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Three-Dimensional Models of Neurotransmitter G-Binding Protein-Coupled Receptors

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SUMMARY

Graphics computer-generated three-dimensional models of all G-binding protein-coupled receptors were built and analyzed. These models were defined using primary sequence comparisons, secondary structure predictions, and three-dimensional homology building, taking bacteriorhodopsin as a template. The transmembrane region of the dopamine D_2, serotonin 5-hydroxytryptamine$_2$, noradrenaline α_2, adrenaline β_2, and acetylcholine m_2 receptors were chosen as prototypes and explored in detail. In this report, we localize the ligand binding site and identify all the residues likely to be responsible for receptor affinity, selectivity, stereospecificity, and efficacy. The precise nature of the important physicochemical interactions between different residue side chains or between the ligand and the adjacent amino acids is also discussed. The models are in agreement with published data obtained from mutagenesis and labeling studies and represent important working hypotheses to direct future mutagenesis studies. They also enable structure-activity relationship studies and more rational drug design.

Receptors represent one of the major focal points for the study of cell and tissue function. They play a pivotal role in the regulation of a variety of physiological processes, particularly within the central nervous, cardiovascular, and endocrine systems. The GPCR represent a very important receptor subclass, and major efforts have been dedicated to their pharmacological characterization.

The discovery of new receptor classes is generally associated with the discovery of new endogenous ligands, although exceptions exist (e.g., benzodiazepines and opioid receptors) (1). More recently, pharmacological, physiological, and structure-activity studies have shown the existence of receptor subtypes that are activated by the same neurotransmitter. For 5-HT receptors, models of the recognition sites have been proposed based on ligand conformational analysis (1, 2). These three-dimensional models highlighted similarities and differences between receptor subtypes. Definitive evidence for the existence of receptor subtypes came from the molecular cloning of numerous receptor classes and subtypes and their identification with human functional receptors (1).

Site-directed mutagenesis and the study of chimeric receptors have contributed to the understanding of the main functional characteristics of hormonal receptors (3). A number of residues and domains that are likely to be involved in the binding of agonists and antagonists, in the coupling with G proteins, and in the desensitization process have been identified. There is also a consensus concerning the existence of seven transmembrane α-helical regions in GPCR. Despite this rapid progress, the major conceptual step of moving from a two-dimensional to a three-dimensional understanding of the precise mechanisms that control the potency and selectivity of ligand binding and the efficacy of signal transduction remains. Unfortunately, the nondegenerative purification of membrane receptors and their crystallization still remain a problem. However, a refined three-dimensional structure of a membrane protein, bacteriorhodopsin, has recently been obtained by cryomicroscopy (4). Although this receptor is not coupled to a G protein, it displays a structure with seven transmembrane-spanning α-helices, which might resemble the GPCR folding. We considered that there are now enough data available to allow the generation of plausible three-dimensional models of GPCR, using molecular modeling techniques.

GPCR primary sequence comparisons. The three-dimensional models were constructed using primary sequence comparison and hydrophobicity-hydrophilicity (hydropathicity) analyses. Primary sequences of 22 GPCR were taken from published data (5). The following receptors were considered: human 5-HT_{1A}, rat 5-HT_{1C}, and rat 5-HT_2 serotonergic (6–8); human D_1, D_2, and D_3 dopaminergic (9–11); rat α_1- and human α_2-, β_1-, β_2-, and β_3-adrenergic (12–19); human m_1, m_2, m_4, and m_5 and pig m_3 muscarinic (20–24); human r, g, b, and d opsins (25, 26); bovine substance k (27); and cannabinol receptors (28). The sequence of bacteriorhodopsin was also included in the study (29).

ABBREVIATIONS: GPCR, GTP-binding protein-coupled receptor(s); G protein; GTP-binding protein; 5-HT, 5-hydroxytryptamine.

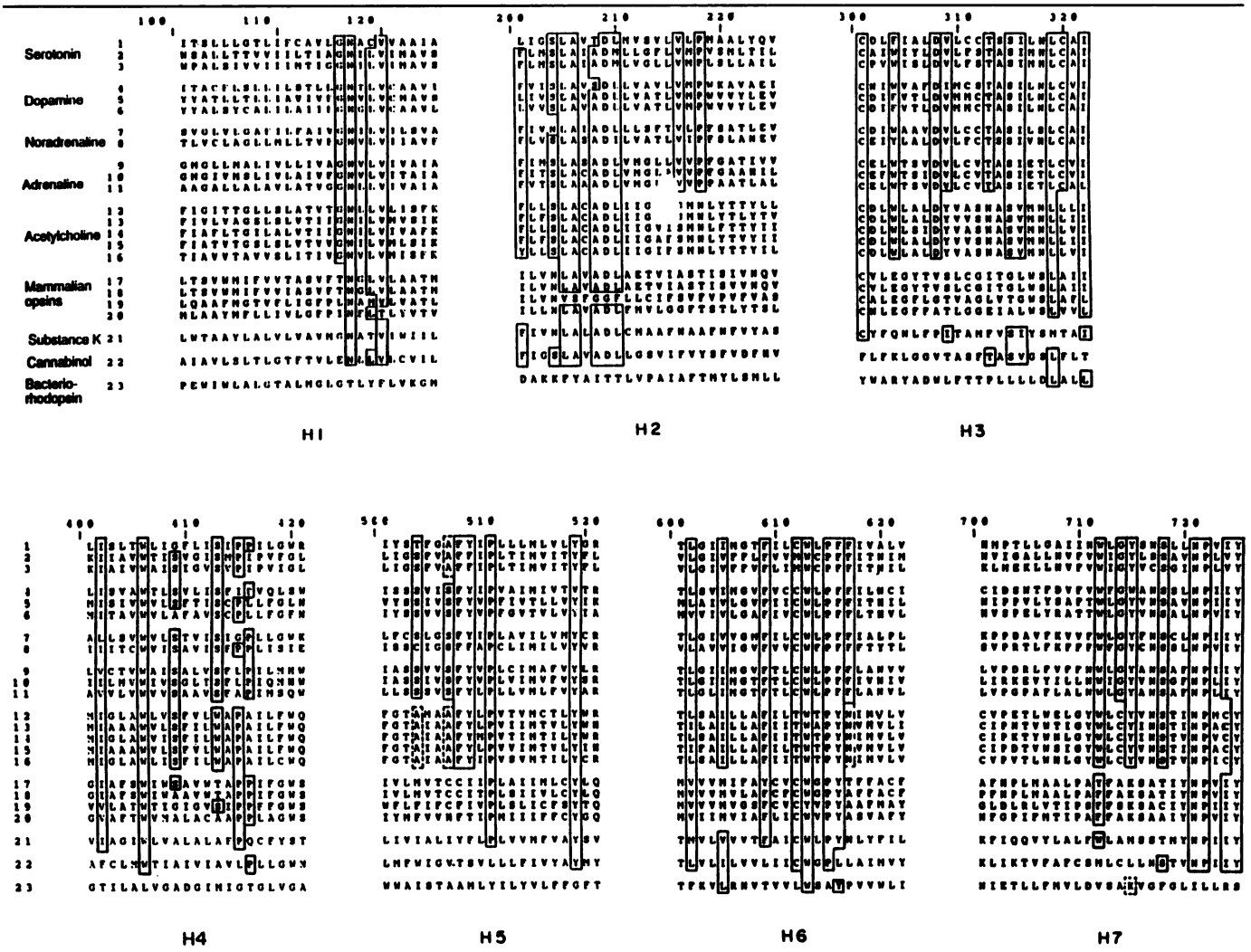
Sequence alignments were performed by the method of Needleman and Wunsch (30), as implemented in the University of Wisconsin Genetic's Computer Group software package (5), using the Dayhoff similarity table for amino acids. Manual refinement was necessary to obtain the optimal multiple alignment. Our proposed alignment of the transmembrane regions and the corresponding residue numbering are given in Table 1. There is a good degree of identity and similarity within the transmembrane regions between the 22 GPCR, suggesting that they belong to the same structural and functional class of receptors. However, the identity with bacteriorhodopsin is very weak (6–11%). Not surprisingly, the highest similarity scores were obtained within receptor subtypes, with the exception of the 5-HT_{1A} serotonin receptor. This receptor has greater identity with the dopaminergic and adrenergic receptors than with 5-HT_2 or 5-HT_{1C} receptors. It is also clear that the neurokinin receptors, the mammalian opsins, and the cannabinol receptor represent distinct subclasses of GPCR.

Hydropathicity predictions for all GPCR and bacteriorhodopsin were studied using Kyte-Doolittle (31) and Goldman-Engelman-Steitz (32) scales. Both methods indicated that the GPCR possess a structure similar to bacteriorhodopsin, with seven transmembrane helices, suggesting a conserved topology throughout the GPCR family. Nevertheless, it was not possible to localize precisely the starting and ending amino acids of the transmembrane regions from these hydropathicity profiles. In particular, transmembrane regions 3 and 7 contain a high number of hydrogen-bonding and charged residues, which induce a marked fluctuation in the hydropathicity plot. Much better evidence for the existence of the seven transmembrane regions is derived from the primary structure alignment, which clearly indicates seven highly conserved hydrophobic regions connected by hydrophilic sequences with only very low conservation. The seventh transmembrane region, which is not clearly defined from the hydropathicity analysis, displays the highest amino acid conservation within the GPCR. Additionally, when this region is drawn as a helical wheel model, all conserved and hydrophilic residues are located on the same face of the helix.

TABLE 1

Alignment of the seven selected regions putatively included in transmembrane domains (labeled H_1 to H_7)

The considered receptors are 5-HT_{1A} (*1*), 5-HT_2 (*2*), 5-HT_{1C} (*3*), D_1 (*4*), D_2 (*5*), D_3 (*6*), α_1 (*7*), α_2 (*8*), β_1 (*9*), β_2 (*10*), β_3 (*11*), m_1 (*12*), m_2 (*13*), m_3 (*14*), m_4 (*15*), m_5 (*16*), r opsin (*17*), g opsin (*18*), b opsin (*19*), d opsin (*20*), substance K receptor (*21*), cannabinol receptor (*22*), and bacteriorhodopsin (*23*), (6–29). For convenience, the residues are numbered 101, 102, etc., in helix H1; 201, 202, etc., in helix H2; etc. Invariant residues in all GPCR or in subclasses are boxed.



Thus, this region is likely to correspond to a membrane-embedded region.

From these analyses, we conclude that there exists a good primary and secondary structure homology within all GPCR. Despite the low primary sequence homology between GPCR and bacteriorhodopsin, they show the same hydropathicity plots, with seven transmembrane helices connected by hydrophilic loops. We propose that their tertiary structures are likely to be similar.

The nature and putative function of conserved residues in some receptor subclasses have already been extensively analyzed and discussed (3, 33). We have extended such a study to 22 GPCR, including the recently published D_1 and D_3 dopaminergic and cannabinol receptors.

Some residues are conserved within all the GPCR, despite major differences in the mechanisms of activation of the receptors (by aryl- or alkylamino neurotransmitters, peptides, alkaloids, or light-activated covalently bound retinal). Other amino acids are found to be conserved only in the cationic neurotransmitter receptor subclass or in receptor subtypes activated by the same ligand or are randomly distributed. The basic assumption, as made by Hulme *et al.* (33), is that amino acid residues conserved within most GPCR transmembrane regions play an essential role in the overall folding or in the function of these receptors (coupling to G protein). In contrast, residues that are conserved only in a subclass of GPCR probably play a role in the binding or in the activation processes that are specific to this subclass. The primary sequences of the 22 GPCR were analyzed on this basis, with an emphasis on the transmembrane regions.

The residues that are conserved in all the considered GPCR are putative α-helix breakers such as proline in helices H_4, H_5, H_6, and H_7; an acidic aspartate residue in H_2; aliphatic hydrophobic residues such as valine, leucine, isoleucine, or alanine in H_1, H_2, H_3, and H_4; and aromatic residues tryptophan, tyrosine, and phenylalanine in H_4, H_5, H_6, and H_7 (see Table 1). Residues that are invariant in only the cationic amine GPCR are also framed in Table 1. Finally, some residues are only conserved within a given receptor subclass. For example, serine residues in H_5 are only found within catecholamine receptors and probably play a role in the binding of the catechol moiety (34). Other residues are different in muscarinic receptors, compared with aromatic neurotransmitter GPCR, serine for valine and asparagine for proline in H_2; tyrosine-valine for valine-leucine, asparagine for threonine, and leucine for cysteine in H_3; tryptophan for serine in H_4; alanine for serine or threonine in H_5; and asparagine for phenylalanine and tyrosine for phenylalanine in H_6.

A number of these residues have been exchanged by site-directed mutagenesis, and hypotheses concerning their function have been formulated, sometimes in a controversial way (see Ref. 3 and 33 and references cited therein). Hulme *et al.* (33) have recently reviewed these data, focusing on the muscarinic receptors. Our primary sequence analysis extended to 22 GPCR is in good agreement with their conclusions concerning the residues likely to be involved in G protein coupling, ligand binding, *N*-glycosylation, palmitoylation, and phosphorylation. In particular, Asp_{308}, which is conserved only in the cationic amine receptors, is probably involved in cationic neurotransmitter binding. Asp_{209}, however, which is conserved in all GPCR, has presumably both a structural and a functional role. In addition to the single Pro_{415} and Pro_{511} in H_4 and H_5, respectively, the sequences Gly_{116}-Asn_{117} in H_1, Pro_{615}-Phe_{616} or Pro-Tyr in H_6, and Asn_{721}-Pro_{722} in H_7 might also represent molecular switches capable of inducing movements in the helices. Interestingly, whereas numerous labeling or mutagenesis studies have led to speculation on the functional role of the aspartate and serine residues in the transmembrane domains, it is more difficult to imagine a precise role for many of the other conserved residues listed above. In particular, the aromatic residues are probably important. However, with the exception of Tyr_{715} (33) and Phe_{617} (35), their role has not been assessed.

Clearly, interesting information can be obtained from experimental data and analysis of primary and secondary structures. However, a three-dimensional model of GPCR is needed to assess the current hypotheses and provide new insights.

GPCR three-dimensional models. The construction of our three-dimensional models was performed in five steps, as follows. (i) As discussed above, the analysis of the hydropathicity and sequence alignment led to selection of the seven sequences displayed in Table 1 as the probable transmembrane α-helical regions. They contain from 21 to 26 residues. (ii) The seven α-helices were constructed (38) with ϕ and ψ values of $-59°$ and $-44°$, respectively, for α-helices in a hydrophobic environment (36). Most conserved amino acids are, thus, distributed on the same face of the α-helices. (iii) The Henderson model of bacteriorhodopsin (4) was used as a template for the positioning of the α-helices main axes. (iv) The helices were oriented in such a way that the conserved residues were located at the inside of the receptor, as were the charged amino acids, in agreement with a similar distribution in bacteriorhodopsin (37). (v) Finally, the geometry of the protein was optimized by energy minimizations, using the Kollmann force field in SYBYL 5.3 (38, 39). The root mean square deviation from the starting structure as well as the ϕ and ψ angles were checked after the minimizations. The root mean square values ranged from 0.6 to 1.1 Å for the different receptors. The ϕ and ψ angles were not significantly altered, with the exception of the amino acid residues adjacent to proline residues on H4, H5, H6, and H7, leading to slightly bent α-helices. This is in full agreement with the refined structure of bacteriorhodopsin and the photosynthetic reaction center, where proline residues lead to only a minor distortion of the α-helical backbone. Three-dimensional models for all the GPCR were thus defined (e.g., Fig. 1).

The three-dimensional characteristics of the GPCR models include seven α-helices, very tightly packed, which define a narrow dihedral cleft. Charged residues are located on the inside of the structure. Most amino acids that are conserved in all GPCR can, indeed, be located around the core of the receptor. Furthermore, amino acids conserved in all or some of the cationic neurotransmitter GPCR are concentrated in the central cleft near the extracellular surface of the receptor, in the neighborhood of Asp_{308}, suggesting that this region is involved in the binding of cationic ligands. Residues that are common to all GPCR and are likely to have a structural or functional role are more randomly distributed over the seven helices. Contrary to previous reports (40), primary sequence analyses, published site-directed mutagenesis, and labeling experiments led us to hypothesize that Asp_{209}, which is present in most GPCR, is probably not directly involved in ligand binding. This is supported by our model, because this aspartate residue is

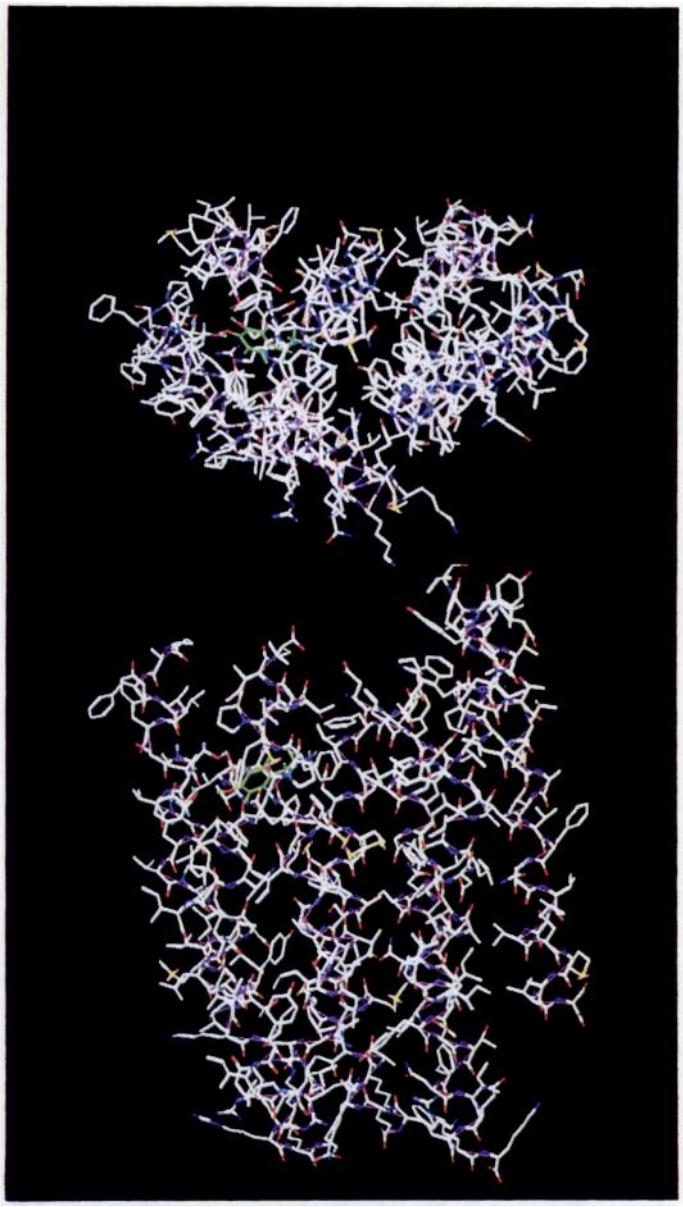


Fig. 1. Display of the complex between the neurotransmitter dopamine (*green*) and the D_2 dopamine receptor transmembrane region (colored by atom types; *white*, carbon; *red*, oxygen; *blue*, nitrogen; *yellow*, sulfur). *Top*, view of the complex from the outside of the cell, in a direction parallel to the axes of the α-helices and perpendicular to the membrane of the cell. *Bottom*, side view of the ligand-receptor complex from a direction perpendicular to the main axes of the seven α-helices. The neurotransmitter binding site is located in a narrow dihedral cleft, near the extracellular side of the transmembrane region and surrounded by α-helices 3, 4, 5, and 6. The activation sites of all GPCR and of bacteriorhodopsin are located in similar homologous parts of the corresponding proteins.

deeply buried in the receptor at about 25 Å from the beginning of the transmembrane region. In contrast, Asp_{308}, which is only conserved in cationic neurotransmitter GPCR, is located in a favorable position on H_3 at the bottom of the cleft and is surrounded by two dissymetrical hydrophobic pockets. Most of the aromatic residues of the protein (from H_4, H_5, and H_6) are concentrated in the first pocket, whereas nonaromatic hydrophobic residues of H_1 and H_2 are in the second pocket. We conclude that, in agreement with experimental data (41–44), the first region corresponds to the main agonist binding site. It is also interesting to notice the similarity of general shape and properties between this site and the recognition site maps defined by the active analogue approach (2). Our study of the receptor-ligand complexes further supports this model.

In the first step, five classical neurotransmitter receptors were selected as prototypes for the study of the differential binding modes, namely the α_2, β_2, m_2, 5-HT_2, and D_2 receptors. The natural ligands were positioned in the active site such that the cationic head of the ligand was located in front of the charged Asp_{308} residue, permitting an electrostatic interaction. The rest of the ligand was docked into the adjacent hydrophobic pocket defined by H_4, H_5, and H_6, which contains the residues conserved only among the cationic neurotransmitter receptors. In the last step, hydrogen bonds and aromatic interactions were optimized. The complete structure of the complexes was then energy minimized by molecular mechanics.

From the models, it was possible to propose the identity of all residues that are likely to be responsible for neurotransmitter binding and specificity. The models of the ligand-receptor complexes can be described as follows (Figs. 2 and 3). For the five receptor-ligand complexes, three highly conserved aromatic residues form a hydrophobic cluster around the aspartate-ammonium ion pair and thus strongly stabilize the complex. These residues are Trp_{304} (or Phe or Tyr), Trp_{613}, and Phe_{616} (or Tyr). This result could not have been postulated from primary sequence analysis. In contrast, as postulated by Hulme *et al.* (33), Tyr_{715}, which is highly conserved only in cationic amine GPCR, can indeed interact with Asp_{308}, but we found that it cannot directly interact with the ligand. The residues defining the hydrophobic area interacting with the nonbasic part of the neurotransmitters are also highly conserved and consist of Trp_{406}, Phe_{508}, and Phe or Asn_{617}.

Trp_{406} and Trp_{613} form aryl-aryl orthogonal interactions with Tyr_{519} and Phe_{609}. Trp_{712} interacts in the same manner with Tyr_{715}. These interactions can stabilize the structure by making a network of interhelical interactions. In addition, it seems possible that the interaction of the neurotransmitter with the adjacent aromatic residues can induce a conformational change that can propagate within the structure via these aromatic connections.

Other invariant residues could possibly form a number of specific interactions. Thus, the analysis of our models leads to the following proposals: Trp_{406} can make an additional interaction with Pro_{511}, Tyr_{725} forms a hydrogen bond to the Asp residue of the conserved DRY sequence in the second outside loop, Asn_{117} stabilizes Asp_{209} on helix H_2, Asn_{721} can form a hydrogen bond with the carbonyl groups of the helix backbone and thus stabilizes the α-helix H_7, and Leu_{119}-Val_{120} can interact with Leu_{205}-Ala_{206} and form a hydrophobic environment around Asp_{209}.

Thus, the three-dimensional models permitted the evaluation of the contribution of most invariant residues to binding, folding, or conformational change processes.

A number of residues that are present only in GPCR subclasses seem to play an important and specific role in the recognition process of the corresponding neurotransmitters. For example, careful analysis of the three-dimensional model for muscarinic receptors suggests that Asn_{617} plays a crucial role in these receptors by interacting specifically with the ester group of acetylcholine. Trp_{413} and Leu_{621} are also specific for this class and complete the hydrophobic environment of the

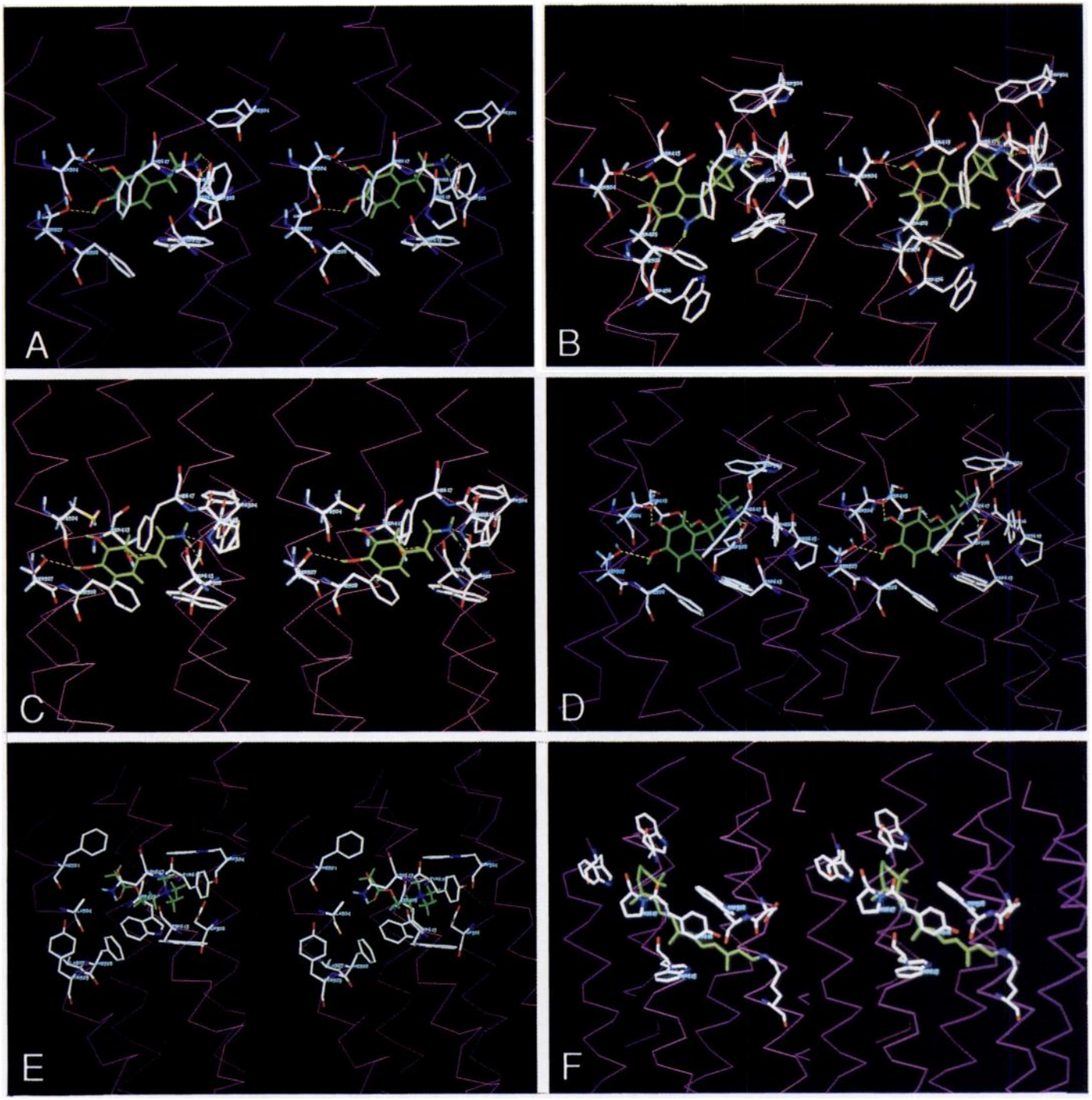


Fig. 2. Neurotransmitters (*green*, carbon; *red*, oxygen; *blue*, nitrogen) in their respective binding sites. Only residues of the binding site (*white*, carbon; *red*, oxygen; *blue*, nitrogen; *yellow*, sulfur) and the trace of the main chain (*magenta*) are displayed. Hydrogen bonds are indicated with *yellow dotted lines*. a, Dopamine D_2; b, serotonin 5-HT_2; c, noradrenaline α_2; d, adrenaline β_2; e, m_2 muscarinic; f, retinal in bacteriorhodopsin (4). The cationic neurotransmitters form ionic interactions with the conserved aspartate on helix 3 (a–e). This ion pair is surrounded by a cluster of three conserved aromatic residues (613, 616, and 304) belonging to helices 6 and 3. Two homologous aromatic residues (613 and 616) are found around retinal in the retinal-bacteriorhodopsin X-ray structure (f) (4). Catecholamine hydroxy substituents can interact with the pair of conserved Ser_{504} and Ser_{501} or Cys_{504} and Ser_{501} residues on helix 5 (a, c, and d). The single hydroxy substituent of serotonin interacts with Ser_{504} (b). The β-hydroxy group of noradrenaline or adrenaline can stereoselectively form a hydrogen bond with Ser_{413} (c, d). The indole nitrogen atom of serotonin can interact with Ser_{409} (b). A conserved aromatic residue, Phe_{617}, can interact with the aromatic nucleus of the aromatic neurotransmitter (a, b, c, and d). In the case of acetylcholine receptors, Phe_{617} is replaced by Asn_{617}, which can form hydrogen bonds with the neurotransmitter ester group. The models were generated with the SYBYL 5.3 molecular modeling package (38) and energy minimized with the Kollmann force field (39). Relaxed stereo views are displayed.

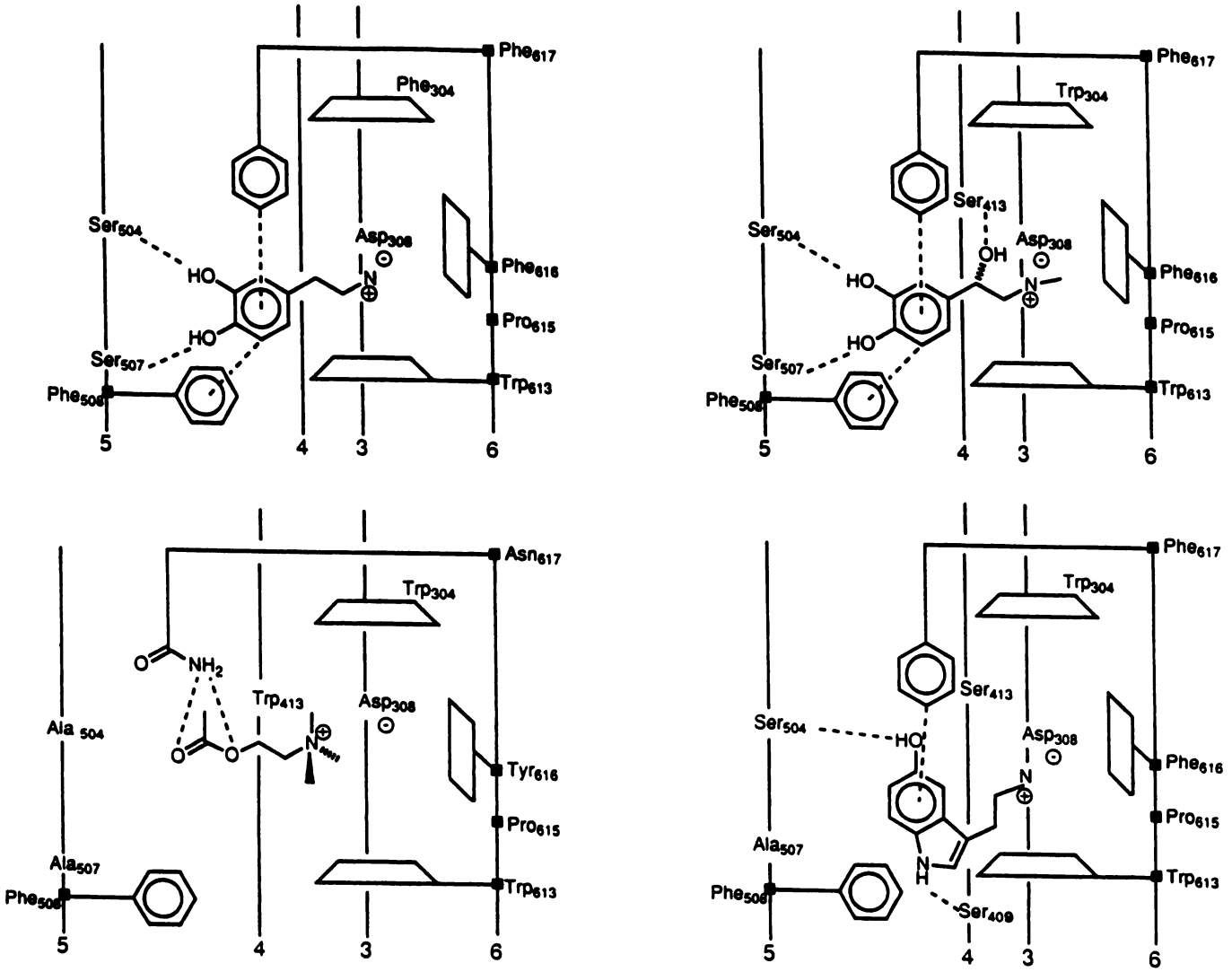


Fig. 3. Schematic representation of the interaction between D_2 (top left), β_2 (top right), 5-HT_2 (bottom right), and m_2 (bottom left), recognition sites and their corresponding neurotransmitters.

ester-Asn_{617} interaction. Ser_{504} (or Cys_{504}) and Ser_{507} are conserved as a pair only in catecholamine receptors. As postulated from site-directed mutagenesis data (34), these two residues can indeed, in our model, form hydrogen bonds to the *para*- and *meta*-hydroxyl groups of the catecholamine moiety of noradrenaline, adrenaline, and dopamine. Ser_{504} (or Thr) is present only in 5-HT receptors and can interact with the 5-hydroxy substituent of serotonin. Another important finding is that Ser_{413} can form a stereospecific interaction with the β-hydroxyl group of noradrenaline and adrenaline, whereas Ser_{409} can form a hydrogen bond with the indole nitrogen atom of serotonin. For the aromatic neurotransmitters, an additional stabilizing T-shaped interaction is provided by Phe_{617}, which is substituted by Asn_{617} in muscarinic receptors.

Thus, the three-dimensional models allowed us to propose a role for residues that are invariant in a given GPCR subclass, with regard to potency, selectivity, and stereospecificity of the binding process.

Finally, the problem of receptor efficacy was addressed. Residues that may be responsible for the induction or the propagation of conformational change in the receptors can be proposed. Three different induction systems are postulated; the first involves the conserved aromatic residues, Trp_{613}, Phe or Tyr_{616}, and Trp, Phe, or Tyr_{304}, the second, the residues Phe_{508} and Asn or Phe_{617}, and the third, Tyr_{715}. The conformation of the side chains of these residues is probably rearranged during the binding process and could directly affect the conformation of the adjacent helices at the level of Pro_{511} and Pro_{615} or affect other helices (H_4 or H_7) by propagation along the chain of interacting conserved aromatic residues. The accessibility of the residues on the cytoplasmic loops would, thus, be modified, affecting the coupling to the G protein.

In particular, Tyr_{715} could transfer the information from the binding site to the domain defined by helices 1, 2, and 7. Tyr_{715} seems able to interact either with Asp_{308} or with Asp_{209} by a simple rotation of its side chain. Such a rotation could be triggered upon ligand binding at Asp_{308} or Na^+ binding at Asp_{209}, as recently suggested by experimental results (45). It can be speculated that modifications of the Asp_{209} environment could be responsible for the cooperativity that is experimentally observed between the two aspartate residues (43, 45). This switch mechanism could induce a conformational change at the

level of the conserved Gly_{116}-Asn_{117} on helix H_1 and Asn_{721}-Pro_{722} on helix H_7 and induce a reorganization of the cytoplasmic part of the receptor.

It seems likely that these complex mechanisms occur in a concerted manner. Appropriate molecular dynamics simulations will be performed to further explore the relevance of these hypotheses.

The weakest starting hypothesis in this study is the choice of bacteriorhodopsin as a template for the packing of the seven α-helices, because there is a very low level of homology between this protein and GPCR primary sequences. However, we could a *posteriori* observe from our models that the neurotransmitters and the terminal part of retinal occupy homologous regions and interact in a similar manner with conserved aromatic residues, Trp_{613} and Tyr_{616} (Fig. 2). In homology with the GPCR, these two residues on H_6 can form favorable π-σ interactions with retinal in its active conformation. This hydrophobic cluster of aromatic residues is completed by Trp_{308}, which occupies the same position as Asp_{308} in cationic neurotransmitter GPCR. This result indicates a high degree of homology within the tertiary structures, at least at the level of the activating sites, and thus provides some additional support to our initial hypothesis.

Recently, a three-dimensional model of the β_2-adrenoceptor was reported (46). In contrast to our study, this model is based on the low resolution structure of bacteriorhodopsin (47) and differs significantly from the model presented here, in particular for the relative position of the seven α-helical domains. A model for muscarinic receptors was also presented (48). This model is based on the most recent structure of bacteriorhodopsin but retains some aspects of the former structure. In addition, all residues likely to be involved in ligand binding were not identified.

The three-dimensional models presented here, in spite of their natural limitations, account for labeling experiments, mutagenesis experimental data, and the traditional structure-activity relationship studies. They represent an additional step towards the understanding of the GPCR structure and function, because residues likely to be responsible for receptor affinity, selectivity, stereospecificity, and efficacy are proposed. For molecular biologists and medicinal chemists, the models constitute an important working hypothesis useful to orient site-directed mutagenesis studies and rationalize drug design.

Acknowledgments

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