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Signal Transduction by a 5-HT_2 Receptor: A Mechanistic Hypothesis from Molecular Dynamics Simulations of the Three-Dimensional Model of the Receptor Complexed to Ligands

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The use of new methods in molecular biology and cell physiology to elucidate the primary structures and physiological functions of cell surface receptors coupled to guanine nucleotide-binding regulatory proteins, the GPCRs,[1-8] has engendered much optimism toward prospects for the design of ligands and modulators of these receptors for therapeutic purposes.[9-13] A key element in these prospects is the development of a three-dimensional (3-D) structural basis for the design efforts. Indeed, such 3-D models were constructed for several GPCRs from their sequences.[14-19] The construction of molecular models of GPCR based on perceived homologies and analogies with bacteriorhodopsin (BR)[20,21] for which the structure is known to consist of seven transmembrane helices (TMH) linked by intracellular and extracellular loops[22] presents some conceptual and practical limitations.[23,24] In spite of such difficulties, the models can suggest hypotheses concerning modes of ligand binding that can be further probed against available data.[3,15,18,25,26] The more complex element in understanding the function of GPCR is the mechanism of signal transduction that connects the ligand binding event to the interaction of the GPCR with the effector (G nucleotide-binding) protein. Thermodynamic models of drug efficacy exist,[27] but to our knowledge, no discrete model for the dynamics of transduction of the binding signal to the effector system has been explored so far at the molecular level.[13] We present here the results of molecular dynamics (MD) simulations carried out on a new 3-D molecular model of the bundle of TMHs in the 5-HT_2 receptor, designed to explore the structural consequences of ligand binding in the recognition site (see Figure 1). A dynamic signal transduction model at molecular detail emerges for the first time from these simulations. The proposed mechanism is consistent with the pharmacological efficacies of the ligands positioned in the recognition site, in that a full agonist (serotonin, 5-HT), but not an antagonist (3-(aminomethyl)-5-hydroxyindole, 5-HGR, representing 5-hydroxygramine) produces a specific structural change localized in the region that is most relevant to receptor/G-protein coupling. The structural changes produced in the region relevant to effector coupling by the binding of the full agonist are larger than those induced by a ligand of lower efficacy (tryptamine, TRYP), while the antagonist 5-HGR does not produce the same changes in receptor structure. The gradual nature of the transition from the pattern of changes induced by the full agonist to that observed for the partial agonist and then the antagonist is in agreement with experimental observations from receptors bearing specific

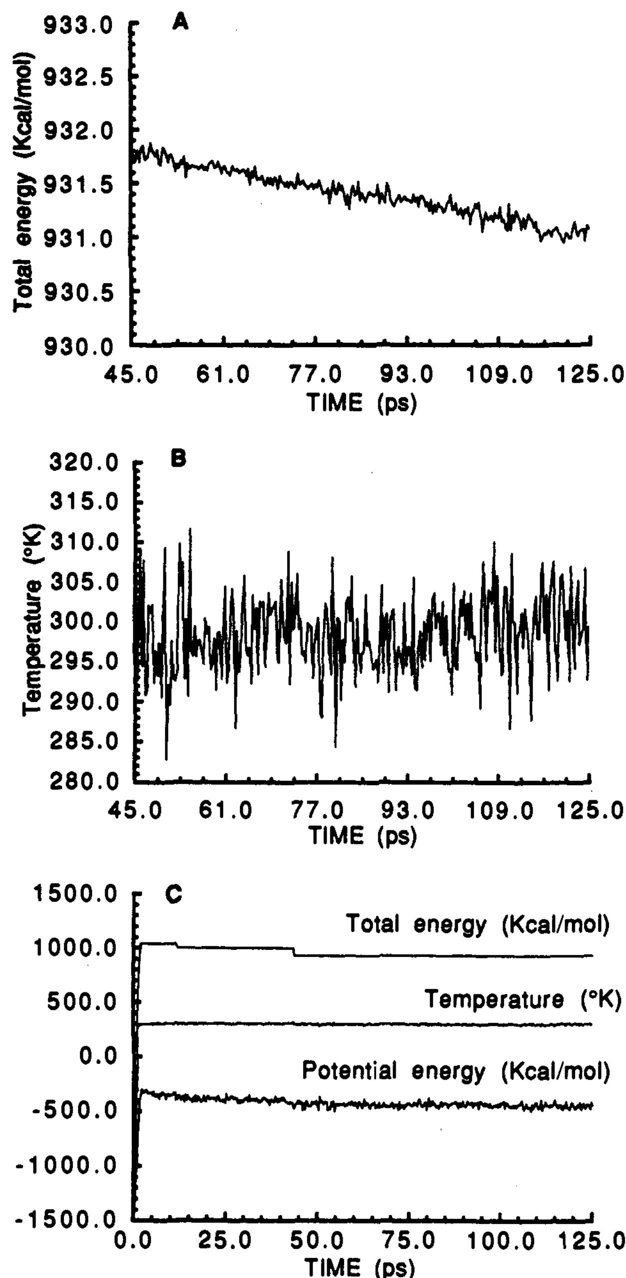

Figure 1. Energy and temperature fluctuations during molecular dynamics simulation of the 5-HT_2 receptor model: (A) the total energy; (B) the temperature; (C) the values for the entire trajectory, including the potential energy. The CHARMm package version V21.2 with the V20 set of parameters was used to carry out the calculations. Each helix of the model was capped by acetamide at its N-terminus and *N*-methylamide at the C-terminus. Ionizable residues in the helices were considered charged if they were in the middle of a helix but were neutralized if they appeared within one turn of the end of a helix. Before dynamics, the structure obtained from graphic modeling was optimized by several runs of energy minimization to a final gradient of 0.1 kcal/mol·Å. This minimization procedure consisted of alternating two runs of steepest descent for 200 steps, and two runs of conjugate gradients for 200 steps, 1000 steps of conjugate gradient, and 1000 steps of Newton–Raphson minimization. The resultant structure constitutes the starting structure for MD simulations carried out with the Verlet algorithm under the following conditions: cutoffs 8 Å for nonbonded interactions (4 Å for hydrogen bonds); pair list update frequency of 5 (steps); step size of 0.5 fs; constraints on bonds with hydrogens by the SHAKE algorithm;[60] and a fixed dielectric constant $\epsilon \approx 4$. The system was heated from 0 to 300 K in 1.5 ps. Up to 50 ps, the frequency for velocity rescaling was set to 50 (steps) and was changed to 400 after the first 50 ps.

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mutations and deletions designed to study structural correlates of receptor activation.

The Molecular Model of the Receptor. The 5-HT_2 receptor (5-HT_2R) model on which the simulations were performed was constructed as described recently[13,26] based on a number of considerations including the amino acid sequence,[28,29] its alignment with those of other GPCR and with BR,[23,28-32] agreement with structure–activity data for 5-HT_2R ligands,[12,33-35] the physicochemical and structural properties of Proline-containing transmembrane proteins,[36-38] the molecular biophysics of helix–helix interactions,[39-45] and a number of restrictive topological considerations pertaining specifically to the transmembrane region of the 5-HT_2 receptor subtype and its interaction with the most selective ligands (for details see ref 13). Our approach to the assembly of TMH[13] differed from those taken by the other published constructions in (i) the use of a specific molecular model of the recognition site obtained earlier from structure–activity considerations and computational probing as a *primary criterion* for the construction of the helix bundle and (ii) the choice of a topological template for the helix bundle that was not identical to that of BR (however, see ref 18). Consequently, the relative positions of TMHs in the present model of the 5-HT_2R differ from those found in the other published models of GPCR. Figure 2 shows the proximity of TMH7 to TMH3 and TMH4 on the one side, and to TMH2 on the other in this model of the 5-HT_2R. These relative positions of the TMH satisfy the constraints imposed by the positioning of the residues defined as the ligand binding site in this model (for the sequence numbering see ref 28, and discussion ref 13): Asp-133 (TMH3), interacting with the protonated amine group of the ligand; the side chains of Phe-218 and Phe-222 (TMH5), interacting with the aromatic rings of the ligand; Met-313 (TMH6), to match the region of the ligand corresponding to N1, C7 of the indole ring in 5-HT; and Ser-350 (TMH7), to interact with the region corresponding to the 5-OH of 5-HT (for details see ref 13).

The 5-HT_2R model used in the MD simulations consists of the TMH bundle without the connecting loops. The minor role of the intracellular and extracellular loops in determining ligand recognition in some GPCR has been demonstrated (see refs 2, 4, 46 and references therein), and the loop sequences were shown to have a negligible role in the association of the TMHs of BR (see ref 45 and references therein). This approximation obviates the difficulty in representing the behavior of the protein at the interface between the membrane environment of the helices and the aqueous environment of the connecting loops (for a discussion of the difficulties in simulating the dynamics of proteins in the membrane and interface environments see refs 13 and 26). The structure of the receptor model was energy optimized with the CHARMm program[47,48] using the parameters and protocols given in the legend to Figure 1.

Dynamics of the TMH Bundle. Figure 1 presents energy and temperature fluctuations during the MD simulations (see the legend for procedural details); the slight drift in the total energy amounts to less than 0.07% of the total. The last 80-ps period of the production run from 45 to 125 ps was divided into four equal segments, and average structures were calculated and energy minimized. The total energies of these structures are very similar to one another, and all are about 200 kcal/mol

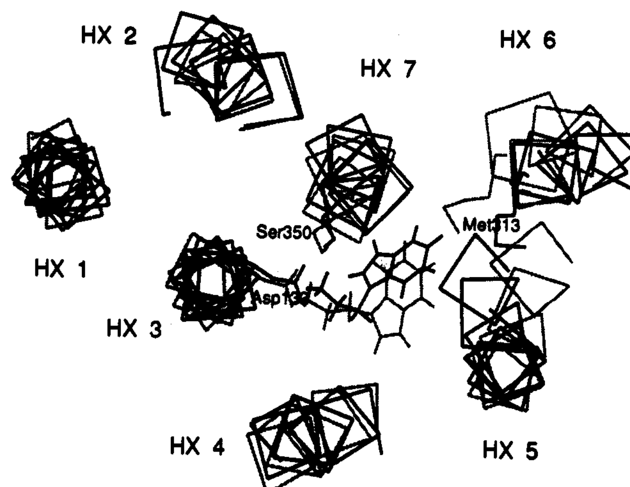

Figure 2. Tryptamine in the binding pocket of the 5-HT2 receptor model. The view is from the extracellular side, perpendicular to the membrane. The structures are shown before (gray) and after (black) the molecular dynamics simulation in the presence of the ligand. The structures of the helices are "clipped" to afford a better view of the ligand in the recognition pocket. Residues in the recognition site are identified, except for Phe-218 and Phe-222 (TMH5) which were omitted from the rendering to avoid crowding. The initial structure (gray) is the energy-minimized 20-ps (65–86 ps) average structure obtained from the MD simulation (see legend of Figure 1) on the receptor alone. The ligand was roughly docked into the putative binding site, and the simulation of the complex followed the same protocol as above (Figure 1) except that a distance constraint of 3 ± 0.5 Å between carboxyl oxygen of Asp-133 and the N of the protonated amine group in a ligand side chain was applied in predynamics energy minimization and for the first 30 ps, with settings of temperature 300 K, scale 10. The scale was reduced to 1 for the period 30–35 ps and completely released after the first 35 ps. The initial structure of tryptamine was generated and energy minimized with CHARMm, and atomic partial charges were obtained from a Natural Population Analysis[61] of the charge distribution in wave functions resulting from ab initio quantum chemical calculations with the 6-31G basis set. The total length of the simulation trajectory was 165 ps. The structure shown in black is the energy minimized average structure over 65–165 ps.

lower than that of the starting structure. The starting structure that served as reference is defined as the result of the minimization procedure described in Figure 1. Root mean square (RMS) differences of the $C\alpha$ positions in the four average structures with respect to the starting structure are all in the range of 2.6–3.0 Å, and the RMS differences among these structures are in the range of 0.7–1.5 Å. Analyses of these average structures with the linear distance plot and distance matrix methods[49] show that the TMHs maintain throughout the trajectories their helical conformations (with the appropriate Pro-kinks) as well as interactions among adjacent helices, much as observed in recent MD simulations of BR carried out under similar conditions.[50] Given the small structural differences among the 20-ps segments, the average structure from the 65–85-ps period was chosen in all subsequent simulations to represent the 5-HT_2R in the absence of ligand (the "reference structure" for calculations of ligand/receptor complexes, see below).

Structural Consequences of Ligand Binding: The Signal Transduction Mechanism. To explore the interactions in the proposed ligand recognition pocket, each of the three ligands—5-HT, TRYP, and 5-HGR—was docked into the receptor model in the *same manner and at an equivalent position*, as detailed in the legend of Figure 2. MD simulations of the ligand/receptor complexes

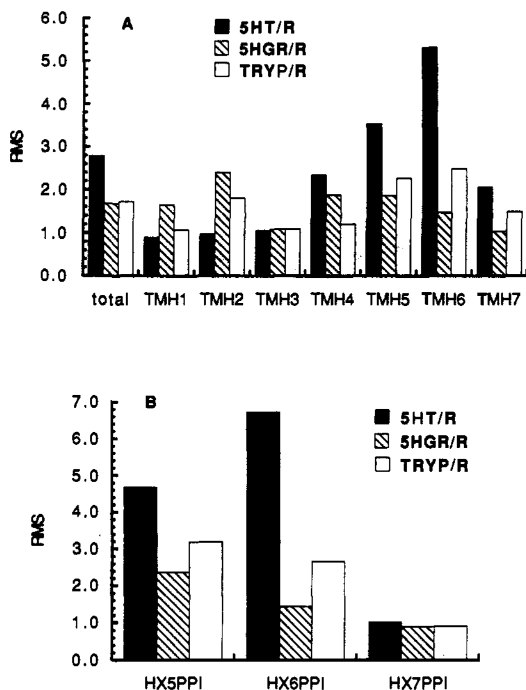

Figure 3. RMS deviations (in angstroms) of the Cα in complexes between the 5-HT_2 receptor model and ligands, relative to the average structure of the 5-HT_2R in the absence of ligands. The structures before and after the simulations in the presence of ligands were obtained as described in the legends to Figures 1 and 2. (A) Results for the complexes with the full agonist 5-HT (complex termed 5HT/R), with the lower efficacy agonist tryptamine (complex termed TRYP/R) and with the antagonist 5-hydroxygramine analog (complex termed 5HGR/R). (B) Same type of calculations, but only for the portions of TMH 5, TMH6, and TMH7 that follow the proline kink in these helices; these helical fragments are termed HX5PPI, HX6PPI, and HX7PPI, respectively.

were performed according to the protocol described in Figure 2. During the simulations, the arrangements of the ligands in the binding pocket changed from their initial positions. The indole ring in 5-HT shifted slightly in orientation to improve interactions with the binding site. In contrast, the indole ring of TRYP flipped during the simulation, resulting in its NH bond occupying a position similar to that of the 5-OH in the 5-HT/receptor complex. These dynamic rearrangements of the two agonists in the binding pocket are in agreement with the physicochemical basis of the interactions predicted for 5-HT congeners from early calculations of their properties,[34,51-54] and with recent suggestions from mutation studies of other receptors that different ligands use a variety of interactions in the same recognition site.[55] The ligand rearrangements correspond to local changes in the receptor, such as the repositioning of the side chain of Ser-350 and Met-313 and the reorientation of Phe-218. Structural changes induced by ligand binding that are propagated distally into the receptor molecule can be identified from the comparative analysis of the dynamic trajectories of ligand/receptor complexes.

The structural effects of ligand binding were analyzed from RMS deviations of the Cα in the resulting energy-minimized average structures of the receptor–ligand complexes, *compared to the same starting structure* of the receptor used in all simulations. This reference structure is the energy-minimized average structure over the 65–85-ps trajectory of the equilibrated 5-HT_2R in the absence of ligand. As shown in Figure 3A, the values of the RMS deviations differ among the TMHs, in a pattern that is not the same for the three complexes. This suggests that ligands with different pharmacological efficacies induce different types of conformational changes of the receptor. In the 5-HT/receptor complex (5HT/R), the RMS values for helices 1–3 are about the same and quite small; however, the RMS values for helices 4–7 show significant differences, with the largest changes induced by 5-HT in TMH6 and TMH5. The pattern of RMS differences produced by the ligand binding in the 5-HGR/receptor complex (5HGR/R) is different from that of 5HT/R in that the changes are roughly comparable in helices 1–3 and 4–7, and the individual RMS values differ more within the first group of helices than within the second (the largest is for TMH2). Note especially that the RMS differences in helices 4–7 are generally smaller than for 5-HT or TRYP and smallest for TMH5 and TMH6. The structural effect of the agonist with lower efficacy, TRYP, as measured by the RMS differences for its TRYP/R complex (Figure 3A), are clearly intermediate between those of 5-HT and the antagonist. Thus, the pattern of RMS in TRYP/R is similar to that of 5HGR/R in helices 1–3 and similar to that of 5HT/R in helices 4–7. Furthermore, when magnitudes are considered, those of TRYP/R are always smaller than those of 5HT/R in the region most relevant to effector coupling (TMH5 and -6). The differences in the patterns of the changes induced by ligand binding make it very unlikely that they are simply artifacts of the positioning of the ligands. Thus, the ligand smallest in molecular weight and volume, TRYP, produces changes that are intermediate between the other two. In addition, the regions of the receptor model most affected by the presence of the ligand are not defined by their proximity to the binding site. Thus, TMH3 and TMH7 are near to, but much less affected than, TMH4 which is farther and TMH5 and TMH6 which are about equidistant. The mechanism of these changes is clearly related to rearrangements in helix–helix interactions and in the intrahelical stabilization factors, as detailed in an analysis to be presented in a subsequent publication.

Both TMH5 and TMH6 contain proline residues. The importance of the Pro residues of a TMH in the structural rearrangements produced by ligand binding has been proposed on the basis of structural as well as energetic considerations.[36,38-39,41] The major changes produced in these two helices as a result of ligand binding are most evident in the cytoplasmic end of each helix that follows Pro.[13,26] Figure 3B shows the distribution of RMS differences produced by ligand binding, calculated for just that part of helices 5–7.

Results in Figures 2 and 3 indicate that the main differences in ligand/receptor complexes with agonists as compared to those with the antagonist are (1) in the intracellular side of TMH5 and TMH6 where the agonist seems to produce the largest change in the regions that have been suggested from a variety of experiments[4] to be important for the interaction of GPCRs with G-proteins[2,4,5,46] and (2) in the group of TMH1–3. A conserved Asp in TMH2 belonging to this group of TMHs has been suggested—based on mutation experiments[56-58]—to be important for regulation of GPCRs by cations, and the antagonist seems to produce here more of an effect than the agonist, albeit still quite small. These differences in the pattern of RMS values representing the conformational changes induced by ligands with efficacies ranging from

agonist to antagonist suggest a correlation between the structural changes and pharmacological properties. Experiments with receptors activated constitutively by a mutation in the region between TMH5 and -6[59] have already demonstrated the gradual nature of the transition from a resting state of the receptor to various degrees of activation caused solely by structural changes. That these structural properties in the putative region connecting TMH5 and TMH6, termed intracellular Loop III, affect ligand affinity was also shown by these elegant experiments.[59] These and other findings (e.g., see refs 2, 5, and 46) point to structural properties in the intracellular Loop III as key factors in the gradual activation of GPCR. The results presented here indicate that the simulated effects of agonist binding produce a structural signal in the same region, indicating a possible mechanism for signal transduction from ligand binding to the region responsible for coupling to the effector. As for the antagonist studied here, it seems to have two effects: (1) it occupies the agonist binding space without inducing the same conformational change as the agonist and (2) it disturbs the structure of TMH1-3. It is noteworthy that the effect of the agonist with lower efficacy was shown to include the conformational changes in the latter group of TMHs, albeit smaller than for the antagonist we studied.

The mechanistic conclusions reached from these first studies of the dynamics of a 3-D model of GPCR in the presence of ligands require further probing with a variety of ligands of varying structures and efficacies. The detailed molecular interactions underlying the observed propagation of the structural changes proposed as the molecular signal transduction mechanism must be identified in order to provide useful insight into the structure-function relations of this receptor, and to make possible the design of specific ligands that produce such changes to a predetermined extent. Methodological improvements and a probing of the effects of the environment, both lipid and aqueous, are required for further validation and quantitative evaluations of the effects we observed. However, it is noteworthy that the nature of the conclusions reached here at the molecular level of detail enables probing at each step by specific mutations and that generalizations to other GPCR can also be explored on that basis.

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Supplementary Material Available: The *xyz* coordinates of each of the three ligands (5-HT, TRYP, 5-HGR) in their final positions in the structures of the respective ligand/receptor complexes (5-HT/R, TRYP/R, 5HGR/R) and of the residues found within 5 Å of any atom of the ligand (12 pages). Ordering information is given on any current masthead page.

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