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Modeling of ligand binding to the dopamine D2 receptor

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Abstract: The dopaminic receptors for a long time have been major targets for the development of new small molecules with high affinity and selectivity to treat psychiatric disorders, neurodegeneration, and drug abuse, and in other therapeutic areas. In the absence of a 3D structure for the human dopamine D2 (HDD2) receptor, the efforts for the discovery and design of new potential drugs rely on comparative models generation, docking and pharmacophore development studies. To obtain a better understanding of the HDD2 receptor binding site and the ligand–receptor interactions, a homology model of the HDD2 receptor based on the X-ray structure of the *β*2-adrenergic receptor was built and used to dock a set of partial agonists of the HDD2 receptor. The main characteristics of the binding mode for the HDD2 partial agonists set are given by the particular folding of a ligand and a complex network of contacts represented by stacking interactions, salt bridge and hydrogen bond formation. The characterization of the binding mode of the partial agonists at the HDD2 receptor provides the information required to generate pharmacophore models, which represent essential information for future virtual screening studies in order to identify new potential HDD2 partial agonists.

Keywords: GPCR; homology modeling; D2 receptor; molecular docking.

INTRODUCTION

Once the link between psychosis and dopamine was established, the modulation of dopaminergic activity intermediated by the dopamine D2 receptors as a possible treatment for schizophrenia continues to remain a challenging research problem. Based on their impact on diagnosis and treatment, the symptoms of schizophrenia are separated into positive symptoms that involve an excess or distortion of normal functions and negative symptoms that are given by a reduction or loss of normal functions. The negative symptoms are present during episodes of low (or absent) positive symptoms and are related with hypoactive

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prefrontal cortex whereas the positive ones are associated with hyperactive dopaminergic transmission in the mesolimbic brain region.

Dopamine D2 receptor antagonists were initially used to treat schizophrenia and related psychiatric disorders. Traditional D2 antagonist antipsychotics have good results in the treatment of the positive symptoms since they block the D2 receptors but an excessive attenuation of brain dopamine neuronal activity is comparable to the neuronal activity recorded in Parkinson's disease. Thus, normalization of dopaminergic activity can be achieved by using dopamine D2 partial agonists. An effective D2 partial agonist would be very efficient in treating the positive symptoms by selectively activating the inhibitory presynaptic D2 autoreceptors while weakly antagonizing the postsynaptic D2 receptors. A partial D2 agonist is theoretically effective in the treatment of positive symptoms based on hyperactivity and in the treatment of negative symptoms based on hypoactivity.

Among the synthesized partial D2 ligands, compounds having Ki_{high} and Ki_{low} values determined for both high- and low-affinity agonist states of the HDD2 receptor ($D2_{high}$ and $D2_{low}$) were selected for this study.[1–3] The set of ligands was docked at the agonist site of the homology model of HDD2 receptor in order to explore the pharmacophoric requirements of HDD2 receptor and to identify the features of the pharmacophore models for HDD2 receptor ligands.

METHODS

Sequence alignment

The sequence of the HDD2 receptor was extracted in Fasta format from the UniProt/Swiss-Prot database[4,5] (P14416) and was automatically aligned using the T-coffee server[6,7] with the sequence of human β2-adrenergic receptor (β2-AR) taken from the RSCB Protein Data Bank (accession code: 2RH1). The resulting alignment was further manually refined according to the template structure. The lysosome T4 fragment was removed from the sequence of the crystal structure of the β2-AR.

Model building

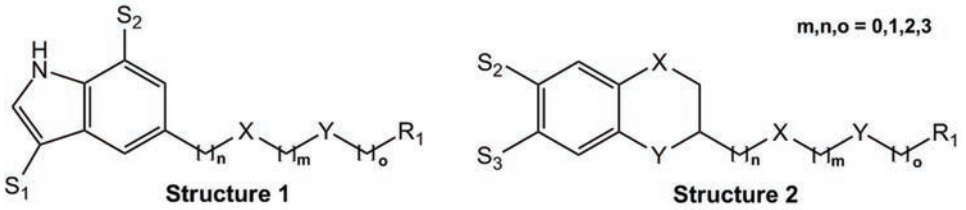
The homology modeling package Modeller[8,9] (version 9v6) was used to generate ten homology models for the HDD2 receptor based on the X-ray structure of β2-AR using the sequence alignment presented in Fig. 1. The C- and N-terminal parts were not modeled. The obtained models were energy-minimized using the standard AMBER99 force field implemented in the HyperChem7.52 package.[10] In the minimization process, the Polak–Ribiere conjugate gradient was used as the optimization algorithm, the stop criterion for the optimization was set to a RMS gradient equal or less than 0.01 kcal/Å·mol. The refined models were stereochemically validated using PROCHECK software.[11,12]

Ligand docking

Ligand setup. A set of previously synthesized and evaluated ligands[1-3] for their affinity and selectivity for high- and low-affinity agonist state of the D2 receptor were chosen for this study (Table I). The structures of the ligands were generated with ISIS Draw[13] and converted into 3D-structures with the Model Builder module of the HyperChem7.52 program.[10] All

ligands were considered in the protonated form and Gasteiger–Marsili atomic partial charges were computed for all of them. The Polak–Ribiere conjugate gradient was used as the optimization algorithm, the stop criteria for the optimization step was set up to a RMS gradient equal or less than 0.01 kcal $Å^{-1}·mol^{-1}$.

TABLE I. General structures **1** and **2** for the selected set of ligands taken in this study



Substituent	Structure
X/Y	CH_2; O; NH
S1	H; Cl; CO–CF_3
S2	H; Cl; OH
S3	H; OH
Ring R1	2-thienyl; 3-thienyl; 2-furyl;3-furyl; cyclohexyl; 4-pyridyl; 4-hydroxyphenyl; 4-fluorophenyl; 4-chlorophenyl; 3-aminophenyl; 1-naphthyl; 2-naphthyl; 1-biphenyl; 1,2,3,4-tetrahydroisoquinoline; 2-indolinyl; 2-indolyl; 2-benzoxazolyl; 3-methyl-1,2-benzo[*d*]isoxazolyl; 3-amino-1-methylphenyl; 3-amino-1-methylphenyl-5-yl; 3-amino-2-chlorophenyl; 2-amino-4-pyrimidinyl

Receptor setup. The 3D structure of the HDD2 receptor was used in the docking process with all the polar hydrogen atoms included. The Kollman united atom charges were also computed.

Docking protocol. The molecular docking was performed with the AutoDock 3.0.5 software package.[14] During the docking simulations, the protein is required to be rigid but the ligands are flexible. The ligand torsional flexibility depends on the number of rotatable bonds from each molecule and it does not apply to bonds in the rings, amide or guanidinium bonds, *etc*. Docking was performed applying a standard protocol, with an initial population of 10 randomly placed individuals, a maximum number of $1.5×10^6$ energy evaluations, a mutation rate of 0.02, a crossover rate of 0.8, and an elitism value of 1. Ten independent docking runs were computed for each ligand. Results differing by less than 0.5 Å in the positional root--mean-square deviation (rmsd) were clustered together and represented by the result with the most favorable free energy of binding. The initial charges were kept and solvation parameters were added to the final protein using the ADDSOL utility of AutoDock 3.0.5.

The grid maps representing the protein in the actual docking process were calculated with AutoGrid. The grids were chosen to be sufficiently large to include not only the active site but also significant portions of the surrounding surface. Thus, the dimensions of the grids were set to 2.44 nm×2.44 nm×2.44 nm, with a spacing of 0.0375 nm between the grid points.

RESULTS AND DISCUSSION

The recently solved structure of the human β2 adrenergic receptor structure (PDB code: 2RH1) was selected as the structural template to model the three-dimensional structure of the HDD2 receptor. The sequence alignment generated with T-coffee server was manually refined using as a guide the three dimensional structure of the template. The refinements were conducted in order to avoid deletions or insertions in the transmembrane domain and to preserve the highly conserved amino acid motifs specific for each transmembrane helix identified based on the conserved residues within the GPCR amino acid sequences. The deletions and insertions were merged into a single piece per loop and moved to the most adequate point according to the template structure. A structural feature common to many GPCR receptors, the formation of a conserved disulfide bond between two cysteine residues placed at the beginning of TM3 and the middle of the second extracellular loop was taken into consideration. The C-terminal part and the second intracellular loop (IL2) were not modeled because these fragments do not have a correspondent in the template structure. The lysosome T4 fragment was removed from the initial structure for ease of use.

The alignment shown in Fig. 1 was used to generate ten 3D models of the HDD2 receptor using the MODELLER software package.[8,9] Each model was further refined with the help of the HyperChem7.52 program as described elsewhere.[15] Shortly, the torsion angles, bond lengths, and peptide-bond planarity were checked with the PROCHECK program and were found to be within the interval of the standard values for nine out of the ten models.

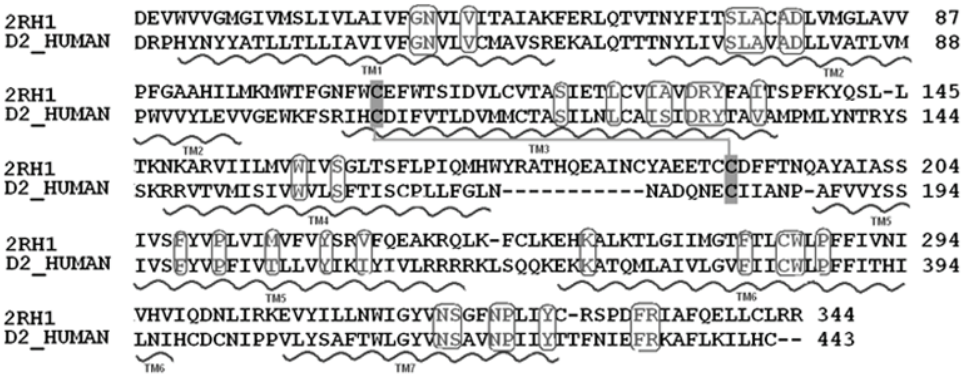


Fig. 1. Sequence alignment of the human beta2-adrenergic receptor and the HDD2 receptor showing a sequence similarity of 59.8 %. The seven transmembranes are underlined with a curved line; the highly conserved residues in the GPCR family are shown in grey and are enclosed in rounded rectangles; the residues involved in the sulfur bridge formation have a grey background.

Site-directed mutagenesis studies showed that Asp114, Ser193, Ser194 and Ser 197 are the most important residues involved in dopamine binding at the active site of the HDD2 receptor.[16] Mutation of Asp114, a highly conserved residue that has been studied in a number of GPCRs, resulted in a marked reduction of ligand binding, emphasizing its importance at the binding site for the agonists and antagonists containing ammonium groups. The serine residues of helix 5: Ser193, Ser194 and Ser 197 are important for binding catechol hydroxyl groups. To check if the present models confirm and support this essential information, docking tests using the endogenous ligand of the HDD2 receptor, dopamine and the nine homology models were performed.

The docking results of two models confirmed the involvement of the previously mentioned residues in the binding of dopamine and the model showing the best stereochemical quality was chosen to be used in further docking experiments (Fig. 2). The three-dimensional coordinates of the final model are provided as Supplemental material to this paper.

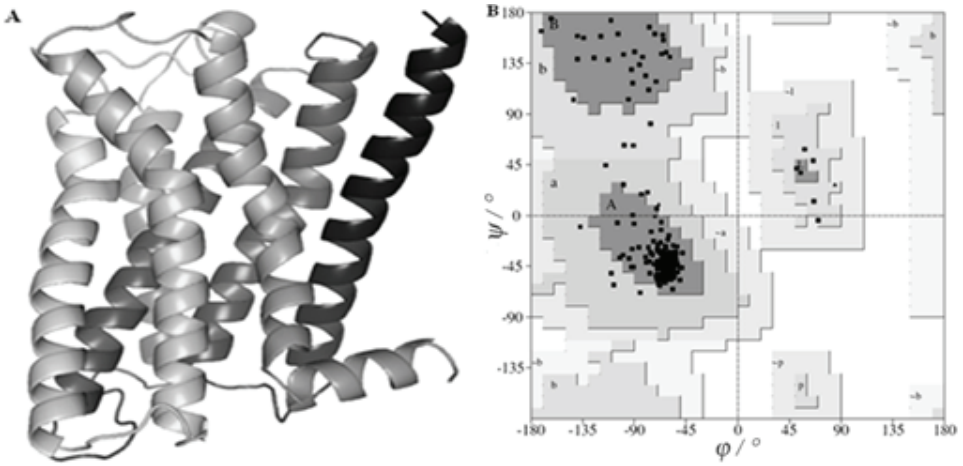


Fig. 2. A) 3D structure of the human dopamine D2 receptor. B) Ramachandran plot for the human dopamine D2 receptor. The final model has 93.6 % amino acids in the most favorable regions, while the other 6.4 % amino acids are placed in the additional allowed regions of the Ramachandran plot.

The positively charged nitrogen atom of dopamine forms a salt bridge with the carboxylate group of the Asp114 residue from helix 3, the distance between the two charged functional groups being 4.26 Å. The *meta*- and *para*-OH groups of the ligand interact with the side-chains of the Ser193 and Ser197 residues, respectively, *via* the formation of two hydrogen bonds. The aromatic ring of dopamine is accommodated in a hydrophobic pocket defined by several aromatic residues found on helix 6: Trp245, Phe248 and Phe249 (Fig. 3).

In order to highlight the molecular characteristics of the active site, a combined docking and pharmacophoric approach was performed using the 3D structure of the HDD2 receptor and a set of HDD2 partial agonists.

The molecular mechanics minimized conformation of each ligand was placed and oriented in the binding site by superposition to the docked dopamine as reference agonist. The rotatable bonds and the torsion angles of the ligands were set up using the AutoDock Tools package. The docking results were analyzed based on their ligand–receptor free binding energies and population clusters. The prediction of the binding affinity for each compound was not very accurate, with a difference of two-log units seen in some cases. Still, the correlation with the experimental results is reasonable if one keeps in mind the dynamics of the binding site and the fact that a homology model was used.

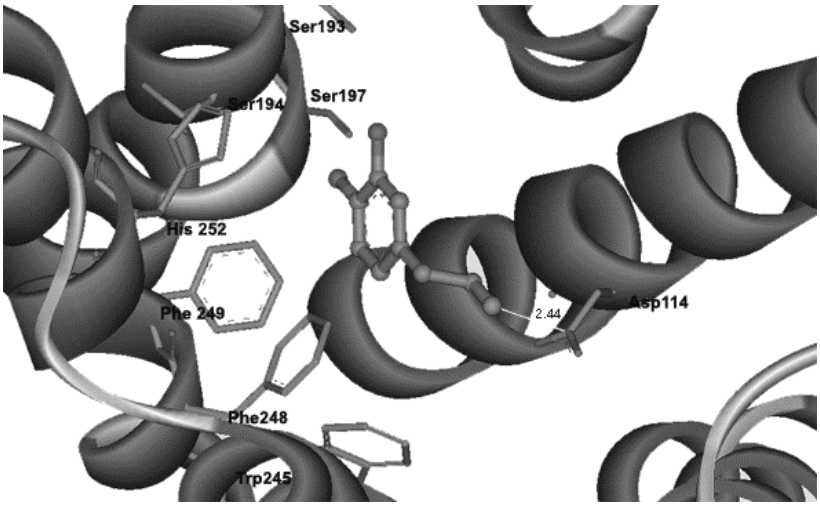


Fig. 3. Molecular model of dopamine in the binding site of the HDD2 receptor.

Analyzing each lowest binding-energy conformation in the binding site, it was observed that the ligands, depending on their size, adopt an L- or a U-shape folded conformation. This folding seems to be very important for biological activity since it allows the formation of one salt bridge between the aspartic acid residue on helix 3, Asp114 and the positively charged nitrogen atoms of the ligands (Fig. 4). Depending on the ligand size, the distance between the two charged functional groups (the carboxyl group from the D2 receptor and the amino function from the ligands) varies between 2.9 and 4.8 Å.

Many compounds showing high affinities against the HDD2 receptor display another important contact, namely a π–π interaction resulting from the stacking of the chromane ring of the ligands on the benzene ring of the Phe248 side-chain. Thanks to this stacked arrangement of the two aromatic rings, another important

interaction for ligand binding could be established. This interaction refers to the formation of a hydrogen bond between the hydroxyl group of one serine residue of helix 5 (usually Ser194) and the hydroxyl group from the chromane ring (Fig. 4). This hydrogen bond seems to be essential for the biological activity of these derivatives against the D2 receptor, being only observed for the *R*-diastereoisomers of the chiral compounds from this set.

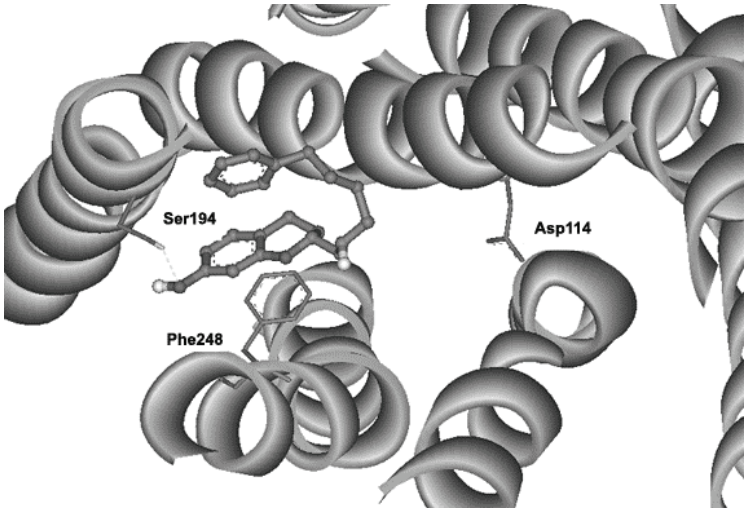
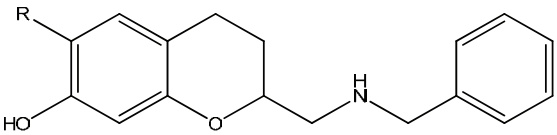


Fig. 4. The best docking pose of a partial D2 agonist in the binding site of the D2 receptor.

According to the experimental data (Table II), the *R*-diastereoisomers are more potent compounds than the *S*-diastereoisomers, allowing the conclusion that substitution at position 2 of the chromane ring is specifically stereoselective (Fig. 5A and B).

TABLE II. Inhibition constants for the R- and S-diastereoisomers

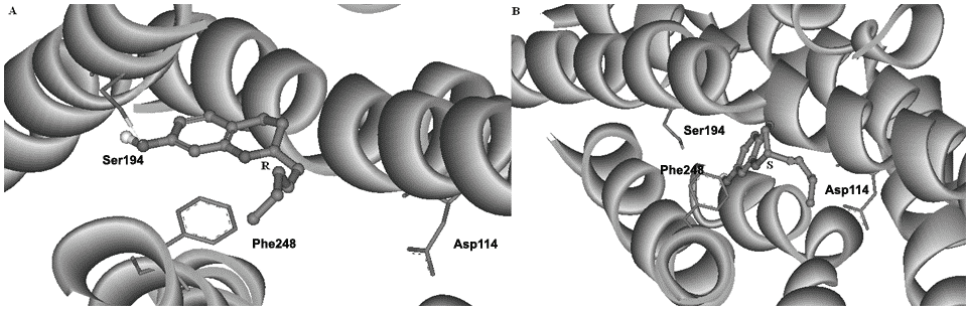


Compound	R	Stereochemistry	Ki_{exp} [a] / nM	Ki_{obs} / μM
1	H	*R*	0.2	1.3
1	H	*S*	12	68.2
2	Cl	*R*	0.6	1
2	Cl	*S*	32	49.6

[a]The values were taken from the literature[1]

The most relevant features that define the binding mode of the studied HDD2 partial agonists are described by the ligand folding and a complex net-

work of interactions represented by stacking interactions, a salt bridge and hydrogen bond formation.



(A) (B)

Fig. 5. View of the *R*-stereoisomer (A) and *S*-stereoisomer (B) in the binding site of the D2 receptor.

CONCLUSIONS

A homology model of the HDD2 receptor based on the X-ray structure of the *β*2-adrenergic receptor was built using comparative modeling. The model has all steric and topologic parameters within the normal range and its accuracy was confirmed by docking experiments using endogenous ligands. In addition, the importance of Asp114, Ser193 and Ser197 residues in ligand binding and affinity at the HDD2 receptor, according to experimental data, was explained *via* docking experiments.

The binding mode of 2-aminochromane derivatives acting as partial agonists against HDD2 receptor is characterized by the following key interactions: 1) a salt bridge formed between Asp114 and one positively charged nitrogen atom of the ligand; 2) one or two hydrogen bonds between the Ser193, Ser194 and/or Ser197 side-chains and hydroxyl group of the chromane ring; 3) a π–π interaction between chromane ring and Phe248. These features of the binding mode of partial agonists at the HDD2 receptor provide the required information to generate pharmacophore models, which represents essential information for future virtual screening studies aimed at identifying new potential HDD2 receptor partial agonists.

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ИЗВОД

МОДЕЛОВАЊЕ ВЕЗИВАЊА ЛИГАНДА НА ДОПАМИНСКИ D2 РЕЦЕПТОР

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Допамински рецептори већ дуже време су главна мета при развоју нових малих молекула који имају велики афинитет и селективност за тај рецептор, а могли би да се примењују за третирање психијатријских сметњи, неуродегенерације, наркоманије и др. Будући да 3D структура хуманог D2 допаминског рецептора (HDD2) није позната, истраживања се заснивају на компаративним моделима. Да би се боље разумело место везивања на HDD2 рецептор, и лиганд–рецептор интеракцоје, конструисан је хомологни модел HDD2. рецептора заснован на рендгенској структури β2-адренергичног рецептора. Одређене су главне карактеристике везивања на HDD2 парцијалних агониста. То даје потребне информације да се добије фармакофорски модел за будућа истраживања и за идентификацију нових потенцијалних HDD2 парцијалних агониста.

(Примљено 8. фебруара, ревидирано 4. априла 2013)

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