0163-769X/00/$03.00/0
Endocrine Reviews 21(1): 90–113

Uncovering Molecular Mechanisms Involved in Activation of G Protein-Coupled Receptors

ULRIK GETHER*

Division of Cellular and Molecular Physiology, Department of Medical Physiology, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark

ABSTRACT

G protein-coupled, seven-transmembrane segment receptors (GPCRs or 7TM receptors), with more than 1000 different members, comprise the largest superfamily of proteins in the body. Since the cloning of the first receptors more than a decade ago, extensive experimental work has uncovered multiple aspects of their function and challenged many traditional paradigms. However, it is only recently that we are beginning to gain insight into some of the most fundamental questions in the molecular function of this class of receptors. How can, for example, so many chemically diverse hormones, neurotransmitters, and other signaling molecules activate receptors believed to share a similar overall tertiary structure? What is the nature of the physical changes linking agonist binding to receptor activation and subsequent transduction of the signal to the associated G protein on the cytoplasmic side of the membrane and to other putative signaling pathways? The goal of the present review is to specifically address these questions as well as to depict the current awareness about GPCR structure-function relationships in general. (*Endocrine Reviews* **21:** 90–113, 2000)

Address reprint requests to: Ulrik Gether, M.D., Division of Molecular and Cellular Physiology, Department of Medical Physiology 12–5-22, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark. E-mail: gether@mfi.ku.dk

*Recipient of an Ole Roemer Associate Research Professorship from the Danish Natural Sciences Research Council.

I. Introduction

G PROTEIN-coupled, seven-transmembrane segment receptors (GPCRs or 7TM receptors) comprise the largest superfamily of proteins in the body. More than 1000 different GPCRs have been identified since the first receptors were cloned more than a decade ago (1). The chemical diversity among the endogenous ligands is exceptional. They include biogenic amines, peptides, glycoproteins, lipids, nucleotides, ions, and proteases. Moreover, the sensation of exogenous stimuli, such as light, odors, and taste, is mediated via this class of receptors (1, 2). GPCRs have been named based on their ability to recruit and regulate the activity of intracellular heterotrimeric G proteins. GPCRs act at the heterotrimeric G proteins as guanine-nucleotide exchange factors; thus, the activated receptor induces a conformational change in the associated G protein α-subunit leading to release of GDP followed by binding of GTP (3). Subsequently, the GTP-bound form of the α-subunit dissociates from the receptor as well as from the stable $\beta\gamma$-dimer. Both the GTP-bound α-subunit and the released $\beta\gamma$-dimer can modulate several cellular signaling pathways. These include, among others, stimulation or inhibition of adenylate cyclases and activation of phospholipases, as well as regulation of potassium and calcium channel activity (4). The complexity of GPCR signaling has recently been further underlined by data indicating that GPCRs may not solely act via heterotrimeric G proteins (5–10). Most intriguingly, it has been suggested that agonist-promoted phosphorylation of the receptors by GRKs (G protein-coupled receptor kinases) (11) and subsequent sequestration of the receptors from the cell surface (11) are not only important for turning off signaling, but also play a key role in switching the receptor from G protein-dependent pathways to signaling cascades normally used by growth factor receptors (5–7, 10). Yet another example illustrating the impressive variability of GPCR function is the

observation that human immune deficiency virus (HIV) utilizes G protein-coupled chemokine receptors as cofactors for their cellular entry (12–15).

It is thus clear that extensive experimental work performed over the last decade has uncovered multiple aspects of GPCR function and challenged many traditional paradigms (reviewed in Refs. 7 and 16–22). However, it is only recently that we are beginning to gain insight into some of the most fundamental questions in GPCR function. How can, for example, so many chemically diverse hormones, neurotransmitters, and other signaling molecules activate receptors believed to share a similar overall tertiary structure? What is the nature of the physical changes linking agonist binding to receptor activation and subsequent transduction of the signal to the associated G protein on the cytoplasmic side of the membrane and to other putative signaling pathways? The goal of the present review is to specifically address these questions as well as to depict the current awareness about GPCR structure-function relationships in general.

II. Structural Classification of G Protein-Coupled Receptors

GPCRs do not share any overall sequence homology (1, 23). The only structural feature common to all GPCRs is the presence of seven transmembrane-spanning α-helical segments connected by alternating intracellular and extracellular loops, with the amino terminus located on the extracellular side and the carboxy terminus on the intracellular side (Fig. 1). Significant sequence homology is found, however, within several subfamilies. The three major subfamilies include the receptors related to the "light receptor" rhodopsin and the β_2-adrenergic receptor (family A), the receptors related to the glucagon receptor (family B), and the receptors related to the metabotropic neurotransmitter receptors (family C) (Fig. 1). Yeast pheromone receptors make up two minor unrelated subfamilies, family D (STE2 receptors) and family E (STE3 receptors). In *Dictyostelium Discoideum* four different cAMP receptors constitute yet another minor, but unique, subfamily of GPCRs (family F) (1).

The subfamily of rhodopsin/β_2 adrenergic receptor-like receptors (family A) is by far the largest and the most studied. Phylogenetically, family A receptors can be subdivided further into six major subgroups as indicated in Fig. 1 (1). The overall homology among all type A receptors is low and restricted to a number of highly conserved key residues (indicated in Fig. 1). The high degree of conservation among these key residues suggests that they have an essential role for either the structural or functional integrity of the receptors. (Fig. 1). The only residue that is conserved among all family A receptors is the arginine in the Asp-Arg-Tyr (DRY) motif at the cytoplasmic side of transmembrane segment (TM) 3 (Fig. 1) (1, 23).

To facilitate comparison of residues between the large number of different receptors belonging to family A there is an obvious need to formulate and use a common numbering scheme. Currently, three different numbering schemes have been suggested but none of them have gained any wide acceptance. The Schwartz and Baldwin numbering schemes are, in principle, identical (24, 25). According to both schemes, the most conserved residue in each helix (*yellow residues* in Fig. 2B and Fig. 3) has been given a generic number describing their predicted relative position in a standard helix of 26 residues (24, 25). A given residue is then described by the helix in which it is located (I–VII) followed by a number indicating its position in the helix. For example, V.16 indicates residue number 16 in TM (transmembrane segment) 5. However, the two numbering schemes are unfortunately incompatible with one another since they do not, except in helix 1, agree on the relative positioning of the conserved residues in the helices (24, 25). This problem is not apparent in the Ballesteros-Weinstein numbering scheme (26). In this scheme, the most conserved residue in each helix has been given the number 50, and each residue is numbered according to its position relative to this conserved residue. For example, 6.55 indicates a residue located in TM 6, five residues carboxy terminal to Pro6.50, the most conserved residue in helix 6 (Fig. 2B and Fig. 3) (26). Since there is no general agreement at this stage in the field on which scheme to use, all residues in this review will be indicated according to the Schwartz scheme followed by the Ballesteros-Weinstein number in superscript.

Family B receptors include approximately 20 different receptors for a variety of peptide hormones and neuropeptides, such as vasoactive intestinal peptide (VIP), calcitonin, PTH, and glucagon (Fig. 1). Except for the disulfide bridge connecting the second (ECL 2) and third extracellular loops (ECL 3), family B receptors do not contain any of the structural features characterizing family A receptors (1) (Fig. 1). Notably, the important DRY motif is absent in family B receptors, and the prolines conserved among the family B receptors are distinct from the ones conserved among the family A receptors (Fig. 1). The most prominent characteristic of family B receptors is a large ($\sim$ 100 residues) extracellular amino terminus containing several cysteines, presumably forming a network of disulfide bridges (27).

Family C receptors are characterized by an exceptionally long amino terminus (500–600 amino acids) (Fig. 1). The receptors include the metabotropic glutamate and γ-aminobutyric acid (GABA) receptors, the calcium receptors, the vomeronasal, mammalian pheromone receptors, and the recently identified putative taste receptors (1, 2). Family C receptors have, like family A and B receptors, two putative disulfide-forming cysteines in ECL 2 and ECL 3, respectively, but otherwise they do not share any conserved residues with family A and B receptors (Fig. 1). The amino terminus of the metabotropic glutamate receptors displays remote sequence homology with bacterial periplasmic binding proteins (PBPs), especially with the leucine/isoleucine/valine binding protein (28). The glutamate binding site has been proposed to be equivalent to the known amino acid binding site of PBPs; therefore, it is believed that the amino terminus of family C receptors contains the ligand-binding site (28, 29).

III. Structural Probing of GPCRs

Due to the inherent difficulties in crystallizing complex membrane proteins, high-resolution structural information

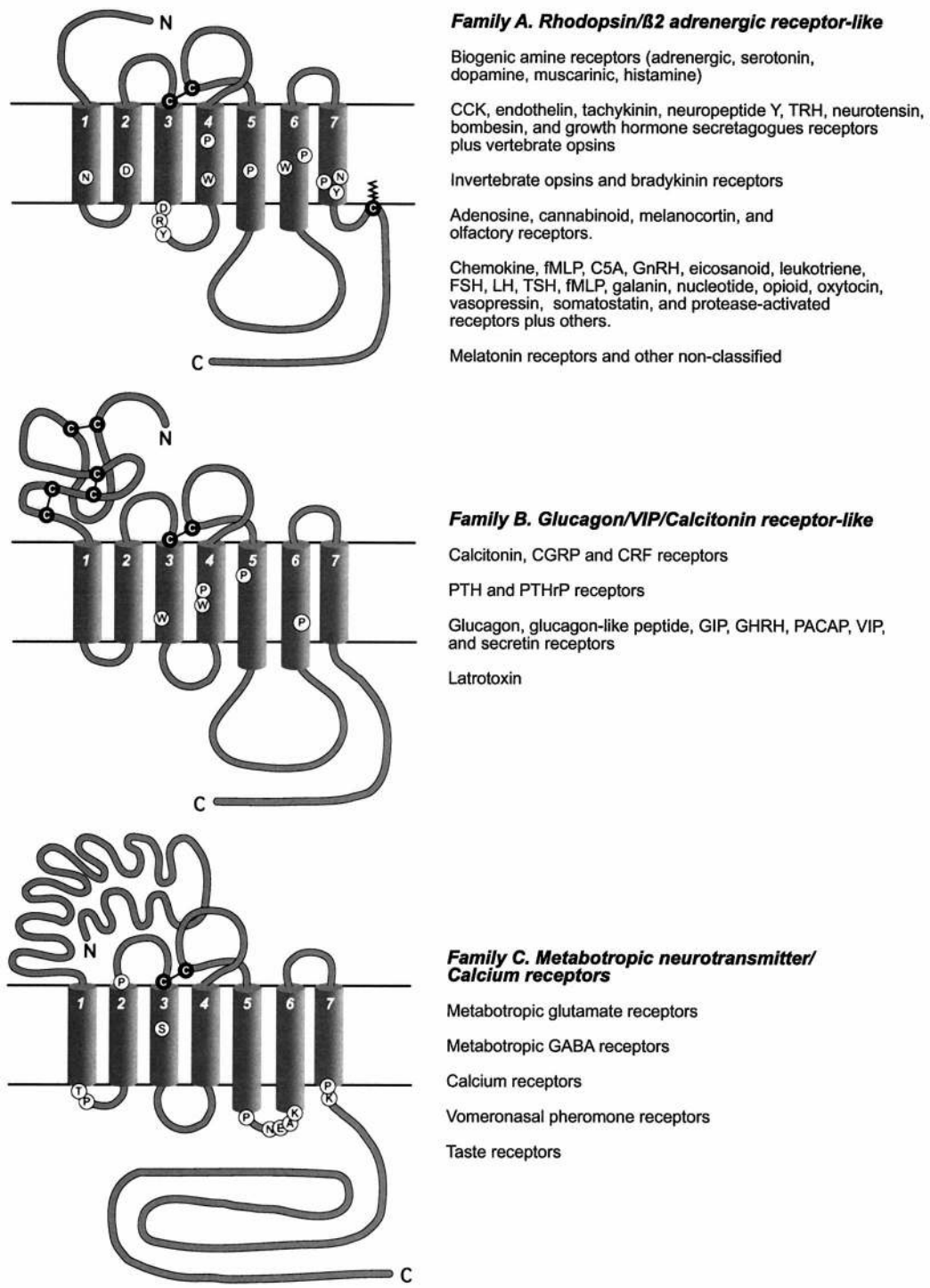

FIG. 1. GPCRs can be divided into three major subfamilies (1). A *snake diagram* for a prototypical member of each subfamily is shown. Family A receptors (*upper panel)* can phylogenetically be subdivided into six subgroups as indicated. Family A receptors are characterized by a series of highly conserved key residues (*black letter in white circles*). In most family A receptors, a disulfide bridge is connecting the second (ECL2) and third extracellular loop (ECL3) (*white letters in black circles*). In addition, a majority of the receptors have a palmitoylated cysteine in the carboxy-terminal tail causing formation of a putative fourth intracellular loop. Family B receptors *(middle panel)* are characterized by a long amino terminus containing several cysteines presumably forming a network of disulfide bridges. The B receptors contain, similar to the A receptors, a disulfide bridge connecting ECL2 and 3. However, the palmitoylation site is missing. Moreover, the conserved prolines are different from the conserved prolines in the A receptors and the DRY motif at the bottom of TM 3 is absent. Family C receptors *(lower panel)* are characterized by a very long amino terminus (~600 amino acids). The amino-terminal domain is thought to contain the ligand-binding site (see *Section IV.F)*. Except for two cysteines forming a putative disulfide bridge, the C receptors do not have any of the key features characterizing A and B receptors. Some highly conserved residues are indicated (*black letter in white circles*). A unique characteristic of the C receptors is a very short and highly conserved third intracellular loop.

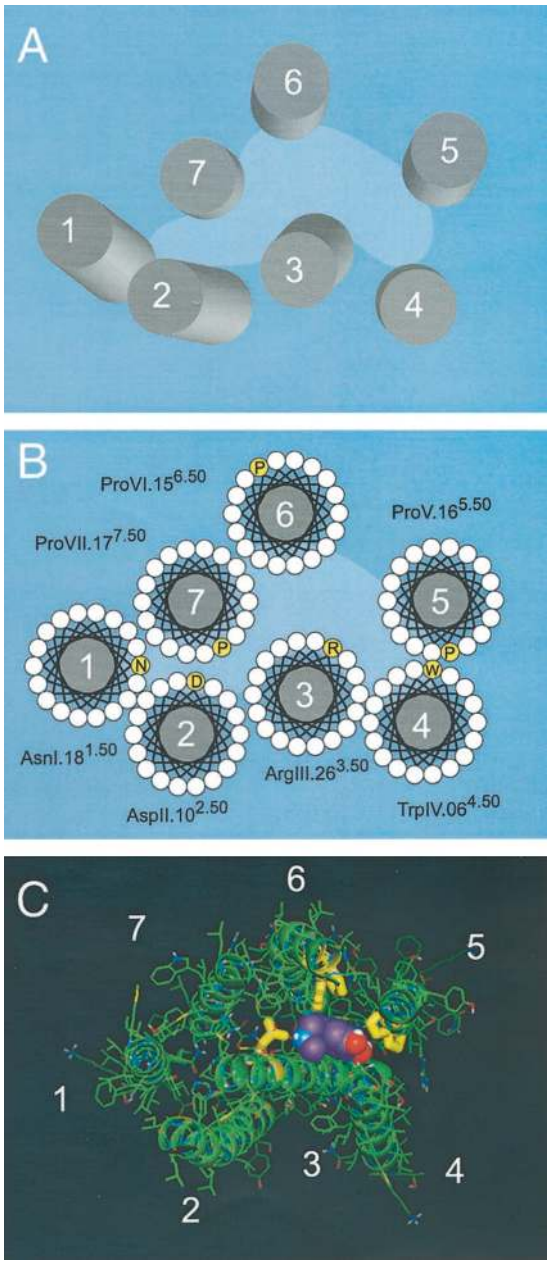

FIG. 2. The predicted structure of rhodopsin-like GPCRs. A, Diagram of a rhodopsin-like GPCR as seen from the extracellular side with each helix represented by a cylinder. The helices are positioned according to the projection maps of frog rhodopsin (37, 38). The helices are organized sequentially in a counterclockwise fashion with helix 3 being almost in the center of the molecule (37, 38). B, "Helical wheel" diagram of a rhodopsin-like GPCR as seen from the extracellular side. The helices are positioned according to the projection maps of frog rhodopsin (37, 38). The conserved fingerprint residues are shown in *yellow*. These residues have been given a general number to facilitate comparison of residues between the receptors. According to the Schwartz numbering scheme, the number is given according to its predicted relative position in the helix (24). For example, ProV.16 indicates residue number 16 in TM 5. In the Ballesteros-Weinstein numbering scheme the most conserved residue in each helix has been given the number 50 (26). The residues are indicated according to the Schwartz scheme followed by the Ballesteros-Weinstein number in superscript. C, Molecular model of the β_2-adrenergic receptor, as seen from the extracellular side, based on the projection map of rhodopsin (26) and structural analysis of multiple GPCR sequences (26). The full agonist epinephrine is shown in the binding crevice with key interactions *highlighted* (see *Section IV.A.2* for further details).

Dr. Juan Ballesteros is thanked for preparing the figure. Susan L. Glick and Julie Bryant from Molecular Simulations, Inc., are thanked for technical assistance.

is not yet available for GPCRs. A high-resolution structure of the light-driven proton pump from *Halobacterium halobium*, bacteriorhodopsin, has been available for several years (30). Since bacteriorhodopsin, similar to the GPCRs, possesses seven-transmembrane α-helices and uses retinal as its chromophore, it has been considered a bacterial homolog of vertebrate rhodopsin. The bacteriorhodopsin structure has accordingly been widely used as a template for tertiary structure models of GPCRs (31–35). However, bacteriorhodopsin is a proton pump, is not linked to a G protein, and does not even display remote sequence homology with any GPCR. Moreover, the structural information that recently has become available for rhodopsin indicated clear differences between bacteriorhodopsin and rhodopsin (30, 36–39). Overall, the use of bacteriorhodopsin as a template for molecular models should now be considered obsolete.

Using electron cryomicroscopy of two-dimensional crystals, Schertler and co-workers (36–39) have succeeded in obtaining low-resolution structures of both bovine and frog rhodopsin. In addition, a low-resolution structure of squid rhodopsin has become available (40). The first projection map of bovine rhodopsin at 9 Å resolution provided the first direct insight into how the predicted seven helices are organized relative to one another in the tertiary structure of the receptor (36). Importantly, a very similar arrangement of the transmembrane helices was found in the projection maps of frog and squid rhodopsin at 7 Å and 8 Å resolutions, respectively (38, 40). The projection maps are characterized by an arc-shaped feature, which has been interpreted as reflecting the presence of three tilted helices (36, 38, 40). Four additional peaks were interpreted as the remaining four transmembrane helices (36, 38, 40). The structural information achieved from aligning multiple receptor sequences permitted assignment of the individual peaks in the projection maps to the individual helices in the receptor (25, 41). As shown in Fig. 2, it is believed that the helices are organized sequentially in a counterclockwise fashion as seen from the extracellular side, with helix 3 being almost in the center of the molecule. Further insight into the packing of the seven-helix bundle and calculation of the tilting angles of the helices have been achieved by detailed analysis of tilted two-dimensional crystals of bovine and frog rhodopsin, allowing generation of the first three-dimensional maps (37, 38). The resolution of the map based on the frog rhodopsin crystals was 7.5 Å in the plane of the membrane and 16.5 Å perpendicular to it (38). According to the map, helices 1, 2, and 3 are tilted 27–30 degrees, helix 5 is tilted 23 degrees, whereas helices 4 and 7 are almost perpendicular to the plane of the membrane (38). Helix 6 appears almost perpendicular to the plane of the membrane in the cytoplasmic half but is bent toward helix 5 on the extracellular side (38). The structure also shows that the helices are tightly packed on the intracellular side with helices 2 and 3 packed between helix 4, 6,

and 7 (38). On the extracellular side the helical arrangement opens up and forms a cavity that serves as a binding pocket for retinal. The cavity is lined by helices 3, 4, 5, 6, and 7 and is closed toward the intracellular side by the tilted helix 3 (38). A recent projection map of bovine rhodopsin with an improved resolution (5 Å) suggests that the two-dimensional crystallography technique may lead to even more detailed understanding of the tertiary structure of GPCRs (39).

Guided by the rhodopsin projection maps and the structural information that has been acquired from extensive analysis of multiple GPCR sequences, several tertiary structure models of receptors belonging to family A have been developed over the last few years (25, 26, 41, 42) (Fig. 2C). The models are, of course, still somewhat uncertain but they do provide a believable general picture of the receptor structure and thus a reliable framework within which the structure and molecular function of GPCRs can be further debated and experimentally explored. Importantly, a large number of experimental studies, aimed at probing tertiary structure relations in GPCRs, have been highly critical for refining and validating the molecular models. First of all, this includes identification of several distance constraints in the receptor structure. The close proximity between TM 1 and 7 has, for example, been established based on rescue of nonfunctional adrenergic α_2/β_2 receptor chimeras and muscarinic M_2/M_5 chimeras (43–46). An important series of helix-helix interactions have also been identified by engineering of histidine zinc(II) binding sites in the neurokinin 1 (NK-1) (substance P) receptor and the κ-opioid receptor (47–49). In the NK-1 receptor bis-zinc(II) binding sites were constructed by introducing pairs of histidines in positions predicted to be in close proximity, and in this way it was possible to define the proximity and orientation of TM 3 relative to TM 2 and 5 (47). The distance constraints inferred from the engineered zinc(II) binding sites, as well as from the rescue of nonfunctional chimeras, strongly supported a counterclockwise organization of the seven helices as seen from the extracellular side (45–47). Additional distance constraints in the tertiary structure of the receptors have been identified by formation of intramolecular disulfide bridges between engineered pairs of cysteines in rhodopsin (50, 51) and lately in the M_3 muscarinic receptor (52). Notably, the use of biophysical techniques has also allowed insight into tertiary structure relationships. Turcatti *et al.* established a system, based on suppression of UAG nonsense codons and the use of modified tRNAs, allowing biosynthetic introduction of a fluorescent, unnatural amino acid at known sites in the tachykinin NK-2 receptor during heterologous expression in *Xenopus* oocytes. In this way, they were able to define a set of distances in the tertiary structure by measurement of fluorescence resonance energy transfer between a fluorescent peptide antagonist and different sites containing the fluorescent amino acid (53).

In the GnRH receptor, the proximity between TM 2 and 7 was suggested based on identification of an evolutionary reciprocal mutation (54). In nearly all family A receptors there is a conserved aspartic acid in TM 2, AspII.10$^{2.50}$ (II.10 according to the Schwartz numbering scheme, 2.50 according to the Ballesteros-Weinstein scheme), and a conserved asparagine in TM 7 (VII.16$^{7.49}$) (Fig. 3), but in the GnRH receptor an asparagine is found in the corresponding position in TM 2 and an aspartic acid in TM 7. Since replacement of the asparagine in TM 2 with aspartic acid eliminated detectable ligand binding, but high-affinity agonist binding was restored by additional mutation of the aspartic acid in TM 2 to asparagine, it was proposed that the two residues are in close spatial proximity (54). The observation is not readily compatible with the receptor model proposed by Baldwin *et al.* (25). In this model the distance between the α-carbons of the two residues is 10.4 Å, which is too large for their side chains to form a direct hydrogen-bonding interaction (25). However, if the proposed kink at ProVII.17$^{7.50}$ also causes a twisting of the helix, the two residues can be in sufficiently close proximity to form a direct interaction (26, 55). Remarkably, the presence of both a kink and twist in helix 7 is experimentally supported by the observed cysteine accessibility pattern in TM 7 (55).

Applying the substituted cysteine accessibility method to the dopamine D_2 receptor has provided further highly useful structural information about GPCRs (55–59). Javitch and coworkers (55–58) have systematically substituted residues in TM 2, 3, 5, 6, and 7 with cysteine and determined their accessibility in the predicted binding crevice by reacting with charged sulfhydryl-specific methanethiosulfonate (MTS) derivatives. Their data have allowed mapping of residues facing the binding crevice and estimation of the relative orientation of individual helices (55–58). The accessibility patterns were consistent with TM 2, 3, 6, and 7 forming regular α-helices in agreement with the predictions from the rhodopsin projection maps (55, 56, 58). In TM 6 and 7, the data also supported the presence of kinks corresponding to the conserved prolines, ProVI.15$^{6.50}$ and ProVII.17$^{7.50}$, respectively (Figs. 2 and 3) (55, 58). The accessibility pattern in TM 5 differed from that observed in the other helices (57). A stretch of 10 consecutive residues in the outer portion of TM 5 were found exposed in the binding crevice, which is inconsistent with the prediction that TM 5, like the other helices, should form a regular helix with one side exposed and one side hidden form the crevice (57). There is no obvious explanation for this observation. One explanation could be that the exposed stretch of residues is nonhelical and loop out into the lumen of the binding crevice, making all the residues accessible to the MTS reagents. Alternatively, the outer portion of TM 5 may be structurally flexible and rapidly shift between different conformations, exposing different sets of residues to the binding crevice (57). In both cases, it is of notable interest that the exposed region contains residues believed to form key contacts with the small-molecule agonists (60).

In rhodopsin, the application of EPR (electron paramagnetic resonance) spectroscopy has provided information about structural features, particularly in the cytoplasmic loop regions. Consecutive residues in the cytoplasmic loops and the carboxy-terminal tail have been substituted with cysteine and each of the cysteine mutants was labeled with sulfhydryl-specific nitroxide spin labels (61–65). By determining the accessibility of the attached nitroxide labels to collisions with paramagnetic probes in solution, information about aqueous/hydrophobic boundary zones and secondary structure relations was obtained. The accessibility pattern in the third intracellular loop connecting TM 5 and 6 provided

important evidence that these two α-helices extend two to three turns beyond the cytoplasmic surface of the membrane (62). In the second intracellular loop connecting TM 3 and 4, the analysis indicated that the TM 3 α-helix extends at least 1.5 turns past the important D/ERY motif (Figs. 1 and 3) and that much of the helix surface at the cytoplasmic side forms contacts with protein rather than with the lipids (61). Analysis of the "fourth intracellular loop" between the cytoplasmic end of TM 7 and the palmitoylation site indicated that helix 7 extends around 1.5 turns beyond the membrane surface and that the remaining part of the loop forms very strong tertiary contacts with the protein (64). It was therefore suggested that the loop beyond the helix may be folded over the body of rhodopsin, allowing interactions with residues in the first loop between TM 1 and 2 (64).

IV. Ligand-Binding Domains

Numerous studies have been carried out to identify domains involved in ligand binding to various subclasses of GPCRs. The binding sites for endogenous "small-molecule" ligands in family A receptors, such as the binding site for the retinal photochromophore in rhodopsin and the binding site for catecholamines in the adrenergic receptors, are perhaps the most well characterized. They have been described in detail several times (16, 17, 19, 21) and will therefore be reviewed here only briefly. It is, however, only recently that we have gained insight into binding domains for other classes of ligands. In particular, the ligand-binding domains in peptide receptors are of interest due to the discovery of many small-molecule nonpeptide ligands that can act with high potency at peptide receptors as antagonists and agonists. Intriguingly, it has appeared that the small-molecule agonists and antagonists of peptide receptors may not necessarily share an overlapping binding site with the endogenous peptide agonist. Since these findings have wide implications for receptor activation models, the current knowledge about ligand-binding domains in peptide receptors will be described in more detail. Specifically, the focus will be on the tachykinin system, which has been extensively investigated and served as an important model system for peptide GPCRs.

A. Rhodopsin and the biogenic amines

1. Rhodopsin. The photochromophore of rhodopsin and the opsins, 11-*cis*-retinal, is unique among the endogenous ligands for GPCRs in that it is *covalently* attached to the receptor within a binding crevice formed by the transmembrane helices (reviewed in Ref. 66). Through formation of a Schiff base, 11-*cis*-retinal is coupled to a lysine in TM 7 (Lys296, VII.10$^{7.43}$). The protonated Schiff base is paired with a glutamic acid (Glu113, III.04$^{3.28}$) in the outer portion of TM 3 (67). Additional interactions are found in TM 3 between the C9 group of retinal and Gly121 (III.12$^{3.36}$) (68), and between retinal and aromatic residues in the outer portion of TM 6 (69). Upon exposure to light, 11-*cis*-retinal undergoes an isomerization to all-*trans*-retinal, which leads to formation of the metarhodopsin II state and thus receptor activation (66). While all-*trans*-retinal behaves like the rhodopsin agonist, 11-*cis*-retinal behaves as an inverse agonist (*i.e.*, an antagonist with negative intrinsic activity), keeping the receptor quiescent in the absence of light (70).

2. Classical small-molecule transmitter family A receptors. The binding sites for the classical "small-molecule" transmitters (epinephrine, norepinephrine, dopamine, serotonin, histamine, and acetylcholine) are contained in a binding crevice formed by the transmembrane helices. The residues involved in binding of agonists and antagonists to the β_2- adrenergic receptor are found in TM 3, 5, 6, and 7 (Figs. 2C and 3). The binding crevice is deeply buried in the receptor molecule as evidenced by spectroscopic analysis of the fluorescent antagonist carazolol bound to the β_2 -adrenergic receptor (71). The energetically most important interaction is most likely a salt bridge between the charged amine of adrenergic ligands and the carboxylated side chain of Asp113 (AspIII.08$^{3.32}$) in TM 3 (72) (Figs. 2C and 3). This aspartic acid is conserved among the biogenic amine receptors and is thought to interact also with the positively charged head group of dopamine (73), serotonin (74, 75), histamine (76), and acetylcholine (77). Additional key interactions of the agonists in the β_2-adrenergic receptor include hydrogen bonding between the hydroxyls of the catechol ring in epinephrine and two serines one α-helical turn apart in TM 5, Ser204 (V.09$^{5.43}$) and Ser207 (V.12$^{5.46}$) (60) (Figs. 2C and 3). In TM 6, Phe290 (VI.17$^{6.52}$) may stabilize the catechol ring (78) while recent evidence suggests that Asn293 (VI.20$^{6.55}$) forms a hydrogen bind with the β-hydroxyl of epinephrine (79) (Figs 2C and 3). In the case of the β_2-adrenergic antagonists, which are structurally related to the endogenous agonists, evidence suggests that they share the Asp113 (III.08$^{3.32}$) ionic interaction with the agonists, but that other key interactions differ. For aryloxyalkylamine antagonists, such as alprenolol and propranolol, an asparagine in TM 7 (Asn312, VII.06$^{7.39}$) has been identified as a critical interaction point (80) (Fig. 3). Even though the majority of ligands for small-molecule transmitter receptors seems to bind deep within the binding crevice, there are indications that some antagonists, which show no structural relationship with their corresponding agonist, may partly interact with residues closer to the surface of the membrane. For example, α_{1B}-antagonists, such as phentolamine and WB4101, may interact with three residues in ECL 2 immediately adjacent to the top of TM 5 (81).

B. The binding domains for peptide ligands in peptide receptors belonging to family A

More than 50 different neuropeptides and peptide hormones have been identified. With only a few exceptions, these peptide messengers all act through receptors belonging to the GPCR superfamily and, at present, more than 100 different peptide GPCRs, including subtypes, have been identified (1). In contrast to the general picture obtained for the small-molecule ligands, mutational mapping of ligand-binding sites in many of the peptide receptors has demonstrated the critical involvement of the extracellular domains for binding of the larger peptide ligands.

1. The tachykinin system. The mammalian tachykinins include substance P, neurokinin A, and neurokinin B, which act at the NK-1 receptor, the neurokinin-2 (NK-2) receptor, and the

neurokinin-3 (NK-3) receptor, respectively (82). In addition, a variant of the NK-3 receptor, NK-3B, has recently been identified (83). These receptors are homologous but display significant differences in their pharmacological profile (82, 84, 85). The initial analyses of chimeric NK-1/NK-2 receptors and NK-1/NK-3 receptors suggested that multiple epitopes scattered throughout the receptor structures contribute to the subtype selectivity of the tachykinin peptides and that different receptor domains contribute in varying degrees to the receptor specificity (84, 85). This suggests that the binding sites for the tachykinin peptides are not fully identical (85). Exchange of extracellular loop segments between the NK-1 and NK-3 receptors revealed the involvement of the extracellular domains in binding of the tachykinins (86, 87). Subsequent point-mutational analysis of the NK-1 receptor identified three residues in the amino terminus (Asn23, Glu24, and Phe25), a residue at the top of TM 3 (His108), and a residue at the top of TM 7 (Tyr287) as putative points of interaction for substance P (Fig. 3) (86–88). The importance of the loop regions in substance P binding has been directly supported by affinity cross-linking of a photolabile and radioactively labeled substance P analog to Met181 in the third extracellular loop (89, 90) (Fig. 3). At present, there is no clear evidence that substance P, like the small-molecule ligands, enters deeply into a binding crevice formed by the transmembrane helices. Despite extensive mutational analysis of residues facing the putative binding crevice, no residues have convincingly been identified as potential sites of interactions for substance P (24, 91).

Mutational analysis of neurokinin A binding to the NK-2 receptor also demonstrated evidence for interactions with residues in the extracellular domains (92, 93). However, the residues affecting neurokinin A binding in the NK-2 receptor differed partly from the residues affecting substance P binding in the NK-1 receptor (92, 93). Moreover, mutation of residues in the transmembrane regions, *e.g.*, Leu202 ($V.09^{5.43}$) in the middle of TM 5, was found to affect neurokinin A binding (92, 93). Thus, neurokinin A may partially enter the transmembrane binding crevice. In agreement with the initial chimeric studies (85), these findings indicate that there may be clear differences in the binding modes even among homologous peptides acting at homologous receptors.

Mutation of four residues situated on the same face of helix 2 in the NK-1 receptor has been reported to substantially impair the ability of substance P to compete for binding of radiolabeled nonpeptide antagonists (88). It was therefore initially concluded that these residues are involved in substance P binding. However, it has later been shown that radiolabeled substance P itself could bind with essentially unaffected affinity to the mutated receptors (94). The most likely explanation is that these mutations, rather than affecting the peptide-binding site, affect the ability of the receptor to freely interchange between distinct receptor conformations, which bind the nonpeptide antagonist and peptide agonist with high affinity, respectively (94). Notably, similar observations have been done in the κ-opioid receptor (95), and recently mutation of yet another residue in the NK-1 receptor (Gly166, $IV.21^{4.65}$) has been shown to affect interconversion between different receptor states that display distinct selectivity for the tachykinin peptides (96). These observations underline the importance of direct determination of binding affinity or testing second messenger coupling ability for an agonist before it is reasonable to consider whether the effect of a mutation reflects a real interaction between the ligand and the receptor or is due to an indirect effect.

2. Other family A peptide receptors. For the majority of receptors studied, there is evidence for major interactions in the amino terminus and predicted extracellular loop regions. This includes the receptors for angiotensin (97–99), neuropeptide Y (100), chemokines (interleukin-8) (101), vasopressin/oxytocin (102), GnRH (103), TRH (104–106), complement factor C5A (107, 108), formyl-Leu-Met-Phe (109), somatostatin (110), opioids (111–115), bradykinins (116), cholecystokinin/gastrin (117–121), and neurotensin (122). Importantly, the significance of the extracellular domains for binding of peptide ligands has been directly documented using affinity cross-linking techniques in the GnRH receptor (103), the bradykinin B2 receptor (116), and the cholecystokinin CCK-A receptor (118, 121).

Evidence indicates that some of the peptides have additional points of interactions in the transmembrane domains and therefore, to different degrees, may enter the transmembrane binding crevice. These include both the small tripeptides TRH (123, 124) and fMLP (125) and larger peptides such as angiotensin (126, 127), endothelin (128–130), somatostatin (131–133), opioids (134), and bradykinin (135). The residues identified are found in the outer portions of TM 2, 3, 5, 6, and 7. They differ considerably among the receptors and are, except in a very few cases (128, 131), different from the key positions believed to interact with the biogenic amines. However, it is remarkable to note that almost all of the residues identified appear to be on the surface of the predicted binding crevice as assessed by the cysteine accessibility method (55–59). This supports a high degree of structural similarity between the receptors, even though they bind chemically very different ligands.

C. The binding domains for nonpeptide ligands in peptide receptors belonging to family A

The large group of peptide receptors represents an impressive pool of potential drug targets; however, until recently this has been an almost unexplored area due to the low bioavailability and metabolic instability of the peptide ligands. It has been a long sought goal to develop small-molecule nonpeptide compounds that are orally active and can act at peptide receptors with high potency. The first and most significant discovery, indicating that this would be feasible, was the identification in the 1970s of a family of peptides, the enkephalins and endorphins, as the endogenous ligands of the opioid receptors (136). Until then, the only known ligands for the opioid receptors were nonpeptide exogenous compounds, such as morphine and naloxone. The finding directly showed that small nonpeptide compounds can act with high affinity at peptide receptors both as agonists and antagonists. It is only within recent years, however, that high-affinity nonpeptide compounds have been discovered for an increasing number of peptide recep-

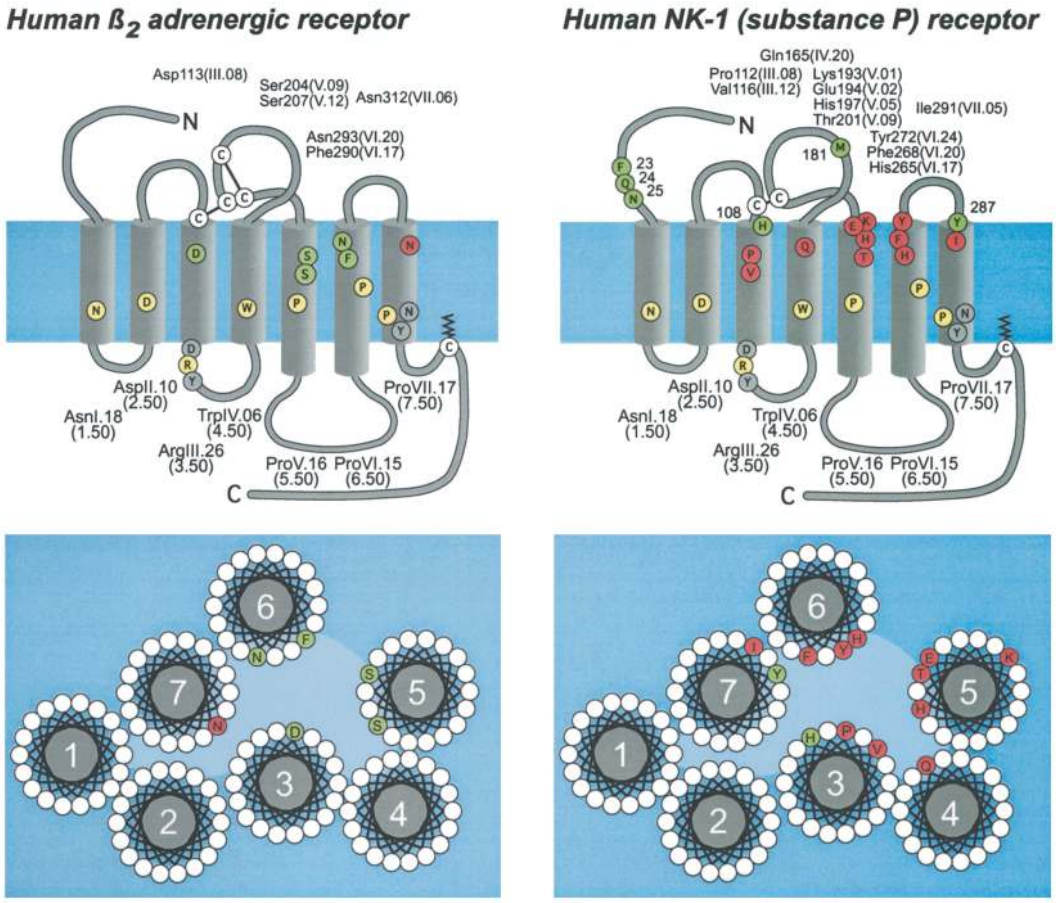

FIG. 3. Comparison of ligand-binding domains in a prototype small-molecule family A receptor (the β_2-adrenergic receptor, β2AR) with a prototype family A peptide receptor (the NK-1 receptor). *Upper panels, Snake diagrams* of the human β2AR and the human NK-1 receptor. *Lower panels,* Helical wheel diagrams of the receptors as seen from the extracellular side. The helices are positioned in a counterclockwise fashion according to the projection map of rhodopsin (25, 36, 38, 41). In the *upper panels* the most highly conserved residue in each helix is indicated in *yellow*. These so-called "finger print" residues have been given a general number to facilitate comparison of residues between the receptors. According to the Schwartz numbering scheme, the number is given according to its predicted relative position in the helix (24). For example, ProV.16 indicates residue number 16 in TM 5. In the Ballesteros-Weinstein numbering scheme, the most conserved residue in each helix has been given the number 50 (26). The numbers for each key residue, according to both numbering schemes, are indicated on this figure below the receptors. Otherwise, the residues shown in the figure are indicated by their "real" number in the receptor followed by the number according to the Schwartz numbering scheme. The amino acids predicted to form the contact points for the agonists are shown in *green* while residues involved in small-molecule antagonist binding are shown in *red* (see text for details). The residues in the β2AR (*left panels*) that form the agonist binding site for the epinephrine are found in a binding crevice between TM 3, 5, and 6 (72, 78, 79). In contrast, the presumed major contact points for the peptide agonist, substance P, in the NK-1 receptor (*right panels*) are found in the extracellular domains or at the top of the helices (86–88). In the β2AR, an asparagine in TM 7 (AsnVII.06) has been shown to interact specifically with aryloxyalkylamine antagonists (80). Notably, the aspartic acid in TM 3 (AspIII.08) (shown in *green*) is a common interaction point for both adrenergic agonists and antagonists (72). The residues shown in *red* in the NK-1 receptor are positions of point mutations shown to affect binding of the prototype nonpeptide antagonist CP 96345 (91, 142–146). Mutation of these residues, clustering in a crevice formed by TM 3, 4, 5, and 6, does not affect peptide agonist binding (91, 142–146).

tors and changed the peptide receptor field into a rapidly expanding area for drug development (24). The majority of the nonpeptide compounds [mostly antagonists but recently, in some cases, also agonists (137)] are developed into high-affinity compounds from "leads" identified by screening of large chemical files (24). In almost all cases, the resulting compounds exhibit no obvious structural similarity to the endogenous peptide ligands, despite an apparent classical competitive mode of action and despite the ability of both the peptide agonist and nonpeptide antagonists to bind with often subnanomolar affinity to the same receptor (24). Interestingly, these nonpeptide compounds have turned out to be valuable for understanding the molecular function of GPCRs.

1. Tachykinin nonpeptide antagonists. An initial series of chimeric NK-1/NK-3 receptors provided the first evidence that the binding mode for the prototype nonpeptide NK-1 receptor antagonist, CP 96,345, was distinct from the binding mode of the endogenous agonist substance P (138). Several chimeric exchanges that dramatically affected CP 96,345 affinity did not affect binding of substance P (138). Overall, the chimeric analyses indicated that CP 96,345 and several other structurally distinct nonpeptide NK-1 receptor antagonists,

but not substance P itself, interact in different ways with a domain located around TM 5 and 6 (138, 139). Moreover, data from a series of NK-1/NK-2 receptor chimeras indicated that SR 48,968, an NK-2 receptor-selective nonpeptide antagonist, has critical interactions in the same region of the NK-2 receptor (140). The different binding modes of the nonpeptide antagonists and the peptide agonists have also been supported by comparing fluorescent analogs of substance P and CP 96,345 bound to the NK-1 receptor. Most significantly, it was found that while the environment surrounding the nonpeptide antagonist was highly hydrophobic and inaccessible to hydrophilic quenchers, the peptide was directly exposed to the solvent (141).

Comprehensive point-mutational analysis has further defined the nonpeptide antagonist binding site in the NK-1 receptor (Fig. 3). The residues predicted to be involved in nonpeptide antagonist binding are located in a transmembrane crevice lined by TM 3, 5, and 6 (91, 142–146), although interactions for some compounds also may occur in TM 4 (147) and 7 (88). The most well documented putative direct interactions of the prototype compound CP 96,345 are Gln165 (IV.20$^{4.64}$) (147), His197 (V.05$^{5.39}$) (142), His265 (VI.17$^{6.52}$) (143, 144), Phe268 (VI.20$^{6.55}$) (91, 146), and Tyr272 (VI.24$^{6.59}$) (145). It should be emphasized here that it is highly difficult with mutational analysis techniques to distinguish direct interactions between the ligand and the receptor from indirect structural effects caused by the mutation. For example, mutation or deletion of Lys193 (V.01$^{5.35}$) and Glu194 (V.02$^{5.36}$) substantially affect CP 96,345 binding affinity (145) (Fig. 3). It is nevertheless unlikely that they participate in a direct interaction since they can be interchanged without affecting CP 96,345 affinity (145). Conceivably, these two residues form a salt bridge that stabilizes the CP 96,345 binding pocket (145). Two other residues, Val116 (III.12$^{3.36}$) and Ile290 (VII.05$^{7.38}$), which are nonconserved between the human and rat receptor, have been shown to be responsible for the species selectivity of CP 96,345 and three other structurally distinct nonpeptide antagonists (148–150) (Fig. 3). It was concluded that these two residues indirectly affected the geometry of a common binding crevice for nonpeptide ligands (148–150). However, Val116 would be predicted to face the binding pocket and could, in fact, be involved in a direct interaction with CP 96,345 (Fig. 3).

Summarized, the studies on the tachykinin receptors suggest the presence of a small-molecule binding pocket, similar to the binding pocket found in the biogenic amine receptors, where structurally distinct nonpeptide compounds can be accommodated through distinct sets of interactions. Surprisingly, this binding pocket is most likely not occupied by substance P, and thus an actual overlap in the binding sites is not required for a competitive mode of action of the nonpeptide antagonists.

2. Nonpeptide ligands for other family A peptide receptors. Considerable differences in binding modes between nonpeptide antagonists and endogenous peptide agonists have been demonstrated in other peptide receptor systems as well. These include the angiotensin (97, 151, 152), opioid (153, 154), CCK/gastrin (155–157), neurotensin (122, 158), and endothelin systems (159). The general conclusions emerging are similar to the ones from the studies of the tachykinin system; hence, the small-molecule nonpeptide compounds interact with residues in the transmembrane binding crevice and, in most cases, there is no evidence that these residues are overlapping with peptide agonist binding. In the neuropeptide Y system, however, there is evidence for several overlapping contact points in the binding site for the peptide agonist and the first available nonpeptide antagonist of the Y1 receptor, BIBP 3226 (160). Similarly, nonpeptide antagonists of the endothelin ET-A and ET-B receptors may share interactions with the endothelin peptides in TM 2 and TM 3 (128, 130). On the other hand, thorough mutagenesis of 18 amino acids in the predicted transmembrane binding crevice of the ET-A receptor revealed no indication of other overlapping contact points between the nonpeptide antagonist bosentan and endothelin-1 (159).

Nonpeptide *agonists* have recently been discovered for the angiotensin receptors. The nonpeptide agonists of the AT-1 angiotensin receptor were found among a series of biphenylimidazole antagonists, of which some turned out to possess agonistic properties (161). Surprisingly, it appeared that the binding mode of the biphenylimidazole agonist differed both from the binding mode of the *peptide* agonist angiotensin, as well as that of the structurally related biphenylimidazole antagonists (161). Mutations in TM 3 and 7, known to severely affect binding of biphenylimidazole antagonists, did not affect binding of the biphenylimidazole agonist. Moreover, binding of the biphenylimidazole agonist was also unaffected by mutation of residues in the extracellular domains known to affect binding of the peptide agonist angiotensin (161).

D. Ligand-binding sites in other family A receptors

While the binding sites for eicosanoids (leukotrienes and prostanoids) and purines mainly are contained within the transmembrane binding crevice (reviewed in Ref. 21), high-affinity binding of glycoprotein hormones such as LH/CG, FSH, and TSH to their receptors occurs in the large extracellular amino terminus that characterizes this receptor subgroup (21, 162–167). It is believed that after the initial binding to the extracellular domain, the amino-terminal part of the hormone undergoes a conformational change leading to secondary contacts with the extracellular loop regions of the membrane-associated part of the receptor and to subsequent receptor activation (21).

The protease-activated thrombin receptors also belong to family A (168, 169). The unique activation mechanism of the thrombin receptor involves cleavage of the amino-terminal segment by thrombin (168). The resulting 33-amino acid amino terminus subsequently acts as tethered peptide ligand, which, through interactions with the extracellular loop regions of the receptor, is able to activate the receptor (170, 171).

E. Ligand-binding domains in family B receptors

Similar to peptide receptors belonging to family A, the binding sites for peptide ligands in family B receptors involve the extracellular domains. The large amino terminus

that characterizes family B receptors seems to play a key role for most ligands, including secretin, VIP, pituitary adenylate cyclase-activating polypeptide (PACAP), glucagon, glucagon-like peptide-1, PTH, and CRF (172–182). The amino terminus is not sufficient for binding of these ligands, and additional interactions are found in the extracellular loops (173, 175, 178, 180, 183–187). However, there is at present no evidence that any of the peptides have interactions deep in a transmembrane binding pocket. Generally, nonpeptide antagonists are still not available for type B receptors. One exception is the CRF receptor for which a few nonpeptide compounds have been recently developed (185, 188). Clear evidence has already been obtained that these may bind very distinctly from the peptide and penetrate into a transmembrane-binding crevice (185).

F. Ligand-binding domains in family C receptors

In the metabotropic glutamate and GABA receptors, the ligand-binding sites are contained within the large extracellular domain characterizing family C receptors, thereby clearly distinguishing this subclass from the biogenic amine family A receptors (28, 189, 190). The calcium-binding site in the calcium-sensing receptors is also found in the large amino terminus (reviewed in Ref. 191). The extracellular amino terminus of the metabotropic glutamate receptors shares remote structural similarity with bacterial periplasmic amino acid-binding proteins (28, 29). A high-resolution x-ray structure of the extracellular glutamate-binding domain of an ionotropic glutamate receptor has recently been published (192). This structure represents the first x-ray structure of a neurotransmitter receptor-binding domain. Based on the x-ray structure, a mechanism was proposed for the propagation of the activation signal in the ionotropic receptors after agonist binding (192). Whether a similar mechanism also accounts for how the signal in metabotropic receptors is transmitted from the extracellular domain to the receptor core region remains elusive.

V. Molecular Mechanisms Involved in Activation of GPCRs

As described in detail in the previous section, the binding modes for agonists acting at GPCRs are almost as diverse as the chemical nature of the ligands. Even agonists acting at the same receptor may not necessarily share an overlapping binding site. Therefore, it seems clear that there are multiple ways of propagating activation of GPCRs or, in other words, there is no common "lock" for all agonists' "keys" (193). Further support for this has been obtained by the identification of receptor-activating antibodies, directed against the extracellular loop regions of the α_1- and β_1-adrenergic receptors, in serum from patients with malignant hypertension and idiopathic dilated cardiomyopathy, respectively (194, 195). The apparent ability of these antibodies to induce receptor activation represents an intriguing example that even in the small-molecule biogenic amine receptors, docking of an activating ligand in the transmembrane-binding crevice is not a prerequisite for ligand-induced receptor activation. Additional examples of activating antibodies, such as monoclonal antibodies against the muscarinic receptors (196) and the bradykinin B2 receptor (197), as well as autoantibodies directed against the extracellular domains of the TSH receptor in Grave's disease (198), also provide strong evidence that there are multiple ways of activating GPCRs. It is still most likely, nevertheless, that the underlying fundamental mechanisms of activation for GPCRs have been conserved during evolution given the ability of the receptors to activate the same intracellular signaling pathways through the same classes of G proteins. In the following section, our current insight into these mechanisms will be discussed.

A. GPCRs are kept silent by constraining intramolecular interactions

An important discovery has been the observation that many GPCRs have a certain basal activity and thus can activate the G protein in the absence of agonists (199–201). Interestingly, it has also been encountered that discrete mutations are able to dramatically increase this constitutive agonist-independent receptor activity (42, 202–205). The majority of the constitutively activating mutations were initially identified after mutational substitutions in the C-terminal part of the third intracellular loop of adrenergic receptors (202–205), but currently activating mutations have been identified in almost any receptor domain in an increasing number of receptors (representative examples in Refs. 42 and 206–218). In a few cases, activating mutations have been found even in the exterior part of the receptors, such as the second extracellular loop of the TSH receptor (214) and the third extracellular loop of the thrombin receptor (213). In the β_2-adrenergic receptor constitutive activation has been observed in a chimeric construct where ECL2 was substituted with the corresponding loop of the α_{1B}-receptor (219). Of interest, some constitutively active mutations have arisen naturally and have been linked to genetic diseases. This includes mutations in the TSH receptor associated with hereditary thyroid adenomas (208, 211, 214); mutations in the LH receptor leading to male precocious puberty (209); and mutations in rhodopsin associated with development of retinitis pigmentosa (210).

A crucial clue about the molecular mechanisms underlying constitutive receptor activation came from a study carried out by Lefkowitz and co-workers in which the naturally occurring Ala293 (VI.0$^{6.34}$) residue in the C-terminal part of third intracellular loop of the α_{1b}-adrenergic receptor was substituted with all other possible residues. They found that substitution of the alanine with any other residue resulted in higher agonist-independent receptor activity (203). This led to the suggestion that constraining intramolecular interactions have been conserved during evolution to maintain the receptor preferentially in an inactive conformation in the absence of agonist. Conceivably, these inactivating constraints could be released as a part of the receptor activation mechanism, either after agonist binding or due to specific mutations, causing key sequences to be exposed to G protein. The hypothesis has been indirectly supported by the recent observation that constitutively activated β_2-adrenergic receptor mutants are characterized by a marked structural instability and enhanced conformational flexibility of the

purified receptor proteins (218, 220). The data imply that the mutational changes have disrupted important stabilizing intramolecular interactions in the tertiary structure, allowing the receptor to undergo conversion more readily between its inactive and active state (218, 220).

Experiments performed in other receptors have also indicated that constraining intramolecular interactions have been conserved during evolution to keep the receptors preferentially silent in the absence of agonists. Hsueh and colleagues (221) obtained evidence using a series of chimeric LH/FSH receptors that stabilizing interactions between TM 5 and 6 are critical for the resistance of the FSH receptor to constitutively activating mutations. A stabilizing role of TM 6 has also been suggested from a random mutagenesis study in the muscarinic M5 receptor where substitutions on one face of the helix conveyed constitutive activity to the receptor (222). Similarly, mutation of polar residues in TM 6 of the α-factor pheromone receptor (STE2p) conveyed constitutive activation to this receptor (223). Molecular modeling and analysis of naturally occurring activating mutations in the LH receptor also strongly point to the importance of the helical packing of TM 6 for maintaining the receptor in an inactive configuration (224). In rhodopsin there is evidence suggesting that opsin, the apoprotein form of rhodopsin, is maintained in an inactive configuration by interactions between a methionine in TM 6 (Met257, VI.05$^{6.40}$) and the conserved NPXXY motif in TM 7 (225), as well as by a salt bridge between Lys296 (VII.10$^{7.43}$) (the retinal attachment site in TM 7) and Glu113 (GluIII.04$^{3.28}$) (the Schiff base counterion in TM 3) (206). Stabilizing interactions between TM 3 and 7 have also been suggested in the angiotensin AT-1 receptor between Asn111 (III.11$^{3.35}$) and Tyr292 (VII.10$^{7.43}$) (226), and in the α_{1b}-adrenergic receptor between the conserved aspartic acid (Asp125, III.08$^{3.32}$) and Lys331 (VII.03$^{7.36}$) (212).

B. Protonation is a key element in GPCR activation

If receptor activation involves disruption of stabilizing intramolecular interaction, an obvious question is how this may be initiated after agonist binding. At present, this question cannot be fully answered; however, substantial evidence suggests that at least one of the key events in the activation process among family A GPCRs involves protonation of the aspartic acid in the highly conserved D/E RY (Glu/Asp-Arg-Tyr) motif at the cytoplasmic side of TM 3 (Fig. 3). The most direct evidence has been obtained by Sakmar and co-workers (227) who compared wild-type rhodopsin and rhodopsin mutated in position Glu134 (III.25$^{3.49}$) by flash photolysis, allowing simultaneous measurement of photoproduct formation and rates of pH changes. Their data strongly suggested that proton uptake of Glu134 (III.25$^{3.49}$) accompanies formation of the metarhodopsin II state (227). The "protonation hypothesis" has been further supported by the observation that charge-neutralizing mutations, which mimics the unprotonated state of the aspartic acid/glutamic acid, cause dramatic constitutive activation of both the adrenergic α_{1b}-receptor and the β_2-adrenergic receptor (42, 218, 228). Similarly, improved coupling has been observed by mutation of the aspartic acid in the GnRH receptor (229). Mutation of the aspartic residue in the M_1 muscarinic receptor resulted in phosphoinositide turnover responses of the mutant that were quantitatively similar to the wild-type despite markedly lowered levels of expression (230). In parallel, constitutive activation was observed in rhodopsin after mutation of the glutamic acid found in the corresponding position of this receptor (231). Finally, it was found that charge-neutralizing mutations of the aspartic acid (Asp130; III.25$^{3.49}$) in the β_2-adrenergic receptor are linked to the overall conformation of the receptor (218). Thus, mutation of Asp130 to asparagine did not only activate the receptor but also caused a cysteine in TM 6 (Cys285, VI.12$^{6.47}$), which is not accessible in the wild-type receptor, to become accessible to methanethiosulfonate ethylammonium (MTSEA), a charged, sulfhydryl-reactive reagent (218). This observation is consistent with a counterclockwise rotation (as seen from the extracellular side) or tilting of TM 6 in the mutant receptor. Importantly, this conformational rearrangement is identical to the movement of TM 6, which biophysical studies have indicated to be essential for agonist-induced receptor activation (see next section).

The experimental data have been supported by molecular modeling and computational simulations. Two distinct hypotheses have been proposed to define the specific role of Asp/GluIII.25$^{3.49}$ protonation in receptor function, the "polar pocket" hypothesis proposed by Scheer *et al.* (42) and the "arginine cage" hypothesis proposed by Ballesteros *et al.* (229). According to the polar pocket hypothesis, the invariably conserved ArgIII.26$^{3.50}$ is in the inactive state of the receptor constrained in a pocket formed by conserved polar residues in TM 1, 2, and 7, including AsnI.18$^{1.50}$, AspII.10$^{2.50}$, AsnVII.16$^{7.49}$, and TyrVII.19$^{7.52}$ (Fig. 3). Upon protonation (or mutation to alanine) of the adjacent AspIII.25$^{3.49}$, the simulation indicated that the arginine shifts out of the polar pocket leading to long-range conformational changes in the receptor molecule (228). In their model, they highlighted that the ionic counterpart of the arginine in the inactive receptor state was the conserved aspartic acid in TM 2 (AspII.10$^{2.50}$, Fig. 3), and that this interaction is broken after receptor activation (228). Alternatively, based on computational simulations in the GnRH receptor, the arginine-cage hypothesis suggests that the ionic counterpart of ArgIII.26$^{3.50}$ in the inactive state of the receptor could be the adjacent AspIII.25$^{3.49}$ and not AspII.10$^{2.50}$ (229). It was hypothesized that during receptor activation, AspIII.25$^{3.49}$ becomes protonated and that AspII.10$^{2.50}$ substitutes for AspIII.25$^{3.49}$ in forming an ionic interaction with ArgIII.26$^{3.50}$ (229). Thus, an ionic interaction between ArgIII.26$^{3.50}$ and AspII.10$^{2.50}$ was associated with the active receptor state instead of with the inactive state as proposed by Scheer *et al.* (42). An indirect support for this alternative hypothesis is the observation in several GPCRs that mutations, which eliminate the charged character of AspII.10$^{2.50}$ and in this way conceivably destabilize the Asp-Arg interaction, also disturb functional coupling of the receptor (232–236). Spectroscopic experiments in rhodopsin have also indicated that AspII.10$^{2.50}$ is more strongly hydrogen bonded upon activation, consistent with its potential interaction with another residue in the active state of the receptor (237).

C. Conformational changes involved in receptor activation

An ultimate understanding of the receptor activation mechanism requires development of techniques that can provide insight into the character of the physical changes accompanying transition of the receptor from the inactive to the active state. Sheikh *et al.* (238) have undertaken an approach where bis-histidine metal ion-binding sites were generated between the cytoplasmic extensions of TM 3 and 6 in rhodopsin. In this way, they were able to show that cross-linking pairs of histidines with Zn^{2+} prevented transducin activation, providing indirect evidence that movements of these two domains are important for activation. Recently, they have obtained similar results in the β_2-adrenergic receptor and in the PTH receptor of which the latter belongs to family B (Fig. 1) (239). This suggests that the activation mechanism may be conserved among both family A and family B receptors (239). Javitch and co-workers (240) have applied the substituted cysteine accessibility method, in which specific advantage was taken of a constitutively activated β_2 adrenergic receptor, CAM. Their main observation in CAM was that a cysteine in TM 6 became accessible in the binding crevice to a charged, sulfhydryl-reactive reagent (240). This indicated a conformational rearrangement of TM 6 with CAM consistent with a counterclockwise rotation or tilting of the helix (240). Assuming that the conformation of CAM mimics the agonist-activated state of the receptor, the data thus indicated that movements of TM 6 are a critical element in the receptor activation mechanism.

Recently, biophysical techniques have also been implemented, allowing direct time-resolved analysis of conformational changes in the receptor molecule. It is not surprising that a majority of the studies initially have been carried out in rhodopsin. There are abundant natural sources of rhodopsin, and the inherent stability of the rhodopsin molecule makes it possible to produce and purify relatively large quantities of recombinant protein. Accordingly, several spectroscopic techniques have been applied to rhodopsin, including Fourier transform infrared resonance spectroscopy (FTIR) (241, 242), surface plasmon resonance (SPR) spectroscopy (243), tryptophan UV-absorbance spectroscopy (244), and EPR spectroscopy (61, 62, 64, 65). All approaches have consistently provided evidence for a significant conformational rearrangement accompanying transition of rhodopsin to metarhodopsin II. Using tryptophan UV-absorbance spectroscopy, Lin and Sakmar (244) were able to obtain the first direct evidence that photoactivation may involve relative movements of TM 3 and 6 (244). Thus, mutation of tryptophans in TM 3 and 6 eliminated the spectral differences in the UV absorbance spectra that distinguished rhodopsin from metarhodopsin II (244).

In a series of very elegant studies, carried out by Khorana, Hubbell, and co-workers (50, 61–65, 245), the use of EPR spectroscopy in combination with multiple cysteine substitutions has led to further insight into the character of conformational changes accompanying photoactivation of rhodopsin. Site-directed labeling of single cysteines inserted at the cytoplasmic side of the transmembrane helices with sulfhydryl-specific nitroxide spin labels provided evidence for movements particularly of the cytoplasmic termination of TM 6 upon light-induced activation of rhodopsin (50, 61–65). The spectroscopic analyses also showed evidence for smaller movements in the loop connecting TM 1 and 2 as well as at the cytoplasmic ends of TM 3 and TM 7 (61, 64, 246). Only minor or no structural changes appeared to occur at the cytoplasmic end of TM 4 and 5 (61, 62). To investigate the character of the conformational changes, Khorana, Hubbell, and co-workers have taken advantage of the magnetic dipole interaction between two nitroxide spin labels causing spectral line broadening if the two probes are less than 25 Å apart (50). Pairs of sulfhydryl-reactive spin labels were incorporated into a series of double-cysteine mutants enabling measurement of changes in relative distance between TM 3 and TM 6 (50). While the movement of TM 3 was interpreted as relatively small, the data pointed to a significant rigid-body movement of TM 6 in a counterclockwise direction (as viewed from the extracellular side) and a movement of the cytoplasmic end of TM 6 away from TM 3 (Fig. 4) (50). Importantly, movements of TM 6 in rhodopsin upon photoactivation have recently been additionally documented by site-selective fluorescent labeling of cysteines inserted at the cytoplasmic termination of the helix (247).

The first direct structural analysis of conformational changes in a GPCR activated by a diffusable ligand was recently carried out in the β_2-adrenergic receptor using fluorescence spectroscopic techniques (220, 248, 249). The spectroscopic technique that initially was applied used the sensitivity of many fluorescent molecules to the polarity of their local molecular environment (248). The sulfhydryl reactive fluorophore IANBD (*N,N′*-dimethyl-*N* (iodoacetyl)-*N′*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) ethylene-diamine) was used to label free cysteine residues in purified detergent-solubilized β_2-adrenergic receptor (248). Both the quantum yield of the emission spectrum and the decreased accessibility to hydrophilic quenchers strongly suggested that one or more of the naturally transmembrane cysteines were labeled. Exposure of IANBD-labeled receptor to agonist led to a reversible and dose-dependent decrease in emission consistent with movements of the fluorophore to a more hydrophilic environment after binding of the full agonist isoproterenol (248). Interestingly, exposure of the IANBD-labeled receptor to inverse agonists (*i.e.,* antagonists with negative intrinsic activity) led to an apparent increase in fluorescence, suggesting that not only agonists but also inverse agonists can promote structural changes in a GPCR (248).

To identify the cysteines labeled with IANBD that gave rise to the spectral changes, a series of mutant β_2 receptors with one, two, or three of the natural cysteines available for fluorescent labeling was generated (249). The fluorescence spectroscopy analysis of the purified and site-selectively labeled mutants showed that IANBD bound to Cys125 (III.20$^{3.44}$) in TM 3 and Cys285 (VI.12$^{6.47}$) in TM 6 were responsible for the observed changes in fluorescence (249). This suggests that movements of TM 3 and 6 may occur during receptor activation (249) (Fig. 4). The possible spatial orientation of IANBD bound to Cys125 (III.20$^{3.44}$) and Cys285 (VI.12$^{6.47}$) in TM 6 was explored in a series of computational simulations to define the character of the putative movements of TM 3 and 6. In a rhodopsin-based model of the

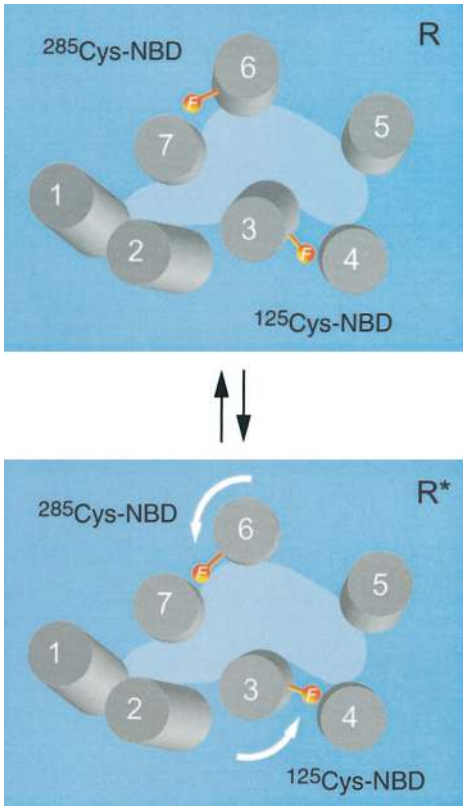

FIG. 4. Predicted conformational changes accompanying activation of family A GPCRs. The figure shows a simplified model of the β_2-adrenergic receptor based on the projection map of frog rhodopsin as seen from the extracellular side (25, 36, 38, 41). The *upper panel* illustrates the inactive receptors' state (R) while the *lower panel* indicates the anticipated conformation of the activated state (R*). The NBD fluorophore (shown in *orange*) bound to Cys125 (III.20$^{3.44}$) in TM 3 is in the inactive state of the receptor predicted to lie at the helix 3–4 interface, oriented predominantly toward the lipid (249). NBD bound to Cys285 (VI.12$^{6.47}$) is predicted to be at the helix 6–7 interface in a boundary zone between the lipid bilayer and the more polar interior of the protein (249). An agonist-induced rigid-body movement of TM 6 involving a counterclockwise rotation and a movement of the cytoplasmic end of the helix away from TM 3, as indicated by the *arrow*, would cause Cys285-NBD to be exposed to a more polar environment in the interior of the protein. Similarly, a counterclockwise rotation and/or tilting of TM 3 would cause Cys125-NBD to be exposed to a more polar face of TM 4 and/or the more polar interior of the receptor as indicated by the *arrow*. These movements explain the observed changes in fluorescence (249) and is consistent with the spin-labeling studies in rhodopsin (50).

β_2–receptor, the preferred conformation of IANBD attached to Cys125 (III.20$^{3.44}$), as defined by the computational simulations, is bounded by the lipid bilayer and the interface of TM 3 and TM 4, while the IANBD attached to Cys285 (VI.12$^{6.47}$) is predicted to be at the helix 6-7 interface in a boundary zone between the lipid bilayer and the more polar interior of the protein (249) (Fig. 4). In the framework of this model, the change in fluorescence of IANBD-labeled β_2-adrenergic receptor can best be explained by a counterclockwise rotation of both TM 3 and TM 6, which would move the IANBD molecules from the nonpolar lipid environment to the more polar interior of the protein (249) (Fig. 4). Of interest, Cys285 (VI.12$^{6.47}$) is situated one α-helical turn below Pro288 (VI.15$^{6.50}$), which is highly conserved among GPCRs and provides a flexible hinge in TM 6. It has been speculated, therefore, that the movement of Cys-NBD to a more polar environment in the protein interior is directly facilitated by this flexible hinge connecting residues involved in agonist binding in the outer part of TM 6 with the putative G protein-coupling domain in the cytoplasmic extension of the helix (249). Notably, site-selective incorporation of the NBD fluorophore in a new series of single-cysteine mutants of the β_2-adrenergic receptor has recently documented significant agonist-promoted conformational changes corresponding to this cytoplasmic extension of TM 6 (A.D. Jensen and U. Gether, to be published).

In summary, the spectroscopic studies in rhodopsin and in the β_2-adrenergic receptor clearly support a critical role of TM 3 and 6 for transition of GPCRs to their activated state (Fig. 3). Importantly, the agreement between the data obtained in rhodopsin and in the β_2-adrenergic receptor also strongly indicates that the activation mechanism in many aspects is similar at least among type A GPCRs. It should, however, be emphasized that the established importance of TM 3 and 6 does not exclude that movements of other domains may contribute to receptor activation. For example, there is evidence based on EPR spectroscopy in rhodopsin that movements of TM 7 may also occur in response to photoactivation (64). The possible importance of TM 7 in receptor activation is also indirectly supported by the very recent observation that an activating metal ion-binding site can be generated between TM 3 and 7 in the β_2-adrenergic receptor (250).

D. How is the activation signal transmitted to the G protein?

A myriad of studies involving chimeric substitutions, various other mutational approaches, and the use of synthetic peptides have in many receptors provided considerable insight into the structural elements important for the interaction with the G protein. The literature describing these studies have been reviewed several times (16, 17, 20, 251–253) and will therefore be discussed only briefly here. Summarized, the studies have established the pivotal roles of the second (ICL2) and the third intracellular (ICL3) loops plus, at least in some receptors, the proximal part of the carboxy terminus in G protein coupling (16, 17, 20, 251–253). Chimeric approaches, applied in the adrenergic and muscarinic systems, clearly defined that ICL3 is the key determinant of coupling specificity among the different G protein α-subunits (16, 17, 20, 251–253). Subsequent point mutational analyses in many receptors have identified residues crucial for selective G protein coupling clustering in the amino-terminal part of ICL3 adjacent to TM 5 (252, 254–257) and in the carboxy-terminal part of ICL3 adjacent to TM 6 (258–260). In contrast to ICL3, ICL2 is less important for determining G protein specificity but is important for the efficiency of G protein activation (16, 17, 20, 251–253). The role of ICL2 has recently been convincingly substantiated by Brann and co-workers, who developed a random mutagenesis approach for their study of muscarinic receptor coupling (215). In ICL2 of the M5 muscarinic receptor, they found that substitution of residues clustering on one side of a presumed ICL2 α-helix extending

from TM 3 caused constitutive activation, while substitutions of residues clustering on the opposite side of the helix compromised G protein coupling. Taken together, the data suggest that the residues on the constitutively activating side were critical for maintaining the receptor in an inactive state, whereas the residues on the opposing side were important for G protein activation (215). It was therefore inferred that ICL 2 could act as a switch that enables G protein coupling (215). Notably, this hypothesis is consistent both with role in receptor activation of the adjacent DRY motif (42, 218, 229) and the predicted movements of TM 3 relative to TM 6 from spectroscopic analyses (50, 249). Interestingly, the aspartic acid of the DRY motif (AspIII.25$^{3.49}$), which is believed to undergo protonation during receptor activation (see *Section V.B.*), is located on the same side of the helix as the residues found to cause constitutive activation (Fig. 2).

Despite the abundance of information acquired over the last decade, the mechanisms by which the signal is transmitted from the activated receptor to the G protein heterotrimer remains, nevertheless, surprisingly elusive. Recently, x-ray crystallography has provided substantial insight into the tertiary structure of the heterotrimeric G proteins (261, 262), but still little is known about the actual points of interactions between the receptor and the G protein and, thus, how the two proteins are oriented relative to one another. So far, only the interaction between the carboxy terminus of the G protein α-subunit and the carboxy-terminal part of IC3 seems reasonably well substantiated from mutagenesis studies (259). Based on the currently available data, an orientation of the G protein relative to the plasma membrane has been proposed placing the nucleotide-binding domain of the α-subunit approximately 30 Å away from the membrane (261–263). According to this, the receptor must induce GDP release from the α-subunit without directly interacting with the nucleotide-binding domain. It has been speculated that the suggested movements of TM 3 and 6 apart from each other during receptor activation (Fig. 4) could allow insertion of the α-subunit carboxy terminus into a cavity in the seven-helix bundle (263). Conceivably, this could trigger structural changes in the adjacent α5-helix and β6-strand that are transmitted to the nucleotide-binding domain via the α5/β6 loop, which is in the immediate vicinity of the guanine nucleotide (263).

E. Receptor dimerization—an artifact or a functional necessity?

It is well known that receptor dimerization is required for signal transduction in other classes of receptors, *e.g.*, receptor tyrosine kinases. An increasing number of studies have shown that many GPCRs also form dimers. For example, formation of receptor *homodimers* has been reported for the β_2-adrenergic receptor (264), the δ-opioid receptor (265), the dopamine D_1, D_2, and D_3 receptors (266–268), the chemokine receptors CCR2b, CCR4, and CCR5 (269, 270), the extracellular calcium-sensing receptor (271, 272), and the metabotropic glutamate receptor 5 (273). It has been demonstrated moreover that functional receptor dimers can be formed by coexpressing two reciprocal nonfunctional chimeras constructed between the α_{2C}-adrenergic receptor and the M_3 muscarinic receptor (274). However, the molecular mechanisms of dimer formation seem to differ considerably among the receptors. In the β_2-adrenergic receptor, dimerization most likely involves interactions between transmembrane segments since a peptide derived from transmembrane segment 6 has been shown to inhibit dimer formation (264). Similarly, peptides derived from the transmembrane domains of the dopamine D_2 receptor dissociated dimers to monomers (266), but a peptide derived from TM 6 of the dopamine D_1 receptor did not affect dimerization of this receptor (268). For the δ-opioid receptor, dimerization was eliminated by deletion of 15 amino acids in the carboxy terminus, indicating the involvement of this part of the receptor in dimerization (265). In contrast, dimerization of the metabotropic glutamate receptors and the extracellular calcium-sensing receptor was found to be dependent on intermolecular disulfide bonds between cysteines in their large amino-terminal domains (271–273).

An intriguing observation has been that agonist can stabilize the dimeric form of several receptors including the β_2-adrenergic receptor (264) and the chemokine receptors CCR2b, CCR4, and CCR5 (269, 270). This suggests that homodimerization could have a role either directly in the receptor activation mechanism or, alternatively, in the subsequent agonist-dependent densitization and internalization process. For the CCR2b receptor, evidence suggests that dimerization does have a direct role in agonist-mediated receptor activation (270). First, it was found that the CCR2b receptor can only be activated by the bivalent form of an agonistic monoclonal antibody directed against the CCR2b receptor and not by the corresponding monovalent Fab fragment (270). Second, it was demonstrated that coexpression of wild-type CCR2b with a coupling-deficient mutant (CCR2/Y139F) eliminated any functional coupling in response to the endogenous agonist of the CCR2b receptor, monocyte chemoattractant protein 1 (MCP-1) (270). Hence, the mutant acted as a dominant negative mutant, indicating that dimerization is a prerequisite for ligand-induced CCR2b signaling (270). For the calcium-sensing receptor there is also experimental support for a role of dimerization in receptor activation (275). In a recent study it was shown that elimination of dimerization, by mutating the two cysteines believed to form intermolecular disulfide bridges between the extracellular domains, resulted in a receptor with lowered calcium affinity and much slower kinetics of the responses to calcium. However, as yet there is no evidence supporting a universal role of dimerization for GPCR activation. In the case of the δ-opioid receptor, it has been observed, for example, that agonists *decrease* the level of dimer formation (265).

Recently, substantial evidence has accumulated demonstrating the possible importance of *heterodimerization* between closely related receptor subtypes (276–279). The $GABA_B$ R1 receptor subtype is mostly retained inside the cell as an immature glycoprotein when expressed in mammalian cells and displays low affinity for agonists (276–279). However, if it is coexpressed with the newly discovered $GABA_B$ R2 receptor, a fully functional and terminally glycosylated receptor can be detected at the cell surface (276–279). The data indicate that heterodimerization can be critical for targeting functional receptors to the cells surface and, thus, that

the *in vivo* functional $GABA_B$ receptor could be a heterodimer of $GABA_B$ R1 and $GABA_B$ R2 (276–279). An additional intriguing example, which indicates a functional relevance of heterodimerization between receptor subtypes, is the observation that formation of heterodimers between two fully functional opioid receptors, δ and κ, results in a new receptor that displays binding and functional properties distinct from those of either of the receptors (280). It is of interest moreover to note that heterodimerization between the wild-type CCR5 receptor and the naturally occurring nonfunctional mutant of the CCR5 receptor, ccr5δ32, has been shown to inhibit targeting of the wild-type receptor to the cell surface after coexpression in HeLa cells (281). Since CCR5 acts as a coreceptor for HIV infection, the inhibition of wild-type receptor surface expression by the mutant was proposed as a molecular explanation for the delayed onset of AIDS in heterozygotic (CCR5/ccr5δ32) individuals (281). Finally, it should be mentioned in this context that a family of accessory single-transmembrane proteins, RAMPs (receptor-activity-modifying proteins), has been identified and found to complex with the calcitonin-receptor-like receptor (CRLR). The association of CRLR with RAMPs was found not only to play a role in targeting the receptor to the cell surface, but also to modify the pharmacological properties of the receptor. While RAMP1 converted CRLR into a calcitonin-gene-related-peptide (CGRP) receptor, RAMP2-associated receptors display the properties of an adrenomedullin receptor (282). To what degree such mechanisms also may account for the function of other GPCRs remains obscure and needs to be clarified in the future.

VI. Models of Receptor Activation

A. The two-state model of receptor activation vs. multistate models of receptor activation

The currently most widely accepted model for GPCR activation is the extended ternary complex model (often referred to simply as the two-state model) (205, 283, 284). This model was proposed in light of the discovery that receptors in the absence of agonist spontaneously can adopt an active conformation and couple to the G protein (205, 283, 284). Importantly, the model both accommodates the phenomenon of agonist-independent receptor activity and the complex behavior of various classes of ligands (agonists, partial agonists, neutral antagonists, and inverse agonists). According to the model, the receptor exists in an equilibrium between an inactive conformation (R) and an active conformation (R*) (205). In the absence of agonist, the inactive R state is prevailing; however, the energy barrier between the R and R* state is sufficiently low, allowing a certain fraction of the receptors spontaneously to assume the R* state. Agonists are predicted to bind with highest affinity to the R* conformation and in this way shift the equilibrium and increase the proportion of receptor in R*. Conversely, inverse agonists (also called negative antagonists), *i.e.*, compounds possessing the ability to inhibit agonist-independent receptor activity, are predicted to stabilize the inactive R state, shifting the equilibrium away from R*. Neutral antagonists, according to the model, are defined as compounds that bind with the same affinity to both R and R* and thus cause no change in the equilibrium (205).

It is becoming increasingly clear that the two-state model cannot sufficiently explain the complex behavior of GPCRs. Several lines of evidence have provided strong support that GPCRs may exist in possibly multiple conformational states (42, 146, 201, 285–288). For example, the nonoverlapping binding sites between peptide agonists and nonpeptide antagonists, proposed for some receptors (see *Section IV.C.*), cannot be reconciled with a simple two-state model (24). Similarly, a two-state model cannot explain how mutation of certain serines in TM 5 of the dopamine D_2 receptor can lead to loss of functional coupling in response to some agonists, but not others, with only modest effect on their affinity (287). Furthermore, different synthetic agonists of the *Drosophila* D_1-like dopamine receptor have been shown to induce selective coupling to distinct second messenger pathways (286). It is also difficult to explain within a simple two-state model how β_2 receptor ligands can act as partial agonists or inverse agonists depending on whether the functional assay is performed in membranes or intact cells (201). An additional interesting finding, strongly supporting the existence of more than one active receptor state, has been the observation that different constitutively active mutants of the α_{1B}-receptor are differentially phosphorylated and internalized although they convey a similar agonist-independent activity to the receptor (288). Finally, more direct structural evidence has been obtained by fluorescence spectroscopy analysis of the purified β_2-adrenergic receptor, which indicated that most ligands promote alterations in receptor structure consistent with the existence of multiple ligand-specific conformational states (146).

Evidently, receptor activation models that incorporate the existence of several or multiple conformational states have recently been suggested (24, 42, 285, 289). In the multistate model proposed by Schwartz *et al.* (24) the receptor is proposed to alternate spontaneously between multiple active and inactive conformations. The key element in this model is that the biological response to a given ligand is determined by the conformation to which the ligand binds with highest affinity. If the preferred conformation is recognized by the G protein as active, the compound would behave like an agonist, and if the preferred conformation is inactive, the ligand would behave like an inverse agonist. The important impact of the model is, obviously, that there is no requirement for a common binding mode for agonist to trigger receptor activation. Even two agonists acting at the same receptor do not have to share (although they probably often would) an overlapping binding site; they both must stabilize an active conformation (24). For example, a peptide agonist may be able to stabilize an active state by interacting with the extracellular loop regions while a small molecule agonist of the same receptor could stabilize the same or another active configuration by penetrating into the transmembrane-binding crevice. Similarly, the model does not require any overlap in binding site between the agonist and a competitive antagonist. The agonist and antagonist can be envisioned simply to stabilize distinct receptor conformations to which the agonist and antagonist bind in a mutually exclusive fashion (24). Kinetically this would be indistinguishable from a classical

competitive situation with overlapping binding sites between the agonist and antagonists (24).

B. Implications from biophysical studies on receptor activation models

The recent biophysical analyses of conformational changes in rhodopsin and in the β_2-adrenergic receptor have provided novel insight into the critical conformational changes accompanying receptor activation. However, the data also raise new interesting questions about molecular modes of agonist-induced receptor activation. As discussed in *Section V.C*, spectroscopic studies of conformational changes in both rhodopsin and the β_2-adrenergic receptor suggest that similar movements are important for activation of both receptors. Otherwise, there are substantial differences underlying activation of rhodopsin compared with the β_2-adrenergic receptor. Rhodopsin is unique in that its ligand, *cis*-retinal, is covalently bound to the receptor as an inverse agonist and upon absorption of a photon isomerizes to an agonist (*trans*-retinal) within the binding pocket (reviewed in Ref. 66). In other words, ligand binding is not part of the activation process. This specialized mechanism of activation may be necessary to facilitate the very rapid response of rhodopsin to light. Thus, formation of the activated metarhodopsin II state occurs essentially within microseconds even in detergent solution in the absence of transducin (290). Interestingly, metarhodopsin II subsequently undergoes a slow ($t_{1/2} \sim 6$ min) transition to the inactive metarhodopsin III (290). During this inactivating transition *trans*-retinal undergoes hydrolysis and release from the binding pocket (291). Remarkably, free *trans*-retinal is not a very effective agonist for opsin, producing only approximately 14% of the response observed for light-activated rhodopsin (292). This shows that efficient activation of rhodopsin by *trans*-retinal requires that *cis*-retinal is prebound and that *cis*-retinal can be rapidly converted to *trans*-retinal by photoisomerization. The less efficient activation of opsin by free *trans*-retinal may more closely reflect the process of activation of other GPCRs.

In contrast to the rapid activation and the slow inactivation kinetics observed for rhodopsin, spectroscopic analyses of the purified β_2-adrenergic receptor labeled with a conformationally sensitive fluorophore revealed slow agonist-induced conformational changes ($t_{1/2} \sim 2$–3 min), significantly slower than the predicted association rate of the agonist (220, 248, 249). However, the reversal of the agonist-induced conformational change was relatively fast ($t_{1/2} \sim 30$ sec) (220, 248, 249). It should be emphasized that the slow activation kinetics now have been observed in several different readouts. Thus, the agonist-induced spectral changes observed after labeling of cysteines introduced at the cytoplasmic side of TM 6 occur with similar kinetics as that observed after labeling of the endogenous cysteines (Cys125 and Cys285) (A.D. Jensen and U. Gether, to be published). It is possible that the differences between rhodopsin and the β_2-adrenergic receptor are caused by differences in the methodological approach. However, since the measurements were performed under similar conditions (in detergent solution in the absence of G protein) it is more likely that they reflect inherent differences between rhodopsin and a receptor activated by a diffusable ligand.

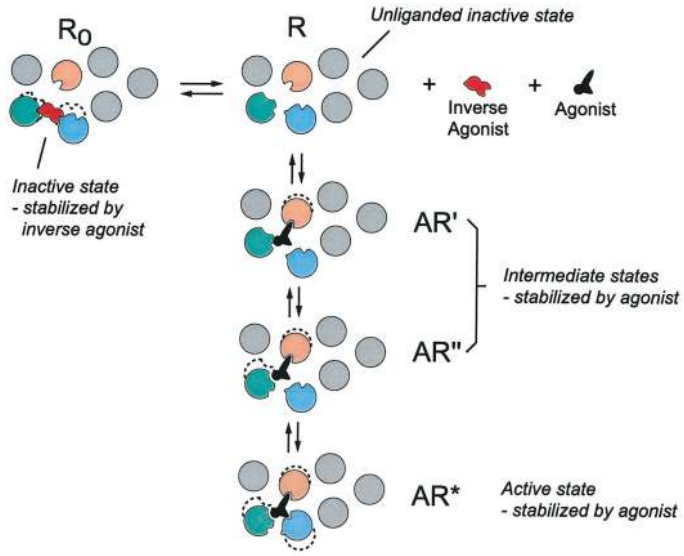

FIG. 5. Sequential binding and conformational stabilization model for the molecular mechanisms of ligand action in GPCRs. The hypothetical receptor is illustrated by seven apparent helices seen from above. The model predicts that the unliganded receptor exists in a unique state R that can undergo transitions to at least two other states R_0 and R*. R_0 is stabilized by inverse agonists and R* is stabilized by agonists. R may undergo spontaneous transitions to the R* state, explaining the high basal activity observed for some GPCRs, and it may undergo spontaneous transition to the R_0. As discussed in the text, binding of the agonist is suggested to occur sequentially, resulting in a series of conformational states that are intermediates (R′ and R″) between R and R*. The agonists are known to have several functionally important sites of interaction with the receptor. Binding may involve an initial interaction between receptor and one structural group of the agonist. After the initial binding of one structural group, binding of the remaining groups occurs in a sequential manner as a result of random and spontaneous movements of TM domains to positions that permit interaction with the functional groups. Each interaction between the receptor and the agonist stabilize one or more TM domains until the receptor has been stabilized in the active R* state. A similar mode of binding can be envisioned for inverse agonists resulting in stabilization of the R_0 state. The model would be consistent both with a rapid association rate for agonists (formation of AR′) and the relatively slow rate of conformational change observed spectroscopically (formation of AR*). Importantly, the G protein may substantially affect the kinetics of the transition from AR′ over AR″ to AR*. Similar to the multistate model described in Ref. 24, the model also readily accommodates the concept of "allosteric competitive antagonism" (24) *i.e.,* that a competitive antagonist does not have to share an overlapping binding site with the agonist. Hence, an "allosteric competitive" antagonist, according to the model, would simply act by stabilizing the receptor in R_0, which would not be expected to bind the agonist. Conversely, the agonist could stabilize the receptor in R*, which would not be expected to bind the antagonist. In this way, by stabilizing different receptor conformations, the agonist and the antagonist can mutually exclude the binding of each other to the receptor.

The observed slow activation kinetics cannot be readily accommodated into a simple "two-state model". According to this model the affinity of a full agonist for the R state is negligible; thus, agonist binding occurs selectively to the activated state R*, thereby pulling the equilibrium toward R*. This would predict that the association rate for agonist binding is limited by the rate of transition from R to R*. This is

not readily compatible with the observation in the β_2-adrenergic receptor that the conformational change, and not the binding event, is the rate-limiting step. We have therefore suggested the "sequential binding and conformational selection" model shown in Fig. 5 (22). This model predicts, similar to the two-state model (205) and the multistate model suggested by Schwartz *et al.* (24), that the receptor spontaneously alternates between different receptor conformations (active and inactive). However, a major difference is that binding of agonist does not occur directly to R* but is suggested to occur sequentially, resulting in a series of conformational states that are intermediates (R′ and R″) between R and R* (Fig. 5). Agonists are known to have several functionally important sites of interaction with the receptor (See *Section IV.A*). As illustrated in Fig. 5, binding may involve an initial interaction between receptor and one structural group of the agonist. After the initial binding of one structural group, binding of the remaining groups occurs in a sequential manner as a result of random and spontaneous movements of TM domains to positions that permit interaction with the functional groups. Each interaction between the receptor and the agonist stabilize one or more transmembrane domains until the agonist finally stabilizes the receptor in the active R* state. Such a model would be consistent both with a rapid association rate for agonists (formation of AR′) and the relatively slow rate of conformational change observed spectroscopically (formation of AR*). Importantly, the G protein may substantially affect the kinetics of the transition from AR′ over AR″ to AR*. The slow kinetics of the agonist-induced conformational change in the absence of G protein strongly suggests the existence of a high activation energy barrier for the transition from AR′ through AR″ to AR*. The R* state can from a thermodynamic point of view be considered a high-energy intermediate that can be stabilized energetically by the G protein and/or the agonist (220). It is conceivable that the G protein stabilizes the AR* state and, in addition, substantially lowers the activation energy barrier, causing the transition from AR′ through AR″ to AR* to occur much faster. The hypothesis awaits experimental evaluation in a reconstituted system with purified receptor and G protein. Nevertheless, it provides an intriguing explanation for the apparent discrepancy between the slow kinetics of agonist-induced conformational changes observed for the purified β_2-adrenergic receptor with the rapid responses to agonist stimulation of GPCRs in cells, such as, for example, activation of ion channels.

VII. Concluding Remarks

The wealth of information gained over the last decade has substantially improved our understanding of GPCR function and changed the way we look at receptors. Importantly, it has been conceptualized that GPCRs are not simple "on/off" switches but highly dynamic structures that exist in equilibriums between active and inactive conformations. In this framework, an agonist is recognized as a molecule that can stabilize an active conformation while an inverse agonist (*i.e.*, an antagonist with negative intrinsic activity) is a molecule that can stabilize an inactive conformation. Thus, it has become clear that not only agonist but also antagonists are capable of actively modulating receptor function. Moreover, it has become evident that neither agonists nor antagonists necessarily have to share an overlapping binding site, even if they act at the same receptor. An important implication of this in clinical endocrinology is the prospect of developing small-molecule antagonists and agonists for, in principle, any GPCR. Recent biophysical studies allowing direct structural analyses of conformational change in the receptor molecule represent an important first step toward a more profound understanding of GPCR function at a molecular level. However, our present knowledge about the physical changes in the receptor structure, distinguishing inactive from active states, is still very limited. Furthermore, our insight into the molecular basis for transmission of the signal to the G protein remains rather poor. The further clarification of these mechanisms represents a daunting task together with efforts aimed at obtaining high-resolution x-ray crystals.

Acknowledgments

Drs. Thue W. Schwartz and Brian K. Kobilka are thanked for genuine inspiration and support. Pejman Ghanouni and Dr. Anne Dam Jensen are thanked for helpful comments on the manuscript. Dr. Juan Ballesteros is thanked for providing the picture of the β_2-receptor model shown in Fig. 2C.

References

1. **Kolakowski Jr LF** 1994 GCRDb: a G-protein-coupled receptor database. Receptors Channels 2:1–7
2. **Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS** 1999 Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 96:541–551
3. **Bourne HR, Sanders DA, McCormick F** 1991 The GTPase superfamily: conserved structure and molecular mechanism. Nature 349: 117–127
4. **Hamm HE** 1998 The many faces of G protein signaling. J Biol Chem 273:669–672
5. **Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, Lefkowitz RJ** 1996 Role of c-Src tyrosine kinase in G protein-coupled receptor- and G$\beta\gamma$ subunit-mediated activation of mitogen-activated protein kinases. J Biol Chem 271:19443–19450
6. **Daaka Y, Luttrell LM, Ahn S, Della Rocca GJ, Ferguson SS, Caron MG, Lefkowitz RJ** 1998 Essential role for G protein-coupled receptor endocytosis in the activation of mitogen-activated protein kinase. J Biol Chem 273:685–688
7. **Lefkowitz RJ** 1998 G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J Biol Chem 273:18677–18680
8. **Hall RA, Premont RT, Chow CW, Blitzer JT, Pitcher JA, Claing A, Stoffel RH, Barak LS, Shenolikar S, Weinman EJ, Grinstein S, Lefkowitz RJ** 1998 The β2-adrenergic receptor interacts with the Na^+/H+-exchanger regulatory factor to control Na+/H+ exchange. Nature 392:626–630
9. **Mitchell R, McCulloch D, Lutz E, Johnson M, MacKenzie C, Fennell M, Fink G, Zhou W, Sealfon SC** 1998 Rhodopsin-family receptors associate with small G proteins to activate phospholipase D. Nature 392:411–414
10. **Luttrell LM, Ferguson SSG, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG, Lefkowitz RJ** 1999 Beta-arrestin-dependent formation of beta2 adrenergic receptor-src protein kinase complexes. Science 283:655–661
11. **Krupnick JG, Benovic JL** 1998 The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu Rev Pharmacol Toxicol 38:289–319
12. **Feng Y, Broder CC, Kennedy PE, Berger EA** 1996 HIV-1 entry

cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272:872–877
13. **Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR** 1996 Identification of a major co-receptor for primary isolates of HIV-1. Nature 381:661–666
14. **Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA** 1996 CC CKR5: a RANTES, MIP-1alpha, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272:1955–1958
15. **Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J** 1996 The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 85:1135–1148
16. **Kobilka B** 1992 Adrenergic receptors as models for G protein-coupled receptors. Annu Rev Neurosci 15:87–114
17. **Savarese TM, Fraser CM** 1992 *In vitro* mutagenesis and the search for structure-function relationships among G protein-coupled receptors. Biochem J 283:1–19
18. **Schwartz TW** 1994 Locating ligand-binding sites in 7TM receptors by protein engineering. Curr Opin Biotech 5:434–444
19. **Strader CD, Fong TM, Graziano MP, Tota MR** 1995 The family of G-protein-coupled receptors. FASEB J 9:745–754
20. **Wess J** 1997 G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. FASEB J 11:346–354
21. **Ji TH, Grossmann M, Ji I** 1998 G protein-coupled receptors. I. Diversity of receptor-ligand interactions. J Biol Chem 273:17299–17302
22. **Gether U, Kobilka BK** 1998 G protein-coupled receptors. II. Mechanism of agonist activation. J Biol Chem 273:17979–17982
23. **Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon SC** 1992 Sequence alignment of the G-protein coupled receptor superfamily. DNA Cell Biol 11:1–20
24. **Schwartz TW, Gether U, Schambye HT, Hjorth SA** 1995 Molecular mechanism of action of non-peptide ligands for peptide receptors. Curr Pharm Design 1:325–342
25. **Baldwin JM, Schertler GF, Unger VM** 1997 An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. J Mol Biol 272:144–164
26. **Ballesteros JA, Weinstein H** 1995 Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein coupled receptors. Methods Neurosci 25:366–428
27. **Ulrich CD, Holtmann M, Miller LJ** 1998 Secretin and vasoactive intestinal peptide receptors: members of a unique family of G protein coupled receptors. Gastroentoerology 114:382–397
28. **O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL, Mulvihill ER** 1993 The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. Neuron 11:41–52
29. **Conn PJ, Pin JP** 1997 Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205–237
30. **Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH** 1990 Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 213: 899–929
31. **Hibert MF, Trumpp-Kallmeyer S, Bruinvels A, Hoflack J** 1991 Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. Mol Pharmacol 40:8–15
32. **Trumpp-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M** 1992 Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. J Med Chem 35:3448–3462
33. **MaloneyHuss K, Lybrand TP** 1992 Three-dimensional structure for the beta 2 adrenergic receptor protein based on computer modeling studies. J Mol Biol 225:859–871
34. **Cronet P, Sander C, Vriend G** 1993 Modeling of transmembrane seven helix bundles. Protein Eng 6:59–64
35. **Roper D, Jacoby E, Kruger P, Engels M, Grotzinger J, Wollmer A, Strassburger W** 1994 Modeling of G-protein coupled receptors with bacteriorhodopsin as a template. A novel approach based on interaction energy differences. J Recept Res 14:167–186
36. **Schertler GF, Villa C, Henderson R** 1993 Projection structure of rhodopsin. Nature 362:770–772
37. **Unger VM, Schertler GF** 1995 Low resolution structure of bovine rhodopsin determined by electron cryo-microscopy. Biophys J 68: 1776–1786
38. **Unger VM, Hargrave PA, Baldwin JM, Schertler GF** 1997 Arrangement of rhodopsin transmembrane α-helices. Nature 389: 203–206
39. **Krebs A, Villa C, Edwards PC, Schertler GF** 1998 Characterisation of an improved two-dimensional p22121 crystal from bovine rhodopsin. J Mol Biol 282:991–1003
40. **Davies A, Schertler GF, Gowen BE, Saibil HR** 1996 Projection structure of an invertebrate rhodopsin. J Struct Biol 117:36–44
41. **Baldwin JM** 1993 The probable arangement of the helices in G protein coupled receptors. EMBO J 12:1693–1703
42. **Scheer A, Fanelli F, Costa T, De Benedetti PG, Cotecchia S** 1996 Constitutively active mutants of the alpha 1B-adrenergic receptor: role of highly conserved polar amino acids in receptor activation. EMBO J 15:3566–3578
43. **Suryanarayana S, Von Zastrow M, Kobilka BK** 1992 Identification of intramolecular interaction in adrenergic receptors. J Biol Chem 267:21991–21994
44. **Pittel Z, Wess J** 1994 Intramolecular interactions in muscarinic acetylcholine receptors studied with chimeric m2/m5 receptors. Mol Pharmacol 45:61–64
45. **Liu J, Schoneberg T, van Rhee M, Wess J** 1995 Mutational analysis of the relative orientation of transmembrane helices I and VII in G protein-coupled receptors. J Biol Chem 270:19532–19539
46. **Mizobe T, Maze M, Lam V, Suryanarayana S, Kobilka BK** 1996 Arrangement of transmembrane domains in adrenergic receptors. Similarity to bacteriorhodopsin. J Biol Chem 271:2387–2389
47. **Elling CE, Schwartz TW** 1996 Connectivity and orientation of the seven helical bundle in the tachykinin NK-1 receptor probed by zinc site engineering. EMBO J 15:6213–6219
48. **Elling CE, Nielsen SM, Schwartz TW** 1995 Conversion of antagonist-binding site to metal-ion site in the tachykinin NK-1 receptor. Nature 374:74–77
49. **Thirstrup K, Elling CE, Hjorth SA, Schwartz TW** 1996 Construction of a high affinity zinc switch in the κ-opioid receptor. J Biol Chem 271:7875–7878
50. **Farrens DL, Altenbach C, Yang K, Hubbell WL, Khorana HG** 1996 Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 274:768–770
51. **Yu H, Kono M, McKee TD, Oprian DD** 1995 A general method for mapping tertiary contacts between amino acid residues in membrane-embedded proteins. Biochemistry 34:14963–14969
52. **Zeng FY, Hopp A, Soldner A, Wess J** 1999 Use of a disulfide cross-linking strategy to study muscarinic receptor structure and mechanisms of activation. J Biol Chem 274:16629–16640
53. **Turcatti G, Nemeth K, Edgerton MD, Meseth U, Talabot F, Peitsch M, Knowles J, Vogel H, Chollet A** 1996 Probing the structure and function of the tachykinin neurokinin-2 receptor through biosynthetic incorporation of fluorescent amino acids at specific sites. J Biol Chem 271:19991–19998
54. **Zhou W, Flanagan C, Ballesteros JA, Konvicka K, Davidson JS, Weinstein H, Millar RP, Sealfon SC** 1994 A reciprocal mutation supports helix 2 and helix 7 proximity in the gonadotropin-releasing hormone receptor. Mol Pharmacol 45:165–170
55. **Fu D, Ballesteros JA, Weinstein H, Chen J, Javitch JA** 1996 Residues in the seventh membrane-spanning segment of the dopamine D2 receptor accessible in the binding-site crevice. Biochemistry 35:11278–11285
56. **Javitch JA, Fu D, Chen J, Karlin A** 1995 Mapping the binding-site crevice of the dopamine D2 receptor by the substituted-cysteine accessibility method. Neuron 14:825–831
57. **Javitch JA, Fu D, Chen J** 1995 Residues in the fifth membrane-spanning segment of the dopamine D2 receptor exposed in the binding-site crevice. Biochemistry 34:16433–16439
58. **Javitch JA, Ballesteros JA, Weinstein H, Chen J** 1998 A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D2 receptor is accessible in the binding-site crevice. Biochemistry 37:998–1006
59. **Javitch JA, Ballesteros JA, Chen J, Chiappa V, Simpson MM** 1999

Electrostatic and aromatic microdomains within the binding-site crevice of the D2 receptor: contributions of the second membrane-spanning segment. Biochemistry 38:7961–7968
60. **Strader CD, Candelore MR, Hill WS, Sigal IS, Dixon RAF** 1989 Identification of two serine residues involved in agonist activation of the beta adrenergic receptor. J Biol Chem 264:13572–13578
61. **Farahbakhsh ZT, Ridge KD, Khorana HG, Hubbell WL** 1995 Mapping light-dependent structural changes in the cytoplasmic loop connecting helices C and D in rhodopsin: a site-directed spin labeling study. Biochemistry 34:8812–8819
62. **Altenbach C, Yang K, Farrens DL, Farahbakhsh ZT, Khorana HG, Hubbell WL** 1996 Structural features and light-dependent changes in the cytoplasmic interhelical E-F loop region of rhodopsin: a site-directed spin-labeling study. Biochemistry 35:12470–12478
63. **Langen R, Cai K, Altenbach C, Khorana HG, Hubbell WL** 1999 Structural features of the C-terminal domain of bovine rhodopsin: a site-directed spin-labeling study. Biochemistry 38:7918–7924
64. **Altenbach C, Cai K, Khorana HG, Hubbell WL** 1999 Structural features and light-dependent changes in the sequence 306–322 extending from helix VII to the palmitoylation sites in rhodopsin: a site-directed spin-labeling study. Biochemistry 38:7931–7937
65. **Altenbach C, Klein-Seetharaman J, Hwa J, Khorana HG, Hubbell WL** 1999 Structural features and light-dependent changes in the sequence 59–75 connecting helices I and II in rhodopsin: a site-directed spin-labeling study. Biochemistry 38:7945–7949
66. **Sakmar TP** 1998 Rhodopsin: a prototypical G protein-coupled receptor. Prog Nucleic Acid Res Mol Biol 59:1–34
67. **Zhukovsky EA, Robinson PR, Oprian DD** 1992 Changing the location of the Schiff base counterion in rhodopsin. Biochemistry 31:10400–10405
68. **Han M, Groesbeek M, Sakmar TP, Smith SO** 1997 The C9 methyl group of retinal interacts with glycine-121 in rhodopsin. Proc Natl Acad Sci USA 94:13442–13447
69. **Nakayama TA, Khorana HG** 1991 Mapping of the amino acids in membrane-embedded helices that interact with the retinal chromophore in bovine rhodopsin. J Biol Chem 266:4269–4275
70. **Han M, Lou J, Nakanishi K, Sakmar TP, Smith SO** 1997 Partial agonist activity of 11-cis-retinal in rhodopsin mutants. J Biol Chem 272:23081–23085
71. **Tota RT, Strader CD** 1990 Characterization of the binding domain of the beta-adrenergic receptor with the fluorescent antagonist carazolol. J Biol Chem 265:16891–16897
72. **Strader CD, Gaffney T, Sugg EE, Candelore MR, Keys R, Patchett AA, Dixon RAF** 1991 Allele-specific activation of genetically engineered receptors. J Biol Chem 266:5–8
73. **Mansour A, Meng F, Meador-Woodruff JH, Taylor LP, Civelli O, Akil H** 1992 Site-directed mutagenesis of the human dopamine D2 receptor. Eur J Pharmacol 227:205–214
74. **Wang CD, Gallaher TK, Shih JC** 1993 Site-directed mutagenesis of the serotonin 5-hydroxytrypamine2 receptor: identification of amino acids necessary for ligand binding and receptor activation. Mol Pharmacol 43:931–940
75. **Ho BY, Karschin A, Branchek T, Davidson N, Lester HA** 1992 The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT1A receptor: a site-directed mutation study. FEBS Lett 312:259–262
76. **Gantz I, DelValle J, Wang LD, Tashiro T, Munzert G, Guo YJ, Konda Y, Yamada T** 1992 Molecular basis for the interaction of histamine with the histamine H2 receptor. J Biol Chem 267:20840–20843
77. **Spalding TA, Birdsall NJ, Curtis CA, Hulme EC** 1994 Acetylcholine mustard labels the binding site aspartate in muscarinic acetylcholine receptors. J Biol Chem 269:4092–4097
78. **Tota RT, Candelore MR, Dixon RAF, Strader CD** 1990 Biophysical and genetic analysis of the ligand-binding site of the beta-adrenoceptor. Trends Pharmacol Sci 12:4–6
79. **Wieland K, Zuurmond HM, Krasel C, Ijzerman AP, Lohse MJ** 1996 Involvement of Asn-293 in stereospecific agonist recognition and in activation of the β2-adrenergic receptor. Proc Natl Acad Sci USA 93:9276–9281
80. **Suryanarayana S, Daunt DA, Von Zastrow M, Kobilka BK** 1991 A point mutation in the seventh hydrophobic domain of the alfa-2 adrenergic receptor increases its affinity for a family of β-receptor antagonists. J Biol Chem 266:15488–15492
81. **Zhao MM, Hwa J, Perez DM** 1996 Identification of critical extracellular loop residues involved in α1-adrenergic receptor subtype-selective antagonist binding. Mol Pharmacol 50:1118–1126
82. **Maggi CA, Patacchini R, Rovero P, Giachetti A** 1993 Tachykinin receptors and tachykinin receptor antagonists. J Auton Pharmacol 13:23–93
83. **Krause JE, Staveteig PT, Mentzer JN, Schmidt SK, Tucker JB, Brodbeck RM, Bu JY, Karpitskiy VV** 1997 Functional expression of a novel human neurokinin-3 receptor homolog that binds [^{3}H]senktide and [125I-MePhe7]neurokinin B, and is responsive to tachykinin peptide agonists. Proc Natl Acad Sci USA 94:310–315
84. **Yokota Y, Akazawa C, Ohkubo H, Nakanishi S** 1992 Delineation of structural domains involved in subtype specificity of tachykinin receptors through chimeric formation of substance P/substance K receptors. EMBO J 11:3585–3591
85. **Gether U, Johansen TE, Schwartz TW** 1993 Chimeric NK-1 (substance P)/NK-3 (Neurokinin-B) receptors: identification of domains determining the binding specificity of tachykinin agonists. J Biol Chem 268:7893–7898
86. **Fong TM, Yu H, Huang RRC, Strader CD** 1992 The extracellular domain of the neurokinin-1 receptor is required for high-affinity binding of peptides. Biochemistry 31:11806–11811
87. **Fong TM, Huang RRC, Strader CD** 1992 Localization of agonist and antagonist binding domains of the human neurokinin-1 receptor. J Biol Chem 267:25664–25667
88. **Huang RRC, Yu H, Strader CD, Fong TM** 1994 Interaction of substance P with the second and seventh transmembrane domains of the neurokinin-1 receptor. Biochemistry 33:3007–3013
89. **Boyd ND, Kage R, Dumas JJ, Krause JE, Leeman SE** 1996 The peptide binding site of the substance P (NK-1) receptor localized by a photoreactive analogue of substance P: presence of a disulfide bond. Proc Natl Acad Sci USA 93:433–437
90. **Kage R, Leeman SE, Krause JE, Costello CE, Boyd ND** 1996 Identification of methionine as the site of covalent attachment of a p-benzoyl-phenylalanine-containing analogue of substance P on the substance P (NK-1) receptor. J Biol Chem 271:25797–25800
91. **Holst B, Zoffmann S, Elling CE, Hjorth SA, Schwartz TW** 1998 Steric hindrance mutagenesis *vs.* alanine scan in mapping of ligand binding sites in the tachykinin NK1 receptor. Mol Pharmacol 53:166–175
92. **Huang RR, Vicario PP, Strader CD, Fong TM** 1995 Identification of residues involved in ligand binding to the neurokinin-2 receptor. Biochemistry 34:10048–10055
93. **Bhogal N, Donnelly D, Findlay JB** 1994 The ligand binding site of the neurokinin 2 receptor. Site-directed mutagenesis and identification of neurokinin A binding residues in the human neurokinin 2 receptor. J Biol Chem 269:27269–27274
94. **Rosenkilde MM, Cahir M, Gether U, Hjorth SA, Schwartz TW** 1994 Mutations along transmembrane segment II of the NK-1 receptor affect substance P competition with non-peptide antagonists but not substance P binding. J Biol Chem 269:28160–28164
95. **Hjorth SA, Thirstrup K, Schwartz TW** 1996 Radioligand-dependent discrepancy in agonist affinities enhanced by mutations in the kappa-opioid receptor. Mol Pharmacol 50:977–984
96. **Ciucci A, Palma C, Manzini S, Werge TM** 1998 Point mutation increases a form of the NK1 receptor with high affinity for neurokinin A and B and septide. Br J Pharmacol 125:393–401
97. **Hjorth SA, Schambye HT, Greenlee WJ, Schwartz TW** 1994 Identification of peptide binding residues in the extracellular domains of the AT1 receptor. J Biol Chem 269:30953–30959
98. **Feng YH, Noda K, Saad Y, Liu XP, Husain A, Karnik SS** 1995 The docking of Arg2 of angiotensin II with Asp281 of AT1 receptor is essential for full agonism. J Biol Chem 270:12846–12850
99. **Heerding JN, Yee DK, Jacobs SL, Fluharty SJ** 1997 Mutational analysis of the angiotensin II type 2 receptor: contribution of conserved extracellular amino acids. Regul Pept 72:97–103
100. **Walker P, Munoz M, Martinez R, Peitsch MC** 1994 Acidic residues in extracellular loops of the human-y1 neuropeptide-y receptor are essential for ligand binding. J Biol Chem 269:2863–2869
101. **Leong SR, Kabakoff RC, Hebert C** 1994 Complete mutagenesis of the extracellular domain of interleukin-8 (IL-8) type A receptor

identifies charged residues mediating IL-8 binding and signal transduction. J Biol Chem 269:19343–19348
102. **Cotte N, Balestre MN, Phalipou S, Hibert M, Manning M, Barberis C, Mouillac B** 1998 Identification of residues responsible for the selective binding of peptide antagonists and agonists in the V2 vasopressin receptor. J Biol Chem 273:29462–29468
103. **Davidson JS, Assefa D, Pawson A, Davies P, Hapgood J, Becker I, Flanagan C, Roeske R, Millar R** 1997 Irreversible activation of the gonadotropin-releasing hormone receptor by photoaffinity cross-linking: localization of attachment site to Cys residue in N-terminal segment. Biochemistry 36:12881–12889
104. **Han B, Tashjian Jr AH** 1995 Importance of extracellular domains for ligand binding in the thyrotropin-releasing hormone receptor. Mol Endocrinol 9:1708–1719
105. **Han B, Tashjian Jr AH** 1995 Identification of Asn289 as a ligand binding site in the rat thyrotropin-releasing hormone (THR) receptor as determined by complementary modifications in the ligand and receptor: a new model for THR binding. Biochemistry 34:13412–13422
106. **Perlman JH, Colson AO, Jain R, Czyzewski B, Cohen LA, Osman R, Gershengorn MC** 1997 Role of the extracellular loops of the thyrotropin-releasing hormone receptor: evidence for an initial interaction with thyrotropin-releasing hormone. Biochemistry 36:15670–15676
107. **Siciliano SJ, Rollins TE, DeMartino J, Konteatis Z, Malkowitz L, Van Riper G, Bondy S, Rosen H, Springer MS** 1994 Two-site binding of C5a by its receptor: an alternative binding paradigm for G protein-coupled receptors. Proc Natl Acad Sci USA 91:1214–1218
108. **Chen Z, Zhang X, Gonnella NC, Pellas TC, Boyar WC, Ni F** 1998 Residues 21–30 within the extracellular N-terminal region of the C5a receptor represent a binding domain for the C5a anaphylatoxin. J Biol Chem 273:10411–10419
109. **Perez HD, Vilander L, Andrews WH, Holmes R** 1994 Human formyl peptide receptor ligand binding domain(s). Studies using an improved mutagenesis/expression vector reveal a novel mechanism for the regulation of receptor occupancy. J Biol Chem 269: 22485–22487
110. **Liapakis G, Fitzpatrick D, Hoeger C, Rivier J, Vandlen R, Reisine T** 1996 Identification of ligand binding determinants in the somatostatin receptor subtypes 1 and 2. J Biol Chem 271:20331–20339
111. **Xue JC, Chen C, Zhu J, Kunapuli SP, de Riel JK, Yu L, Liu-Chen LY** 1995 The third extracellular loop of the mu opioid receptor is important for agonist selectivity. J Biol Chem 270:12977–12979
112. **Wang WW, Shahrestanifar M, Jin J, Howells RD** 1995 Studies on mu and delta opioid receptor selectivity utilizing chimeric and site-mutagenized receptors. Proc Natl Acad Sci USA 92:12436–12440
113. **Valiquette M, Vu HK, Yue SY, Wahlestedt C, Walker P** 1996 Involvement of Trp-284, Val-296, and Val-297 of the human δ-opioid receptor in binding of δ-selective ligands. J Biol Chem 271: 18789–18796
114. **Varga EV, Li X, Stropova D, Zalewska T, Landsman RS, Knapp RJ, Malatynska E, Kawai K, Mizusura A, Nagase H, Calderon SN, Rice K, Hruby VJ, Roeske WR, Yamamura HI** 1996 The third extracellular loop of the human δ-opioid receptor determines the selectivity of delta-opioid agonists [published erratum appears in Mol Pharmacol 1997 Aug;52(2):335]. Mol Pharmacol 50:1619–1624
115. **Pepin MC, Yue SY, Roberts E, Wahlestedt C, Walker P** 1997 Novel "restoration of function" mutagenesis strategy to identify amino acids of the δ-opioid receptor involved in ligand binding. J Biol Chem 272:9260–9267
116. **AbdAlla S, Jarnagin K, Muller-Esterl W, Quitterer U** 1996 The N-terminal amino group of [Tyr8]bradykinin is bound adjacent to analogous amino acids of the human and rat B2 receptor. J Biol Chem 271:27382–27387
117. **Kennedy K, Gigoux V, Escrieut C, Maigret B, Martinez J, Moroder L, Frehel D, Gully D, Vaysse N, Fourmy D** 1997 Identification of two amino acids of the human cholecystokinin-A receptor that interact with the N-terminal moiety of cholecystokinin. J Biol Chem 272:2920–2926
118. **Ji Z, Hadac EM, Henne RM, Patel SA, Lybrand TP, Miller LJ** 1997 Direct identification of a distinct site of interaction between the carboxyl-terminal residue of cholecystokinin and the type A cholecystokinin receptor using photoaffinity labeling. J Biol Chem 272:24393–24401
119. **Silvente-Poirot S, Escrieut C, Wank SA** 1998 Role of the extracellular domains of the cholecystokinin receptor in agonist binding. Mol Pharmacol 54:364–371
120. **Gigoux V, Escrieut C, Silvente-Poirot S, Maigret B, Gouilleux L, Fehrentz JA, Gully D, Moroder L, Vaysse N, Fourmy D** 1998 Met-195 of the cholecystokinin-A receptor interacts with the sulfated tyrosine of cholecystokinin and is crucial for receptor transition to high affinity state. J Biol Chem 273:14380–14386
121. **Hadac EM, Pinon DI, Ji Z, Holicky EL, Henne RM, Lybrand TP, Miller LJ** 1998 Direct identification of a second distinct site of contact between cholecystokinin and its receptor. J Biol Chem 273: 12988–12993
122. **Labbe-Jullie C, Botto JM, Mas MV, Chabry J, Mazella J, Vincent JP, Gully D, Maffrand JP, Kitabgi P** 1995 [^{3}H]SR 48692, the first nonpeptide neurotensin antagonist radioligand: characterization of binding properties and evidence for distinct agonist and antagonist binding domains on the rat neurotensin receptor. Mol Pharmacol 47:1050–1056
123. **Perlman JH, Laakkonen L, Osman R, Gershengorn MC** 1994 A model of the thyrotropin-releasing hormone (TRH) receptor binding pocket. Evidence for a second direct interaction between transmembrane helix 3 and TRH. J Biol Chem 269:23383–23386
124. **Perlman JH, Thaw CN, Laakkonen L, Bowers CY, Osman R, Gershengorn MC** 1994 Hydrogen bonding interaction of thyrotropin-releasing hormone (TRH) with transmembrane tyrosine 106 of the TRH receptor. J Biol Chem 269:1610–1613
125. **Mills JS, Miettinen HM, Barnidge D, Vlases MJ, Wimer-Mackin S, Dratz EA, Sunner J, Jesaitis AJ** 1998 Identification of a ligand binding site in the human neutrophil formyl peptide receptor using a site-specific fluorescent photoaffinity label and mass spectrometry. J Biol Chem 273:10428–10435
126. **Yamano Y, Ohyama K, Kikyo M, Sano T, Nakagomi Y, Inoue Y, Nakamura N, Morishima I, Guo DF, Hamakubo T, Inagami T** 1995 Mutagenesis and the molecular modeling of the rat angiotensin II receptor (AT_1). J Biol Chem 270:14024–14030
127. **Monnot C, Bihoreau C, Conchon S, Curnow KM, Corvol P, Clauser E** 1996 Polar residues in the transmembrane domains of the type 1 angiotensin II receptor are required for binding and coupling. Reconstitution of the binding site by co-expression of two deficient mutants. J Biol Chem 271:1507–1513
128. **Lee JA, Brinkmann JA, Longton ED, Peishoff CE, Lago MA, Leber JD, Cousins RD, Gao A, Stadel JM, Kumar CS, Ohlstein EH, Gleason JG, Elliott JD** 1994 Lysine 182 of endothelin B receptor modulates agonist selectivity and antagonist affinity: evidence for the overlap of peptide and non- peptide ligand binding sites. Biochemistry 33:14543–14549
129. **Lee JA, Elliott JD, Sutiphong JA, Friesen WJ, Ohlstein EH, Stadel JM, Gleason JG, Peishoff CE** 1994 Tyr-129 is important to the peptide ligand affinity and selectivity of human endothelin type A receptor. Proc Natl Acad Sci USA 91:7164–7168
130. **Webb ML, Patel PS, Rose PM, Liu EC, Stein PD, Barrish J, Lach DA, Stouch T, Fisher SM, Hadjilambris O, Lee H, Skwish S, Dickinson KE, Krystek Jr SR** 1996 Mutational analysis of the endothelin type A receptor (ETA): interactions and model of selective ETA antagonist BMS-182874 with putative ETA receptor binding cavity. Biochemistry 35:2548–2556
131. **Strnad J, Hadcock JR** 1995 Identification of a critical aspartate residue in transmembrane domain three necessary for the binding of somatostatin to the somatostatin receptor SSTR2. Biochem Biophys Res Commun 216:913–921
132. **Kaupmann K, Bruns C, Raulf F, Weber HP, Mattes H, Lubbert H** 1995 Two amino acids, located in transmembrane domains VI and VII, determine the selectivity of the peptide agonist SMS 201–995 for the SSTR2 somatostatin receptor. EMBO J 14:727–735
133. **Ozenberger BA, Hadcock JR** 1995 A single amino acid substitution in somatostatin receptor subtype 5 increases affinity for somatostatin-14. Mol Pharmacol 47:82–87
134. **Befort K, Tabbara L, Kling D, Maigret B, Kieffer BL** 1996 Role of aromatic transmembrane residues of the δ-opioid receptor in ligand recognition. J Biol Chem 271:10161–10168
135. **Fathy DB, Mathis SA, Leeb T, Leeb-Lundberg LM** 1998 A single

position in the third transmembrane domains of the human B1 and B2 bradykinin receptors is adjacent to and discriminates between the C-terminal residues of subtype-selective ligands. J Biol Chem 273:12210–12218
136. **Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR** 1975 Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577–580
137. **Rohrer SP, Birzin ET, Mosley RT, Berk SC, Hutchins SM, Shen DM, Xiong Y, Hayes EC, Parmar RM, Foor F, Mitra SW, Degrado SJ, Shu M, Klopp JM, Cai SJ, Blake A, Chan WW, Pasternak A, Yang L, Patchett AA, Smith RG, Chapman KT, Schaeffer JM** 1998 Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. Science 282:737–740
138. **Gether U, Johansen TE, Snider RM, Lowe JA, Nakanishi S, Schwartz TW** 1993 Different binding epitopes on the NK1 receptor for substance-P and a non-peptide antagonist. Nature 362:345–348
139. **Gether U, Emondsalt X, Breliere JC, Fujii T, Hagiwara D, Pradier L, Garret C, Johansen TE, Schwartz TW** 1994 Evidence for a common molecular mode of action for chemically distinct nonpeptide antagonists at the neurokinin-1 (Substance-P) receptor. Mol Pharmacol 45:500–508
140. **Gether U, Yokota Y, Emonds-Alt X, Breliere J-C, Lowe III J, Snider RM, Nakanishi S, Schwartz TW** 1993 Two nonpeptide tachykinin antagonists act through epitopes on corresponding segments of the NK-1 and NK-2 receptors. Proc Natl Acad Sci USA 90:6194–6198
141. **Turcatti G, Zoffmann S, Lowe III JA, Drozda SE, Chassaing G, Schwartz TW, Chollet A** 1997 Characterization of non-peptide antagonist and peptide agonist binding sites of the NK1 receptor with fluorescent ligands. J Biol Chem 272:21167–21175
142. **Fong TM, Cascieri MA, Yu H, Bansal A, Swain C, Strader CD** 1993 Amino aromatic interaction between histidine-197 of the neurokinin-1 receptor and CP-96345. Nature 362:350–353
143. **Zoffmann S, Gether U, Schwartz TW** 1993 Conserved His(VI-17) of the NK-1 receptor is involved in binding of non-peptide antagonists but not substance P. FEBS Lett 336:506–510
144. **Fong TM, Yu H, Cascieri MA, Underwood D, Swain CJ, Strader CD** 1994 The role of histidine-265 in antagonist binding to the neurokinin-1 receptor. J Biol Chem 269:2728–2732
145. **Gether U, Nilsson L, Lowe III JA, Schwartz TW** 1994 Specific residues at the top of transmembrane segment V and VI of the neurokinin-1 (NK-1) receptor involved in binding of the nonpeptide antagonist CP 96,345. J Biol Chem 269:23959–23964
146. **Gether U, Lowe III JA, Schwartz TW** 1995 Tachykinin non-peptide antagonists: binding domain and molecular mode of action. Biochem Soc Trans 23:96–102
147. **Fong TM, Yu H, Cascieri MA, Underwood D, Swain CJ, Strader CD** 1994 Interaction of glutamine 165 in the fourth transmembrane segment of the human neurokinin-1 receptor with quinuclidine antagonists. J Biol Chem 269:14957–14961
148. **Jensen CJ, Gerard NP, Schwartz TW, Gether U** 1994 The species selectivity of chemically distinct tachykinin nonpeptide antagonists is dependent on common divergent residues of the rat and human neurokinin-1 receptors. Mol Pharmacol 45:294–299
149. **Fong TM, Yu H, Strader CD** 1992 Molecular basis for the species selectivity of the neurokinin-1 receptor antagonist-CP-96,345 and antagonist-RP67580. J Biol Chem 267:25668–25671
150. **Sachais BS, Snider RM, Lowe JA, Krause JE** 1993 Molecular basis for the species selectivity of the substance-P antagonist, CP-96,345. J Biol Chem 268:2319–2323
151. **Schambye HT, Hjorth SA, Bergsma DJ, Sathe G, Schwartz TW** 1994 Differentiation between binding sites for angiotensin II and nonpeptide antagonists on the angiotensin II type 1 receptors. Proc Natl Acad Sci USA 91:7046–7050
152. **Ji H, Zheng W, Zhang Y, Catt KJ, Sandberg K** 1995 Genetic transfer of a nonpeptide antagonist binding site to a previously unresponsive angiotensin receptor. Proc Natl Acad Sci USA 92:9240–9244
153. **Kong H, Raynor K, Yano H, Takeda J, Bell GI, Reisine T** 1994 Agonists and antagonists bind to different domains of the cloned κ opioid receptor. Proc Natl Acad Sci USA 91:8042–8046
154. **Xue JC, Chen C, Zhu J, Kunapuli S, DeRiel JK, Yu L, Liu-Chen LY** 1994 Differential binding domains of peptide and non-peptide ligands in the cloned rat κ opioid receptor. J Biol Chem 269:30195–30199
155. **Beinborn M, Lee Y-M, McBride EW, Quinn SM, Kopin AS** 1993 A single amino acid of the cholecystokinin-B/gastrin receptor determines specificity for non-peptide antagonists. Nature 362:348–350
156. **Silvente-Poirot S, Wank SA** 1996 A segment of five amino acids in the second extracellular loop of the cholecystokinin-B receptor is essential for selectivity of the peptide agonist gastrin. J Biol Chem 271:14698–14706
157. **Blaker M, Ren Y, Gordon MC, Hsu JE, Beinborn M, Kopin AS** 1998 Mutations within the cholecystokinin-B/gastrin receptor ligand 'pocket' interconvert the functions of nonpeptide agonists and antagonists. Mol Pharmacol 54:857–863
158. **Labbe-Jullie C, Barroso S, Nicolas-Eteve D, Reversat JL, Botto JM, Mazella J, Bernassau JM, Kitabgi P** 1998 Mutagenesis and modeling of the neurotensin receptor NTR1. Identification of residues that are critical for binding SR 48692, a nonpeptide neurotensin antagonist. J Biol Chem 273:16351–16357
159. **Breu V, Hashido K, Broger C, Miyamoto C, Furuichi Y, Hayes A, Kalina B, Loffler BM, Ramuz H, Clozel M** 1995 Separable binding sites for the natural agonist endothelin-1 and the non-peptide antagonist bosentan on human endothelin-A receptors. Eur J Biochem 231:266–270
160. **Sautel M, Rudolf K, Wittneben H, Herzog H, Martinez R, Munoz M, Eberlein W, Engel W, Walker P, Beck-Sickinger AG** 1996 Neuropeptide Y and the nonpeptide antagonist BIBP 3226 share an overlapping binding site at the human Y1 receptor. Mol Pharmacol 50:285–292
161. **Perlman S, Schambye HT, Rivero RA, Greenlee WJ, Hjorth SA, Schwartz TW** 1995 Non-peptide angiotensin agonist. Functional and molecular interaction with the AT1 receptor. J Biol Chem 270:1493–1496
162. **Nagayama Y, Russo D, Wadsworth HL, Chazenbalk GD, Rapoport B** 1991 Eleven amino acids (Lys-201 to Lys-211) and 9 amino acids (Gly-222 to Leu-230) in the human thyrotropin receptor are involved in ligand binding. J Biol Chem 266:14926–14930
163. **Nagayama Y, Wadsworth HL, Chazenbalk GD, Russo D, Seto P, Rapoport B** 1991 Thyrotropin-luteinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. Proc Natl Acad Sci USA 88:902–905
164. **Moyle WR, Bernard MP, Myers RV, Marko OM, Strader CD** 1991 Leutropin/beta-adrenergic receptor chimeras bind choriogonadotropin and adrenergic ligands but are not expressed at the cell surface. J Biol Chem 266:10807–10812
165. **Thomas D, Rozell TG, Liu X, Segaloff DL** 1996 Mutational analyses of the extracellular domain of the full-length lutropin/choriogonadotropin receptor suggest leucine-rich repeats 1–6 are involved in hormone binding. Mol Endocrinol 10:760–768
166. **Osuga Y, Kudo M, Kaipia A, Kobilka B, Hsueh AJ** 1997 Derivation of functional antagonists using N-terminal extracellular domain of gonadotropin and thyrotropin receptors. Mol Endocrinol 11:1659–1668
167. **Dufau ML** 1998 The luteinizing hormone receptor. Annu Rev Physiol 60:461–496
168. **Vu TK, Hung DT, Wheaton VI, Coughlin SR** 1991 Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 64:1057–1068
169. **Ishihara H, Connolly AJ, Zeng D, Kahn ML, Zheng YW, Timmons C, Tram T, Coughlin SR** 1997 Protease-activated receptor 3 is a second thrombin receptor in humans. Nature 386:502–506
170. **Gerszten RE, Chen J, Ishii M, Ishii K, Wang L, Nanevicz T, Turck CW, Vu TKH, Coughlin SR** 1994 Specificity of the thrombin receptor for agonist peptide is defined by its extracellular surface. Nature 368:648–651
171. **Nanevicz T, Ishii M, Wang L, Chen M, Chen J, Turck CW, Cohen FE, Coughlin SR** 1995 Mechanisms of thrombin receptor agonist specificity. Chimeric receptors and complementary mutations identify an agonist recognition site. J Biol Chem 270:21619–21625
172. **Couvineau A, Gaudin P, Maoret JJ, Rouyer-Fessard C, Nicole P, Laburthe M** 1995 Highly conserved aspartate 68, tryptophane 73 and glycine 109 in the N-terminal extracellular domain of the human VIP receptor are essential for its ability to bind VIP. Biochem Biophys Res Commun 206:246–252
173. **Buggy JJ, Livingston JN, Rabin DU, Yoo-Warren H** 1995 Glucagon.glucagon-like peptide I receptor chimeras reveal domains

that determine specificity of glucagon binding. J Biol Chem 270: 7474–7478
174. **Holtmann MH, Ganguli S, Hadac EM, Dolu V, Miller LJ** 1996 Multiple extracellular loop domains contribute critical determinants for agonist binding and activation of the secretin receptor. J Biol Chem 271:14944–14949
175. **Stroop SD, Nakamuta H, Kuestner RE, Moore EE, Epand RM** 1996 Determinants for calcitonin analog interaction with the calcitonin receptor N-terminus and transmembrane-loop regions. Endocrinology 137:4752–4756
176. **Unson CG, Cypess AM, Wu CR, Goldsmith PK, Merrifield RB, Sakmar TP** 1996 Antibodies against specific extracellular epitopes of the glucagon receptor block glucagon binding. Proc Natl Acad Sci USA 93:310–315
177. **Wilmen A, Van Eyll B, Goke B, Goke R** 1997 Five out of six tryptophan residues in the N-terminal extracellular domain of the rat GLP-1 receptor are essential for its ability to bind GLP-1. Peptides 18:301–305
178. **Hashimoto H, Ogawa N, Hagihara N, Yamamoto K, Imanishi K, Nogi H, Nishino A, Fujita T, Matsuda T, Nagata S, Baba A** 1997 Vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide receptor chimeras reveal domains that determine specificity of vasoactive intestinal polypeptide binding and activation. Mol Pharmacol 52:128–135
179. **Dautzenberg FM, Wille S, Lohmann R, Spiess J** 1998 Mapping of the ligand-selective domain of the *Xenopus laevis* corticotropin-releasing factor receptor 1: implications for the ligand- binding site. Proc Natl Acad Sci USA 95:4941–4946
180. **Clark JA, Bonner TI, Kim AS, Usdin TB** 1998 Multiple regions of ligand discrimination revealed by analysis of chimeric parathyroid hormone 2 (PTH2) and PTH/PTH-related peptide (PTHrP) receptors. Mol Endocrinol 12:193–206
181. **Mannstadt M, Luck MD, Gardella TJ, Juppner H** 1998 Evidence for a ligand interaction site at the amino-terminus of the parathyroid hormone (PTH)/PTH-related protein receptor from cross-linking and mutational studies. J Biol Chem 273:16890–16896
182. **Adams AE, Bisello A, Chorev M, Rosenblatt M, Suva LJ** 1998 Arginine 186 in the extracellular N-terminal region of the human parathyroid hormone 1 receptor is essential for contact with position 13 of the hormone. Mol Endocrinol 12:1673–1683
183. **Couvineau A, Rouyer-Fessard C, Maoret JJ, Gaudin P, Nicole P, Laburthe M** 1996 Vasoactive intestinal peptide (VIP)1 receptor. Three nonadjacent amino acids are responsible for species selectivity with respect to recognition of peptide histidine isoleucine-amide. J Biol Chem 271:12795–12800
184. **Bergwitz C, Jusseaume SA, Luck MD, Juppner H, Gardella TJ** 1997 Residues in the membrane-spanning and extracellular loop regions of the parathyroid hormone (PTH)-2 receptor determine signaling selectivity for PTH and PTH-related peptide. J Biol Chem 272:28861–28868
185. **Liaw CW, Grigoriadis DE, Lorang MT, De Souza EB, Maki RA** 1997 Localization of agonist- and antagonist-binding domains of human corticotropin-releasing factor receptors. Mol Endocrinol 11:2048–2053
186. **Du K, Nicole P, Couvineau A, Laburthe M** 1997 Aspartate 196 in the first extracellular loop of the human VIP1 receptor is essential for VIP binding and VIP-stimulated cAMP production. Biochem Biophys Res Commun 230:289–292
187. **Bisello A, Adams AE, Mierke DF, Pellegrini M, Rosenblatt M, Suva LJ, Chorev M** 1998 Parathyroid hormone-receptor interactions identified directly by photocross-linking and molecular modeling studies. J Biol Chem 273:22498–22505
188. **Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley III FD, Winston EN, Chen YL, Heym J** 1996 CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc Natl Acad Sci USA 93: 10477–10482
189. **Takahashi K, Tsuchida K, Tanabe Y, Masu M, Nakanishi S** 1993 Role of the large extracellular domain of metabotropic glutamate receptors in agonist selectivity determination. J Biol Chem 268: 19341–19345
190. **Tones MA, Bendali N, Flor PJ, Knopfel T, Kuhn R** 1995 The agonist selectivity of a class III metabotropic glutamate receptor, human mGluR4a, is determined by the N-terminal extracellular domain. Neuroreport 7:117–120
191. **Brown EM, Pollak M, Hebert SC** 1998 The extracellular calcium-sensing receptor: its role in health and disease. Annu Rev Med 49:15–29
192. **Armstrong N, Sun Y, Chen GQ, Gouaux E** 1998 Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature 395:913–917
193. **Schwartz TW, Rosenkilde MM** 1996 Is there a 'lock' for all agonist 'keys' in 7TM receptors? [see comments]. Trends Pharmacol Sci 17:213–216
194. **Fu ML, Herlitz H, Wallukat G, Hilme E, Hedner T, Hoebeke J, Hjalmarson A** 1994 Functional autoimmune epitope on alpha 1-adrenergic receptors in patients with malignant hypertension. Lancet 344:1660–1663
195. **Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J** 1994 Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the β1-adrenoceptor with positive chronotropic effect. Circulation 89:2760–2767
196. **Leiber D, Harbon S, Guillet JG, Andre C, Strosberg AD** 1984 Monoclonal antibodies to purified muscarinic receptor display agonist-like activity. Proc Natl Acad Sci USA 81:4331–4334
197. **abu Alla S, Quitterer U, Grigoriev S, Maidhof A, Haasemann M, Jarnagin K, Muller-Esterl W** 1996 Extracellular domains of the bradykinin B2 receptor involved in ligand binding and agonist sensing defined by anti-peptide antibodies. J Biol Chem 271:1748–1755
198. **Nagayama Y, Wadsworth HL, Russo D, Chazenbalk GD, Rapoport B** 1991 Binding domains of stimulatory and inhibitory thyrotropin (TSH) receptor autoantibodies determined with chimeric TSH-lutropin/chorionic gonadotropin receptors. J Clin Invest 88:336–340
199. **Costa T, Herz A** 1989 Antagonists with negative intrinsic activity at delta-opioid receptors coupled to GTP-binding proteins. Proc Natl Acad Sci USA 86:7321–7325
200. **Samama P, Pei G, Costa T, Cotecchia S, Lefkowitz RJ** 1994 Negative antagonists promote an inactive conformation of the β(2)-adrenergic receptor. Mol Pharmacol 45:390–394
201. **Chidiac P, Hebert TE, Valiquette M, Dennis M, Bouvier M** 1994 Inverse agonist activity of β-adrenergic antagonists. Mol Pharmacol 45:490–499
202. **Allen LF, Lefkowitz RJ, Caron MG, Cotecchia S** 1991 G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the α 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. Proc Natl Acad Sci USA 88:11354–11358
203. **Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG, Lefkowitz RJ** 1992 Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. J Biol Chem 267:1430–1433
204. **Lefkowitz RJ, Cotecchia S, Samama P, Costa T** 1993 Constitutive activity of receptors coupled to guanine nucelotide regulatory proteins. Trends Pharmacol Sci 14:303–307
205. **Samama P, Cotecchia S, Costa T, Lefkowitz RJ** 1993 A mutation-induced activated state of the β2-adrenergic receptor: extending the ternary complex model. J Biol Chem 268:4625–4636
206. **Robinson PR, Cohen GB, Zhukovsky EA, Oprian DD** 1992 Constitutively active mutants of rhodopsin. Neuron 9:719–725
207. **Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L, Baack E, Mountjoy KG, Cone RD** 1993 Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72:827–834
208. **Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G** 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature 365: 649–651
209. **Shenker A, Laue L, Kosugi S, Merendino JJ, Minegishi T, Cutler GB** 1993 A constitutively activating mutation of the lutenizing hormone receptor in familial male precocious puberty. Nature 365:652–654
210. **Rao VR, Cohen GB, Oprian DD** 1994 Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. Nature 367:639–642
211. **Parma J, Van Sande J, Swillens S, Tonacchera M, Dumont J, Vassart G** 1995 Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning

thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3′,5′-monophosphate and inositol phosphate-Ca2+ cascades. Mol Endocrinol 9:725–733
212. **Porter JE, Hwa J, Perez DM** 1996 Activation of the α1b-adrenergic receptor is initiated by disruption of an interhelical salt bridge constraint. J Biol Chem 271:28318–28323
213. **Nanevicz T, Wang L, Chen M, Ishii M, Coughlin SR** 1996 Thrombin receptor activating mutations. Alteration of an extracellular agonist recognition domain causes constitutive signaling. J Biol Chem 271:702–706
214. **Duprez L, Parma J, Costagliola S, Hermans J, Van Sande J, Dumont JE, Vassart G** 1997 Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. FEBS Lett 409:469–474
215. **Burstein ES, Spalding TA, Brann MR** 1998 The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. J Biol Chem 273:24322–24327
216. **Gaudin P, Maoret JJ, Couvineau A, Rouyer-Fessard C, Laburthe M** 1998 Constitutive activation of the human vasoactive intestinal peptide 1 receptor, a member of the new class II family of G protein-coupled receptors. J Biol Chem 273:4990–4996
217. **Hjorth SA, Orskov C, Schwartz TW** 1998 Constitutive activity of glucagon receptor mutants. Mol Endocrinol 12:78–86
218. **Rasmussen SG, Jensen AD, Liapakis G, Ghanouni P, Javitch JA, Gether U** 1999 Mutation of a highly conserved aspartic acid in the β2 adrenergic receptor: constitutive activation, structural instability, and conformational rearrangement of transmembrane segment 6. Mol Pharmacol 56:175–184
219. **Zhao MM, Gaivin RJ, Perez DM** 1998 The third extracellular loop of the beta2-adrenergic receptor can modulate receptor/G protein affinity. Mol Pharmacol 53:524–529
220. **Gether U, Ballesteros JA, Seifert R, Sanders-Bush E, Weinstein H, Kobilka BK** 1997 Structural instability of a constitutively active G protein-coupled receptor. Agonist-independent activation due to conformational flexibility. J Biol Chem 272:2587–2590
221. **Kudo M, Osuga Y, Kobilka BK, Hsueh AJW** 1996 Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. J Biol Chem 271:22470–22478
222. **Spalding TA, Burstein ES, Henderson SC, Ducote KR, Brann MR** 1998 Identification of a ligand-dependent switch within a muscarinic receptor. J Biol Chem 273:21563–21568
223. **Dube P, Konopka JB** 1998 Identification of a polar region in transmembrane domain 6 that regulates the function of the G protein-coupled α-factor receptor. Mol Cell Biol 18:7205–7215
224. **Lin Z, Shenker A, Pearlstein R** 1997 A model of the lutropin/choriogonadotropin receptor: insights into the structural and functional effects of constitutively activating mutations. Protein Eng 10:501–510
225. **Han M, Smith SO, Sakmar TP** 1998 Constitutive activation of opsin by mutation of methionine 257 on transmembrane helix 6. Biochemistry 37:8253–8261
226. **Groblewski T, Maigret B, Larguier R, Lombard C, Bonnafous JC, Marie J** 1997 Mutation of Asn111 in the third transmembrane domain of the AT1A angiotensin II receptor induces its constitutive activation. J Biol Chem 272:1822–1826
227. **Arnis S, Fahmy K, Hofmann KP, Sakmar TP** 1994 A conserved carboxylic acid group mediates light-dependent proton uptake and signaling by rhodopsin. J Biol Chem 269:23879–23881
228. **Scheer A, Fanelli F, Costa T, De Benedetti PG, Cotecchia S** 1997 The activation process of the alpha1B-adrenergic receptor: potential role of protonation and hydrophobicity of a highly conserved aspartate. Proc Natl Acad Sci U S A 94:808–813
229. **Ballesteros J, Kitanovic S, Guarnieri F, Davies P, Fromme BJ, Konvicka K, Chi L, Millar RP, Davidson JS, Weinstein H, Sealfon SC** 1998 Functional microdomains in G-protein-coupled receptors. The conserved arginine-cage motif in the gonadotropin-releasing hormone receptor. J Biol Chem 273:10445–10453
230. **Lu ZL, Curtis CA, Jones PG, Pavia J, Hulme EC** 1997 The role of the aspartate-arginine-tyrosine triad in the m1 muscarinic receptor: mutations of aspartate 122 and tyrosine 124 decrease receptor expression but do not abolish signaling. Mol Pharmacol 51:234–241
231. **Cohen GB, Yang T, Robinson PR, Oprian DD** 1993 Constitutive activation of opsin: influence of charge at position 134 and size at position 296. Biochemistry 32:6111–6115
232. **Strader CD, Sigal IS, Candelore MR, Rands E, Hill WS, Dixon RA** 1988 Conserved aspartic acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. J Biol Chem 263: 10267–10271
233. **Chung FZ, Wang CD, Potter PC, Venter JC, Fraser CM** 1988 Site-directed mutagenesis and continuous expression of human beta-adrenergic receptors. Identification of a conserved aspartate residue involved in agonist binding and receptor activation. J Biol Chem 263:4052–4055
234. **Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL** 1991 Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol 39:733–739
235. **Surprenant A, Horstman DA, Akbarali H, Limbird LE** 1992 A point mutation of the α2-adrenoceptor that blocks coupling to potassium but not calcium currents. Science 257:977–980
236. **Bihoreau C, Monnot C, Davies E, Teutsch B, Bernstein KE, Corvol P, Clauser E** 1993 Mutation of Asp(74) of the rat angiotensin-II receptor confers changes in antagonist affinities and abolishes G-protein coupling. Proc Natl Acad Sci USA 90:5133–5137
237. **Rath P, DeCaluwe LL, Bovee-Geurts PH, DeGrip WJ, Rothschild KJ** 1993 Fourier transform infrared difference spectroscopy of rhodopsin mutants: light activation of rhodopsin causes hydrogen-bonding change in residue aspartic acid-83 during meta II formation. Biochemistry 32:10277–10282
238. **Sheikh SP, Zvyaga TA, Lichtarge O, Sakmar TP, Bourne HR** 1996 Rhodopsin activation blocked by metal-ion-binding sites linking transmembrane helices C and F. Nature 383:347–350
239. **Sheikh SP, Vilardarga JP, Baranski TJ, Lichtarge O, Iiri T, Meng EC, Nissenson RA, Bourne HR** 1999 Similar structures and shared switch mechanisms of the β2-adrenoceptor and the parathyroid hormone receptor. Zn(II) bridges between helices III and VI block activation. J Biol Chem 274:17033–17041
240. **Javitch JA, Fu D, Liapakis G, Chen J** 1997 Constitutive activation of the beta2 adrenergic receptor alters the orientation of its sixth membrane-spanning segment. J Biol Chem 272:18546–18549
241. **Rothschild KJ, Cantore WA, Marrero H** 1983 Fourier transform infrared difference spectra of intermediates in rhodopsin bleaching. Science 219:1333–1335
242. **Garcia-Quintana D, Francesch A, Garriga P, de Lera AR, Padros E, Manyosa J** 1995 Fourier transform infrared spectroscopy indicates a major conformational rearrangement in the activation of rhodopsin. Biophys J 69:1077–1082
243. **Salamon Z, Wang Y, Brown MF, Macleod HA, Tollin G** 1994 Conformational changes in rhodopsin probed by surface plasmon resonance spectroscopy. Biochemistry 33:13706–13711
244. **Lin SW, Sakmar TP** 1996 Specific tryptophan UV-absorbance changes are probes of the transition of rhodopsin to its active state. Biochemistry 35:11149–11159
245. **Kim JM, Altenbach C, Thurmond RL, Khorana HG, Hubbell WL** 1997 Structure and function in rhodopsin: rhodopsin mutants with a neutral amino acid at E134 have a partially activated conformation in the dark state. Proc Natl Acad Sci USA 94:14273–14278
246. **Klein-Seetharaman J, Hwa J, Cai K, Altenbach C, Hubbell WL, Khorana HG** 1999 Single-cysteine substitution mutants at amino acid positions 55–75, the sequence connecting the cytoplasmic ends of helices I and II in rhodopsin: reactivity of the sulfhydryl groups and their derivatives identifies a tertiary structure that changes upon light-activation. Biochemistry 38:7938–7944
247. **Dunham TD, Farrens DL** 1999 Conformational changes in rhodopsin. Movement of helix f detected by site-specific chemical labeling and fluorescence spectroscopy. J Biol Chem 274:1683–1690
248. **Gether U, Lin S, Kobilka BK** 1995 Fluorescent labeling of purified beta2-adrenergic receptor: evidence for ligand-specific conformational changes. J Biol Chem 270:28268–28275
249. **Gether U, Lin S, Ghanouni P, Ballesteros JA, Weinstein H, Kobilka BK** 1997 Agonists induce conformational changes in transmembrane domains III and VI of the β2 adrenoceptor. EMBO J 16:6737–6747
250. **Elling CE, Thirstrup K, Holst B, Schwartz TW** 1999 Exchange of agonist site with metal-ion chelator site in the β2 adrenergic receptor. Proc Natl Acad Sci USA 96:12322–12327

251. **Dohlman HG, Thorner J, Caron MC, Lefkowitz RJ** 1991 Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 60:653–688
252. **Strader CD, Fong TM, Tota MR, Underwood D, Dixon RAF** 1994 Structure and function of G protein-coupled receptors. Annu Rev Biochem 63:101–132
253. **Wess J** 1998 Molecular basis of receptor/G-protein-coupling selectivity. Pharmacol Ther 80:231–264
254. **Bluml K, Mutschler E, Wess J** 1994 Identification of an intracellular tyrosine residue critical for muscarinic receptor-mediated stimulation of phosphatidylinositol hydrolysis. J Biol Chem 269:402–405
255. **Bluml K, Mutschler E, Wess J** 1994 Functional role of a cytoplasmic aromatic amino acid in muscarinic receptor-mediated activation of phospholipase-C. J Biol Chem 269:11537–11541
256. **Hill-Eubanks D, Burstein ES, Spalding TA, Brauner-Osborne H, Brann MR** 1996 Structure of a G-protein-coupling domain of a muscarinic receptor predicted by random saturation mutagenesis. J Biol Chem 271:3058–3065
257. **Burstein ES, Spalding TA, Brann MR** 1996 Amino acid side chains that define muscarinic receptor/G-protein coupling. Studies of the third intracellular loop. J Biol Chem 271:2882–2885
258. **Burstein ES, Spalding TA, Hill-Eubanks D, Brann MR** 1995 Structure-function of muscarinic receptor coupling to G proteins. Random saturation mutagenesis identifies a critical determinant of receptor affinity for G proteins. J Biol Chem 270:3141–3146
259. **Liu J, Conklin BR, Blin N, Yun J, Wess J** 1995 Identification of a receptor/G-protein contact site critical for signaling specificity and G-protein activation. Proc Natl Acad Sci U S A 92:11642–11646
260. **Liu J, Blin N, Conklin BR, Wess J** 1996 Molecular mechanisms involved in muscarinic aetylcholine receptor-mediated G protein activation studied by insertion mutagenesis. J Biol Chem 271:6172–6178
261. **Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA, Gilman AG, Sprang SR** 1995 The structure of the G protein heterotrimer Gi α1 β1 γ2. Cell 83:1047–1058
262. **Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB** 1996 The 2.0 A crystal structure of a heterotrimeric G protein. Nature 379:311–319
263. **Iiri T, Farfel Z, Bourne HR** 1998 G-protein diseases furnish a model for the turn-on switch. Nature 394:35–38
264. **Hebert TE, Moffett S, Morello JP, Loisel TP, Bichet DG, Barret C, Bouvier M** 1996 A peptide derived from a β2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. J Biol Chem 271:16384–16392
265. **Cvejic S, Devi LA** 1997 Dimerization of the delta opioid receptor: implication for a role in receptor internalization. J Biol Chem 272: 26959–26964
266. **Ng GY, O'Dowd BF, Lee SP, Chung HT, Brann MR, Seeman P, George SR** 1996 Dopamine D2 receptor dimers and receptor-blocking peptides. Biochem Biophys Res Commun 227:200–204
267. **Nimchinsky EA, Hof PR, Janssen WGM, Morrison JH, Schmauss C** 1997 Expression of dopamine D3 receptor dimers and tetramers in brain and in transfected cells. J Biol Chem 272:29229–29237
268. **George SR, Lee SP, Varghese G, Zeman PR, Seeman P, Ng GY, O'Dowd BF** 1998 A transmembrane domain-derived peptide inhibits D1 dopamine receptor function without affecting receptor oligomerization. J Biol Chem 273:30244–30248
269. **Rodriguez-Frade JM, Vila-Coro AJ, Martin A, Nieto M, Sanchez-Madrid F, Proudfoot AE, Wells TN, Martinez AC, Mellado M** 1999 Similarities and differences in RANTES- and (AOP)-RANTES-triggered signals: implications for chemotaxis. J Cell Biol 144:755–765
270. **Rodriguez-Frade JM, Vila-Coro AJ, de Ana AM, Albar JP, Martinez AC, Mellado M** 1999 The chemokine monocyte chemoattractant protein-1 induces functional responses through dimerization of its receptor CCR2. Proc Natl Acad Sci USA 96:3628–3633
271. **Bai M, Trivedi S, Brown EM** 1998 Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. J Biol Chem 273:23605–23610
272. **Ward DT, Brown EM, Harris HW** 1998 Disulfide bonds in the extracellular calcium-polyvalent cation-sensing receptor correlate with dimer formation and its response to divalent cations *in vitro*. J Biol Chem 273:14476–14483
273. **Romano C, Yang WL, O'Malley KL** 1996 Metabotropic glutamate receptor 5 is a disulfide-linked dimer. J Biol Chem 271:28612–28616
274. **Maggio R, Vogel Z, Wess J** 1993 Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. Proc Natl Acad Sci USA 90:3103–3107
275. **Pace AJ, Gama L, Breitwieser GE** 1999 Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys → Ser mutations at Cys101 and Cys236. J Biol Chem 274:11629–11634
276. **White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH** 1998 Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature 396:679–682
277. **Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA, Gerald C** 1998 GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. Nature 396:674–679
278. **Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B** 1998 GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature 396:683–687
279. **Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A, Kornau HC** 1999 Role of heteromer formation in GABAB receptor function. Science 283:74–77
280. **Jordan BA, Devi LA** 1999 G-protein-coupled receptor heterodimerization modulates receptor function. Nature 399:697–700
281. **Benkirane M, Jin DY, Chun RF, Koup RA, Jeang KT** 1997 Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by CCR5δ32. J Biol Chem 272:30603–30606
282. **McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM** 1998 RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393:333–339
283. **Bond RA, Leff P, Johnson TD, Milano CA, Rockman HA, McMinn TR, Apparsundaram S, Hyek MF, Kenakin TP, Allen LF, Lefkowitz RJ** 1995 Physiological effects of inverse agonists in transgenic mice with myocardial expression of the β2-adrenergic receptor. Nature 374:272–276
284. **Leff P** 1995 The two-state model of receptor activation. Trends Pharmacol Sci 16:89–97
285. **Riitano D, Werge TM, Costa T** 1997 A mutation changes ligand selectivity and transmembrane signaling preference of the neurokinin-1 receptor. J Biol Chem 272:7646–7655
286. **Reale V, Hannan F, Hall LM, Evans PD** 1997 Agonist-specific coupling of a cloned *Drosophila melanogaster* D1-like dopamine receptor to multiple second messenger pathways by synthetic agonists. J Neurosci 17:6545–6553
287. **Wiens BL, Nelson CS, Neve KA** 1998 Contribution of serine residues to constitutive and agonist-induced signaling via the D2S dopamine receptor: evidence for multiple, agonist-specific active conformations. Mol Pharmacol 54:435–444
288. **Mhaouty-Kodja S, Barak LS, Scheer A, Abuin L, Diviani D, Caron MG, Cotecchia S** 1999 Constitutively active α-1b adrenergic receptor mutants display different phosphorylation and internalization features. Mol Pharmacol 55:339–347
289. **Scaramellini C, Leff P** 1998 A three-state receptor model: predictions of multiple agonist pharmacology for the same receptor type. Ann NY Acad Sci 861:97–103
290. **Farahbakhsh ZT, Hideg K, Hubbell WL** 1993 Photoactivated conformational changes in rhodopsin: a time-resolved spin label study. Science 262:1416–1419
291. **Farrens DL, Khorana HG** 1995 Structure and function in rhodopsin. Measurement of the rate of metarhodopsin II decay by fluorescence spectroscopy. J Biol Chem 270:5073–5076
292. **Han M, Lin SW, Minkova M, Smith SO, Sakmar TP** 1996 Functional interaction of transmembrane helices 3 and 6 in rhodopsin. Replacement of phenylalanine 261 by alanine causes reversion of phenotype of a glycine 121 replacement mutant. J Biol Chem 271: 32337–32342