Ligand-Binding Modes in Cationic Biogenic Amine Receptors

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The binding site in G protein-coupled cationic biogenic amine receptors is formed in the cleft of the seven transmembrane segments. Upon binding the ligand, the receptors are activated or inactivated through the conformational changes of the transmembrane segments. G protein-coupled receptors bind four functionally distinct ligands; inverse agonists, antagonists, partial agonists, and full agonists. Hence, putative structural models for biogenic amine receptors corresponding to the ligand function (inverse agonist-, antagonist-, partial agonist-, and full agonist-bound receptor models) were built by using photointermediate models in the rhodopsin photocascade (M. Ishiguro et al. ChemBioChem. ***2004**, 5, 298–310). The ligand–receptor recognition of each was examined by modeling receptor–ligand complexes with functional ligands. The complex models suggested that each functional ligand binds the corresponding receptor structure and that ligand-specific interactions contribute to stabilization of the corresponding receptor structure.*

Introduction

G protein-coupled receptors (GPCRs), which are heptahelical transmembrane-integrated proteins, transduce a large number of signals across the cell membrane by binding signaling molecules, such as ions, odorants, biogenic amines, lipids, peptides, and proteins on the extracellular side of the membrane. Heterotrimeric GTP-binding protein (G protein) can then initiate a wide variety of intracellular biochemical events through interactions with the activated GPCR. Recent X-ray crystallographic analysis of rhodopsin, a typical GPCR, revealed the topology of the seven transmembrane helices, and the three-dimensional structures helped to elucidate the roles of highly conserved GPCR residues.[1–3]

GPCRs, members the rhodopsin family, share seven hydrophobic transmembrane regions and they share a few highly conserved residues in each α-helical transmembrane segment (TM). Mutational analysis of several GPCRs has indicated that the extracellular region of the transmembrane helices forms the ligand-binding pocket[4] for cationic biogenic amine ligands, acetylcholine, adrenaline, dopamine, histamine, and serotonin, while the intracellular loops mediate receptor–G protein coupling. Mutations in several transmembrane segments give rise to constitutively active receptor mutants,[5,6] whereas other mutations produce mutants that bind agonists, but fail to activate G proteins.[7,8] Some mutations affect agonist binding but not antagonist binding,[9] and vice versa.[10] These mutational analyses and the observation of the rigid-body motion of the TMs[11,12] in the photoactivation process of rhodopsin suggest the presence of multiple structures in inactive and active GPCRs. A two-state model, consisting of both inactive and active states, has been discussed.[13,14] Recent analysis of the structural changes in the fluorescence-labeled adrenergic receptor upon ligand binding suggested that the partial-agonist-bound receptor structure is distinct from that of the full-agonist-bound receptor.[15] The recent report on κ-opioid-receptor ligands suggested that full agonist binding involves the rigid-body rotation of TM6.[16] Despite the progress in understanding pharmacological events, the structural basis for controlling the potency and selectivity of ligands and the efficacy of signal transduction at the atomic level remained unclear due to a lack of information on the three-dimensional structure of the receptors, with the exception of rhodopsin.[1–3]

Rhodopsin, a typical GPCR, changes from the inactive form to the active form, metarhodopsin II, through the photochemical isomerization of the retinylidene chromophore. A motion of TM3 at an early stage of the structural change[17] and the subsequent rigid-body rotation of TM6[11] give rise to the fully activated form. The roles of the highly conserved residues in the structural changes of the helical arrangement have been investigated by modeling the photoactivated intermediate structures in the rhodopsin photocascade (Scheme 1).[18] Metarhodopsin I does not bind the G protein transducin and thus is totally inactive, whereas the subsequent intermediate, metarhodopsin Ib binds but does not activate transducin.[19,20] The motion of TM3 provoked by the photoisomerization of the retinylidene chromophore and the concomitant motion of TM4 in the formation of lumirhodopsin and metarhodopsin I appeared to be insufficient for the intracellular loop 2 (IL2) to bind transducin, whereas the motion of TM3 and TM4 in the subsequent formation of metarhodopsin Ib is sufficient for G protein binding.[18–20] Opsin, on the other hand, only weakly activates transducin in the absence of the prosthetic retinylidene chromophore under physiological conditions;[22] this indicates that it may be analogous to the protein moiety of metarhodopsin Ib.[18] The protein structure of metarhodopsin I_{380},[23] a photointermediate in the pathway to metarhodopsin II, appeared

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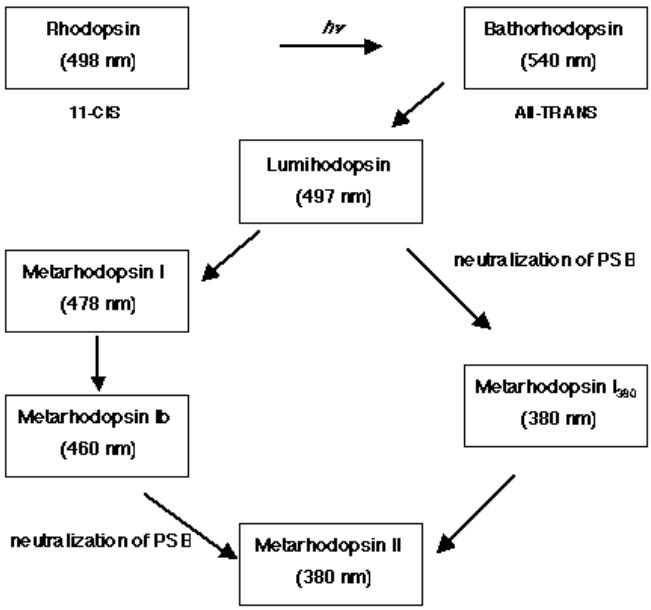


Scheme 1. *The photocascade of rhodopsin photointermediates. Rhodopsin binds 11-*cis *retinylidene chromophore and isomerizes to the all-*trans *chromophore bathorhodopsin. Neutralization of the Schiff base (PSB) occurs at the lumirhodopsin-to-metarhodopsin I_{380} transition under physiological conditions or at metarhodopsin Ib-to-metarhodopsin II transition at lower temperatures. The UV absorption maximum for each of the photointermediates is indicated in parenthesis.*

to be analogous to a constitutively active Glu113(3.28)Gln rhodopsin mutant under physiological conditions.[6, 18] The mutant is constitutively more active than opsin, yet still only partially active, it exhibits full activity upon binding exogenous all-*trans* retinal.[6] Thus, the mutant is expected to have a structure analogous to a partial agonist-bound receptor, and the formation of the fully active form is thought to necessitate rotational motion of TM6.The aim of this investigation is to elucidate the structural relationship between the function of ligands and the multiple receptor structures involved in ligand–receptor recognition by modeling ligand–receptor complex structures. Herein, four distinct structures of each of the five cationic biogenic amine receptors—acetylcholine (M_2), histamine (H_2), serotonin ($5HT_{2A}$), dopamine (D_2), and adrenaline (β_2)—were built by using structural models of the photointermediates—metarhodopsins I, Ib (opsin), I_{380} (the rhodopsin mutant), and II[18]—as templates for putative inverse agonist-, antagonist-, partial agonist-, and full agonist-bound structures, respectively. Docking studies of ligands at the binding cleft of the receptor models suggested that each functionally distinct ligand binds a different receptor structure, which corresponds to each photointermediate. In particular, the results suggested that partial agonists bind a receptor structure that differs from that bound by the full agonist-bound receptor structure.

Results and Discussion

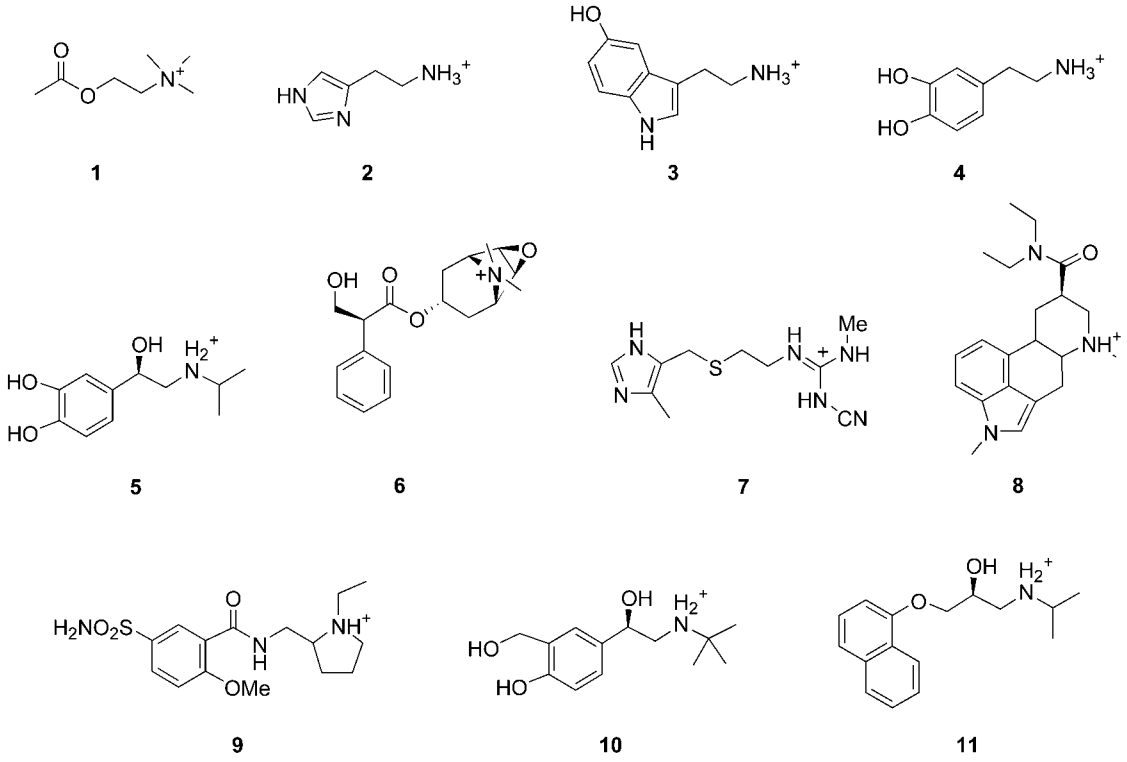
Two numbering schemes for amino acid residues in TMs were used. Amino acid residues in the biogenic amine receptors were identified by their sequence numbers. In addition, the generic numbering scheme of amino acid residues in putative TMs proposed by Ballesteros and Weinstein[24] was used for comparison among rhodopsin and the five biogenic amine receptors. According to this nomenclature, residues in putative TMs were assigned two numbers ($N_1.N_2$). N_1 refers to the TM number and N_2 to the residue number in each TM, with 50 assigned to the most conserved residue in each TM and numbers decreasing toward the N terminus and increasing toward the C terminus. The following are the most conserved residues in each TM of rhodopsin: Asn55(1.50) in TM1, Asp89(2.50) in TM2, Arg135(3.50) in TM3, Trp161(4.50) in TM4, Pro215(5.50) in TM5, Pro267(6.50) in TM6, and Pro303(7.50) in TM7.

The ligand-binding cleft consisted of the conserved structural motifs including the transmembrane segments and extracellular loop 2 (EL2). Since the deletion and insertion sites were located at the junctions between the transmembrane segments and the loops, these sites were not involved in the ligand-binding cleft.

Receptor–ligand complex models for muscarinic acetylcholine

Acetylcholine (**1**) was docked into the ligand-binding cleft of the fully activated form of the M_2 receptor models constructed from the metarhodopsin II model. Acetylcholine favored the gauche conformation at the Cβ–O bond (70°) in the binding cleft of the model structure. The quaternary cationic group remained at Asp103(3.32) in TM3 within 3.8 Å of the salt bridge, while the carbonyl oxygen of the acetyl group and the ester oxygen formed hydrogen bonds to Tyr403(6.51) in TM6 and Ser107(3.36) in TM3, respectively (Figure 1). In addition, Thr190(5.42) in TM5 was hydrogen bonded to the acetyl group. The rigid-body rotation of TM6[11, 18] enabled Tyr403(6.51) to form a network of hydrogen bonds between the full agonist and Thr190(5.42), whereas Tyr403(6.51) in the other three forms of the receptor models—partially active, physiologically inactive, and fully inactive—was distant from Thr190(5.42), and thus hydrogen bonds with acetylcholine were not formed (not shown). The hydrogen-bond network in the complex model appears to be particularly important in the stabilization of the rotated conformation of TM6. These findings were consistent with previous reports that Thr190(5.42) and Tyr403(6.51) play critical roles in agonist binding,[9, 10, 25] and that Asp103(3.32) binds the cationic moiety of acetylcholine.[26]

The complex model of *N*-methylscopolamine (**6**), an M_2 receptor antagonist, in the binding cleft of the physiologically inactive form of the M_2 receptor models constructed from the metarhodopsin Ib model suggested that the ester group of the antagonist forms hydrogen bonds to Ser107(3.36) and Asn404(6.52). The complex model further suggested that the hydroxymethyl and epoxy oxygen atoms are proximal to Asn108(3.37) and to Asn428(7.45), respectively (Figure 2). On the other hand, Tyr403(6.51) was not involved in antagonist binding in the physiologically inactive form of the M_2 receptor model. Coincidentally, the M_2 antagonists interact with Asp103(3.32) and Asn404(6.52) of the M_2 receptor, whereas, according to experiment, Tyr403(6.51) does not appear to con-



tribute to antagonist binding.[10,25,27] The phenyl group of the antagonist was accommodated in the hydrophobic pocket formed by Val111(3.40), Phe195(5.47), and Trp400(6.48) in the complex model. The interactions between the antagonist and the residues Trp400(6.48) and Asn404(6.52) would contribute to the stabilization of the physiologically inactive (antagonist-bound) form of the M_2 receptor. Although the this M_2 model preferentially bound the M_2 antagonist, the ligand-binding cleft of the fully inactive model (inverse agonist-bound form) of the receptor was too small to favorably accommodate *N*-methylscopolamine, since the space of the ligand-binding cleft in the fully inactive model of the receptor corresponding to the metarhodopsin I model is smaller than in the antagonist-bound form.[18]

Figure 3 illustrates the superimposed transmembrane helical regions of the fully inactive and physiologically inactive M_2 receptor models and the transmembrane helical region of the fully active M_2 receptor model. The size of the ligand-binding space of the fully inactive receptor model is about 50 Å^3 smaller than that of the physiologically inactive receptor model, although the binding cleft is lined by the same residues in TM3 through TM7. On the other hand, the ligand-binding surface of

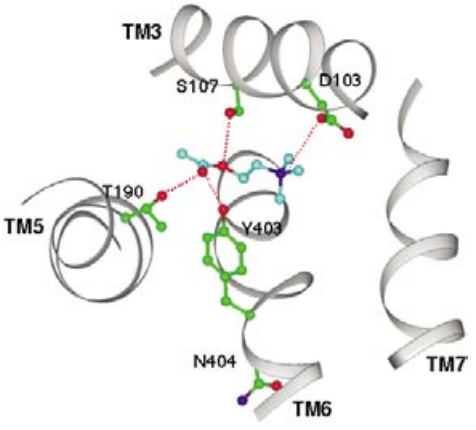


Figure 1. *Complex model of acetylcholine at the binding cleft of the fully active form of the M_2 receptor models. All of the figures of complex models are viewed from the extracellular site. Specific amino acid residues are indicated by their one letter codes with sequence numbers. Transmembrane helical regions (TM) at the binding clefts are shown with gray ribbon. Hydrogen bonds are indicated with red dotted lines. Oxygen and nitrogen atoms are red and dark blue, respectively. Carbon atoms are green or light blue to indicate protein residues and ligands, respectively.*

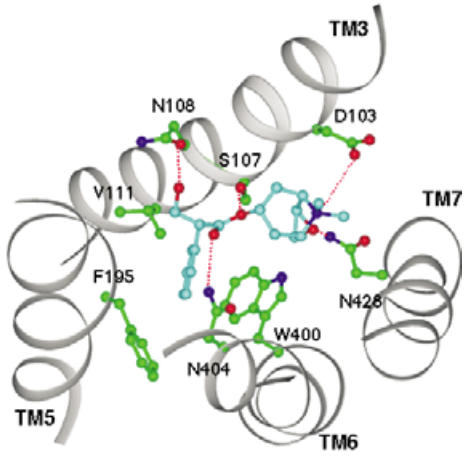


Figure 2. *Complex model of* N*-methylscopolamine at the binding cleft of the physiologically inactive form of the M_2 receptor models.*

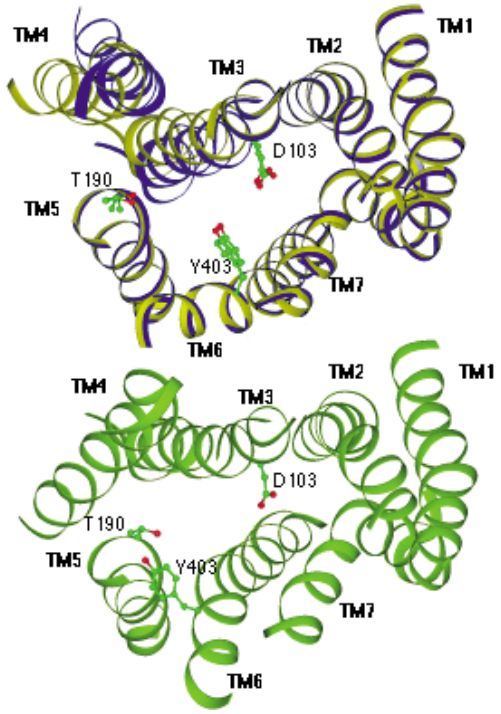


Figure 3. *Superimposed transmembrane regions of the fully inactive (blue ribbon) and physiologically inactive (yellow ribbon) forms of the M_2 receptor models (top) and the transmembrane regions of the fully active form of the M_2 receptor models (green ribbon) (bottom). Only the three residues (D103, T190, and Y404) that constitute the ligand-binding cleft are shown.*

the fully active receptor model is quite different from those of the inactive forms, mainly due to the altered conformation of TM6 caused by the rigid-body rotation.[11,18]

Receptor-ligand complex models of histamine receptor (H_2)

Tyr250(6.51) of the histamine H_2 receptor, analogous to Tyr403(6.51) of the M_2 receptor, appears to be involved in the stabilization of the active form of the receptor models constructed from the metarhodopsin II model, since the residue was proximal to Asp186(5.42) in the fully active form of the H_2 receptor models. Unlike in the acetylcholine-receptor-complex model, Tyr250(6.51) did not directly interact with the imidazole group of histamine (**2**), but a hydrogen bond between the imidazole group and Asp186(5.42) yielded the proximity of Asp186(5.42) to Tyr250(6.51) in the complex model (Figure 4). Thus, it is conceivable that histamine showed only partial activity in an Asp186(5.42)Ala mutant,[28] since Ala186(5.42) would not contribute to the stabilization of the rotated conformation of TM6 in the fully active form of the receptor models. The cationic amine maintained a salt bridge with Asp98(3.32) of the complex model. Although the Thr190(5.46) residue, where hydrophilic residues are well conserved among the cationic biogenic amine receptors, was distant from the imidazole group, this residue was hydrogen bonded to Asp186(5.42) in the complex model. In the histamine H_1 receptor model, Nε_2 of the imidazole group formed a hydrogen bond with Asn198(5.46) at a

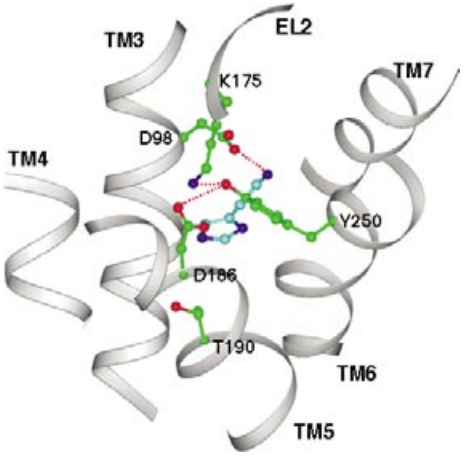


Figure 4. *Complex model of histamine at the binding cleft of the fully active form of the H_2 receptor models.*

position analogous to the Thr190(5.46) residue of the H_2 receptor (not shown). Coincidentally, Asn198(5.46) of the H_1 receptor plays a critical role in agonist binding,[29] whereas Thr190(5.46) of the H_2 receptor is not directly involved in agonist binding.[28]

Cimetidine (**7**), an H_2 receptor antagonist, bound in the binding cleft of the physiologically inactive form of the H_2 receptor models derived from the metarhodopsin Ib model. The imidazole group of cimetidine was proximal to Asp186(5.42) in accord with the mutational experiments on Asp186(5.42),[28] and the methyl group on the imidazole group was accommodated in a hydrophobic pocket lined with Val99(3.33) and Leu149(4.56). On the other side of the ligand, the sterically less hindered CH_3NH group of the guanidino moiety preferentially formed a hydrogen bond with the conserved Asp98(3.32) (Figure 5). The *N*-cyano-*N*′-methylguanidino moiety was sandwiched by Tyr250(6.51) and Trp247(6.48) in the complex model. This sandwich structure might contribute to the stabilization of the physiologically inactive form of the H_2 receptor models. On the other hand, cimetidine was not well docked in the fully active receptor model due to its bulkier guanidino moiety and the longer chain connecting the imidazole and guanidino groups (data not shown).

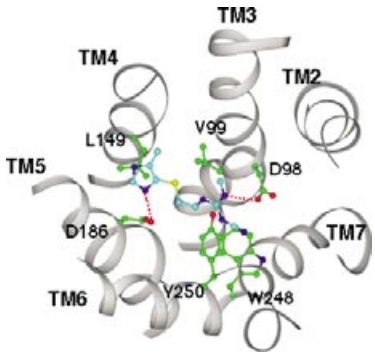


Figure 5. *Complex model of cimetidine at the binding cleft of the physiologically inactive form of the H_2 receptor models.*

Receptor–ligand complex models of the 5-hydroxytryptamine receptor (5-HT_{2A})

5-Hydroxytryptamine (5-HT, serotonin, **3**) was accepted into the binding cleft of a fully active form of the 5-HT_{2A} receptor models constructed from the metarhodopsin II model. The cationic amine moiety was doubly hydrogen bonded to Asp155(3.32) and Ser159(3.36). This was consistent with the previous report indicating that these two residues are involved in binding 5-HT.[30,31] On the other hand, the 5-hydroxyl group did not hydrogen bond to any particular residues, but lay near Gly238(5.42) in the complex model. Incidentally, Ser198(5.42) in 5-HT_{1A}, at a position analogous to Gly238(5.42) in 5-HT_{2A} binds 5-HT,[32] and the space surrounding Ser198(5.42) are well conserved in 5-HT_{2A}. Thus, we assumed that the hydroxyl group of Ser198(5.42) in 5-HT_{1A} is substituted by a water molecule in 5-HT_{2A}. Then, the water molecule was placed at the position of the hydroxyl oxygen atom of the Ser198(5.42) residue. The optimized position of the water molecule in the 5-HT_{2A} receptor model suggested that the water molecule mediates a hydrogen-bond network between the adjacent Ser239(5.43) residue and 5-HT (Figure 6), although 5-HT would not directly interact with Ser239(5.43). This hydrogen-bond network would account for the reduction in the binding affinity with 5-HT by the mutation of Ala for Ser239(5,43).[33]

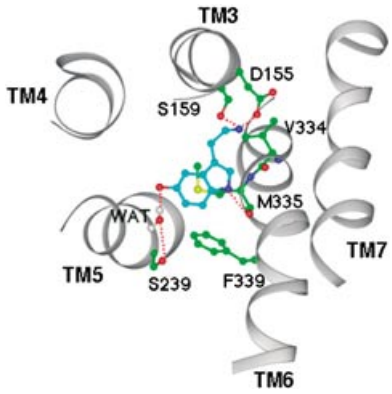


Figure 6. *Complex model of serotonin (5-HT) at the binding cleft of the fully active form of the 5HT_{2A} receptor models. Sulfur atoms are colored yellow. Water molecule is indicated by WAT.*

The N1 atom of the indole moiety was proximal to the backbone carbonyl of Met335(6.47), which was exposed to the ligand-binding cleft in the complex model. Thus, the backbone carbonyl could serve as a hydrogen-bond acceptor at the site in which the highly conserved Pro338(6.50) residue forms a kink. The interactions between 5-HT and TM6 in the fully active form of the 5-HT_{2A} receptor models appear to correspond to the interaction between the acetyl group of acetylcholine and Tyr403(6.51) in the fully active form of the M_2 receptor models and would thus contribute to the stabilization of the fully active form of the 5-HT_{2A} receptor models. Coincidentally, *N*-alkylated ligands such as *N*-isopropyl-5-methoxytryptamine show a partial agonist activity.[34] N1-alkyl substitution of the indole moiety would disturb the hydrogen bond between the backbone carbonyl and the N1 proton and would no longer stabilize the fully active form of the receptor models. Thus, the N1-alkyl substitution could provoke a different binding mode of N1-alkylated ligands.

Lysergic acid diethylamide (**8**), a partial agonist of 5-HT_{2A}, was snugly docked into the partially active form of the 5-HT_{2A} receptor models constructed from the metarhodopsin I_{380} model, which showed an ionic interaction between the tertiary amine moiety and Asp155(3.32) (Figure 7). The ligand-binding

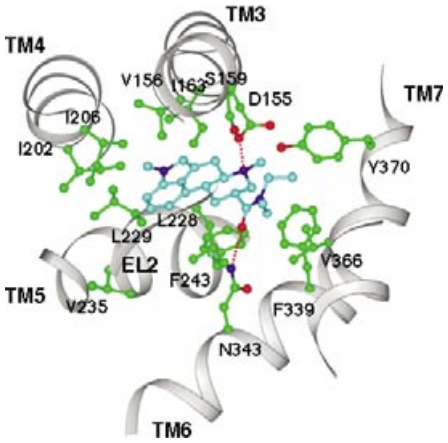


Figure 7. *Complex model of lysergic acid* N,N*-diethyl amide at the binding cleft of the partially active form of the 5HT_{2A} receptor models. The residues, Leu228, Phe339, and Val366, are not shown for clarity.*

space around Asp155(3.32) of the fully active or the physiologically inactive form of the 5-HT_{2A} receptor models was too narrow to accommodate the fairly bulky tertiary amine moiety of ligand **8**. The cationic tertiary amine of ligand **8** has only one proton at the nitrogen atom; therefore, it did not interact with Ser159(3.36). The amide carbonyl of ligand **8** was proximal to Asn343(6.55), and the *N*,*N*-diethyl moiety was accommodated in a hydrophobic pocket formed by Leu228 in EL2, Phe339(6.51), and Val366(7.39). Since the indole moiety formed an edge-to-face aromatic interaction with Phe340(6.52) in the complex model, this aromatic interaction would contribute to stabilizing the partial agonist-bound form of the receptor models.[35] The *N*-methyl group of the indole moiety was directed towards Ser242(5.46) in the complex model. The interactions between Ser242(5.46) and the *N*-alkyl groups of the indole moiety of ergoline partial agonists have been demonstrated in rat 5-HT_{2A} mutants at Ala242(5.46).[34]

Since neither the fully active nor physiologically inactive form of the 5-HT_{2A} receptor models provided enough space around Asp155(3,32) for the bulky N-substituents to bind, the partial agonist was thought to bind the partially active form.

The ligand-binding modes of dopamine receptor

The conserved Asp residue in TM3 of the dopamine receptor (D_2) is crucial to facilitate the binding of the cationic moiety in dopamine.[37] Dopamine (**4**) was docked in the fully active form

of the D_2 receptor models constructed from the metarhodopsin II model, with its aromatic ring accommodated parallel to the transmembrane helices. The *para*-hydroxyl group of the catechol moiety was located within hydrogen-bonding distance of Ser194(5.43), and the *meta*-hydroxyl group was proximal to Ser193(5.42), Ser197(5.46), and the backbone carbonyl group of Cys385(6.47) (Figure 8). These observations were in good agreement with earlier reports that the interaction between the *para*-hydroxyl group and Ser194(5.43) is essential for receptor activation.[37] The hydrogen bond formed between the *meta*-hydroxyl group and the backbone carbonyl group of Cys385(6.47) is thought to contribute to the stabilization of the fully activated form of the receptor models. This was because the backbone carbonyl group was accessible to the hydroxyl group only in the fully active form of the receptor models. Thus, it is conceivable that tyramine is not a full agonist but a partial agonist,[37] since tyramine has no *meta*-hydroxyl group to stabilize the fully active form of the receptor models through hydrogen bonding with the backbone carbonyl group.

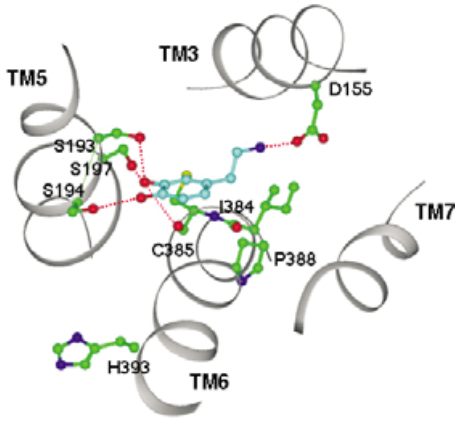


Figure 8. *Complex model of dopamine at the binding cleft of the fully active form of the D_2 receptor models. Only the backbone amides for I384 and C385 are shown.*

His393(6.55) plays an important role in benzamide-type antagonist binding; however, it is not involved in agonist binding.[38] His393(6.55) was exposed to the ligand-binding cleft of the physiologically inactive form of the receptor models constructed from the metarhodopsin Ib model, but not the fully active form; this suggests that His393(6.55) is involved in binding the antagonists, but not the agonist. Figure 9 illustrates that sulpiride (**9**), a benzamide-type antagonist, interacts with His393(6.55), Ser193(5.42), and Ser197(5.46) at the phenylsufonylamide moiety in the binding cleft of the physiologically inactive form of the D_2 receptor model.

The ligand-binding modes of the adrenergic receptor

A full agonist (*R*)-isoproterenol (**5**) formed a salt bridge between Asp113(3.32) and the cationic amine and a characteristic hydrogen bond between the β-hydroxyl group and the backbone carbonyl group of Leu284(6.46), which lies at the kink site of TM6 in the fully active form of the β_2 adrenergic receptor models constructed from the metarhodopsin II model (Figure 10). It is difficult to confirm the hydrogen bond between the β-hydroxyl group and the backbone carbonyl group of Leu284(6.46) as it is impossible to investigate this hydrogen bond by using mutational experiments. However, evaluation of its enantiomeric isomer, (*S*)-isoproterenol, revealed that it is not a full agonist but a partial agonist.[39] This might be because the opposite configuration of the β-hydroxyl group in (*S*)-isoproterenol would not properly contribute to the stabilization of the fully active form of the receptor models. Furthermore, modification of the β-hydroxyl group of the full agonists to deoxy, methyl, and methoxyl groups converts the derivatives to partial agonists.[40] This might be because these modifications would break the hydrogen bond formed with the backbone carbonyl in TM6 of the fully active form of the receptor models. This is reminiscent of the conversion of the agonists to the partial agonists of 5-HT_{2A} and D_2 receptors by eliminating the hydrogen-bond donors such as the N1 proton of 5-HT and the *meta*-hydroxyl group of dopamine.[34,37]

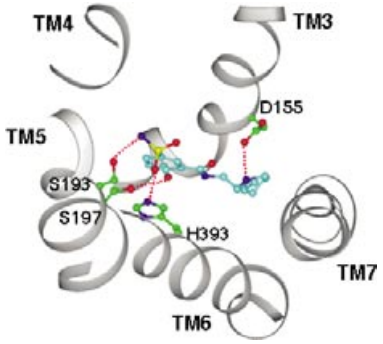


Figure 9. *Complex model of sulpiride at the binding cleft of the physiologically inactive form of the D_2 receptor models.*

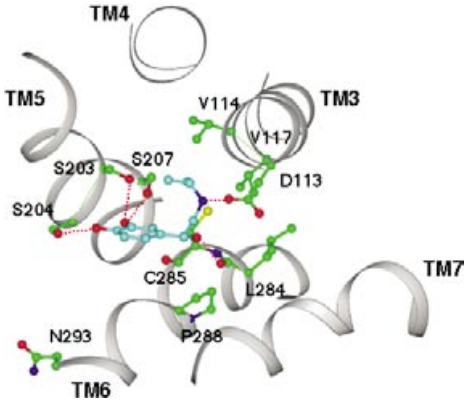


Figure 10. *Complex model of (R)-isoproterenol at the binding cleft of the fully active form of the β_2 receptor models.*

It has been suggested that Asn293(6.55) is a determinant of the configuration of the β-hydroxyl group of the agonists through site-directed mutation[40] and derivation of the β-hy-

droxyl group of the full agonists.[40] However, the substitution of Leu for Asn293(6.55) does not affect full- and partial-activation potentials towards (*R*)- and (*S*)-isoproterenol, respectively,[41] whereas modification of the β-hydroxyl group converts the full agonists to partial agonists.[40] Thus, the β-hydroxyl group is expected to be involved in the full activation of the receptor. However, Asn293(6.55) is not involved in the activation of the receptor, but only participates in the binding affinity. Therefore, it is not definite that Asn293(6.55) of the β_2 adrenergic receptor directly interacts with the β-hydroxyl group of the agonist. In addition, the Asn293(6.55)Leu mutant showed a rather small alteration (~9 times) in the affinity of (*R*)-isoproterenol (**5**) and similar alterations in the affinities of (*R*)- and (*S*)-epinephrine and (*R*)- and (*S*)-norepinephrine.[39] These experimental data suggest that Asn293(6.55) does not directly interact with the β-hydroxyl group of the full-agonist ligands, although it affects the binding affinity with (*R*)-isoproterenol. In the fully activated receptor model, Asn293(6.55) was moved far away from the β-hydroxyl group by the rigid-body motion of TM6 and was unable to directly interact with the β-hydroxyl group, while this residue was able to interact with His296(6.58). Since these two residues are located in TM6, a putative hydrogen bond could be possible in any states of the receptor structure. In the structural models of the physiologically or partially active receptor, His296(6.58) was proximal to the Glu188 residue in EL2 but not in the fully active form of the receptor models. Provided that, in the Asn293(6.55)Leu mutant, His296(6.58) do not interact with Leu293(6.55) but forms a hydrogen bond with Glu188 in the physiologically or partially active receptor, this hydrogen bond would interfere with the rigid-body rotation of TM6 in the formation of the fully active receptor structure, but not the physiologically and partially active receptor structures. Thus, the His296(6.58)Leu mutant would reduce the binding affinity of the agonists. However, to be certain of this, we shall have to wait for experiments focused on the roles of His296(6.58) in TM6 and Glu188 in EL2 in agonist and antagonist binding.

The present complex model shows a clear contrast with the β_2-adrenergic receptor–ligand complex models constructed by de novo methods, which predicted the direct interaction between Asn293(6.55) and the β-hydroxyl group of agonists.[41,42] As mentioned above, however, the role of Asn293(6.55) in agonist binding is not definite, since the mutation of Leu for Asn293(6.55) did not alter the full activation potential for isoproterenol, whereas the modifications of the β-hydroxyl group altered the activation potential of the native receptor,[40] and the mutant did not largely alter the recognition in the stereospecificity of the β-hydroxyl group of agonists other than isoproterenol.[39]

The *para*-hydroxyl group of the catechol moiety recognized Ser204(5.43), while the *meta*-hydroxyl group formed hydrogen bonds with Ser203(5.42) and Ser207(5.46) in the binding cleft of the fully active form of the β_2-receptor models. Although the *meta*- and *para*-hydroxyl groups interact with the three serine residues in TM5,[43–45] either mutation of one of the serine residues or removal of one of the hydroxyl groups of the catechol moiety results in a reduction of not only the affinity but also the efficacy of the receptor activation.[44] This indicates that, in the partially active form of the receptor, the *meta*- and *para*-hydroxyl groups interact with Ser204(5.43) and Ser207(5.46), respectively. Thus, it remains unknown which catechol hydroxyl group of the full agonists interacts with Ser204(5.43) or Ser207(5.46) of the fully activated form of the receptor. The complex model suggested that the *para*- and *meta*-hydroxyl groups of (*R*)-isoproterenol (**5**) bind at Ser204(5.43) and Ser207(5.46), respectively, in a fashion similar to that of the D_2 receptor, in which the *para*-hydroxyl group interacts with Ser194(5.43) at a position analogous to Ser204(5.43) of the β_2 receptor.

The binding of the bulky *tert*-butyl group of salbutamol (**10**), a typical partial agonist, at the conserved Asp113(3.32) residue necessitated a wide space around Asp113(3.32) in the partially active form of the receptor models constructed from the meta-rhodopsin I_{380} model. The *tert*-butyl group was bound to the hydrophobic pocket formed by Val114(3.33), Val117(3.36), Phe208(5.47), and Trp286(6.48). The hydrophobic interactions and the salt bridge between the cationic amine and Asp113(3.32) oriented the β-hydroxyl group of salbutamol toward Asn293(6.55). Thus, the *para*-hydroxyl and *meta*-hydroxymethyl groups were directed toward Ser203(5.42) and Ser204(5.43) in the complex model, respectively (Figure 11).

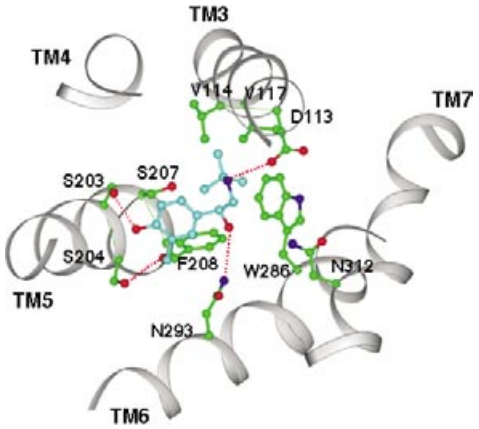


Figure 11. *Complex model of salbutamol at the binding cleft of the partially active form of the β_2 receptor models.*

These findings are in good agreement with the previous finding that Ser204(5.43) but not Ser207(5.46) is involved in ligand recognition.[46] Salbutamol has a structure modified from that of the full agonist ((*R*)-isoproterenol) in that the *tert*-butyl and hydroxymethyl groups have been replaced with isopropyl and *meta*-hydroxyl groups, respectively. However, these findings suggested that minor structural modifications at the *N*-alkyl and the catechol hydroxyl groups could invoke significant differences in the mode of binding.

The binding of the catechol moiety to the serine residues in TM5 resulted in the β-hydroxyl group of the full agonist **5** interacting with the backbone carbonyl of TM6 in the fully active form of the β_2 receptor models and vice versa. Although propranolol (**11**), an inverse agonist, has the same *N*-isopropyl

ethanolamine moiety, with the same configuration at the β-carbon, as (*R*)-isoproterenol (**5**), the bulky hydrophobic naphthoxymethyl group appears to drive the β-hydroxyl group to form a hydrogen bond with Asn312(7.39) in the cleft of the fully inactive form of the receptor models constructed from the metarhodopsin I model (Figure 12). The isopropyl amino

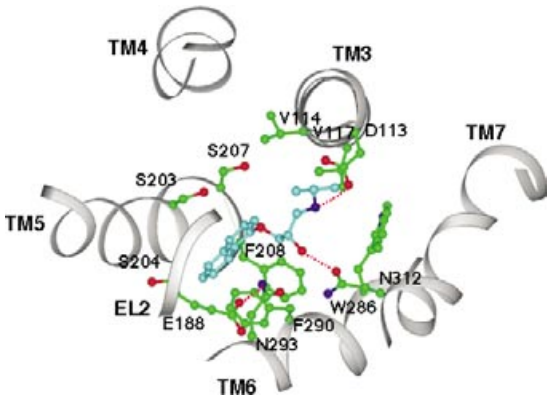


Figure 12. *Complex model of propranolol at the binding cleft of the fully inactive form of the β_2 receptor models.*

group interacted with the residues in TM3 in a fashion similar to the isopropyl amino and *tert*-butyl amino groups of (*R*)-isoproterenol and salbutamol (**10**), but the β-hydroxyl group of propranolol (**11**) interacted with neither the backbone carbonyl nor the Asn293(6.55) residue. Although an Asn312(7.39)Ala (or Phe) mutant largely reduced an affinity with propranolol, the role of this residue in ligand binding is still unclear.[47] Asn386(7.39) in 5-HT_{1A}, a receptor subtype-specific residue among the 5-HT receptor family, would be located at a position analogous to Asn312(7.39), contributing to the recognition of the β-hydroxyl group of the aryloxypropanolamino β-adrenergic receptor antagonists.[48] Since both the Asn residues at positions analogous to Asn293(6.55) and Asn312(7.39) of the β_2 receptor are conserved in the $5HT_{1A}$ receptor, the binding mode of the aryloxypropanolamine antagonists is expected to be common among the β_2 and $5HT_{1A}$ receptors. The hydrophobic naphthalene moiety was housed in a pocket lined with residues, Val114(3.33), Val117(3.36), Tyr199(5.38), Phe208(5.47), and Phe290(6.52) in the complex model. These findings are consistent with the finding that hydrophilic residues in TM5 such as Ser203(5.42), 204(5.43), and 207(5.46) are not necessary for inverse agonist binding.[39]

Active and inactive forms of four distinct receptor structures

The biogenic amine receptor–ligand-complex models suggest that the ligands select the receptor structure according to their function (inverse agonist, antagonist, partial agonist, or full agonist). The partial agonists, in particular, are thought to bind a receptor structure that differs from the full agonist-bound receptor structure. This is in accordance with the recent finding that the partial-agonist-bound structure of the β-adrenergic receptor is distinct from the full-agonist-bound structure.[15]

The rigid-body motion of TM6 is thought to provoke a considerable change at the ligand-binding surface of this TM. Thus, the agonists bearing hydrogen-bond donating groups would interact with the backbone carbonyl groups in TM6, whereas those bearing hydrogen-bond accepting groups would interact with residues bearing hydrogen-bond donating groups in TM6. The agonist-specific interactions between TM6 and agonists would stabilize the full-agonist-bound structures. Namely, the acetyl group of acetylcholine would form a hydrogen bond with Tyr403(6.51) of the M_2 receptor (Figure 1). Tyr250(6.51) would form a hydrogen-bond with Asp186(5.42) of the H_2 receptor, which would interact with the imidazole group of histamine (Figure 4). The agonist-bound structural models of the 5-HT_{2A}, D_2, and β_2 receptors suggested that the backbone carbonyl groups in TM6 form hydrogen bonds with the N1 proton of 5-HT (Figure 6), the *meta*-hydroxyl group of dopamine (Figure 8), and the β-hydroxyl group of isoproterenol (Figure 10), respectively.

The inverse-agonist-, antagonist- or partial-agonist-bound models of the M_2, 5-HT_{2A}, and D_2 receptors also suggested that the interactions between the residues in TM6 and the ligands contribute to the stabilization of the ligand-bound structures. The ligand-binding space in the structural model for the antagonist-bound receptor, which would correspond to that of the rhodopsin photointermediate metarhodopsin Ib, increases by about 50 $Å^3$ from the model structure of the inverse-agonist-bound receptor, which would correspond to that of metarhodopsin I. On the other hand, the ligand-binding space of the structural model for the partial-agonist-bound receptor, which would correspond to that of metarhodopsin I_{380}, is similar to that of the inverse-agonist-bound receptor, although the ligand-binding space around the conserved Asp(3.32) residue for the partial-agonist-bound receptor was larger than that for the inverse-agonist-bound receptor. Thus, the difference of the ligand-binding space in the three forms of the receptor models would contribute to the ligand recognition. Figure 13 illustrates the superimposed binding clefts of the fully inactive, physiologically inactive, and partially active M_2 receptor

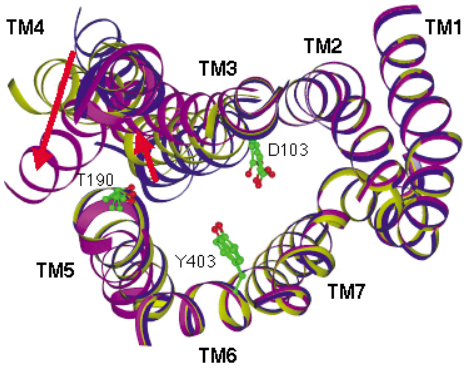
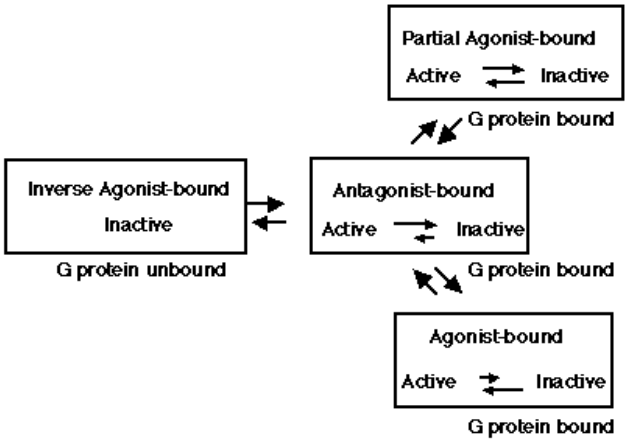


Figure 13. *Superimposed transmembrane regions of the fully inactive (blue ribbon), physiologically inactive (yellow ribbon) and partially active forms of the M_2 receptor models (magenta ribbon). Arrows indicate the direction of the motion of the intracellular site of TM3 and 4.*

models. The superimposed structures indicate that the residues in TM3, 5, and 6 exposed to the binding cleft are almost same, but that the size of the motion of TM3 and 4 of the partially active form of the receptor models is the largest of the three receptor models.

Not only the fully activated form of rhodopsin (metarhodopsin II), but also metarhodopsin Ib (opsin-like) and the Glu113(3.32)Gln rhodopsin mutant (metarhodopsin I_{380}-like) bind transducin. Contrastingly, metarhodopsin I does not bind this G protein. In metarhodopsin II, an ionized form (metarhodopsin IIa, inactive) of Glu134(3.49) of the ERY triplet at the intracellular site of TM3 is in equilibrium with the protonated form (metarhodopsin IIb, active) at the cytoplasmic site.[49] Our previous report on the metarhodopsin II model suggested that the outward swing of the C-terminal end of TM3 transfers the Glu134(3.49) residue from a polar to an apolar environment and enables the protonation of Glu134(3.49); this leads to a conformational change in Arg135(3.50), which facilitates the GDP–GTP exchange in G proteins (G-protein activation).[18] Provided that the outward motion of TM3 determines the equilibrium rate, the larger range of motion of TM3 affords a higher ratio of the protonated form of Glu134(3.49) to the deprotonated form. Namely, the fully activated form (metarhodopsin II-like) of the GPCR is thought to predominate in the protonated state of the Asp residue of the D(E)RY triplet, whereas the ionized form of the Asp residue is thought to predominate in a physiologically inactive (metarhodopsin Ib-like) structure. In the case of a highly but not fully active structure (partially active form, metarhodopsin I_{380}-like), the protonated form would be an intermediate in the equilibrium reaction. On the other hand, the fully inactive form (metarhodopsin I-like) would not exhibit an equilibrium reaction, as it would not bind G protein (Scheme 2). Thus, this scheme includes four distinct arrangements of the transmembrane segments, each of which consist of two states; the ionized and protonated forms of the Asp residue in the DRY triplet. The inverse agonist-bound structure, however, would consist of a single (inactive) state. In this unique "multiple two-state structural model", the G protein dissociates from the antagonist-bound form to the inverse-agonist-bound form, and the partial-agonist-bound form is independent from the agonist-bound form.



Scheme 2. *Putative structural changes of GPCR upon binding each functional ligand. With the exception of the inverse agonist-bound form, all forms bind G proteins. The favorable equilibrium conformation between the active and inactive states of each receptor are indicated by the double arrows.*

Conclusion

The four distinct structures of the five cationic biogenic amine receptors are well adapted to the full-agonist-, partial-agonist-, antagonist-, and inverse-agonist-binding modes. The full agonists are thought to stabilize the fully rearranged (fully active) receptor structure through interaction with specific residues or the backbone carbonyl groups in TM6, whereas the partial agonists are thought to stabilize the incompletely rearranged (partially active) receptor structure. The antagonists are thought to stabilize the transmembrane arrangement of the unrearranged (physiologically inactive) receptor structure. Antagonists as well as partial agonists are thought to interact with the residues in TM6 different from those in TM6 of the fully active form. The inverse agonists are thought to induce a structural change to stabilize the metarhodopsin I-like (fully inactive) structure, which prevents them from binding G proteins. Thus, the ligand-binding cleft of receptors would be sterically and electronically altered according to the binding of the functionally different ligands. Considering the activated and inactivated states that correspond to the protonated or unprotonated forms of the highly conserved Asp (Glu) residue in the D(E)RY triplet at the intracellular site of TM3, the multiple two-state structure model is expected to be applicable to ligand recognition in GPCRs of the rhodopsin family.

A further examination of other ligand binding of GPCRs would elucidate more information on the mechanism of receptor–ligand recognition. Modeling of these receptors in their ligand-bound states will be the focus of future investigation.

Computational Methods

Previously constructed three-dimensional structural models of metarhodopsin I, metarhodopsin Ib (opsin), metarhodpsin I_{380} (Glu113(3.28)Gln rhodopsin mutant), and metarhodopsin II[18] were used to construct the structural models of the putative inverse agonist-, antagonist-, partial agonist-, and full agonist-bound forms of the human receptors for cationic biogenic amines. The replacement of side-chains was carried out by using a Homology module installed within Insight II (2000 version, Molecular Simulations Inc. San Diego, CA, USA) according to a sequence homology alignment similar to that reported by Trumpp-Kallmeyer et al.[50] The insertions and deletions at the extracellular site were set at the junctions of the loop and transmembrane segments. Extra portions longer than IL2 and 3 of the rhodopsin photointermediate models were deleted. The molecular-dynamics calculations for the backbone amides and side-chains were performed at 298 K by using the cell-multipole method, a distance-dependent dielectric constant, and a time step of 1 fs for 100 ps, sampling at 1 ps intervals with Discover 3 (2000 version, Molecular Simulations Inc.). The hundred conformations were minimized until the final root-mean-square deviation (rmsd) was less than 0.1 kcal mol^{-1} $Å^{-1}$. The lowest-energy conformation was selected for the ligand-docking study.

The ligands were manually docked into the ligand-binding cleft of the corresponding receptors, guided by a salt bridge between the cationic amine and the conserved Asp residue in TM3. The initial complex model was minimized and then optimized by using the molecular dynamics/minimization procedure without constraints between the ligands and the receptors. The lowest-energy structure was selected as an energy-refined complex model.

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Keywords: amines · G protein-coupled receptors · modeling · molecular recognition · partial agonist · rhodopsin

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