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REVIEW —*Current Perspective*—

β-Adrenoceptors: Three-Dimensional Structures and Binding Sites for Ligands

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ABSTRACT—Recent progress in analyzing the structures and functions of G-protein coupled receptors (GPCRs) including β-adrenoceptors (β-ARs) has been made by pharmacological, physiological and molecular biological techniques. The three-dimensional (3D) structures, interaction sites with ligands and conformational changes of these receptor subtypes due to ligand binding are now better understood by the simulation of these receptors using computer-aided molecular modeling. Based on these techniques, numbers and conformations of amino acid sequences of each subtype (β_1-, β_2- and β_3-ARs) were defined and also interaction sites or modes of interaction between ligands and β-ARs could be analyzed three-dimensionally. In addition, simulation of 3D structures of β-ARs by molecular modeling could clearly determine the limited size, space or pocket for fitting with ligands. These studies will give some clues for the clarification of other GPCRs. Thus, this review summarizes current findings on chemical structures of ligands, amino acid sequences, 3D structures and important amino acids of β-AR subtypes for interacting with ligands obtained from mutagenesis, chimeric studies and molecular modeling techniques.

Keywords: β-Adrenoceptor subtype, β_1-Adrenoceptor, β_2-Adrenoceptor, β_3-Adrenoceptor, Three dimensional structure, Interaction site, Molecular modeling

Introduction

The β-adrenoceptors (β-AR) belong to a large family of G-protein-coupled receptors GPCRs that are characterized by seven transmembrane helices. Three subtypes of β-ARs (β_1, β_2 and β_3) have been characterized by pharmacological, biochemical and molecular biological cloning approaches (1), while Kaumann et al. reported the existence of β_4-ARs in the mammalian heart (2). Granneman used the β_3-AR agonist CGP 12177 to define a novel atypical β-AR subtype, the putative β_4-AR (3). In addition, molecular modeling techniques are also giving us much information on chemical interactions between ligands and receptors. Thus, tremendous and epochal studies using these multiple approaches will contribute to elucidating the structures and functions of β-ARs. As β-AR ligands (agonists and antagonists) are clinically becoming very important for treating many kinds of diseases (4 – 7), this review will focus on the structure of β-adrenoceptors and their ligands, their interaction sites as well as transduction of signals from the receptors to the G proteins.

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Structure and structure-activity relationship of β-AR ligands

The first trial to clarify the structure-function relationship of β-AR ligands was done by Lands et al. (8). The basic structures of β-AR ligands are 1) phenylethanolamine and 2) phenoxypropanolamine. The compounds of phenylethanolamine series are consisting of a benzene ring and an ethylamine side chain which contains a hydroxyl group at the β-carbon. Most of these compounds (epinephrine, isoproterenol, BRL 37344, etc.) exhibit agonistic properties towards $\beta_1/\beta_2/\beta_3$-AR. On the other hand, the compounds of phenoxypropanolamine series are consisting of a phenoxy group attached to a β-hydroxypropanolamine side chain. All of these compounds show antagonism towards β_1- and β_2-AR, but they are agonists of β_3-AR, and only few (ICI 118551, CGP 20712A, bupranolol, etc.) exhibit β_3-AR antagonistic activity. There are, however, a few exceptions and those compounds are dichloroisoprenaline, pronethalol, sotalol, etc., which possess the ethanolamine side chain, but show antagonistic activity towards β_1- and

β_2-AR. Thus, the important conformation involved in β_2-AR activity consisted of the dimensional chemical arrangement of catechol OH, OH in the beta position, and N-H binding (9).

In recent years, as mentioned above, agonists (BRL 37344A, BRL 35135A) with strong selectivity for β_3-ARs have been reported (10). Blin et al. reported structural characteristics of the three pharmacological classes of β-ARs. The selective or potent β_3-AR ligands show common structural characteristics, i.e., 18 – 20-carbon backbone length, a substituted or unsubstituted aromatic ring, and an (oxy)hydroxylalkylamine chain ending in an indol function or a phenyl carrying hydroxyl, ether or acid functions, which increase steric bulk and moderate lipophilicity (10). These conformational characteristics of these subtype ligands are almost the same as those of β_1- and β_2-ARs, but only differences in these subtypes are the aromatic ring and bulky and long substituents of amino side chains. However, there are three types of agonists and antagonists for β_3-ARs. Those are 1) the potent $\beta_1/\beta_2/\beta_3$-AR agonists (BRL 37344, LY 79771), 2) β_3-agonist, β_1/β_2-antagonists (alprenolol, oxprenolol) and 3) $\beta_1/\beta_2/\beta_3$-antagonists (bupranolol, CGP 20712A, ICI 118551). In addition, β_3-AR agonists also show two kinds of chemical structures: 1) phenylethanolamine (BRL 37344, LY 79771) and 2) phenoxypropanolamine (bucindolol, ICI 201651) series. These compounds have different length with respect to the number of carbons. Therefore, in 1993, Blin et al. suggested that several compounds can adopt either folded or extended conformations for best fit into their respective receptor surface for interactions, and these authors used molecular dynamics modeling to analyze the three-dimensional (3D) structures of compounds. They also suggested that the folded conformations of pindolol, cyanopindolol and CGP 12177A are in large part responsible for their β_1/β_2-antagonists activity, whereas extended conformations of these compounds are responsible for the β_3-agonist activity (10). Recently, Konkar et al. reported the anomalous biphasic effects of CGP 12177 on β_1-AR. Low concentrations of CGP 12177A potently blocked isoproterenol-induced stimulation of β_1-AR, whereas high concentration of this compound stimulated the β_1-AR (11).

Thus, ligand conformation plays an important role in the efficacy of ligands to interact with different receptor subtypes for agonistic or antagonistic activity. The addition of a hydrophobic group to the amino end of the β-AR ligand pharmacophore can result in molecules that are capable of existing in either extended or folded conformation in space. For small molecules, transconformation is mainly due to rotation around the C_α-C_β bond of the hydroxyethyl amine chain but for large molecules, rotation occurs around both C_α-C_β and C_α-$C_{\beta'}$ bonds of the hydroxyethyl amine chain.

The 3D interatomic distances between the reacting atoms in a ligand are important for eliciting pharmacological effects (10). In different conformational adoption (extended and folded) of molecules, this distance between involved atoms will vary. Thus, a particular conformation of a ligand will induce pharmacological effects at a particular receptor subtype, and this will resemble other ligands at the 3D level. Figure 1 shows the schematic representation of the β_3-AR minimal pharmacophore in postulated extended and folded conformation. Interatomic distances vary markedly in different conformations, as can be assumed from the figure.

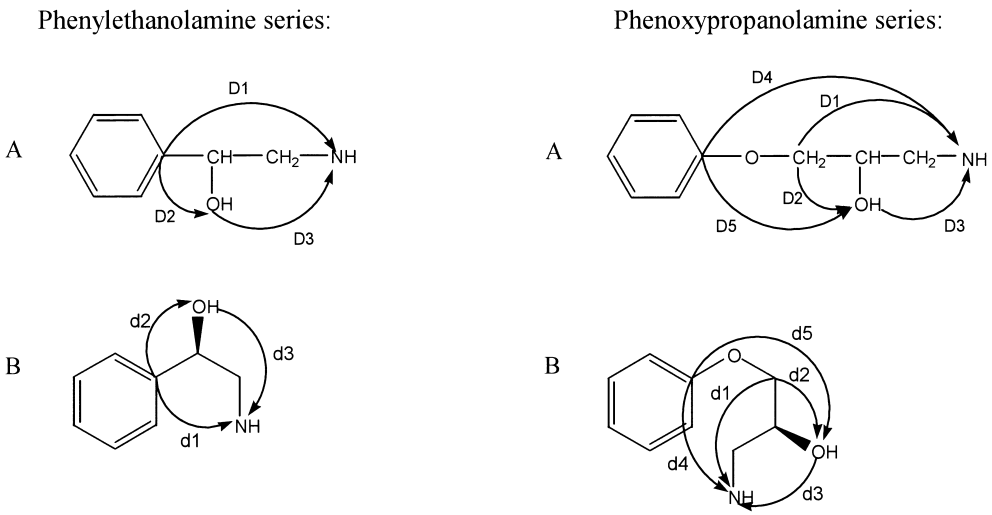


Fig. 1. Schematic representation of the β-AR minimal pharmacophore. Differences in apparent 3D distances of the postulated folded and extended conformations. Conformations A denote extended forms and conformations B denote folded forms. Folded forms show minimal interatomic distances. Extended forms are cited in reference 12.

Structure of β-AR subtypes

β-ARs are one of the GPCRs. Kobilka et al. were able to determine the entire structure of a β_2-AR, which is now known to be composed of 413 amino acids; and the structure of a human β_1- and β_3-AR have also been determined, each being composed of 477 and 408 amino acids, respectively (12). The human β_3-AR was shown to have 49% and 51% overall homology at the amino acid sequence to human β_2-AR and β_1-AR, respectively (13, 14).

Important interaction sites between ligands and β-AR subtypes

Mutagenesis studies

Deletion mutagenesis of the β_2-AR revealed that the ligand binding domain for small AR agonists and antagonists resides within the conserved hydrophobic core of the receptor (15). As mentioned above, it is considered that there are several functional groups in their structures for interactions with β-ARs (Table 1).

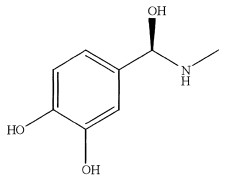
1) The *meta*- and *para*-hydroxyl groups: these groups form hydrogen bonds with Ser^{204} and Ser^{207}, respectively, on TM5 of β_2-AR (16). If agonist interaction with the receptor involves the formation of specific hydrogen bonds with serine residues on TM5, then the binding might cause conformational changes in this helix that could be transmitted to the residues at the bottom of the helix, catalyzing the interaction of this region with Gs. Antagonists that do not appear to interact with Ser^{204} and Ser^{207} would not be expected to promote this conformational change.

2) beta-Hydroxyl group: the hydroxyl group of the ethanolamine side-chain interacts with Ser^{161} on TM4 of β_2-AR by a hydrogen bond (17). However, a hydrogen bond is also formed when β_3-AR agonist interacts with Ser^{169} on TM7 of β_3-AR (1).

3) Charged amine group: this charged amine interacts with the carboxylated side chain of Asp^{113} on TM3 of β_2-AR (18, 19). Structure-activity analysis of AR ligands has shown the amine moiety of both agonists and antagonists to be essential for the interaction with the β-ARs (20, 21). Strader et al. reported that substitution of Asp^{113} with a glutamic acid residue resulted in a mutant β-AR that recognizes several known β-AR antagonists (alprenolol,

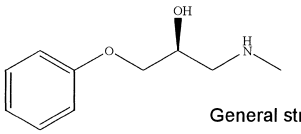
Table 1. Ligand binding sites on β_2- and β_3-AR

Agonists



Phenylethanolamine series

General structure of β_2-AR agonists

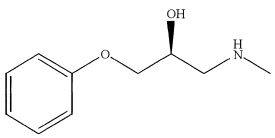


Phenoxypropanolamine series

General structure of β_3-AR agonists (with β_2-AR agonist structure)

Functional groups on ligands	Amino acid of β_2-AR bound	Position in TM	Ref.	Amino acid of β_3-AR bound	Position in TM	Ref.
Charged amino	Asp^{113}	TM3	(19)	Asp^{117}	TM3	(1)
meta-Hydroxyl	Ser^{204}	TM5	(16)	Ser^{209}	TM5	(1)
para-Hydroxyl	Ser^{207}	TM5	(16)	Ser^{212}	TM5	(1)
beta-Hydroxyl	Ser^{161}	TM4	(17)	Ser^{169}	TM4	(1)
Aryloxy ether				Asn^{312}	TM6	(37)
Ligand aromatic ring	Phe^{289}	TM6	(24)	Phe^{309}	TM6	(1)
	Phe^{290}	TM6				
	Phe^{208}	TM5				

Antagonist



Phenoxypropanolamine series

General structure of β_2-AR and β_3-AR antagonists

Functional groups on ligands	Amino acid of β_2-AR bound	Position in TM	Ref.
Charged amino	Asp^{113}(ionic)	TM3	(19)
	Val^{117}(hydrophobic)	TM3	(23)
beta-Hydroxyl	Asn^{312}	TM7	(38)
Aryloxy ether	Asn^{312}	TM7	(37)
Ligand aromatic ring	Trp^{109}	TM3	(39)
	Tyr^{308}	TM7	

pindolol, propranolol) as partial agonists. These results suggest the existence of overlapping binding sites for agonists and antagonists on the β-ARs (22).

4) Aromatic catechol ring: this aromatic ring can interact Phe^{289} and Phe^{290} in TM6 (23). Thus, these catechol ring and hydroxyl group interactions would serve to specifically orient the catechol ring of the agonist in the binding site of the receptor (24). Several findings reveal that the aryloxy end of antagonist interacts with TM6 and TM7. Suryanarayana and Kobilka proposed that Asn^{312} in TM7 forms a hydrogen bond with the aryloxy oxygen of the antagonist pharmacophore (25).

On the other hand, a β_3-AR agonist (BRL 37344) differs significantly from the other subtype agonists in terms of pharmacology and this recognized several β_3-AR compounds acting as potent β_1- and β_2-AR antagonists. The amino acids that were involved in the binding of the ligands were also identified by site-directed mutagenesis and photoaffinity labeling of the β_3-ARs. As shown in Table 1, those were Asp^{117} in TM3 (hydrogen bond with amine), Ser^{169} in TM4 (hydrogen bond with the hydroxyl group of the ethanolamine side-chain), Ser^{209} and Ser^{212} in TM5 (hydrogen bond with the hydroxyl groups of the catechol side chain) and Phe^{309} in TM5 (hydrophobic interaction with the aromatic ring of the catecholamine), respectively (1, 26, 27). The interactive amino acids of β-AR subtypes with the functional groups of the ligands are shown in Fig. 2.

Chimeric studies

The TM4 was largely responsible for determining β_1- vs β_2-AR properties with respect to agonist binding by using chimeric β_1/β_2-AR gene expression. TM6 and TM7 play an important role in determining binding of β_1- and β_2-selective antagonists (28). Isogaya et al. also suggested that the major amino acid of β_2-AR interacting with the β_2-selective binding of salmeterol (β_2-AR agonist) was Tyr^{308} in TM7 and that the position of the ether oxygen in the side chain was also important for β_2-selective binding (29).

Photoaffinity label studies

The interactions between β-AR antagonists and the β_2-AR were studied with the use of photoaffinity labels (30). These authors indicated that the aryloxy end of the β_2-AR antagonist pharmacophore is highly constrained within TM6 and TM7 by using the ^{125}I-iodocyanopindololdiazarene (ICYPdz), whereas the amino terminus is much less constrained and able to assume multiple conformations. These photoaffinity labeling studies data were in agreement with the results of the mutagenesis data, which suggest the involvement of regions within TM2 and TM7 in antagonist binding to the β-AR (31, 32).

Fluorescence probe analysis

Tota and Strader showed that the antagonist binds to the β-AR in a rigid hydrophobic environment which is buried deep within the core of the protein by the method of carazolol (a high affinity β-AR antagonist) fluorescence probe analysis (33).

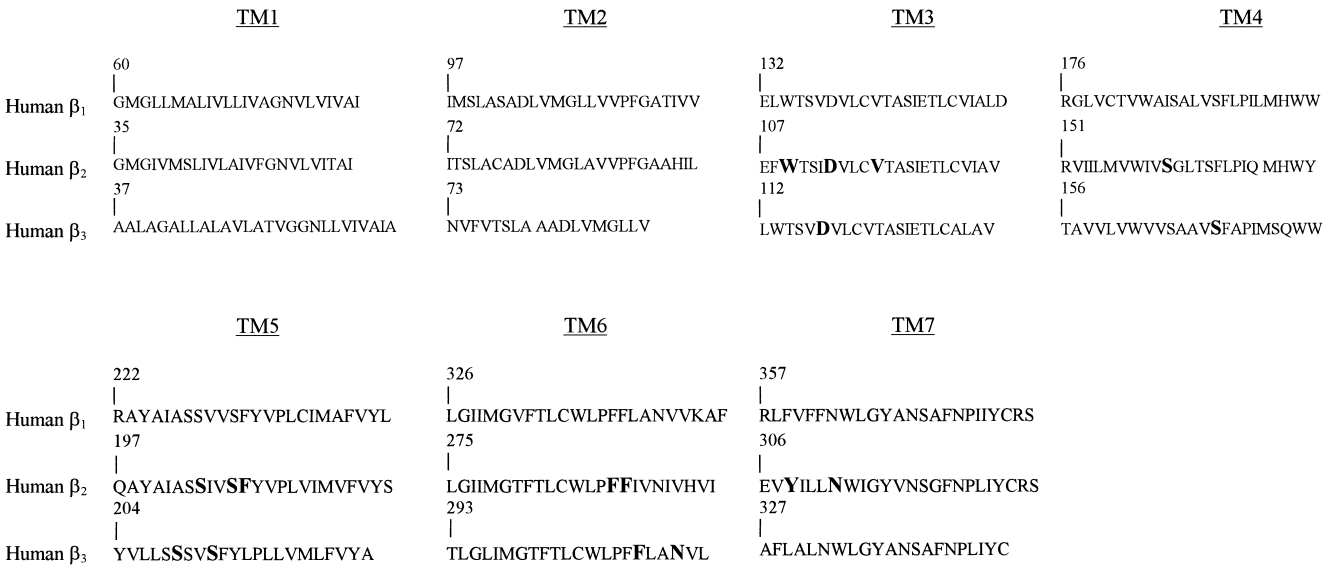


Fig. 2. Amino acid sequences in TM1 to TM7 of β-AR subtypes. Bold letters represent interactive amino acids with groups of ligands.

Analysis of binding sites in β-AR subtypes by molecular modeling

Molecular modeling technique is useful for analyzing the 3D structures of compounds at β-AR subtypes because the β-AR ligands exist in either a folded or extended conformation in the pockets of β-ARs. This molecular dynamics simulation could predict that ^{125}I-iodoazidothiophenyl-alprenolol (IABP), ^{125}I-iodoazidophenyl CGP 12177A (IAPCGP) and ICYPdz favor a folded conformation, with both ends close together (30). These authors also indicated that derivatization of TM6 and TM7 by these photolabelled compounds suggests the folded conformation of these compounds in the ligand binding pocket. On the other hand, our laboratory deduced 3D structures of human β-ARs and profiles of β-AR antagonists binding by computer simulation based on the electron density map of rhodopsin (34, 35). This modeling analysis supported the results of molecular biological-/pharmacological-experiments and further gave us some novel interesting suggestions. We assumed that the amine, benzoic acid, indole methyl, *t*-butyl, phenyl and indole functional groups of bopindolol possibly interact with Asp^{138} (TM3), Ser^{190} (TM4), Ala^{343} (TM6), Val^{137} (TM3), Pro^{339} (TM6), Cys^{336} (TM6), Leu^{237} (TM5) and Pro^{236} (TM5) of β_1-AR, respectively, by either hydrogen bonding or hydrophobic interactions (35). Thus, the analysis of interaction between ligands and receptors by this computer simulation will give us newer information of highly and more precise 3D structures of β-AR subtypes and/or different interactions in these subtypes.

Conformational changes induced by agonists and antagonists

Binding of ligand is presumed to induce a change in conformation in the receptor, which in case of the agonist is transmitted as a signal for activation of the G_S. During agonist binding to β_2-AR, certain conformational changes to the receptor occur. As for example, when catechol compounds bind with the receptor, the Ser^{204} and Ser^{207} residues of TM5 contribute to ligand binding through its catechol moiety and Ser^{165}(36)/Ser^{161}(21) residue of TM4 contributes through its beta-hydroxyl group. Meanwhile, the Tyr^{316} side chain of TM7 moves from Asp^{113} of TM3 to the Asp^{79} residue of TM2 to avoid steric hindrance with the agonist molecule and to favor hydrogen bond interaction. This conformational modification by the agonist molecule could thus initiate signal processing through other polar side chains found near helices 1 – 3 and 7 on the cytoplasmic side of the receptor (36).

On the other hand, during antagonist binding to β_2-AR, the change in receptor conformation is slightly different from that occurring in the case of agonist binding. Most β_2-AR antagonists are phenoxy propanolamine compounds. The Asp^{113} of TM3 participates in a favorable attraction with the cationic amino group and Ser^{165} of TM6 (36) or Asn^{312} of TM7 (37), with the ether group of the ligand. Due to the particular position of the antagonist ligand inside the groove, the N-part of this molecule points deeper inside the signal region and pushes the tyrosine side-chain previously bound to the Asp^{113} residue on TM3 towards the Asn^{312} residue on TM7. In such a position, a hydrogen bond between the phenol hydroxyl and the amino side-chain can be formed, thus preventing the interaction between the Tyr^{316} and Asp^{79} residue of TM2.

Transduction from binding sites with β-ARs to G protein

The interaction between receptors and G proteins presumably occurs at the inner surface of the plasma membrane of the cell where the G proteins are located. For this reason it was predicted that the internal loops of the receptors should be involved in the activation of G protein (38). Strader et al. proposed that the agonist-specific hydrogen bonding interactions are localized in the TM5 of β-ARs that suggests a mechanism for agonist activation of the receptor. The interaction of the receptor with G protein has been postulated to involve residues within TM3 of the receptor, which connects the TM5 and TM6. Specifically, the interaction of the β-AR with Gs requires a stretch of residues at the N-terminal portion of this loop, predicted to form a cytoplasmically exposed amphipathic α-helix located at the bottom of the TM5 (39).

Perspectives of GPCR including β-AR study

However even though GPCRs, especially adrenoceptors, have been investigated from many viewpoints, they are still attractive targets of study with respect to their 3D structures and relationships between their structures and functions. Structural biology will be a very useful approach to describe physiological events from the molecular point of view following the genome project. As a matter of fact, the number of articles about structure-function relationships have multiplied in these recent years. Very recently, the crystal structure of rhodopsin was determined (40). The members of GPCR are believed to share the same arrangement of the membrane-embedded parts. Therefore, the determination of the rhodopsin structure should be a major breakthrough. However, the loops connecting the helices, especially the intracellular loops, and N- and C-terminals have low homologies with each GPCR and their lengths are varied. The variation in the regions may reflect the specificities of each GPCR. In the near future, the structures of other GPCRs will also be determined, which will give us useful and logical information to describe the relationships of structure-specificity and conformational change-function and the mode of ligand binding. It will advance the developments of therapeutic drugs.

On the other hand, orphan GPCRs have been and also will be found following the genome project. The physiological functions and endogenous ligands of orphan GPCRs are also interesting from pharmacological and therapeutic points of view. Recently, a novel mechanism that modulates GPCR functions has been reported. Oligomerization between GPCRs could change their affinities to ligands and efficacies. In addition, GPCR and other types of hormonal receptors also could form a heterodimer and change their functions (41). This modulation might reflect pharmacological diversities of GPCRs. Ophan GPCRs and oligomer of GPCRs point to additional targets for the development of therapeutic drugs.

Determination of rhodopsin structure will greatly advance structural biological analyses of GPCRs. Cooperation between molecular biology and structure biology encourages making dynamic structure-function relationships of GPCRs clear. The relationships would describe the mechanisms of GPCRs as a switch to relay the signal to G proteins and various states of GPCRs binding full, partial and inverse agonists. Furthermore, it would contribute to the discovery of the endogenous ligands, help identify the function of orphan receptors, and elucidate, the modulation of functions by oligomerization.

We now face a new situation of GPCR studies and the study of GPCRs will be more exciting.

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