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ORIGINAL PAPER

Docking studies on a refined human β_2 adrenoceptor model yield theoretical affinity values in function with experimental values for R-ligands, but not for S-antagonists

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Abstract G-protein coupled receptors (GPCR) belong to the largest group of membrane proteins involved in signal transduction. These receptors are implicated in diverse physiological and pathological events. The human β_2 adrenergic receptor (hβ_2AR) is one of the few GPCRs whose 3-D structures are available on the Protein Data Bank. Because there is great interest by drug developers for hβ_2AR as a target, it is necessary to study its ligand-recognition process at the atomic level. The hβ_2AR can recognize both R/S enantiomeric ligands, R-agonists result in a greater activation than do S-agonists (eutomers and distomers for activation, respectively), according to experimental results. In this work is reported the ligand recognition on a refined hβ_2AR-structure of a set of well-known R/S-ligands by means of *docking* studies. Data obtained *in silico* were analyzed and compared with those reported *in vitro*. The theoretical affinity values were reproduced for agonists, but not for antagonist (or inverse agonists). However, theoretical data for R-antagonists are in function to experimental data. The theoretical results confirm the role of amino acids previously reported by mutagenesis studies due to their important roles in drug affinity and stereoselectivity.

Keywords β_2 adrenergic receptor · *Docking* · Eutomer · Stereoselectivity

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Introduction

The human β_2 adrenergic receptor (hβ_2AR) is included among G protein-coupled receptors (GPCRs) which have been a major focus of pharmaceutical research for many years [1]. The hβ_2AR-agonist medications, such as albuterol and salmeterol, are widely used to treat asthma [2]. Due partly to the lack of reliable receptor structures, drug discovery efforts have been largely ligand-based. The recently determined X-ray structure of the human beta2-adrenergic receptor (hβ_2AR) offers an opportunity to investigate the advantages and limitations inherent in a structure-based approach to ligand discovery against this and related GPCR targets [1].

Studying hβ_2AR ligand recognition using computational tools is useful because it allows one to visualize the ligand mechanism at the atomic level. There are several theoretical works regarding hβ_2AR that attempt to elucidate the key factors for understanding structural phenomena by using

theoretical methods [3]; however, the ligand recognition including either R or S chiral centers has not been explored in structural detail.

For other hand, through site-directed mutagenesis, the amino acids responsible for the stereoselective recognition of ligands have been suggested in hβ_2AR under experimental methods [4, 5]. Besides revealing structure-activity relationships, computational assays have led to the discovery of some essential moieties of specific ligands that are important for interactions in the hβ_2AR recognition process [6, 7]. Also, there is evidence that some key amino acids are responsible for triggering hβ_2AR biological effects; however, identifying these amino acids *via* experimental methods is difficult and expensive. Additional limitations have also been demonstrated: hβ_2AR shows several conformational states and these different states have been implicated in different signaling outputs to selectively activate ligands; this is in accord with the concept of the 'receptosome' complex described recently for GPCR [8].

At the moment, several models of the hβ_2AR are available at protein data bank (PDB codes: 2rh1, 2r4r, 2r4s, 3d4s) obtained by X-ray crystallography. Consequently, these 3-D structures enable the generation of reliable *docking* simulation and give rise to insights regarding the well-know agonist ligand recognitions on hβ_2AR [8]. With the aim of clarifying the action mechanism of GPCR, there have been several recent studies using computational tools that allow one to observe the hβ_2AR dynamic behavior in 3-D and support the applicability of GPCR modeling to experiments and to drug discovery [7, 9]. These studies have allowed for the identification of key amino acids involved in the ligand recognition process as well as the implication of water's role in hydrogen bond interactions with the ligand-receptor [10], and the existence of energy barriers among different conformational states generated for antagonists -and agonist- bonded states [6]. Theoretical studies have also provided insights into the identification of key changes in specific residues since, for example, different ligand stabilize some conformations by breaking the ionic bonds between Arg131 of TM3, Glu268 of TM6, and the rotamer toggle switch of Trp286 on TM6 [11]. Moreover, molecular dynamic simulations with R-adrenaline were carried out for studying stereochemistry by comparison with the S-carazolol hβ_2AR complex by experimental methods [7]. These theoretical studies are in agreement with experimental evidence, showing that ligand recognition is carried out as a multistep process involving various specific amino acids at the ligand binding site [7, 8, 12–14]. All these results must be taken into account for studying specific interactions in the hβ_2AR-ligand complex.

The stereoselective recognition can be explored at atomic level by using *docking* simulations. It is widely supported that R-enantiomer agonists act as eutomers to produce a relaxant effect in smooth muscle. In this sense, Asn293 have been strongly implicated in stereoselectivity of hβ_2AR [4], but its role have been poorly studied by theoretical methods.

In this sense, the aim of the present work is to determine the theoretical affinity values of known hβ_2AR ligands and indentify key hβ_2AR residues involved in either R or S-enantiomeric recognition by *docking* studies to compare with those obtained by experimental methods. These theoretical studies were carried on a refined hβ_2AR 3-D model which was built from a model characterized previously by X-ray crystallography.

Methods

Hardware

Modeling and *docking* studies were performed in a cluster of 6 nodes using a Kernel Open Mosix, running in Linux operating system with a 1.8 GHz processor (each node), 12 Gb RAM, 620 Gb hard drive and an AT RADEON X1200 graphics card.

Receptor refinement

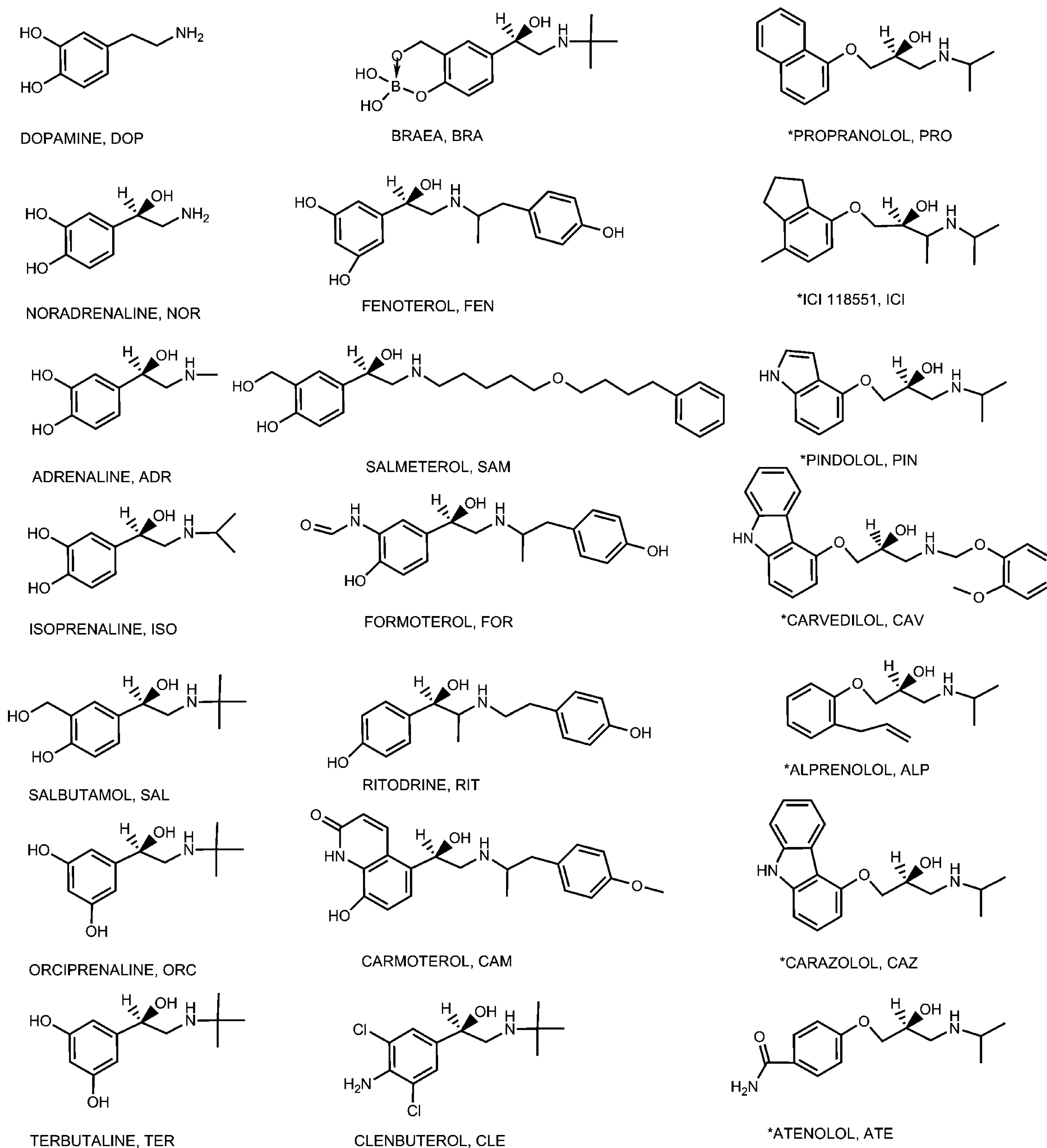
The receptor used for this study was obtained from that recently obtained by Cherezov et al. (PDB code: 2rh1, crystal resolution = 2.40 Angstroms) [14]. Before *docking* analysis, the ligands, the T4-lysozyme and co-crystallized water molecules were removed from the hβ_2AR, the protein's hydrogens were added. Then, the entire protein structure was minimized *in vacuo* during 10000 steps at 0 K using the steepest descendent protocol employing the CHARMM27 parameters implemented in the NAnoscale Molecular Dynamics (NAMD 2.6) program [15]. The obtained model was analyzed by 3-D superimposition on its template; RMSD was calculated and Ramachandran plot was built, both by using VMD 1.8.6 program [16].

Methodology for ligand-receptor recognition study

To identify the hβ_2AR recognition site and determine the ligands' affinities on this receptor, *docking* simulations were performed using 3-D ligand/receptor structures. Previously, for corroborating availability of the putative binding site in the refined model, a binding site prediction was carried out by using Q-site Finder program [17].

Ligands retrieval

A set of 41 ligands (full, partial, and inverse agonists and an antagonist) was tested (Scheme 1), including a recently synthesized compound reported as an hβ_2AR agonist by



Scheme 1 R and S-ligands tested on hβ_2AR models. The chiral center depicted was considered for building isomeric forms (R/S). Asterisk marks in antagonist or inverse agonists. Names are in complete form and in abbreviations used in charts

our-group [18]. For each ligand, except for dopamine (which does not contain a chiral center), the R and S-enantiomeric structures were built from their 2-D structures which were downloaded from the DrugBank [http://www.drugbank.ca/drugs]. Only the β carbon was considered as chiral center (Scheme 1). Then, the 3-D structures of the ligands were geometrically optimized at the B3LYP/6-31G* level using Gaussian 98 [19].

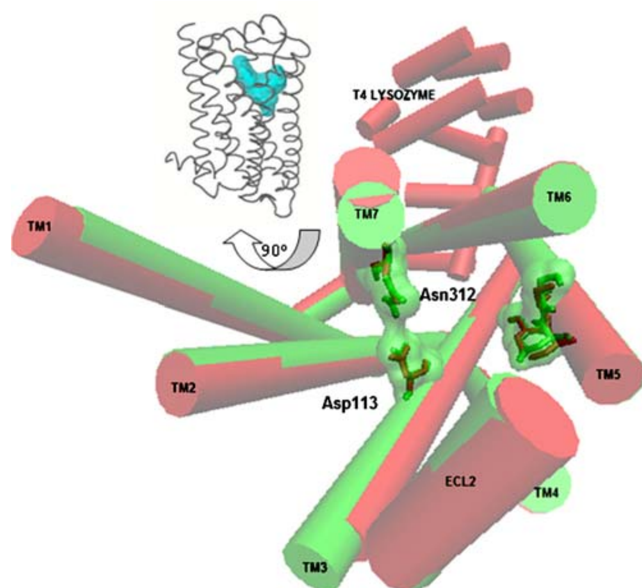


Fig. 1 Superimposition of original (PDB code: 2rh1, red) and refined (green) models in cartoon representation. In the last model, great proximity is notable between TM3 and TM7. In both models, amino acids Asp113, Asn312, Ser203, Ser204, and Ser207 are represented in tube. In the ribbon representation is depicted the binding site calculated by Q-site Finder server on the refined model

Docking methodology

All rigid/flexible bonds, partial atomic charges (Gasteiger-Marsili formalism), and non-merge hydrogens of the ligands were assigned. The Kollman partial charges for all atoms in the hβ_2AR, its solvatation parameters, and the non-mergen hydrogens were added using AutoDock Tools 1.4.5 while maintaining the other program's default parameters [20]. *Docking* simulations were performed using a commonly-used search algorithm (hybrid Lamarckian Genetic) implemented in AutoDock 3.0.5 [20]. The initial population was 100 randomly placed individuals, and the maximum number of energy evaluations was 10 million. To avoid interaction on inaccessible lateral faces of the lipid bilayer membrane or intracellular faces, the input initializations of the ligand structures and hβ_2AR-binding-site definitions were carried out using a GRID-based procedure [21]. A 60×60×60 Å point grid with 0.375-Å spacing was used, centered at Cα of Asp113 (residue of the binding site) [4, 22]. Docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest free-energy cluster returned for each compound was used for further analysis using Autodock Tools 1.4.5. *Docking* results (hβ_2AR-ligand complexes) were visualized using VMD 1.8.6 [16].

Results and discussion

The hβ_2AR refined model

The hβ_2AR refined model showed to be very similar to the inverse-agonist S-carazolol linked model obtained by X-ray crystallography (PDB code: 2rh1), however, there exists greater proximity between TM3 and TM7 implicated in the ligand recognition process. Additionally, Ser203, Ser204 and Ser207 in TM5 are slightly nearer to the core formed by the seven transmembranal domains than 2rh1 model (Fig. 1). The RMSD between both models were 0.445 Å, and the Ramachandran plots were similar, with 100% of amino acids in allowed areas for the crystal structure and 98.9% for the refined model (Fig. 2). This means that despite TM3 and TM7 greatest movements, the 3-D structure was maintained.

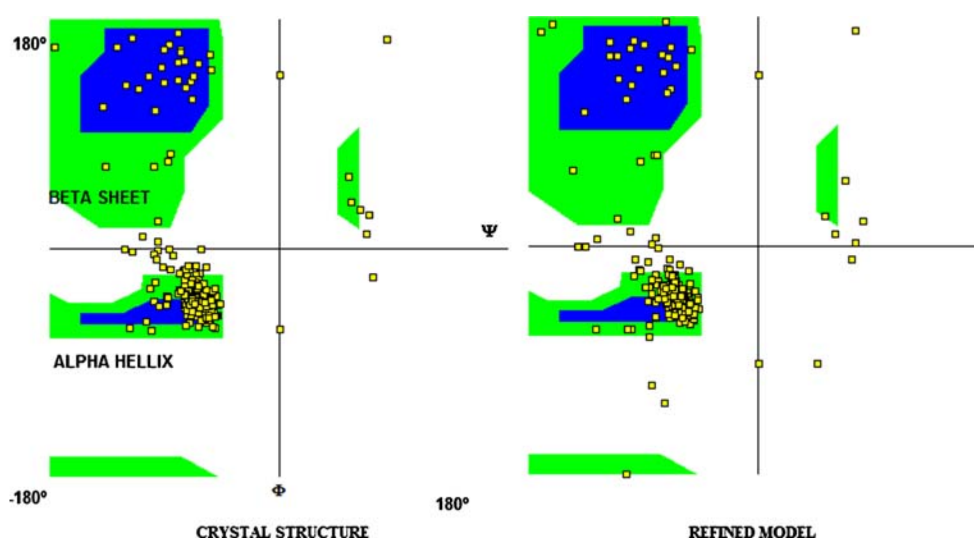


Fig. 2 Ramachandran plots for the crystal structure and the refined model of hβ_2AR. 86.07% of amino acids are in favoured areas for the crystal structure (on left) and 89.05% for the refined model (on right)

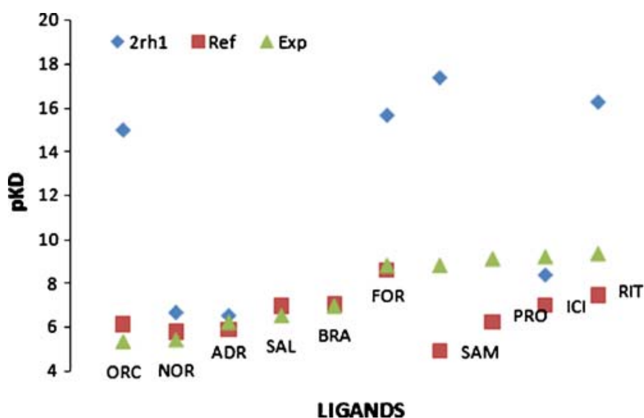


Chart 1 Affinity values (pK_D) experimentally reported (triangles, Exp) and correlation with data obtained from simulation on X-ray obtained (rhombs, 2rh1) and refined (squares, Ref) models. See scheme 1 for abbreviations

Docking results on the hβ_2AR models and correlation with in vitro reported data

The experimental data were collected or calculated from its kD values from previous *in vitro* binding assays [14, 23–26].

The affinity values calculated from original structure have no correlation with experimental data (Chart 1). However, the pK_D values obtained from refined model were similar to the experimental data reported for conventional agonists (Charts 1, 2, and 3) and a linear function for predicting the affinity of R-antagonists and inverse agonist (but not for S-forms) was determined (Shown as equation in Chart 4). Salmeterol and ritodrine for agonists and atenolol for antagonists were exceptions to these respective functions. However, the pK_D experimental values for these agonists can be approximated by using the function obtained for R-antagonists (see Chart 1).

Ligand-hβ_2AR binding site

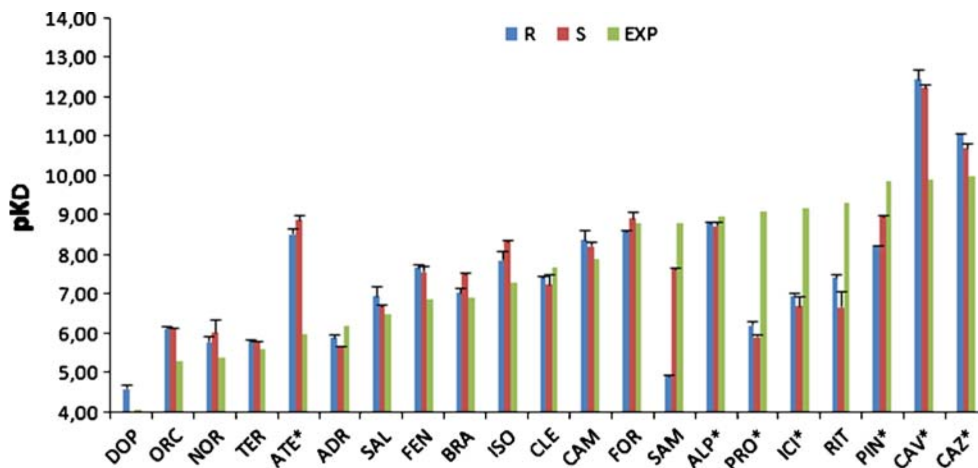
The analysis by using Q-site Finder program showed ten binding sites; however, only the first putative binding site (462 cubic Angstroms of volume) was between amino acids included in TM3 to TM7 (Fig. 1). This site was similar to that described for previous theoretical assays [1, 22]. On the other hand, based on the *docking* simulations on the refined hβ_2AR structure, a common binding site for ligands was found (similar to the predicted by Q-site Finder), and singular interactions depending on the ligand-binding mode (at the same or nearby site) were identified. From these data, a map of this binding pocket was built (Scheme 2). In this pocket, residues which have been suggested under punctual residue mutations, with structural and ligand/hβ_2AR-binding properties [4, 5] are included and disposed accord towith the experimental results.

Close-up on R or S ligands hβ_2AR recognition

As is mentioned above, through site-directed mutagenesis, the amino acids responsible for the ligands recognition have been suggested in hβ_2AR; this is an interesting phenomenon that can be explored using theoretical methods and compared with previously reported experimental data [22].

The stereoselective recognition is a key point for studying under theoretical methods, that is because it is widely supported that R-enantiomers (also named (–) or levo for endogen adrenergic ligands) act as eutomers -for example- to produce a relaxant effect in smooth muscle, while distomers (its S-enantiomers) show less potency as an agonist on hβ_2AR [27]. In relation to stereoselectivity of hβ_2AR, Asn293 have been the most implicate residue according to experimental data [27]; Asn293Asp mutation abolishes agonist-induced activity, but not constitutive

Chart 2 pK_D values (mean of five ligand-receptor complexes with lowest free-energy) calculated for each enantiomer (R and S) and experimentally (EXP) obtained for racemic mixtures of tested ligands on the refined hβ_2AR model. See scheme 1 for abbreviations. Asterisk marks in antagonists or inverse agonists



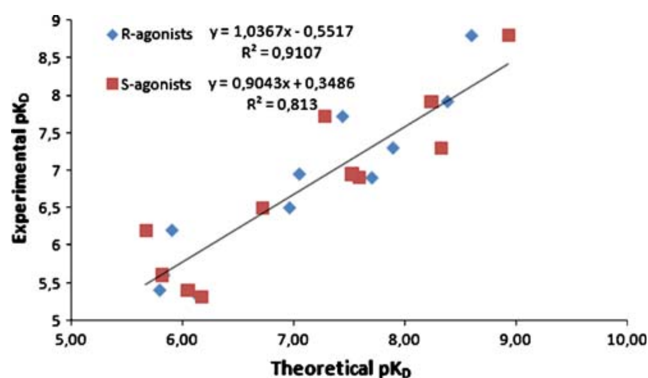


Chart 3 Relation and linear function between theoretical pK_D values determined for R(rhombs)/S(squares)-agonists on the refined hβ_2AR model (x-axis) and experimental reported data (y-axis). The regression line for S-agonists was omitted for clarity

activity [28] and Asn293Leu mutation showed a loss of hβ_2AR stereoselective binding and full-agonist affinity [4, 27, 28]. In the same form, other residue mutations have evidenced disruption in hβ_2AR-selectivity and activation [4, 5, 29, 30], one of them is Tyr308, which results are very important for the ligand selective binding [29].

Interestingly, in our *docking* simulations it was identified that hydroxyl groups located at chiral carbons common for all agonists form hydrogen bonds with a bipolar region formed by the Asp113, Asn312 and Tyr308 side chains, but not with Asn293. With Asn293, only some p-hydroxyl of the aromatic ring of R and S adrenaline, noradrenaline and the non chiral ligand dopamine form hydrogen bonds. Asp113 (preferably with R-ligands) and Asn312 form hydrogen bonds and electrostatic interactions with the amino group of the ligands, respectively, as was reported recently by using theoretical simulations [31]. In addition, the phenol-hydroxyl group of Tyr308 is toward the chiral carbon center making hydrogen bonds with the hydroxyl group at the chiral center with the S-ligands but not for R-ligands (Fig. 3). Also, Tyr308 showed hydrophobic interactions with the second methyl group presented in the second chiral carbon of R-fenoterol and R-formoterol and with aromatic rings of S-ligands. This means that Tyr308 could be a very important residue involved in the hβ_2AR stereoselectivity.

Other interactions were different between R and S enantiomers. Thus, Trp286 and Phe193 show hydrophobic (and π–π) interactions with the aromatic ring of the R-enantiomers whereas only terbutaline and propranolol S-enantiomers make these interactions with these aromatic residues (Scheme 2, Fig. 3). In this sense, should be mentioned, despite the differences between calculated affinities for R or S antagonists subsets, no different key interactions were identified between them (Scheme 2).

Theoretical/experimental-coupling assumption for hβ_2AR-stereoselectivity

In these *docking* simulations no interactions between Asn293 with the hydroxyl group at chiral center common for ligands were observed. Further, neither in the crystal hβ_2AR structures with S-carazolol or S-pindolol (PDB codes: 2rh1, 3d4s), nor in model obtained with R-Adrenaline [7] are interactions between an atom bonded to the chiral center with Asn293 or Tyr308 (Fig. 3). That is in disagreement with that found by Wieland et al. in previous simulations; however, the loss of stereoselectivity with Asn293 mutation was observed [27].

We trust in theoretical simulations for elucidating key interactions in hβ_2AR-stereoselectivity, such as have been possible for other receptors [32]. We support the idea that Asn293 and Tyr308 residues are implicated in hβ_2AR ligand stereoselectivity [27–29], but this can be a previous step to the ligand-coupling at binding pocket, this can explain why several ligand-receptor change results in indefinite stereoselectivity insights [27, 28]. Thus, Asn293 located slightly above the binding site, appears to play a role as a ‘filter’ for the ligand arriving to the binding site (Fig. 4), due to in the recent theoretical and X-ray models no interactions have been observed. Moreover, bulky chemical groups, greater than isopropyl, appear to lack the functionality of this filter. This may be because the steric impediment increases the distance between TM3, TM4, TM5, TM6, and TM7 for interactions with residues of the same transmembrane domains before the ligand reaches this region (of Asn293) at the receptor. This may explain why in the Asn293Leu mutant receptor does not alter the affinity and activity for partial agonists and antagonists with bulky substituent bonds to that amino group; nor by ligands lacking a β-hydroxyl group [27]. However, the hβ_2AR enantioselectivity for this kind of ligand exists, which

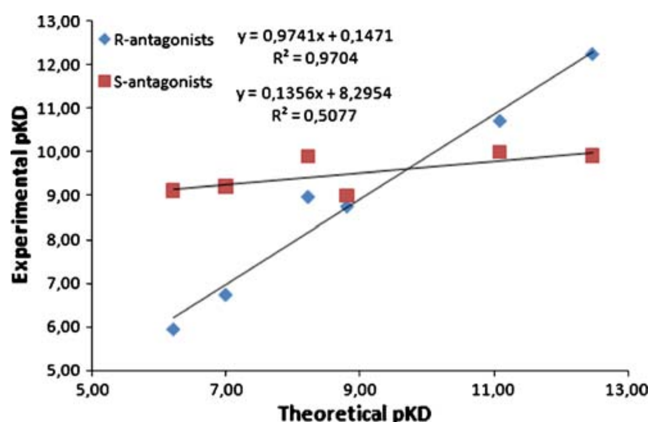
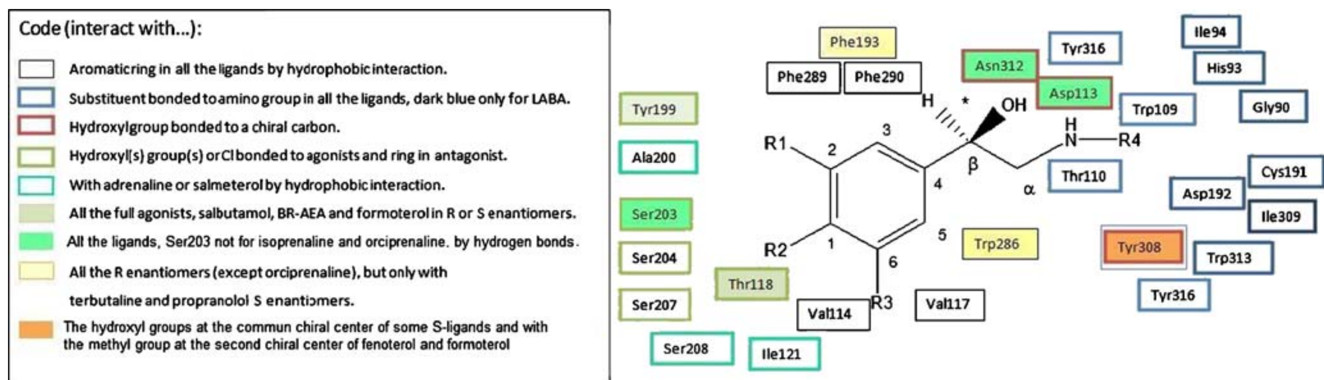


Chart 4 Relation and linear function between theoretical pK_D values determined for R(rhombs)/S(squares)-antagonists on the refined hβ_2AR model (x-axis) and experimental reported data (y-axis)



Scheme 2 Interactions found between the set of all ligands tested on the refined hβ_2AR model by *docking* simulations

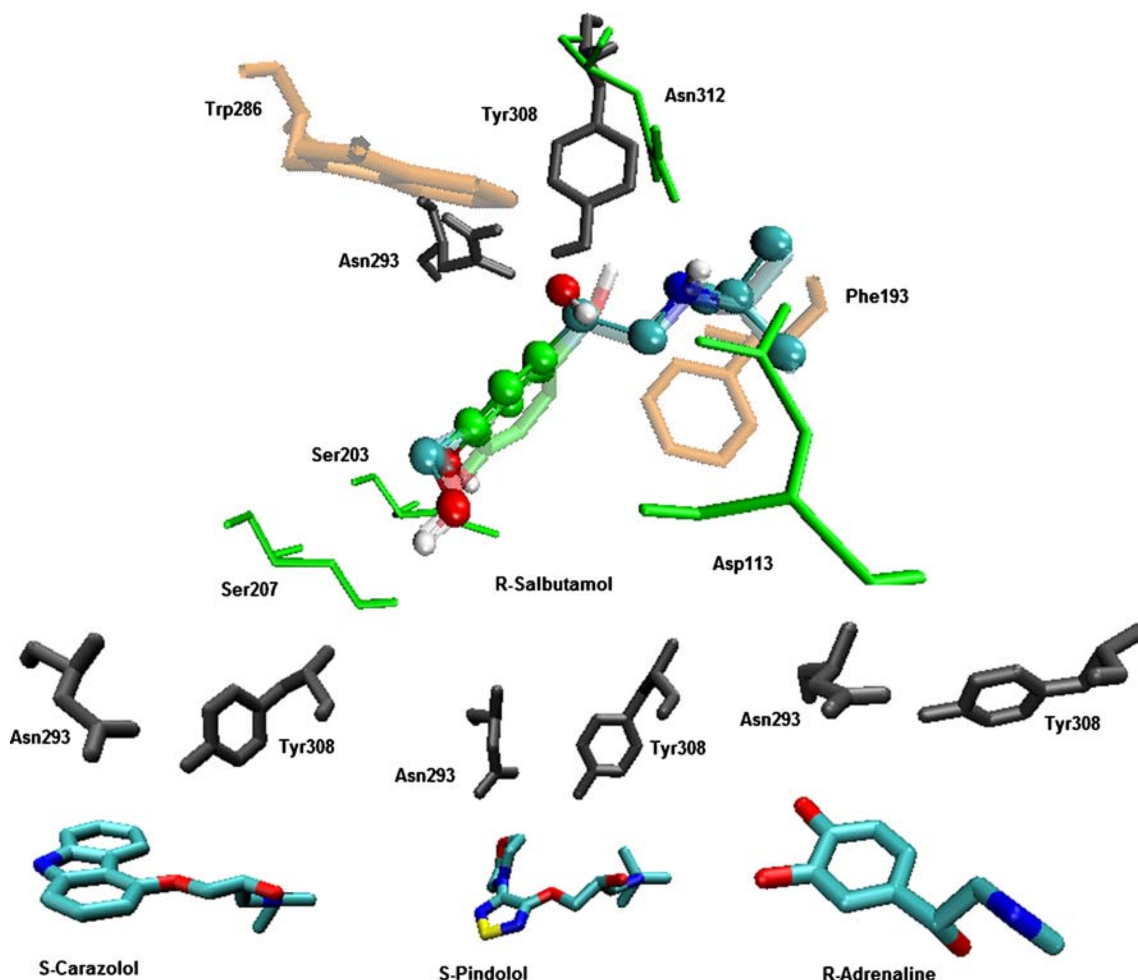


Fig. 3 Interactions found between salbutamol as R-enantiomer ligand (balls) and S-enantiomer ligand (tubes) on hβ_2AR 3-D refined by *docking* simulations. All R-enantiomers showed interaction distances ≥6.57 Å with Asn293, Trp286, Asp113 whereas S-enantiomers showed more interactions with Tyr308 and Phe193. In both cases were interactions with Ser207 and Ser203 at aromatic ring and with Asp113 and Asn312 at amine nitrogen. Additionally, is shown the disposition of Asn293 and Tyr308 with original coordinates in X-ray structures (PDB codes: 2rh1 with S-carazolol, 3d4s with s-pindolol) and from the estimated for Huber et al. with R-adrenaline [7]; in these cases the distances with oxygen atom of hydroxyl group in Tyr308 is greater than 7.36 Å, and with Asn293 greater than 10.2 Å

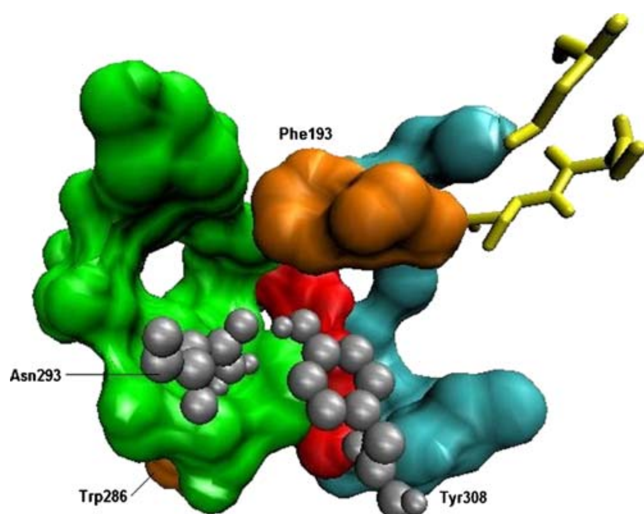


Fig. 4 A hypothetical way to stereoselectivity: A near extracellular view of the entrance to the binding site showing Asn293, Tyr308, and Phe193 as residues that could participate in selection of the entrance of ligand to reach binding site (surface representation). Besides, together with the previous residues mentioned, Trp286 can play an essential role to form the binding pocket for R or S form of a ligand hβ_2AR. Additionally, the disulphide bridges formed between Cys190-Cys184 and Cys191-Cys106 (which could interfere in the spatial disposition of the second extracellular loop) are depicted (yellow bonds)

suggests that they have an additional effect on the residues that form the binding site. Taking into account that the binding process is a multistep conformational phenomenon, it is possible that the same amino acids may have a central role. This is in agreement with recent studies which suggest that Asn293 and Tyr308 play important roles in conformational stabilization of the hβ_2AR active form by means of hydrogen bond interactions [31]. Additionally, Tyr308 can play a supplementary role in the strain action since it has been demonstrated that its mutation alters the affinity for agonists with bulky moieties, but not for isoproterenol [29]. In the binding site, the *docking* results showed that there are predominant π–π interactions between the R-enantiomers with Trp286 and Phe 193 (Fig. 3). Trp286 is an amino acid included in the toggle switch [33]. This is favorable for the total G-protein mediated activity and likely stabilizes the receptor in this conformation, which avoids other possible conformational states associated with other activation pathways. Furthermore, Trp286 together with those residues that make interactions at the hydroxyl group place at chiral center carbon, could be the key to allow a partial or total conformational changes to make them partial or total agonist effects as has been described for other protein targets [33]. Only indirect evidence is available regarding ligand interaction with Phe193. There is evidence provided by the recent 3-D model that Phe193 is likely to significantly contribute to the energy of ligand-receptor complex formation. The position of this residue may allow it to act as a gate that contributes to the unusually slow dissociation of the ligand [11]. Finally, should be mentioned that there were not interactions with -the neighbor residues to the binding site- Cys190 and Cys191 which have been involucrate in hβ_2AR ligand binding [34], which form disulphide bonds with Cys184 and Cys106, respectively. These bonds have an effect on second extracellular loop disposition which appears to be important for ligand binding [4, 11, 33, 35]. These residues may help stabilize the extracellular helical segment in the second extracellular loop [11, 34]. This segment, also found in the β_1AR, may be a common feature in those GPCRs that bind their ligands rapidly and reversibly and participate in the selectivity or generation of some active-inactive conformational states, as has been proposed for similar domains [36].

Conclusions

In these computational experiments, we obtained a refined model from the hβ_2AR crystal structure by means of a straightforward optimization procedure, which although is a static model (and represents a conformational state of the hβ_2AR), allows one to predict the ligand interactions in the binding site for the majority of hβ_2AR R and S agonist and R-antagonists tested. These results support previous theoretical works which suggest it is possible to obtain 3-D model capability to be used in prediction of hβ_2AR ligands recognition [9, 31]. Additionally, in this model, are identified some key residues, singly or in cluster during the recognition, and maybe for enantioselectivity in hβ_2AR activation. These *docking* simulations suggest selectivity appears to be a property of hβ_2AR determined by a specific distribution of amino acids that limit access to the binding site region, such as Asn293 and Tyr308, which also could participate in the binding site for each R or S-ligand and the effectors involved in the biological response after the ligand-receptor complex is formed. This 'restriction of entrance' can be comparable to that recently described by Mustafi et al. [37] for the opening of the retinal binding site in opsin. More theoretical and experimental evidence is necessary to support or discard this assumption. Analysis of impact of site-directed mutations on the specific R/S ligands should be studied. Besides, computational tools can be used for analysis of hβ_2AR-dynamics behavior on the R/S ligands recognition. A better understanding of this point could allow for the development of drugs with more selectivity than those currently available.

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References

1. Kolb P, Rosenbaum DM, Irwin JJ, Fung JJ, Kobilka BK, Shoichet BK (2009) Structure-based discovery of beta2-adrenergic receptor ligands. Proc Natl Acad Sci USA 106:6843–6848
2. Yu IW, Bukaveckas BL (2008) Pharmacogenetic tests in asthma therapy. Clin Lab Med 28:645–665
3. Rubenstein LA, Zauhar RJ, Lanzara RG (2006) Molecular dynamics of a biophysical model for beta2-adrenergic and G protein-coupled receptor activation. J Mol Graph Model 25:396–409
4. Savarese TM, Fraser CM (1992) In vitro mutagenesis and the search for structure-function relationships among G protein-coupled receptors. Biochem J 283:1–19
5. Chelikani P, Hornak V, Eilers M, Reeves PJ, Smith SO, RajBhandary UL, Khorana HG (2007) Role of group-conserved residues in the helical core of beta2-adrenergic receptor. Proc Natl Acad Sci USA 104:7027–7032
6. Bhattacharya S, Hall SE, Li H, Vaidehi N (2008) Ligand stabilized conformational states of human beta 2 adrenergic receptor: insight into G protein coupled receptor activation. Biophys J 94:2027–2042
7. Huber T, Menon S, Sakmar TP (2008) Structural basis for ligand binding and specificity in adrenergic receptors: implications for GPCR-targeted drug discovery. Biochem 47:11013–11023
8. Audet M, Bouvier M (2008) Insights into signaling from the β_2-adrenergic receptor structure. Nat Chem Biol 4:397–403
9. Costanzi S (2008) On the applicability of GPCR models to Computer-aided Drug Discovery: a comparison between in silico and crystal structure of the β2-adrenergic receptor. J Med Chem 51:2907–2914
10. Spijker P, Vaidehi N, Freddolino LP, Hilbers PAJ, Goddard WA (2006) Dynamic behavior of fully solvated β_2-adrenergic receptor, embedded in the membrane with bound agonist or antagonist. Proc Natl Acad Sci USA 103:4882–4887
11. Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK (2007) GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science 318:1266–1273
12. Swaminath G, Xiang Y, Lee TW, Steenhuis J, Parnot C, Kobilka BK (2004) Sequential binding of agonists to the β2 adrenoceptor. J Biol Chem 279:686–691
13. Ambrosio C, Molinari P, Cotecchia S, Costa T (2000) Catechol-binding serines of beta(2)-adrenergic receptors control the equilibrium between active and inactive receptor states. Mol Pharmacol 57:198–210
14. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318:1258–1265
15. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K (2005) Scalable molecular dynamics with NAMD. J Comp Chem 26:1781–1802
16. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14:33–38
17. Laurie AT, Jackson RM (2005) Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. Bioinf 21:1908–1916
18. Soriano-Ursúa MA, Valencia-Hernández I, Arellano-Mendoza MG, Correa-Basurto J, Trujillo-Ferrara JG (2009) Synthesis, pharmacological and in silico evaluation of 1-(4-di-hydroxy-3, 5-dioxa-4-borabicyclo[4.4.0]deca-7, 9, 11-trien-9-yl)-2-(tert-butyl-amino)ethanol, a compound designed to act as a β2 adrenoceptor agonist. Eur J Med Chem 44:2840–2846
19. Frisch MJ, Trucks GW, Schlegel HB et al (1998) Gaussian 98, Version A.7. Gaussian Inc, Pittsburgh
20. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. J Comp Chem 19:1639–1662
21. Goodford PJ (1985) A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J Med Chem 28:849–857
22. Swaminath G, Deupi X, Lee TW, Zhu W, Thian FS, Kobilka TS, Kobilka B (2005) Probing the β_2 adrenoceptor binding site with catechol reveals differences in binding and activation by agonist and partial agonists. J Biol Chem 280:22165–22171
23. Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450:383–387
24. January B, Siebold A, Whaley B, Hiplin RW, Lin D, Schonbrunn A, Barber R, Clark RB (1997) beta(2)-adrenergic receptor desensitization, internalization and phosphorylation in response to full and partial agonists. J Biol Chem 272:23871–23879
25. Liapakis G, Chan WC, Papadokostaki M, Javitch JA (2004) Synergistic contributions of the functional groups of epinephrine to its affnity and efficacy at the beta(2)-adrenergic receptor. Mol Pharmacol 65:1181–1190
26. Baker JG (2005) The selectivity of beta-adrenoceptor antagonists at the human beta1, beta2 and beta3 adrenoceptors. Br J Pharmacol 144:317–322
27. Wieland K, Zuurmond HM, Krasel C, Ijzerman AP, Lohse MJ (1996) Involvement of Asn-293 in stereospecif agonist recognition and in activation of the beta 2-adrenergic receptor. Proc Natl Acad Sci USA 93:9276–9281
28. Hannawacker A, Krasel C, Lohse MJ (2002) Mutation of Asn293 to Asp in transmembrane helix VI abolishes agonist-induced but not constitutive activity of the beta(2)-adrenergic receptor. Mol Pharmacol 62:1431–1437
29. Kikkawa H, Isogaya M, Nagao T, Kurose H (1998) The role of the seventh transmembrane region in high affinity binding of a beta 2-selective agonist TA-2005. Mol Pharmacol 53:128–134
30. O'Dowd BF, Hnatowich M, Regan JW, Leader WM, Caron MG, Lefkowitz RJ (1988) Site-directed mutagenesis of the cytoplasmic domains of the human beta 2-adrenergic receptor. Localization of regions involved in G protein-receptor coupling. J Biol Chem 263:15985–15992
31. De Graaf C, Rognan D (2008) Selective structure-based virtual screening for full and partial agonist of the β2 adrenergic receptor. J Med Chem 51:4978–4985
32. Brooks WH, Daniel KG, Sung SS, Guida WC (2008) Computational validation of the importance of absolute stereochemistry in virtual screening. J Chem Inf Model 48:639–645
33. Kobilka BK (2007) G protein coupled receptor structure and activation. Biochim Biophys Acta 1768:794–807
34. Fraser CM (1989) Site-directed mutagenesis of beta adrenergic receptors. Identification of conserved cysteine residues that independently affect ligand binding and receptor activation. J Biol Chem 264:9266–9270
35. Fatakia SN, Costanzi S, Chow CC (2009) Computing highly correlated positions using mutual information and graph theory for G protein-coupled receptors. PLoS One 4:e4681
36. Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF (2008) Structure of a β1 adrenergic G-protein coupled receptor. Nature 454:486–492
37. Mustafi D, Palczewski K (2009) Topology of class A G protein-coupled receptors: insights gained from crystal structures of rhodopsins, adrenergic and adenosine receptors. Mol Pharmacol 75:1–12