through another, slightly higher minimum that is displaced approximately 120° from the lowest one. In local minimum #2, two additional minima were identified. The comparison between the electrostatic and SCF results is summarized in Table 3. The rank order of energies obtained from electrostatic scans agrees very well with that obtained from the SCF

calculations, with a correlation coefficient of 0.962; only one case of near degeneracy is observed between two rotational minima in local minimum #2. Thus, the electrostatic scans seem to be sufficiently reliable for the prediction of relative energies of the position and orientation of preferred interaction geometries between 5-HT and the PTM.

Similar electrostatic scans were performed with the other forms of the PTM, the TS (column 2 in Table 2), and the IM/AM$^+$ complex (column 3 in Table 2). The isoenergy contour maps of these scans are shown in Figs. 4 and 5, respectively. Comparisons of these maps with the one obtained with the IM$^+$/AM form of the PTM (Fig. 3) reveal a significant change both in the shape of the isoenergy contours and in their values at the local minima. Whereas the scan with IM$^+$/AM (Fig. 3) has an orientational character, the scans with TS (Fig. 4) and with the IM/AM$^+$ (Fig. 5) forms of the PTM are more circular, with the AM portion of the PTM positioned in the center.

The change in the nature of the electrostatic scan maps is caused by the charge redistribution induced by the transfer of the proton from the IM to the AM position of the PTM. Two effects contribute to this change: First, the movement of the proton toward AM reduces the charge on IM and increases the charge on AM simply because of the displacement of the proton (see Table 2). Secondly, in the IM$^+$/AM, the positive charge is delocalized in the IM and determines the directional character of PTM with 5-HT (Fig. 3). The transfer of the charge toward the AM position generates a localized positive charge, which produces a circular electrostatic interaction map with the electrostatic potential of 5-HT (1, 2). The localization of the positive charge also increases the electrostatic interaction energy; this is clearly demonstrated in the values of the isoenergy contours in Figs. 4 and 5. In spite of the differences in interaction energies that the electrostatic scan maps exhibit with the movement of the proton in the PTM, one position remains distinct both in its orientation and in its strong interaction. This is local minimum #2 in Figs. 3–5. This minimum agrees with the position and orientation obtained from the electrostatic scan of 5-HT with IM$^+$ (6). We therefore selected local minimum #2 in each case to investigate the mechanism of the proton transfer triggered by 5-HT.

The Mechanism of Triggering the Proton Transfer by 5-HT

SCF calculations of the PTM systems interacting with 5-HT were performed for the selected geometries from the electrostatic scan maps and compared with those calculated in the absence of 5-HT to assess the role that the 5-HT molecule plays in triggering the proton transfer from the IM to the AM. The results are presented in Fig. 6.

For the purpose of comparison, the upper curve in Fig. 6a is a schematic representation of the results shown in Fig. 2. The energy of proton transfer from IM to AM is shown relative to the IM$^+$/AM form of the PTM (see value in Table 1). The transfer of the proton requires traversing a barrier of 11.2 Kcal/mole and results in a structure, IM/AM$^+$, that is preferred over the starting point, IM$^+$/AM, by 2.1 Kcal/mole. The lower curve of the same figure (Fig. 6a) presents the energy profile for the same process occurring in the presence of 5-HT. The interaction of 5-HT with IM$^+$/AM stabilizes the complex by 8.5 Kcal/mole with respect to the separated molecules. However, the IM/AM$^+$ form of the PTM is stabilized in the complex with 5-HT by 15.3 Kcal/mole with respect to the separated molecules. In the presence of 5-HT the preference for the IM/AM$^+$ is increased to 8.9 Kcal/mole as compared with 2.1 Kcal/mole in its absence. This additional stabilization of the products over the reactants, which results from the interaction of the localized charge on the AM with the negative electrostatic potential of 5-HT, can be considered as the *driving force* for the proton transfer process. The interaction of the PTM with 5-HT also changes the barrier to proton transfer: in the presence of 5-HT the barrier is reduced to 7.8 Kcal/mole as compared with 11.2 Kcal/mole in its absence. This 3.4 Kcal/mole reduction in the barrier to proton transfer comes from a similar effect as the stabilization of the IM/AM$^+$ form of the PTM, namely, from the interaction of the more localized charge in the PTM with the negative electrostatic potential of the 5-HT. Thus, the effect of 5-HT on the proton transfer process is twofold: it generates a significant driving force by enhancing the preference of the products over the reactants by 6.8 Kcal/mole, and it facilitates this process by lowering the barrier by 3.4 Kcal/mole.

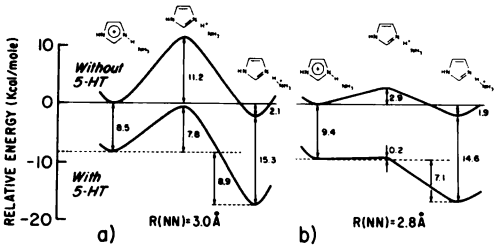

Fig. 6. Energy diagram of the proton process in the absence of 5-HT (*upper curves*) and in its presence (*lower curves*) at two *N . . . N* distances in the PTM: (a) *N—N* = 3.0Å; (b) *N—N* = 2.8Å. Energies are from calculations with the LP-3G basis set.

As was demonstrated before for a large number of different systems (for a review see Refs. 16 and 17), a different way to lower the barrier to proton transfer is to bring the proton donor closer to the proton acceptor (see Fig. 2). The upper curve of Fig. 6b is a schematic representation of the results shown in Fig. 2b, with the PTM configured at an N(imidazole) to N(ammonia) distance of 2.8Å. Shortening the distance between IM and AM to 2.8Å in the absence of 5-HT is energetically favored by 5.0 Kcal/mole. The complex still maintains a preference of 1.9 Kcal/mole for the IM/AM$^+$ form of the PTM over the IM$^+$/AM, but the change in the barrier is more dramatic. The barrier changes from 11.2 Kcal/mole in the PTM with N-N distance of 3.0Å to 2.9 Kcal/mole when the distance is 2.8Å. The lower curve of Fig. 6b shows the effect of the interaction with 5-HT with the PTM on the process of proton transfer. *First*, the stabilization energy of IM$^+$/AM with 5-HT is 9.4 Kcal/mole with respect to the separated molecules, compared with a stabilization of only 8.5 Kcal/mole in the complex with the PTM at an N-N distance of 3.0Å. The stabilization by 5-HT is 0.9 Kcal/mole greater than in the PTM with N-N distance of 3.0Å because the interaction between 5-HT and the PTM with an N-N distance of 2.8Å is favored by 5.9 Kcal/mole. Thus, 5-HT favors a shortening of the N-N distance and could help induce it by providing additional energy that may be required to overcome the structural constraints inside the protein that are responsible for a longer N-N distance. *Second*, 5-HT generates a driving force for the proton transfer by preferring the product, IM/AM$^+$, over the reactant, IM$^+$/AM, by 7.1 Kcal/mole. The mechanism of preferred stabilization of the products over the reactants is the same as for the PTM

with the longer N-N distance. *Third,* the 5-HT has a dramatic effect on the barrier to proton transfer. From the comparison of the lower curves of Fig. 6a and b, it is clear that the interaction of 5-HT with the PTM in which the N-N distance has been shortened causes a virtual elimination of the barrier to proton transfer.

A dynamic picture of the interaction of 5-HT with the PTM emerges from these studies. Initially, the interaction of 5-HT with the PTM is governed by electrostatic forces that align the two molecules and contribute to the recognition process of 5-HT by the PTM. The favorable electrostatic interaction can help induce a shortening of the N-N distance in the PTM for which the stabilization energy is greater. At the shorter distance, which is preferred for the interaction with 5-HT, the barrier is lower and is further reduced by the additional stabilization that comes from the negative electrostatic potential of 5-HT. The result is a virtual elimination of the barrier to the transfer of the proton. The charge redistribution associated with the proton transfer and the resulting localization of the charge on the AM gives rise to an increased preference for the IM/AM^+ form over the IM^+/AM form of the PTM. This serves as a driving force for the process of proton transfer.

Conclusions

The studies of the interaction of 5-HT with the proton transfer model make it possible to formulate a link between the elements of recognition of a molecule at a biological receptor and the mechanism by which the recognized molecule activates the receptor. The receptor activation is the result of changes induced in the recognition site, which can trigger molecular changes that may eventually lead to the biological response. The recognition of 5-HT by the PTM is controlled by molecular properties determined by the charge distribution and reflected in the electrostatic characteristics of these molecules. One of the important properties that controls the geometry of interaction is the tendency of 5-HT to align its electrostatic directionality (1) along the positive charge distribution in the PTM so as to maximize the electrostatic interaction between them. In this aligned geometry, the interaction between 5-HT and the PTM triggers a sequence of events that could be considered as the primary molecular events in the process of receptor activation. We have shown that in the absence of 5-HT, this process of proton transfer is prevented by a high barrier and a minimal driving force. When 5-HT approaches the PTM, the interaction lowers the barrier and generates an appreciable driving force for the proton transfer.

It is premature to conclude from these results that the actual receptor contains an IM^+ (e.g., from histidine) hydrogen bonded to a proton acceptor such as an AM (e.g., from lysine) even though such arrangements in proteins are not uncommon. These results should rather be viewed as pointing to a plausible molecular mechanism for the recognition and the primary steps of the activation of a receptor in a specific molecular realization that was unavailable heretofore. Because this mechanism is formulated in terms of molecular properties such as charge distributions and the resulting molecular electrostatic potentials, and depends on the nature of the interaction between the molecules and the effects of this interaction of their charge redistributions, the hypothesis is not constrained by the specific identity of the molecules. Rather, the resulting molecular processes identify the relevant properties of the model that should be responsible for its ability to discriminate between molecules that can be recognized by the receptor and those that show no affinity for it. The model should also be able to discriminate between agonists, i.e., molecules that are recognized and can trigger the molecular event, and antagonists, i.e., those that are recognized but are unable to trigger the response. These properties of the model are now being tested.

Acknowledgments

A generous grant of computer time from the University Computer Center of the City University of New York is gratefully acknowledged. Data analysis and curve fitting were done on the PROPHET computer system, a national computational resource sponsored by the National Institutes of Health through the Division of Research Resources.

References

1. Weinstein, H., D. Chou, S. Kang, C. L. Johnson, and J. P. Green. Reactivity characteristics of large molecules and their biological activity: indoleakylamines on the LSD/serotonin receptor. *Int. J. Quantum Chem.* **QBS3:**135–150 (1976).
2. Weinstein, H., R. Osman, W. D. Edwards, and J. P. Green. Theoretical models for molecular mechanisms in biological systems: tryptamine congeners acting on an LSD-serotonin receptor. *Int. J. Quantum Chem.* **QBS5:**449–461 (1978).
3. Weinstein, H., J. P. Green, R. Osman, and W. D. Edwards. QuaSAR: quantitative structure activity relationship of analgesics, narcotic antagonists and hallucinogens. *NIDA Research Monograph No. 22.* (G. Barnett, M. Trsic, and R. E. Willette, eds.) National Institute on Drug Abuse, Washington, D. C., 333–358 (1978).
4. Weinstein, H., R. Osman, and J. P. Green. The molecular basis of structure activity relationships: quantum chemical recognition mechanisms in drug-receptor interactions, in *Computer Assisted Drug Design: ACS Symposium Series* (E. C. Olson and R. E. Christoffersen, eds.), Vol. 112. American Chemical Society, Washington, D. C., 161–187 (1979).
5. Weinstein, H., R. Osman, S. Topiol, and J. P. Green. Quantum mechanical studies on molecular determinants of drug action. *Ann. N. Y. Acad. Sci.* **367:**434–451 (1981).
6. Weinstein, H., R. Osman, J. P. Green, and S. Topiol. Electrostatic potentials as descriptors of molecular reactivity: the basis for some successful predictions of biological activity, in *Chemical Applications of Atomic and Molecular Electrostatic Potentials* (P. Politzer and D. G. Truhlar, eds.) Plenum Publishing, New York, 309–334 (1981).
7. Shinitzky, M., and E. Katchalski. Complexes between indole and imidazole derivatives of the charge transfer type, in *Molecular Associations in Biology* (E. B. Pullman, ed.) Academic Press, New York, 361–376 (1968).
8. Morokuma, K. Molecular orbital studies of hydrogen bonds. III. C:O ... H-O hydrogen bond in H_2CO ... H_2O and H_2CO ... $2H_2O$. *J. Chem Phys.* **55:**1236–1244 (1971).
9. Kitaura, K., and K. Morokuma. A new energy decompositions scheme for molecular interactions within the Hartree-Fock approximation. *Int. J. Quantum Chem.* **10:**325–340 (1976).
10. Osman, R., S. Topiol, H. Weinstein, and J. E. Eilers. Theoretical studies of molecular complexes: a probe into basis set and correlation effects. *Chem. Phys. Lett.* **73:**399–403 (1980).
11. Osman, R., H. Weinstein, S. Topiol, and L. Rubenstein. A molecular theory of recognition and activation at a 5-HT receptor based on a quantum chemical approach to structure activity relationships. *Clin. Physiol. Biochem.* **3:**80–88 (1985).
12. Topiol, S., G. G. Mercier, R. Osman, and H. Weinstein. Theoretical models for proton transfer in $[CH_3OH.H.NH_3]^+$. *J. Comput. Chem.* **6:**581–586 (1985).
13. Osman, R., S. Topiol, and H. Weinstein. Electron density redistribution in the stabilization of a molecular stacking complex: the nature and correction of basis set superposition errors. *J. Comput. Chem.* **2:**73–82 (1981).
14. Topiol, S., and R. Osman. On the use of minimal valence basis sets with the coreless Hartree-Fock effective potential *J. Chem. Phys.* **73:**5191–5196 (1980).
15. Topiol, S., J. W. Moskowitz, and C. F. Melius. Atomic coreless Hartree-Fock pseudopotentials for atoms Li through Ne. *J. Chem. Phys.* **70:**3008–3011 (1979).
16. Schuster, P., G. Zundel, and C. Sandorfy, eds. *The Hydrogen Bond—Recent Developments in Theory and Experiments,* Vols. I–III. North Holland, Amsterdam (1976).
17. Scheiner, S. Theoretical studies of proton transfers. *Acc. Chem. Res.* **18:**174–180 (1985).
18. Hehre, W. J., R. F. Stewart, and J. A. Pople. Self consistent molecular orbital methods. I. Use of gaussian expansions of slater-type atomic orbitals. *J. Chem. Phys.* **51:**2657–2664 (1969).

Send reprint requests to: Dr. Roman Osman, Department of Physiology and Biophysics, Mount Sinai School of Medicine, Box #1218, One Gustave L. Levy Place, New York, NY 10029.