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Role of Threonine 342 in Helix 7 of the 5-Hydroxytryptamine Type 1D Receptor in Ligand Binding: An Indirect Mechanism for Receptor Selectivity

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SUMMARY

Recent mutations of the 5-hydroxytryptamine (5-HT)$_{1B}$ and 5-HT$_{1D}$ receptor subtypes suggest that a threonine in the seventh transmembrane helix may be responsible for the selectivity of these receptors. A molecular dynamics simulation of a three-dimensional model of the 5-HT$_{1D}$ receptor interacting with a selective agonist, sumatriptan, shows that, although Thr^{342} in helix 7 does not have a direct interaction with sumatriptan, it contributes to the selectivity of this receptor through an indirect mechanism. The hydrogen bond between O_γ-H of Thr^{342} and the backbone C=O of Phe^{338} stabilizes a bent conformation of the helix that is formed due to the interaction between sumatriptan and Asp^{339} at one end and Tyr^{346} at the other end. The indirect mechanism may explain the small change in the affinity for the selective agonist sumatriptan of the receptor in which Thr^{342} was mutated to asparagine.

5-HT, a neurotransmitter found in the central nervous system, activates several subtypes of serotonergic receptors (1). These receptor subtypes belong to the family of G protein-coupled receptors and mediate a great variety of physiological responses. The cloning of the 5-HT$_{1B}$ receptor subtype in rodent brain (2), followed by the isolation of the gene for the human 5-HT$_{1B}$ subtype (3), offered an opportunity to focus on the identity of the amino acid residues that may be responsible for their different selectivities. The human receptor (also designated 5-HT$_{1D\alpha}$) (4) is 93% identical to the rodent receptor. Of the 32 replacements, only eight are in the putative transmembrane helical portions, raising the question of the identity of the specific amino acid residues that confer selectivity to these receptors. Three recent works (5–7) focused on the role of threonine in Hx 7 in determining the specificity of the human receptor, compared with the rodent receptor, which has an asparagine in the same position. A mutation of Thr^{355} in the human receptor did not change the affinities for 5-HT or 5-carboxamidotryptamine but increased the affinity for pindolol by a factor of approximately 500. The mutated receptor also bound metergoline, methysergide, and sumatriptan with 5–15 times lower affinity than the wild-type receptor. Because sumatriptan is a selective agonist for the human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (8), a detailed understanding of the specific role of Thr^{355} may help in formulating the molecular basis for the selectivity of these receptors. Such an understanding may also help in the search for new selective drugs.

Materials and Methods

To address the role of Thr^{355}, we have performed MD simulations of a complex between sumatriptan and a three-dimensional structural model of the human 5-HT$_{1D\alpha}$ receptor. The three-dimensional model was constructed based on the known sequence of the 5-HT$_{1D\alpha}$ subtype (9). The α and β subtypes of 5-HT$_{1D}$ receptors are highly homologous. Hx 3 and Hx 7, which serve as the major structures involved in ligand recognition, are virtually identical. Because of several deletions in the loops connecting the Hx, the numbering is different in the two subtypes. Thr^{355} in the 5-HT$_{1D\beta}$ receptor is identical to Thr^{342} in the 5-HT$_{1D\alpha}$ receptor. Throughout the manuscript the numbering system for 5-HT$_{1D\alpha}$ is used.

With the aid of extensive computational modeling techniques,[1] the receptor model was constructed in three stages. First, the putative transmembrane portions were built individually as ideal α-Hx and their structures were optimized by energy minimization and relaxed by 40–60 psec of MD simulation at 300°K. The stabilization of the total energy and RMSD were used to determine the simulation time required for the Hx to approach a stable structure. In the second stage, averaged and minimized structures from the last 10 psec of the simulation of individual Hx were used to construct an initial structure of a helical

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[1] A. Smolyar and R. Osman. Three-dimensional model of a selective recognition site in the human 5-HT$_{1D}$ receptor. Manuscript in preparation.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; Hx, helix or helices; MD, molecular dynamics; RMSD, root mean square deviation; BRD, bacteriorhodopsin.

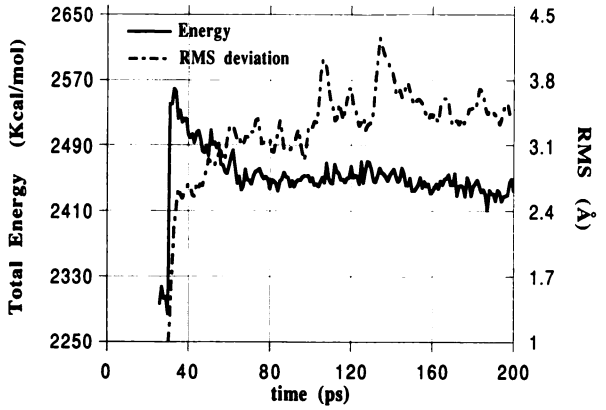

Fig. 1. Total energy and RMSD fluctuations during MD simulations of the complex of the 5-HT$_{1D}$ receptor with sumatriptan.

TABLE 1

Analysis of interaction energies between sumatriptan and receptor

Residue	Interaction energies			
	Before dynamics	140–160-psec average	160–180-psec average	180–200-psec average
	kcal/mol			
Leu^{115} (Hx 3)	−11.35	−9.29	−9.42	−9.38
Asp^{118} (Hx 3)	−47.29	−47.13	−45.77	−46.33
Phe^{317} (Hx 6)	−0.15	−3.85	−2.95	−3.23
Asp^{339} (Hx 7)	−19.25	−29.71	−29.33	−29.64
Thr^{342} (Hx 7)	−12.64	−2.61	−2.58	−2.58
Trp^{343} (Hx 7)	−4.69	−7.91	−5.72	−6.51
Tyr^{346} (Hx 7)	−7.24	−18.27	−17.55	−18.63

bundle of the seven transmembrane helical portions, using the general topology of BRD (10). The backbone of each Hx in the sequence of the 5-HT$_{1D\alpha}$ receptor was fitted to the backbone of the corresponding Hx of BRD, using the conserved residues to define the orientation of the Hx towards the inside of the helical bundle (11). At the end of the construction the RMSD of the superimposed backbones of the 5-HT$_{1D}$ receptor and BRD was 3.37 Å, indicating that the backbones of the two structures are not identical. In the third stage, the structure of the entire helical bundle was optimized through energy minimization and sumatriptan was docked inside the helical bundle by positioning the protonated side chain amine next to the carboxylate of Asp^{118} in Hx 3, the N-H group of the sulfonamide of sumatriptan next to Asp^{339} in Hx 7, and the S-O group next to the O$_\gamma$-H of Thr^{342}. The choice of Thr^{342} as an anchoring point to S-O was made based on recent experimental results (5–7), which demonstrated changes in the binding affinity of 5-HT$_{1D}$-selective ligands. Finally, the structure of the sumatriptan-receptor complex was optimized by energy minimization and then heated to 300°K, followed by 200 psec of constant-temperature MD simulation at 300°K with a distance-dependent dielectric function. At the end of the simulation the RMSD between the backbones of the receptor and BRD was 4.22 Å. All calculations were performed with AMBER 4.0 (12, 13).

Results and Discussion

The variations of the total energy of the complex and the RMSD from the minimized structure in the course of the MD simulation, shown in Fig. 1, indicate that the fluctuations are approximately around an average, suggesting that the structure has reached a stable point along the trajectory. The trajectories from the three last 20-psec intervals were averaged and the resultant structures were minimized. These structures were used to calculate the interaction energies between sumatriptan and the neighboring residues. The data shown in Table 1 clearly illustrate that the variations in the interaction between sumatriptan and the receptor are small, suggesting that the complex has reached a stable point. An inspection of a zone of 3.5 Å around sumatriptan shows that the binding site includes groups from 14 different residues. Of these, only seven residues (listed in Table 1) interact directly with sumatriptan with energetic contributions to the total interaction energy exceeding 2.5 kcal/mol. The binding site made up of these residues is shown in Fig. 2. The cationic side chain of sumatriptan forms an ionic interaction with the carboxylate of Asp^{118}. Direct hydrogen bonds are formed with the backbone C═O of Leu^{115} (to indole N-H of sumatriptan), the carboxylate of Asp^{339} (to the sulfonamide N-H), and the O-H of Tyr^{346} (to the sulfonamide S-O). Two hydrophobic interactions can also be observed, one a stacking interaction with Trp^{343} and the other with Phe^{317} (data not shown; see Table 1).

Surprisingly, the interaction with the O$_\gamma$-H of Thr^{342}, which was built into the initial structure, was effectively replaced by that with the O-H of Tyr^{346}. Inspection of the MD trajectory indicated that in the course of the simulation the O$_\gamma$-H of Thr^{342} turned to form an interaction with the backbone C═O of Phe^{338}. An analysis of the time evolution of the interaction energies between sumatriptan and four residues with major contributions to interaction energy (Asp^{339}, Thr^{342}, Trp^{343}, and Tyr^{346}) is shown in Fig. 3, A and B. The data show that after a brief equilibration the interaction energies between sumatriptan and Asp^{339}, Trp^{343}, or Tyr^{346} stabilize around the final values observed in Table 1 (Fig. 3A). The interaction energy of sumatriptan with Thr^{342} shows a different behavior (Fig. 3B); the

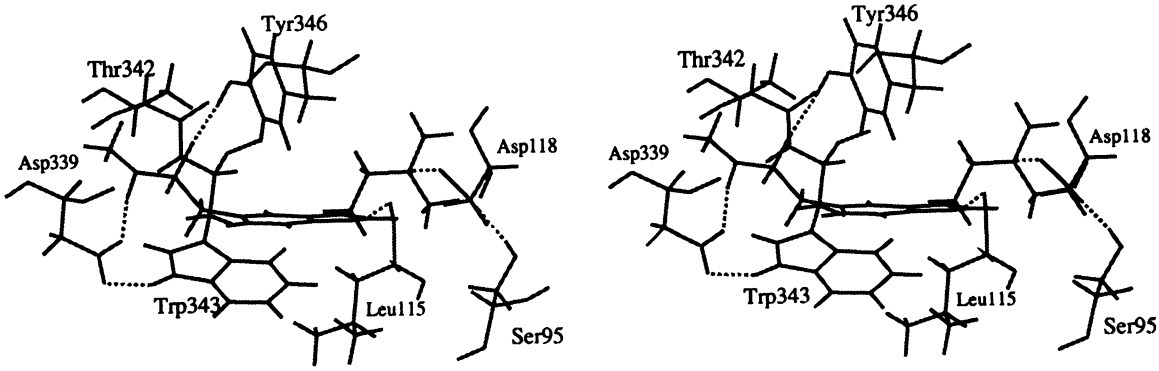

Fig. 2. Stereo view of the binding site, showing sumatriptan in the center (unlabeled) surrounded by the amino acids that make direct contact with it.

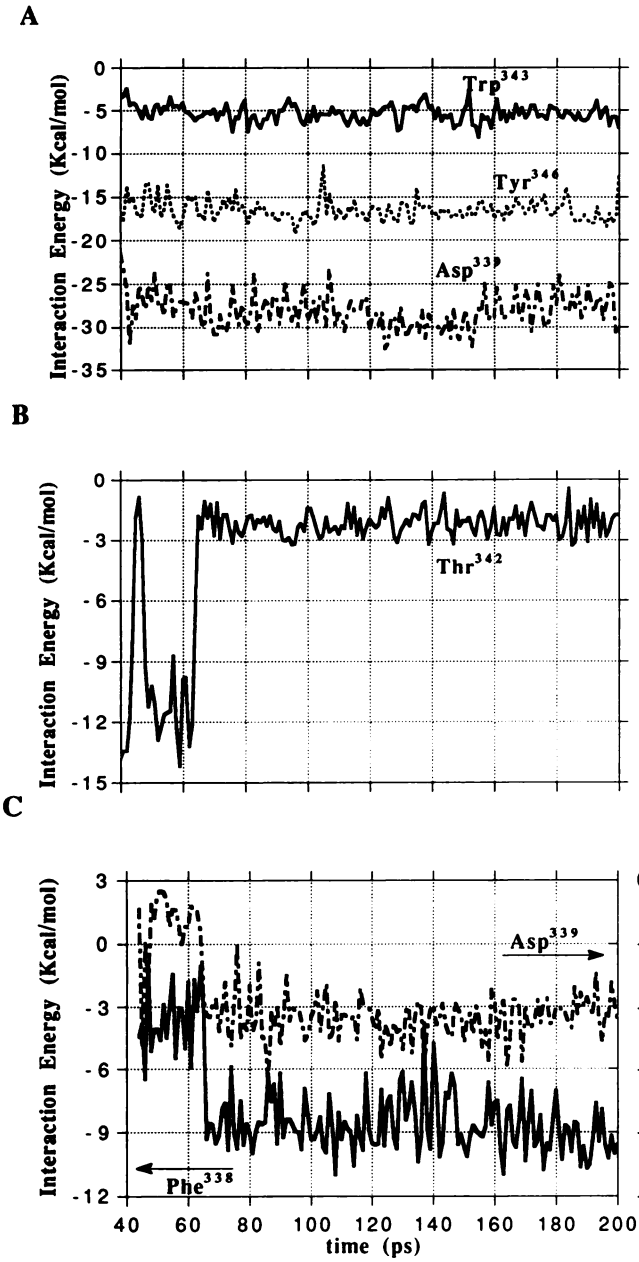

Fig. 3. Time evolution of interaction energies during the MD simulation. A, Interaction energies between sumatriptan and three residues that are part of the binding site, i.e., Asp^{339}, Trp^{343}, and Tyr^{346}. B, Interaction energy between sumatriptan and Thr^{342}. C, Interaction energies between Thr^{342} and two residues in its neighborhood, i.e., Phe^{338} and Asp^{339}.

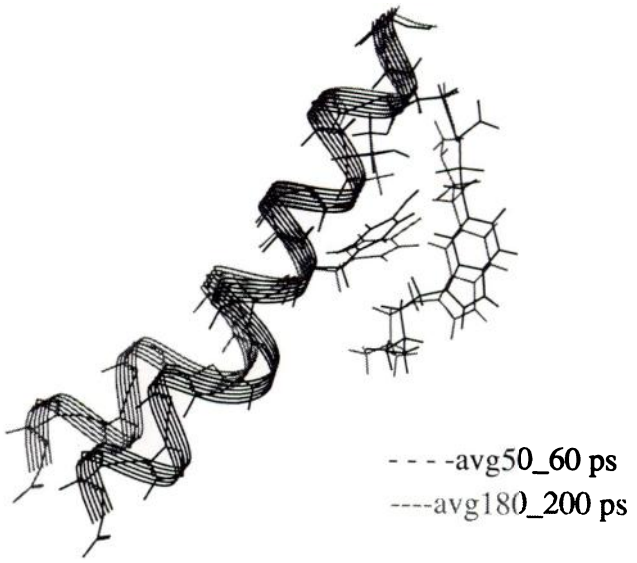

Fig. 4. Superposition of two averaged and minimized structures of Hx 7 with sumatriptan. The *structure in bold face* is from the 50–60-psec interval, which is before the rotation of Thr^{342}. The *other structure* is from the 180–200-psec interval, which is after the rotation of Thr^{342}. The structures were superimposed by a least squares fit of the backbone of Thr^{342}. Note the large deviations of the intracellular ends of the Hx between the two structures.

interaction between the O-H of Thr^{342} and sumatriptan is maintained for the first 42 psec but is briefly interrupted because of a fluctuating rotation of the hydroxyl around the C_β-O_γ bond. At 64 psec, a rotation around C_α-C_β turns the methyl group and the O-H group of Thr^{342} permanently away from sumatriptan. As a result, the O_γ-H forms a hydrogen bond with the backbone C=O of Phe^{338}. The interaction of a threonine side chain with the backbone is well known (14) and is believed to be the consequence of the steric collision between the threonine methyl group and the backbone of the Hx.

To further analyze the behavior of Thr^{342}, the interactions of Thr^{342} with residues in its environment were calculated as a function of time. These are illustrated in Fig. 3C. Simultaneously with the loss of the interaction between Thr^{342} and sumatriptan, Thr^{342} interacts with Phe^{338} and Asp^{339}, respectively. A detailed analysis of these interactions shows that they are made up primarily of an interaction between the side chain of Thr^{342} and the backbone of Phe^{338} and both the side chain and the backbone of Asp^{339}. The character of those interactions is mostly electrostatic. Analysis of the energetic changes shows that the loss of interaction energy between Thr^{342} and sumatriptan due to side chain rotation is perfectly compensated for by the interactions of Thr^{342} with Asp^{339} and Phe^{338}. This energy transfer, which takes place in the course of the MD simulation, could be considered as the exchange of a local interaction between the ligand and the receptor for favored interaction between threonine and the backbone of the Hx that stabilizes the ligand-receptor complex.

To examine the role of the hydrogen bond between the O_γH of Thr^{342} and the backbone of the Hx (C=O of Phe^{338}), we have compared the structure of Hx 7 before and after the rotation of Thr^{342}. Two averaged and minimized structures of the ligand-receptor complex obtained from the MD simulation were superimposed by a least squares fit to the backbone of Thr^{342}. One was from the 50–60-psec interval, representing a structure before the rotation, and the other was from the 180–200-psec interval, representing a structure after the rotation of Thr^{342}. The superimposed structures, restricted to Hx 7, are shown in Fig. 4. An analysis of the changes in the Hx shows that an immediate result of the rotation of the side chain of Thr^{342} is the displacement of the C=O of Phe^{338}, with which it forms a hydrogen bond, by 0.61 Å. The C=O of Asp^{339} is also displaced by 0.56 Å, making it possible for the backbone N-H of Thr^{342} to create an additional hydrogen bond with C=O of Asp^{339}, in addition to its usual hydrogen bond with C=O of Phe^{338}. This rearrangement results in a movement of C_α of Asp^{339} by 0.78 Å and of O_δ by 0.83 Å. However, because O_δ of Asp^{339} interacts with the sulfonamide N-H of sumatriptan, this movement also

displaces sumatriptan. Because the other side of the sulfonamide, the S-O, interacts with the O-H of Tyr^{346}, the displacement results in the movement of the C_α of Tyr^{346} by 1.24 Å. These local changes in the binding site of sumatriptan are further propagated along the Hx. For example, the displacement of the backbone of the residues on the same side of the Hx as Tyr^{346} increases in the same direction; the C_α of Leu^{350} is displaced by 1.42 Å, the C_α of Ile^{354} is displaced by 3.21 Å, and finally, at the bottom of Hx 7, the C_α of Thr^{357} is displaced by as much as 4.27 Å. The resultant overall bending of Hx 7 can be clearly seen in Fig. 4. An examination of the positions of the neighboring Hx shows that they move as well; in fact, the relative positions of all Hx have been affected by the rotation of the side chain of Thr^{342}. Thus, although Thr^{342} does not interact directly with sumatriptan, its side chain assists in the stabilization of a bent backbone, which changes the position of other Hx. It is possible that the mutation of threonine to asparagine does not allow the formation of the intrahelical interaction, which changes the affinity of sumatriptan for the mutated receptor by only 1–1.5 kcal/mol. The effect of this mutation on the affinity of sumatriptan (and other ligands) (5–7) is therefore indirect. This is in clear distinction from the effect of this mutation on the affinity of pindolol, which increases by 3.5–4 kcal/mol. This suggests that asparagine interacts directly with pindolol.

The present study presents a proposition for the composition of the binding site in the 5-HT$_{1D}$ receptor and the basis of the selectivity conferred by threonine in Hx 7. These possibilities, such as the explicit role of Asp^{339} and Tyr^{346}, which interact with the sulfonamide, and especially the hypothesized indirect role of Thr^{342} in determining selectivity, could be tested by site-directed mutagenesis. Recently, two closely related receptors have been cloned and their pharmacological profiles have been studied. One subtype, denoted 5-HT$_{1E}$ (15, 16), has a low affinity for sumatriptan (K_i of approximately 2500 nM), whereas the other, denoted 5-HT$_{1F}$ (17, 18), has a K_i of approximately 20 nM. An inspection of the sequences shows that significant variability can be found in Hx 7 around the area explored in this study. For example, the beginning of Hx 7 in the new subtypes has a glutamate instead of the alanine in the 5-HT$_{1D}$ subtypes. In the place of Phe^{338} in 5-HT$_{1D}$, which plays an important role in forming a hydrogen bond to Thr^{342}, 5-HT$_{1E}$ has an alanine and 5-HT$_{1F}$ a serine. Furthermore, the 5-HT$_{1F}$ subtype has an asparagine in place of Asp^{339} and an alanine in place of Thr^{342}. Clearly, the variability of the sequences in this area can be the basis for their differential selectivity with respect to other ligands. Further molecular biological experiments and MD simulations could provide a precise delineation of the binding pocket in these receptors.

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