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Molecular modeling of a putative antagonist binding site on helix III of the β-adrenoceptor

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SUMMARY

In recent biochemical studies it was demonstrated that residue Asp^{113} of the β-adrenoceptor (β-AR) is an indispensable amino acid for the binding of β-AR antagonists. Earlier fluorescence studies showed that a tryptophan-rich region of the β-AR is involved in the binding of propranolol, the prototype β-AR antagonist. Bearing these two biochemical findings in mind, we explored the β-AR part containing Asp^{113}, for an energetically favorable antagonist binding site. This was done by performing molecular docking studies with the antagonist propranolol and a specific β-AR peptide which included, besides Asp^{113}, two possibly relevant tryptophan residues. In the docking calculations, the propranolol molecule was allowed to vary all its internal torsional angles. The receptor peptide was kept in an α-helix conformation, while side chains relevant to ligand binding were flexible to enable optimal adaptations to the ligand's binding conformation. By means of force-field calculations the total energy was minimized, consisting of the *intra*molecular energies of both ligand and receptor peptide, and the *inter*molecular energy. We found an antagonist binding site, consisting of amino acids Asp^{113} and Trp^{109}, which enabled energetically favorable interactions with the receptor-binding groups of propranolol. According to these results, binding involves three main interaction points: (i) a reinforced ionic bond; (ii) a hydrogen bond; and (iii) a hydrophobic/charge transfer interaction. The deduced binding site shows a difference in affinity between the levo- and dextrorotatory isomers of propranolol caused by a difference in ability to form a hydrogen bond, which is in conformity with the experimentally observed stereoselectivity. Moreover, it also provides an explanation for the β_1-selectivity of *p*-phenyl substituted phenoxypropanolamines like betaxolol. The *p*-phenyl substituent of betaxolol was shown to be sterically hindered upon binding to the β_2-AR peptide, whereas this hindrance is very likely to be much less with the β_1-AR peptide. Finally, the proposed antagonist binding site is discussed in the light of some recent biochemical findings and theories.

INTRODUCTION

The β-AR is a membrane-bound glycoprotein, coupled to the enzyme adenylate cyclase. The

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activation of the cyclase by the β-AR, in the presence of an agonist, is regulated through the guanine nucleotide regulatory protein G_s [1]. The most important function of this system is the transmission of adrenergic impulses to effector organs. Two subtypes of β-ARs exist, termed β_1 and β_2, which have distinct pharmacological properties and subserve different physiological functions [2].

Recently, the genes and complementary DNAs (cDNAs) for the hamster [3] and human β_2-AR [4], the avian β_1-AR [5], and the cDNA for the human β_1-AR [6] have been cloned, and the amino acid sequences derived. These results revealed a significant homology in primary amino acid sequence between these receptors and rhodopsin, a G-protein-coupled membrane protein, located in the retina of the eye. The structural homology is most evident in seven stretches of 20–28 hydrophobic amino acids, which likely represent membrane-spanning α-helices in the β-ARs, analogous to those determined for rhodopsin [1, 7].

In an attempt to locate the ligand binding site on the β-AR, Dixon et al. expressed a series of deletion mutant genes of the hamster β_2-AR in mammalian cells [8]. Deletion of amino acids 274–330 (including putative helices VI and VII) resulted in a virtually complete loss of antagonist binding, as determined with the radioligand [^{125}I]-cyanopindolol. In a following study, however, the same group reported oligonucleotide-directed mutagenesis studies of the same hamster gene [9]. Several mutant β_2-ARs were expressed, all having a particular charged amino acid substituted by an uncharged amino acid. Replacement of Asp^{113}, present in putative helix III, by an uncharged Asn residue resulted in an almost complete loss of [^{125}I]-cyanopindolol binding. The important role of Asp^{113} in both antagonist and agonist binding was again recently highlighted [10]. Furthermore, trypsin-mediated cleavage of the β-AR and subsequent photoaffinity labeling showed that the binding site of antagonists is restricted to helices I–IV [7].

The binding of a ligand to the β-AR is generally accepted to involve a three-point interaction, consisting of an ionic interaction between the positively charged amino function in the aliphatic side chain of the ligand and an anionic amino acid residue, a hydrogen bond (H-bond) of the ligand's β-hydroxyl group with an H-bond acceptor in the β-AR, and a hydrophobic interaction of the ligand's aromatic moiety with an aromatic part of the β-AR [11]. Fluorescence studies by Cherksey et al. indicated that this hydrophobic interaction probably involves one or more tryptophan residues of the β-AR [12], which was further stressed by us in a recent study, in which the higher affinity of pindolol versus propranolol could be explained by an interaction with a tryptophan moiety [13] (for various chemical structures of the ligands, see Fig. 1).

In the last-cited study, we explored possible antagonist binding sites on stretches VI and VII, and found an energetically favorable interaction site on helix VII. Glu^{306}, Ile^{309} and Trp^{313} were the active amino acids, serving as anchor points for the ionic, H-bond and hydrophobic interaction, respectively. However, no stereoselectivity was observed for this binding site, as no energy difference was found between *R*- and *S*-propranolol. Therefore, we considered it worthwhile to examine other regions of the β-AR as well. Here, we report a search for a putative antagonist binding site on stretch III (for sequence see Fig. 2), in which Asp^{113} is involved in the ionic interaction with the protonated amino function of propranolol. This site shows stereoselectivity for β-AR ligands, and, moreover, it provides an explanation for β-AR subtype selectivity of certain ligands.

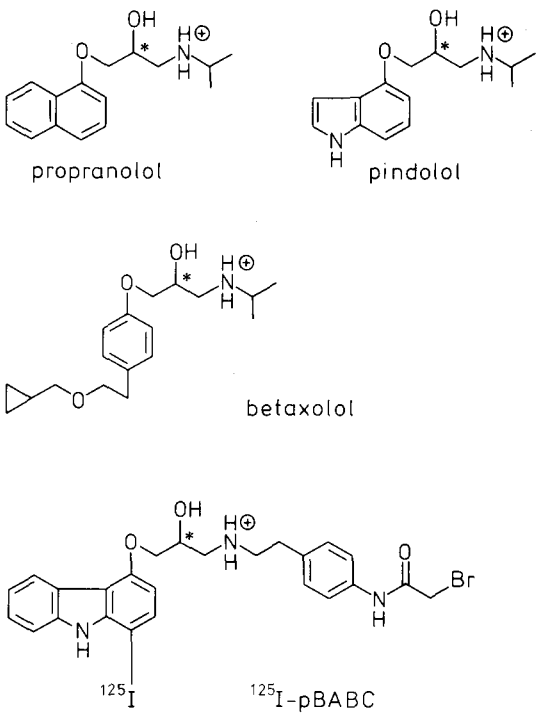

Fig. 1. Chemical structures of various β-AR ligands mentioned in this paper. Note: all compounds are almost fully protonated at physiological pH; *: chiral center.

METHODS

Construction of the ligands and the receptor

In this study, the molecular modeling system Chem-X was used [14]. The structure of *S*-propranolol was retrieved from the Cambridge Crystallographic Database [15]. *R*-Propranolol was constructed by inverting the chirality of the β-carbon atom in *S*-propranolol. *S*-Betaxolol was built by first replacing the naphthyl moiety of *S*-propranolol with a phenyl group and subsequently at-

						105				109				113								
HAMβ_2AR	N	F	G	N	F	W	C	E	F	W	T	S	I	D	V	L	C	V	T	A	S	120
HUMβ_2AR	T	F	G	N	F	W	C	E	F	W	T	S	I	D	V	L	C	V	T	A	S	120
THURβ_1AR	L	W	G	S	F	L	C	E	C	W	T	S	L	D	V	L	C	V	T	A	S	128
HUMβ_1AR	E	Y	G	S	F	F	C	E	L	W	T	S	V	D	V	L	C	V	T	A	S	145

Fig. 2. Amino acid sequences of a part of the third hydrophobic domain of the hamster β_2-AR (HAMβ_2AR), human β_2-AR (HUMβ_2AR), turkey β_1-AR (TURβ_1AR) and human β_1-AR (HUMβ_1AR). The sequences are aligned for maximum homology [6, 9, 27]. Important amino acids are indicated with sequence numbers corresponding to the HAMβ_2AR and HUMβ_2AR.

taching the 2-(cyclopropylmethoxy)ethyl substituent. Charge distributions of all compounds were calculated with the semiempirical molecular orbital package MOPAC [16], followed by a structural optimization with molecular mechanics (MM2 [17]). The receptor model was constructed by building the amino acids of helix III of the hamster β_2-AR in an α-helix conformation ($\varphi = -60°$, $\psi = -60°$). The thus-constructed receptor was too large to employ a MOPAC calculation, and therefore the charge distribution of the peptide was calculated with the Gasteiger and Marsili method [18]. This was followed by a structural optimization of the amino acid residues with molecular mechanics, in order to prevent strong steric hindrances within the peptide.

Docking procedure

In order to save computer time, we first interactively positioned the propranolol molecule near the peptide in such a way that the interacting atoms of propranolol were close to the putative interacting amino acid residues of the peptide. This also prevented the ligand from moving into nonrelevant local minima while approaching the receptor. In the docking procedure, the total van der Waals (VdW) repulsion energy, which consists of the intramolecular energies of the ligand and the receptor, and the intermolecular 'interaction' energy between the ligand and the receptor, was minimized. The VdW repulsion-energy force-field includes parameters for bond lengths, bond angles, torsion angles, VdW 1–3 interactions and nonbonded interactions. The calculated energy values do not correspond to the real energetic values in an absolute way. They can only be compared to each other in terms of 'more' or 'less' favorable states. The energy values cannot be used to calculate exact values of affinities between molecules out of interaction energies, since changes in entropy are not taken into account.

We started the docking procedure with a distance restraint of 1.9 Å between atom pairs of ligand and receptor that might interact by a H-bond. The distance restraint was removed as soon as the two atoms were within suitable hydrogen-bonding distance (1.7–2.2 Å), and the subsequent docking was continued without the distance restraint.

During the docking procedure, every torsion angle in the propranolol molecule (except those in the naphthyl ring) was allowed to be flexible, and the molecule as a whole could translate and rotate in every direction. The torsion angles of the amino acids involved in binding, determining the positions of their functional groups, were also flexible, allowing an 'induced fit'. A dielectric constant (ε) of 5 was used in the docking calculations, in order to account for the membrane environment to some extent. It appeared that smaller values of ε exaggerated, and larger values underestimated, the contribution of the ionic interaction points to the total interaction energy.

This docking procedure was repeated several times with different initial orientations of the ligand, in order to obtain the global energy minimum rather than a local minimum. The calculations were performed on a VAX 11/785 of the CAOS/CAMM Center in Nijmegen.

RESULTS AND DISCUSSION

Design of the β-adrenoceptor ligand binding site

First, the β-AR model was constructed by placing the amino acids of stretch III into an α-helix conformation. In this peptide, two tryptophan residues are present in the vicinity of Asp^{113}, viz.,

Trp^{109} (distance $\approx$ 4.5 Å) and Trp^{105} (distance $\approx$ 10.5 Å), which are feasible candidates for the hydrophobic interaction point. Both tryptophan residues are located on the same side of the α-helix as Asp^{113}, allowing easy access for ligands, and an interaction orientated in a parallel way to the α-helix. Thereupon, the levorotatory *S*-propranolol was flexibly docked to the specified binding site. A hydrophobic interaction of the *S*-propranolol naphthyl moiety with Trp^{105} would require an H-bond acceptor located in a proper position between Asp^{113} and Trp^{105}. This can only be accomplished by a carboxamide oxygen of the peptide backbone. Interaction with Trp^{105}, however, is strongly affected by Trp^{109}, which protrudes between Asp^{113} and Trp^{105}, and thus prevents an energetically favorable interaction. A second possibility is the formation of an H-bond with Asp^{113} itself. In this case, one oxygen of Asp^{113} is involved in the ionic interaction with the positively charged nitrogen, while the other oxygen serves as an H-bond acceptor. Such a receptor-propranolol complex can well be formed, and additionally it appeared that Trp^{109} is in a correct position to interact with the naphthyl moiety.

The energetically most favorable receptor-ligand complex found in the docking studies is shown in Fig. 3. Upon binding, both the receptor and propranolol change their conformations, in order to maximize the interaction strength. Since we started the docking procedure with the receptor and the ligand in their energetically optimized conformations, these changes led to increases in intramolecular energy content, namely 12.5 kcals/mol for the receptor and 1.5 kcals/mol for propranolol. The interaction energy of the complex is −35.6 kcals/mol, resulting in a decrease in total van der Waals repulsion energy of 21.6 kcals/mol.

These energy values indicate an energetically favorable binding, yet they are not to be considered as absolute values, as explained in 'METHODS'. According to the Gibbs free-energy function ($\Delta G = -RT \ln K_i$), the binding of *S*-propranolol to the β_2-AR (K_i about 1 nM [19]) involves a to-

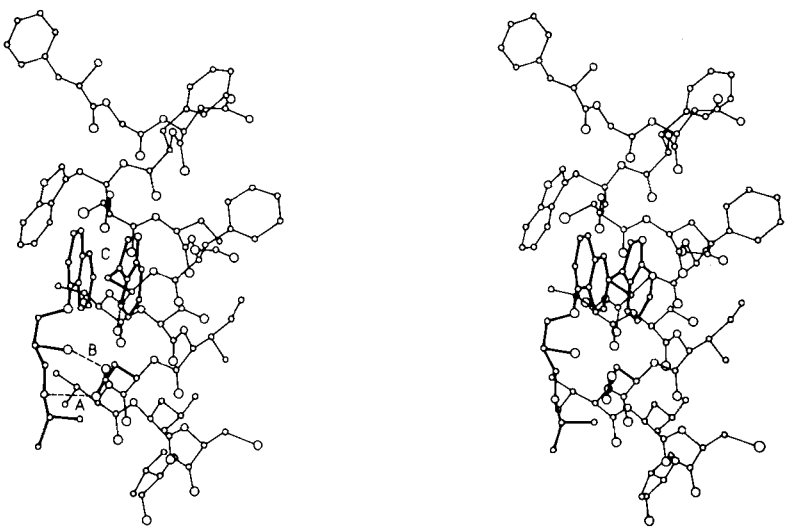

Fig. 3. The interaction complex of *S*-propranolol and helix III of the hamster β_2-AR (stereo drawing). Hydrogen atoms of the peptide are not shown for reasons of clarity. A, B, and C indicate the ionic interaction, the hydrogen bond, and the hydrophobic/charge transfer interaction, respectively.

tal energy decrease of approximately 12.3 kcals/mol, indeed deviating from our calculated value (21.6 kcals/mol). It should be kept in mind that ΔG values comprise both changes in enthalpy and entropy; unfortunately, the latter term cannot be included in the energy calculations.

Although they provide measures on a relative scale only, the calculated energy values, nevertheless, appear to be very useful in studying receptor-ligand interactions. The relatively small increase in intramolecular energy of *S*-propranolol upon binding is in accordance with results earlier published by Andrews et al., indicating that propranolol interacts with the β-AR with a binding energy which is above average [20]. This favorable difference can be ascribed to a conformation of receptor-bound propranolol that is very close to its lowest energy state. Table 1 describes the various interactions between the receptor model and *S*-propranolol.

TABLE I

CHARACTERIZATION OF THE INTERACTIONS OF *S*-PROPRANOLOL WITH ITS PUTATIVE BINDING SITE ON HELIX III OF THE HAMSTER β_2-AR

Functional group in *S*-propranolol	Interaction with	Interaction type	Orientation/distance
Positively charged nitrogen	Asp^{113}	reinforced ionic bond[a]	nitrogen-oxygen (Asp): 3.4 Å
β-hydroxyl group	Asp^{113}	hydrogen bond	angle O-H---O: 156° distance H---O: 2.1 Å
Naphthyl ring	Trp^{109}	charge transfer[a] hydrophobic interaction	parallel stacking, ring–ring distance: 3.6 Å

[a]These interaction types are discussed in Ref. 26.

Stereoselectivity

A striking feature, which increases the probability of this model complex, is the appearance of stereoselectivity. Docking of the dextrorotatory *R*-isomer of propranolol resulted in a maximal van der Waals repulsion energy reduction of 15 kcals/mol, 6 kcals/mol less than with *S*-propranolol. Again, on a relative scale, this difference fairly agrees with the experimentally observed difference of 2 pK_i units between the two isomers [19]. It appears that the *R*-isomer is not able to form both an H-bond and a strong ionic interaction. Formation of an H-bond resulted in an unfavorable increase in the distance between the protonated nitrogen and Asp^{113}, and vice versa (Fig. 4).

β-Adrenoceptor subtype selectivity

The deduced antagonist binding site also explains the β_1-selective properties of *p*-phenyl substituted phenoxypropanolamines, like betaxolol (Fig. 5). It has been proposed that the 2-(cyclopro-

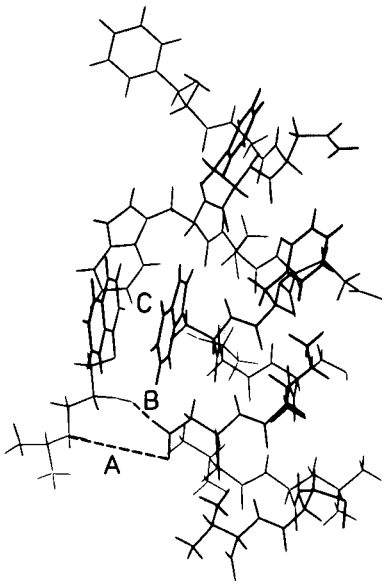

Fig. 4. The interaction complex of *R*-propranolol and helix III of the hamster β_2-AR. In the binding orientation shown here the ligand has formed the favorable H-bond (B), and thereby disables the molecule to position its positively charged nitrogen close to Asp^{113} in an energetically favorable way (A). Moving of the nitrogen to Asp^{113} results in a strong increase of intramolecular energy of *R*-propranolol. The hydrophobic/charge transfer interaction (C) is relatively unaffected. Note: In this figure Trp^{105} seems to penetrate the naphthyl moiety of *R*-propranolol. In fact it is located behind this moiety, and no steric hindrance occurs whatsoever.

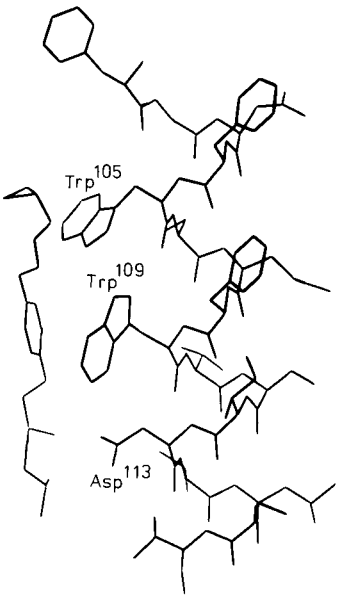

Fig. 5. The interaction complex of *S*-betaxolol with the helix III peptide of the hamster β_2-AR. The steric hindrance between the long *p*-phenyl substituent of betaxolol and the Trp^{105} residue is evident. It can be seen that this steric hindrance additionally causes the hydrophobic/charge transfer interaction between the phenyl moiety of betaxolol and Trp^{109} to be partially disrupted (no H atoms shown).

pylmethoxy)ethyl *p*-substituent in betaxolol is causing the β_1-selectivity, as its presence causes a strong reduction in affinity at the β_2-AR and only a small decrease in affinity at the β_1-AR, when compared to the non-substituted compound [21]. Therefore, it was proposed that this selectivity is probably caused by steric hindrance, which is considerably stronger on the β_2-AR than on the β_1-AR. In our binding model, this could well be studied by comparing the amino acid sequences of the β_1-AR and the β_2-AR in the para-region of the receptor-bound ligand. In this region a number of differences exist (Fig. 2), but the most probable cause of the selectivity is the presence of Trp^{105} in the β_2-AR. In the corresponding positions of the avian and human β_1-AR are located Leu^{113} and Phe^{130}, respectively, which are both considerably smaller amino acids than the tryptophan residue. To examine whether this difference can cause such a selectivity, we docked betaxolol to the proposed binding site on helix III. It appeared that, upon binding to the β_2-AR, the *p*-substituent of betaxolol was positioned very close to Trp^{105}, and slight variations in φ and ψ angle values of the receptor helix resulted in a strong steric hindrance, caused by a penetration of betaxolol's cyclopropyl moiety into the Trp^{105} residue. Since such φ/ψ variations continuously occur in a realistic dynamic system, this steric hindrance is very likely to be the origin of the observed ligand

selectivity. The corresponding Leu^{113} and Phe^{130} residues in the two β_1-ARs can accommodate the *p*-substituent much better by their smaller size and larger conformational flexibility.

Agonist versus antagonist binding

This binding site should also be able to bind agonists, as agonists competitively displace antagonists from their binding sites. Since the agonist molecule part comprising the protonated nitrogen and the β-hydroxyl group is similar to the corresponding part of antagonists, identical interactions (ionic and H-bond) can be accomplished with Asp^{113}. The aromatic moiety of agonists, however, is located more closely to the β-hydroxyl and protonated nitrogen, because most agonists lack the extra oxymethylene bridge of most antagonists. As a consequence, for an optimal hydrophobic interaction, the aromatic moiety of agonists cannot reach the indole ring of Trp^{109} as adequately as the antagonists, and therefore this third interaction point will be less strong than in antagonist binding. This feature was recognized earlier in QSAR studies [11], in which it was concluded that the aromatic moiety of agonists contributed less to affinity than the same moiety in antagonists.

Comparison with other models

The antagonist binding site proposed in this paper deviates from other ideas. Since the ligand binding site is known for retinal, the endogenous ligand for rhodopsin, it has been proposed that β-AR agonists and antagonists interact in a similar manner with the β-AR; that is, they intercalate between the hydrophobic transmembrane helices [1, 3, 8]. In our model, the ligand is not intercalated among the transmembrane helices, but has its principal interaction points located on one helix only. The intercalated binding model, however, is solely based on the analogy of retinal interacting with opsin. Retinal itself is a compound that strongly differs from β-AR agonists and antagonists. It has no charged function of its own. Only upon (covalent) binding does it form a protonated Schiff base. Furthermore, no hydroxyl or other hydrogen-donating groups are present in its side chain. Additionally, the binding site of retinal involves Lys^{296}, located in stretch VII, while Dohlman et al. found that the antagonist binding site of β-ARs is restricted to stretches I–IV [7]. For these reasons, extrapolation of the interaction of retinal with opsin to the β-AR antagonist binding site topology seems highly speculative.

Recently, Dohlman et al. identified a binding site peptide of the β_2-AR [22]. Treatment of the receptor with *p*-(bromoacetamido)benzyl-1-[^{125}I]iodocarazolol (^{125}I-pBABC, Fig. 1) resulted in covalent incorporation of this ligand into the ligand binding domain. From subsequent degradation experiments of the ^{125}I-pBABC-labeled receptor, a peptide fragment was identified to which the ligand was covalently bound. This peptide, consisting of amino acid residues 83–96, is located in putative helix II of the β_2-AR, and is not included in the binding site proposed in this paper.

These findings, however, are well compatible with the antagonist binding site proposed by us. The bromoacetamido moiety of ^{125}I-pBABC, responsible for the covalent incorporation into the β_2-AR, is in a position rather remote from the classic aryloxypropanolamine fragment that was used to define our antagonist binding site. The length of the (bromoacetamido)benzyl substituent is estimated to be approximately 11 Å. Therefore it is likely that this covalent bond is formed in a peptide region neighboring the propranolol binding site. Upon degradation, only the covalent

bond will 'survive', whereas hydrophobic and ionic interactions are too weak to persist under these circumstances. For this reason, the identification of peptide 83–96 to be involved in the ligand-receptor interaction does not preclude the hypothesis that the carazolol entity of ^{125}I-pBABC interacts with our deduced binding site on helix III. Indeed, the aromatic moiety of carazolol will favorably interact with tryptophan residues like Trp^{109}, as we already described for the indole function of pindolol [13]. Thus, on interaction of ^{125}I-pBABC with the proposed binding site on helix III, the covalent bond can well be formed with a residue on helix II. These ideas are strengthened as it appears probable that both our proposed binding site and the peptide found by Dohlman et al. are located close to each other. First, they are part of transmembrane helices III and II respectively, two α-helices that are probably adjacent, as can be inferred from secondary and tertiary models developed for rhodopsin [1, 23, 24]. Second, they are both located in transmembrane helix parts neighboring the extracellular surface [1, 22].

The binding and subsequent directing of the ligand via other aspartate residues, a theory recently proposed by Wheatley et al. for the muscarinic receptor [25], does not apply to the β-AR binding site suggested by us. In our opinion, the three-point interaction is too strong and specific to allow a movement of an antagonist molecule along several ionic interaction points.

Of course, our proposed β-AR antagonist binding site can only be an approximation of biochemical reality. Besides the main binding residues, other parts of the receptor and membrane components are likely to have subtle interactions with the ligand in its binding site, altogether determining the detailed conformation of the receptor-ligand complex. Nevertheless, this study gives useful information on a possible mode of antagonist binding to the β-AR, and the main amino acid residues involved in it.

CONCLUSIONS

The principal finding of the present study is that a probable antagonist binding site, with interacting amino acid residues Asp^{113} and Trp^{109}, is found on the β-AR, which accounts for the β_1-selectivity of certain compounds, and may explain the stereoselectivity of β-AR ligands.

This information can be taken into account in future biochemical and computer graphics studies. Additionally, new results of other β-AR studies can be used to refine our model of the antagonist binding site, which then may enable optimization of β-AR ligands with respect to affinity and β_1/β_2-selectivity.

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ABBREVIATIONS

β-AR: β-adrenergic receptor.
cDNA: complementary DNA.
H-bond: hydrogen bond.
VdW: van der Waals.
QSAR: quantitative structure-activity relationship.
^{125}I-pBABC: *p*-(bromoacetamido)benzyl-1-[^{125}I]iodocarazol.