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A molecular graphics study exploring a putative ligand binding site of the β-adrenoceptor

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SUMMARY

The recent elucidation of the primary structure of the cell membrane-bound β-adrenoceptor has prompted us to explore putative ligand binding sites on this physiologically important receptor. By minimizing the energies of the 'prototype' ligand propranolol, (part of) the receptor and the proposed ligand-receptor complex with the aid of force field and quantum chemical calculations, we identified amino acid residue Trp^{313} as a highly probable candidate for interaction with the aromatic moiety of propranolol. The charge distribution on the indole nucleus of another β-blocker, pindolol, with higher affinity for the β-adrenoceptor, enables an even stronger interaction with the tryptophan residue. The carboxylic amino acid residue Glu^{306}, located near the extracellular space of the cell membrane, interacts favorably with the positively charged nitrogen atom in the aliphatic side chain of the ligands. Finally, this putative model is discussed in the light of recent findings in mutagenesis studies, and compared to other ideas with respect to ligand-receptor interactions.

INTRODUCTION

The β-adrenoceptor is part of the adenylate cyclase system, an efficient trans-membrane signalling system, located in the wall of a variety of cell types. This system is further composed of a regulatory guanine nucleotide-binding protein (named N_s or G_s) and a catalytic moiety, altogether providing a means of translating extracellular signals into intracellular events, i.e., the stimulation of the production of cAMP [1]. Thus, dependent on cell type and tissue, β-adrenoceptors, which are subdivided into two types, viz., β_1 and β_2 [2], are involved in a number of physiological processes, e.g., cardiac contractility, lipolysis, glycogenolysis and smooth muscle relaxation [3].

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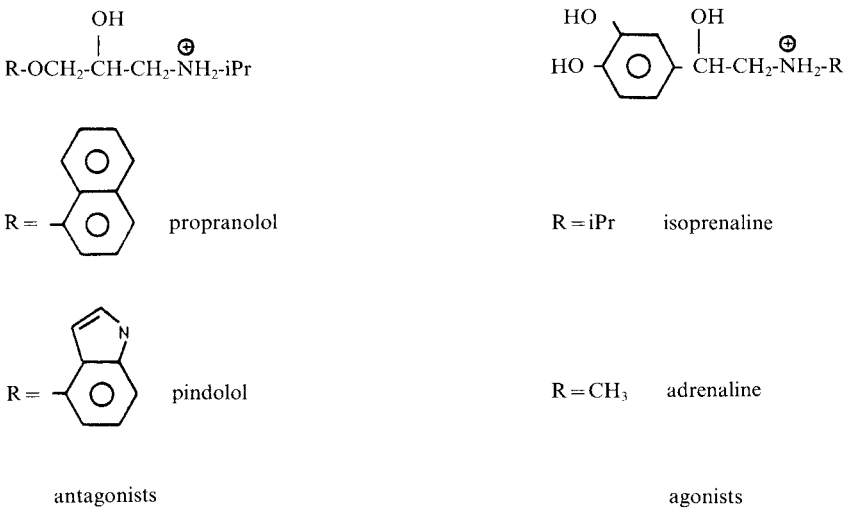

Fig. 1. Chemical structures of β-adrenoceptor ligands (iPr = isopropyl).

With respect to the topology of the ligand binding site on the β-adrenoceptor, some features have been derived. From radioligand binding studies performed at various pH values, it could be concluded that the cation of β-adrenoceptor agonists and antagonists is the active ionic species [4, 5]. Consequently, the amino function in propranolol (see Fig. 1), the 'prototype' β-adrenoceptor antagonist, is protonated. Furthermore, Cherksey et al. convincingly demonstrated that the aromatic nucleus of propranolol is located in a tryptophan-rich region [6]. By means of QSAR analysis and, secondly, a distance geometry approach, it was further established, that the aromatic nuclei of phenoxypropanolamines (usually β-adrenoceptor antagonists) and phenylethanolamines (usually β-adrenoceptor agonists), respectively, are in different positions on the β-adrenoceptor [7, 8].

Recently, the genes and cDNAs for the human β_2- [9], the hamster β_2- [10] and the turkey erythrocyte β_1-adrenoceptor [11] have been cloned, and the amino acid sequences derived. All these receptors are peptides of over 400 amino acids and appear to share some homology with bovine and human rhodopsins. These are integral membrane proteins, located in the retina of the eye, and essential as visual pigments. This homology is most evident in seven stretches of 20–28 hydrophobic amino acids, which likely represent membrane spanning helices in all these proteins [12]. In an attempt to define the ligand binding site, Dixon et al. expressed a series of deletion mutant genes of the hamster β_2-adrenoceptor in mammalian cells [13]. Deletion of amino acids 274–330 (including the putative stretches VI and VII, see Fig. 2) resulted in a virtually complete loss of binding capacity, as determined with the radioligand [^{125}I]cyanopindolol, a β-adrenoceptor antagonist.

Bearing all these data, as summed up in this INTRODUCTION section, in mind, we explored this particular amino acid sequence by means of computer-aided force field and quantum chemical calculations and graphical display. The results, in terms of a possible mode of interaction between propranolol and the β_2-adrenoceptor, are reported and discussed in this paper.

Thr^{274}-Leu-Gly-Ile-Ile-Met-Gly^{280}-Thr-Phe-Thr-Leu-Cys-Trp-Leu-Pro-Phe-Phe^{290}-Ile-Val-Asn-Ile-Val-His-Val-Ile-Gln-Asp^{300}-Asn-Leu-Ile-Pro-Lys-Glu-Val-Tyr-Ile-Leu^{310}-Leu-Asn-Trp-Leu-Gly-Tyr-Val-Asn-Ser-Ala^{320}-Phe-Asn-Pro-Leu-Ile-Tyr-Cys-Arg-Ser-Pro^{330}-

274–298 helix VI
299–306 extracellular turn
307–330 helix VII

Fig. 2. Amino acid sequence 274–330 of the hamster β_2-adrenoceptor.

METHODS

As mentioned in the INTRODUCTION, we studied the interaction of the 'prototype' β-adrenoceptor antagonist propranolol with the receptor fragment containing amino acids 274–330.

The receptor: amino acids 274–330

The peptide consisting of amino acids 274–330 was constructed in the modify/cursor mode of the molecular modeling system CHEM-X [14]. Amino acids 274–298 (stretch VI) were built in an α-helix ($\varphi = -52°$, $\psi = -53°$), amino acids 299–306 were constructed to form a large turn, and amino acids 307–330 (stretch VII) were built in an α-helix antiparallel to the first α-helix, according to the hypothesized structure for the β-adrenoceptor [12]. Since the conformation of the extracellular turn consisting of amino acids 299–306 is not known, we constructed a random U-shaped turn. The peptide was then divided into several parts to enable charge distribution calculations with the semi-empirical MOPAC method [15]. Thereafter, the parts were fused together to form the original peptide. In order to assure that no functional groups of the amino acids were sterically hindered, we performed a molecular mechanics optimization (MM2) of the peptide [16].

The ligands: propranolol and pindolol

The structure of *S*-propranolol was retrieved from the Cambridge Crystallographic Database. *R*-propranolol was then constructed by inverting the chirality of the β-carbon atom in *S*-propranolol. *S*-pindolol was constructed by replacing the naphthyl moiety of *S*-propranolol with an indole group. Charge distributions were calculated with MOPAC [15], and all compounds were structurally optimized with molecular mechanics (MM2P) [17].

The interaction: propranolol with the peptide

To study the most favorable interaction of propranolol with the peptide we used the VdW (Van der Waals) energy minimization mode 'minimize' in CHEM- X. We minimized the total energy of the system, which consists of the intramolecular energies of propranolol and the peptide, and the intermolecular 'interaction' energy of propranolol with the peptide. In the VdW energy minimization, potential functions, electrostatic and torsional terms are taken into account [18]. These calculated energies are not to be considered as absolute values, however, they provide measures on a relative scale. In order to save CPU 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. We then minimized the total VdW energy of

the system, allowing every torsion angle in the propranolol molecule (except the naphthyl ring) to be flexible, and the propranolol molecule as a whole to translate and rotate in every direction. The torsion angles of the interacting amino acids, determining the positions of their functional groups, were also flexible, allowing an 'induced fit'. Since in this docking procedure no parameters for hydrogen bonding are available, we set a distance restraint of 1.9 Å between the atoms which might interact by such a bond.

A dielectric constant of 1.0 was used, since CHEM-X does not provide a distance-dependent dielectric constant. However, on increasing the dielectric constant, the total interaction energy remained strongly negative, although less than with the default value of 1.0.

RESULTS

In Fig. 3, the optimized structure of *S*-propranolol is graphically represented in blue. The molecule is in an almost extended conformation, the distance between the positively charged nitrogen and the center of the naphthyl ring being ca. 8 Å. β-Adrenergic ligands are thought to interact with the β-adrenoceptor by a three-point attachment [19], in which the aromatic nucleus, the β-hydroxy group and the positively charged nitrogen of the ligands are involved. Thus, complementary moieties on the β-adrenoceptor are likely to be aromatic, hydrogen accepting and negatively charged, respectively.

In view of the findings by Cherksey et al. [6], as outlined in the INTRODUCTION, two likely 'candidates' for aromatic anchoring are Trp^{286} in helix VI and Trp^{313} in helix VII (cf. Fig. 2). Negative-

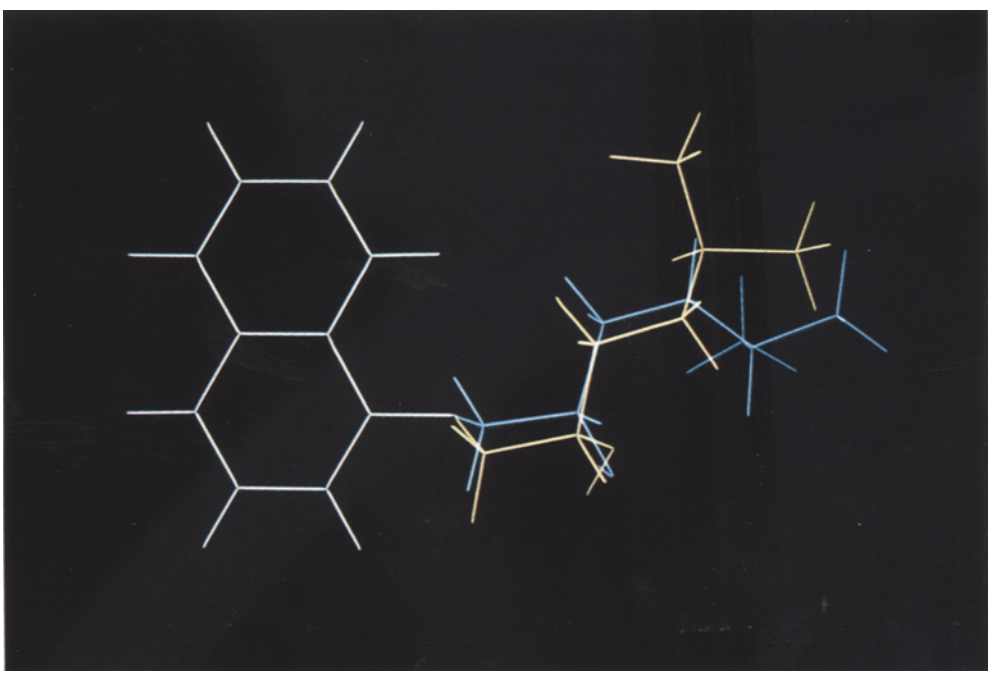

Fig. 3. Energetically optimized (blue) and receptor bound (yellow) conformations of *S*-propranolol.

ly charged residues in the neighborhood of these tryptophan residues are Asp^{300}, located close to helix VI, and Glu^{306} in proximity of helix VII. After structural optimization of helix VI, the distance between Trp^{286} and Asp^{300} was calculated, being ca. 21 Å. Analogously, in helix VII the distance between Trp^{313} and Glu^{306} equals 15 Å. Bearing the length (8 Å) of *S*-propranolol in mind, we thus considered helix VII as the more probable binding domain, and we explored it further.

The optimized structure of helix VII (before binding propranolol) is shown in Fig. 4 in which Trp^{313} and Glu^{306} are indicated. In this selected peptide only two hydrogen bond acceptors are in a proper orientation, viz., the carboxamide oxygen atoms of the backbone skeleton of Glu^{306} and Ile^{309}, respectively.

The results of the docking procedure are represented in Figs. 5 and 6. Figure 5 is a stick drawing of the ligand-receptor complex in which the interacting groups on the receptor peptide are in blue, and the ligand in yellow. The two combinations of white/green VdW dot surfaces represent the interaction between the positively charged nitrogen and Glu^{306}, and the proposed hydrogen bond, respectively. Figure 6 is a CPK model representation of the complex, in which the parallel stacking of the two aromatic rings is evident. From this docking procedure, it appeared that Ile^{309} is better accommodated for hydrogen bonding than Glu^{306}.

In Fig. 7, the conformations of the receptor in the free and bound situation, respectively, are superimposed. From this representation, it is evident that the positions of Trp^{313} and Glu^{306} are changed when propranolol binds. The two residues are directed towards each other (distance ca. 11 Å), ensuring a better interaction with the β-adrenoceptor ligand. Similarly, the conformation of *S*-propranolol changes upon binding, which is represented in Fig. 3.

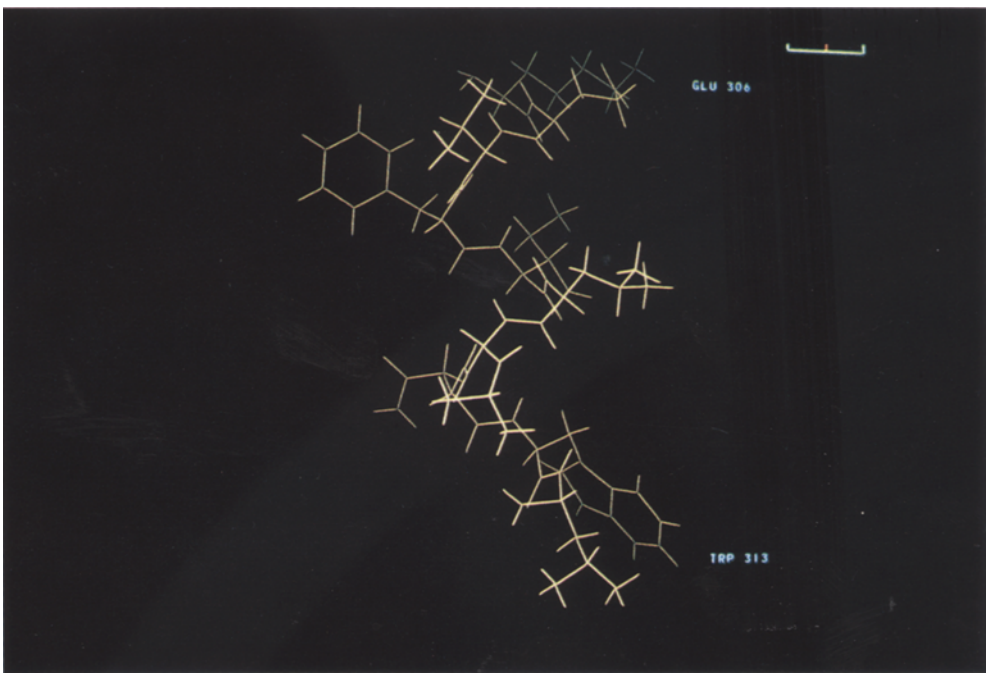

Fig. 4. Energetically optimized structure of helix VII of the β-adrenoceptor.

TABLE 1
INTERACTION OF *S*-PROPRANOLOL WITH ITS PUTATIVE BINDING SITE ON THE HAMSTER β_2-ADRENOCEPTOR

Functional group in propranolol	Interaction with	Interaction type	Orientation/distance
Postively charged nitrogen	Glu^{306}	reinforced ionic bond [20]	nitrogen-oxygen (Glu) = 2.9 Å
β-Hydroxyl group	carboxamide oxygen of Ile^{309}	hydrogen bond	angle O-H---O = 170° distance H---O = 2.1 Å
Naphthyl ring	Trp^{313}	– charge transfer [20] – hydrophobic interaction	parallel stacking ring–ring distance = 3.8 Å

Is the formation of the ligand-receptor complex energetically favorable? Of course, the intramolecular VdW repulsion energies of both the receptor and the ligand increase upon binding, but only to a slight extent, viz., 9 kcal/mol for the receptor, and 2 kcal/mol for propranolol. The intermolecular interaction energy, however, resulting from electrostatic and VdW attraction, decreased considerably by 71 kcal/mol. Thus, in the final ligand-bound situation, the decrease in total VdW repulsion energy of the system is approximately 60 kcal/mol.

Finally, in Table 1, the various interactions between the receptor and the ligand are described.

DISCUSSION

Two aspects will be discussed in this section, viz., the characteristics of the deduced binding site and, secondly, its significance in view of some other recent findings.

As visualized in the RESULTS section there are two amino acid residues, viz., residues Asp^{300} and Glu^{306}, in the amino acid sequence, which bear negatively charged carboxylic moieties. These are located between the two hydrophobic helices, facing the extracellular space. Therefore, as ligands approach from outside the cell, both residues are important candidates for the interaction with the protonated amino function of propranolol, and could serve as a first point of anchoring for this antagonist.

In earlier studies, we concluded that the contribution of the aliphatic side chain to the β-adrenoceptor affinity is larger than the contribution of the aromatic moiety in β-adrenoceptor ligands [7], which stresses the role of the amino-carboxylic reinforced ionic bond as a first point of recognition and interaction. Since *S*-propranolol (as all the other levorotatory β-adrenoceptor ligands) has far higher affinity for the β-adrenoceptor than its dextrorotatory *R*-isomer, the specific orientation of the hydroxyl group on the β-carbon atom in the side chain is of importance as well. In our model, this functionality interacts with the backbone oxygen atom of Ile^{309}, and this hydrogen bond may constitute the second important site of interaction. Modeling of *R*-propranolol in the deduced binding site, however, shows that the naphthyl-Trp^{313} and the hydrogen bond with Ile^{309} still can be formed, resulting in an interaction energy comparable to the binding of *S*-propranolol. Upon binding, the rest of the side chain is in a different position, which is especially reflected in the ori-

entation of the *N*-isopropyl group (results not shown). Whether this shifted orientation will induce stereoselectivity (e.g., due to hindrance of the other amino acid sequences) is a question yet to be answered.

The third point of attachment is found in the interaction between the indole ring structure of Trp^{313} and the naphthyl moiety of propranolol. What is the nature of this interaction? Due to the presence of an ether linkage between the aromatic nucleus and the propanolamine side chain in propranolol, the charges on the naphthyl nucleus are not evenly distributed, which gives rise to a (small) dipole moment. Similarly, the presence of a nitrogen atom in the indole nucleus of Trp^{313} induces an uneven charge distribution as well, again the cause of a dipole vector (Fig. 8). On binding to the β-adrenoceptor, the naphthyl nucleus is superimposed on the indole moiety in such a way that the more negative five-membered ring of the indole nucleus interacts with the more positive part of the naphthyl function, and vice versa (with opposite dipole moments). Thus, in addition to 'hydrophobic bonding' of the two conjugated ring systems, there is a further contribution to binding due to 'electron-' or 'charge-transfer'. Now the even higher affinity of indoloxypropanolamines [21], like pindolol (Fig. 1), is easily understood. The charge transfer in this case is even more prominent as, on superposition, the indole nucleus of pindolol is in a reversed position with respect to the same entity of Trp^{313} (Fig. 8).

The energy content of propranolol in its bound conformation is only slightly higher (ca. 2 kcal/mol) than in the conformation with minimal energy. This finding is in accordance with the ideas of Andrews et al. [22]. By analyzing functional group contributions to drug-receptor interactions, they concluded that the binding energy of propranolol is above average. This favorable difference can be ascribed to the minimal deviation in the structure of receptor-bound propranolol from propranolol in its lowest energy state.

Since β-adrenoceptor agonists are capable of displacing radiolabeled antagonists competitively, it seems reasonable to assume that agonists interact to a greater or lesser extent with the postulated binding site as well. Unfortunately, direct proof of this assumption is hard to obtain, as radiolabeled agonists with sufficiently high affinity are not available.

How does the thus deduced binding site compare with other ideas?

Since the ligand binding site of retinal, the endogenous ligand for all opsin proteins, is known, some speculations on the nature of the β-adrenoceptor binding site have already been made [10, 12, 13]. Retinal, upon binding, forms a Schiff base with the ε-amino function of amino acid residue Lys^{296}, which is in the middle of the seventh transmembrane helix, and thereby becomes positively charged [23]. This feature would be energetically unfavorable in this hydrophobic domain without a counterion. Now, helices II and III in all opsins contain a negatively charged aspartyl and glutamyl residue, respectively, which may serve as possible counterions.

Analogously, Applebury and Hargrave [24] have suggested that aspartyl residues in helices II and III of the β-adrenoceptor are similar counterions for the protonated amines of ligands like adrenaline, an endogenous analog of the synthetic isoprenaline (see Fig. 1). When this paper was in preparation, Strader et al. [25] reported oligonucleotide-directed mutagenesis studies of the hamster gene encoding the β-adrenoceptor. They stressed the importance of Asp^{113} present in helix III, as its replacement by the uncharged Asn^{113} resulted in the almost complete loss of [^{125}I]cyanopindolol binding. Thus, in the opinion of these authors, Asp^{113} is an integral part of the ligand binding site, and interacts with the protonated amino function of the ligands.

In these ideas, β-adrenoceptor ligands are intercalated among the hydrophobic transmembra-

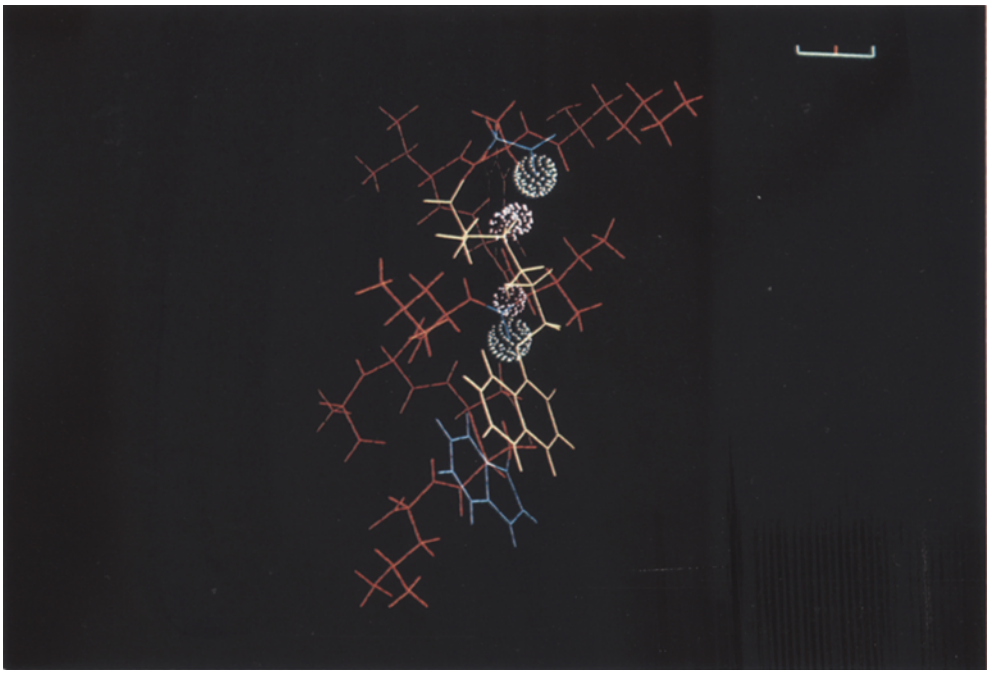

Fig. 5. The interaction between *S*-propranolol and helix VII of the β-adrenoceptor. Blue: Glu^{306}, Ile^{309}, Trp^{313}; yellow: *S*-propranolol; white dots: VdW surfaces of interacting hydrogen atoms of *S*-propranolol; and green dots: VdW surfaces of interacting oxygen atoms of the receptor (Ile^{309} and Glu^{306}, respectively).

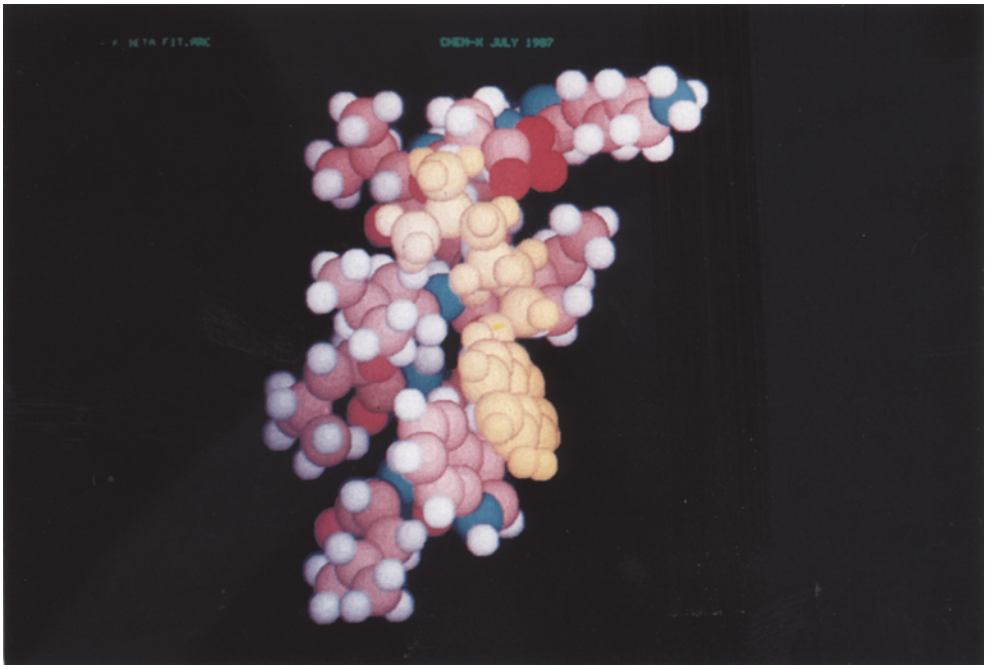

Fig. 6. CPK representation of the propranolol-β-adrenoceptor complex (yellow: *S*-propranolol).

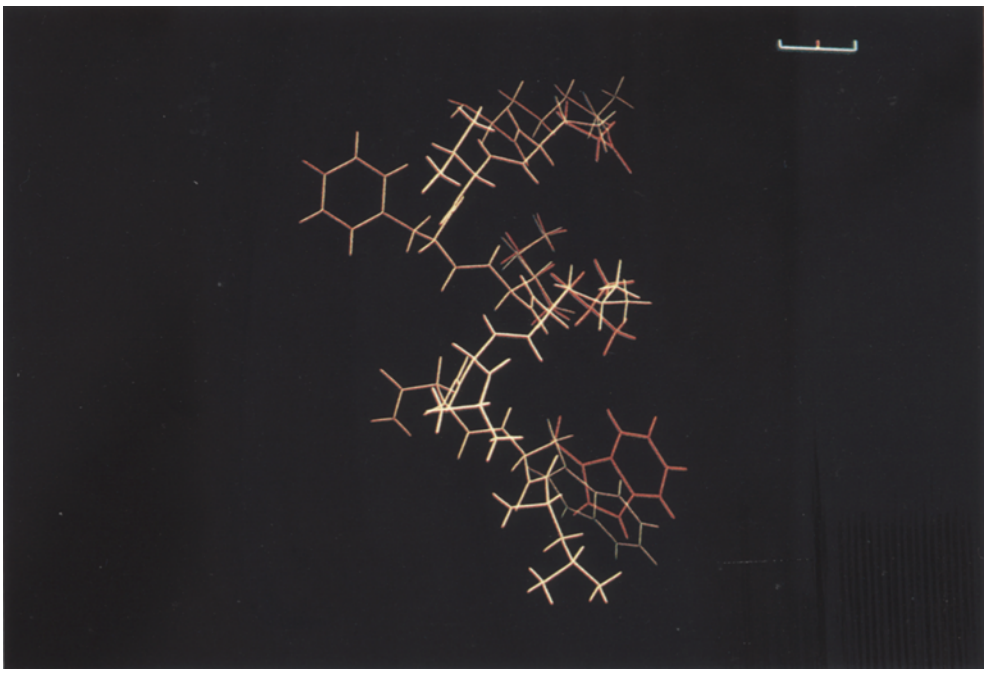

Fig. 7. Energetically optimized (yellow) and ligand-bound (red) conformations of helix VII of the β-adrenoceptor.

ne helices [10, 12, 13], and this picture clearly deviates from the binding site hypothesized in this paper.

Although a definite answer favoring one of the two models has yet to come (by e.g. Roentgen diffraction analysis of the crystalline ligand-protein complex), some remarks have, however, to be made. Retinal itself is a very lipophilic compound which can easily penetrate within the hydrophobic core of an opsin. Only after reaction with lysyl residue 296 a positively charged complex emerges, which is effectively neutralized by carboxylic residues as described above. β-Adrenoceptor ligands are far more hydrophilic due to their protonated amino function, and this moiety of the ligands will not have easy access to the hydrophobic segments.

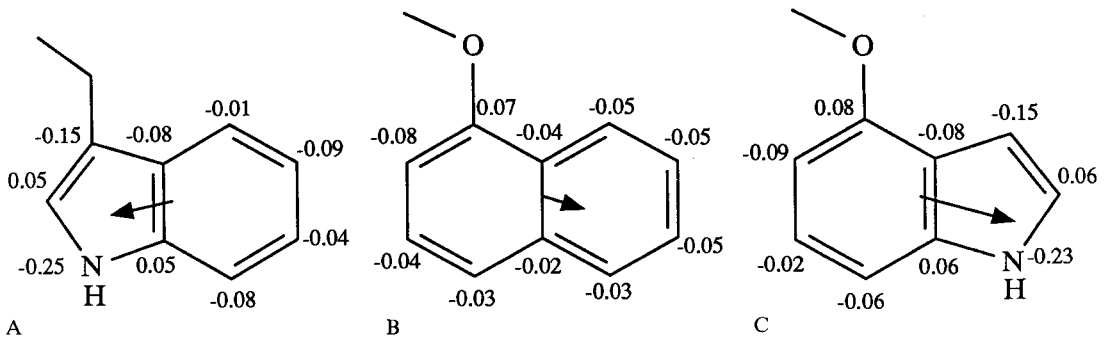

Fig. 8. Charge distributions on ring atoms, and dipole moments for the aromatic moieties of tryptophan (A), propranolol (B) and pindolol (C).

Thus, in our opinion, a model in which the charged amino function is anchored in a position close to the extracellular space, seems more likely. Unfortunately, the 'intercalated' model cannot be analyzed by molecular graphics, as the tertiary structure of the β-adrenoceptor as a whole is not yet known.

Since tryptophan residues are present in helix III as well, in close proximity of Asp^{113}, we are currently examining this region as another putative ligand binding site.

In conclusion, the principal finding from the present study is that tryptophan residue Trp^{313} is a highly probable point of interaction for propranolol and pindolol. With respect to the other β-adrenoceptor antagonists (like the ortho-substituted phenoxypropanolamines penbutolol and alprenolol), it seems likely that Trp^{313} plays a role in the interaction as well. In the deduced binding site is ample space available to accommodate the aliphatic ortho substituents.

Finally, computer-assisted molecular modeling is a valuable tool in the analysis of putative binding sites on receptors. As structural information with regard to receptors becomes more available [26], this tool will be of increasing significance.

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