Pharmacogenomic and Structural Analysis of Constitutive G Protein–Coupled Receptor Activity

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Abstract

G protein–coupled receptors (GPCRs) respond to a chemically diverse plethora of signal transduction molecules. The notion that GPCRs also signal without an external chemical trigger, i.e., in a constitutive or spontaneous manner, resulted in a paradigm shift in the field of GPCR pharmacology. The discovery of constitutive GPCR activity and the fact that GPCR binding and signaling can be strongly affected by a single point mutation drew attention to the evolving area of GPCR pharmacogenomics. For a variety of GPCRs, point mutations have been convincingly linked to human disease. Mutations within conserved motifs, known to be involved in GPCR activation, might explain the properties of some naturally occurring, constitutively active GPCR variants linked to disease. In this review, we provide a brief historical introduction to the concept of constitutive receptor activity and the pharmacogenomic and structural aspects of constitutive receptor activity.

1. INTRODUCTION

G protein–coupled receptors (GPCRs) form one of the most versatile families of proteins and respond to a chemically diverse plethora of signal transduction molecules. Hence, for many years this receptor family has been the subject of study for therapeutics of humans. Many top-selling drugs from the past and present target membrane-bound GPCRs, and the pipelines of most pharmaceutical industries are filled with GPCR-targeting molecules. With the notion that GPCRs can also signal without an external chemical trigger, i.e., in a constitutive or spontaneous manner, a paradigm shift in the field of GPCR pharmacology was recently initiated. In this review, we give a brief historical introduction to the development of the concept of constitutive receptor activity, whereafter we indicate the importance of constitutive GPCR activity in relation to current ideas on the structural basis of GPCR (de)activation and on aspects of human GPCR pharmacogenomics.

1.1. Early Receptor Concepts and the Molecular Basis of Drug Action

GPCRs have been the subject of study since the early days of pharmacology and many of these investigations were instrumental in the development of modern concepts of receptor theory. The term receptors was initially introduced by Langley (1) and Ehrlich (2) to explain the action of nicotine and toxins, respectively. Applying the lock-key model as introduced by Emil Fischer (3) for describing enzyme-substrate interactions in biochemistry, the founders of early pharmacology suggested the existence of receptive substances to explain the biological actions of exogenous chemicals on cells. This concept matured with the seminal contribution of Clark, stating that the effect of an agonist is proportional to the number of occupied receptors. His occupancy theory (4, 5) also readily accommodated the difference between agonists and antagonists, following the lock-key principle of Fischer.

In the 1960s, Ariëns and coworkers published their book *Molecular Pharmacology* (6), in which the work of Clark was extended. Ariëns et al. introduced the concept of intrinsic activity to explain the observation that not every agonist of a given receptor induced the same maximum effect. Compounds reaching the maximum were referred to as full agonist (intrinsic activity $= 1$) and other agonists were named partial agonist, having an intrinsic activity between 0 and 1. Competitive antagonists were proposed to have an affinity for the receptor but possess an intrinsic activity of 0. The Clark-Ariëns model was extended first by Stephenson (7) and later by Furchgott (8, 9), with the introduction of drug efficacy and the system-independent concept of intrinsic efficacy.

The concepts noted above have had a great impact in the area of pharmacology and drug discovery, especially as the mathematics applied were simple and made it easy to calculate the affinity and the activity of agonists as well as the affinity of antagonists. Looking back, it is most remarkable that the ideas about receptor activation were developed during a period of approximately 75 years, when no real information on the biochemical nature of receptors was available, let alone the molecular mechanisms

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involved in the generation or transfer of a signal. In the "Introduction" to *Molecular Pharmacology* (6), a receptor was compared with a beautiful lady, "you may write a letter to her, sometimes she answers but she never shows up, though some day she may do so." Moreover, during a conference of the N.Y. Academy of Sciences in 1967, Ariëns admitted in a very clear way, "when speaking about receptors I am talking about something I do not know anything about" (10).

A medicinal chemist (Nauta)—not a pharmacologist—first proposed in 1968 that a GPCR family member might be a protein adopting a helical conformation, using the receptor for histamine as a model (11). Based on this purely hypothetical model, Nauta proposed that reversible interactions between a ligand and the amino acid side chains of the receptor protein were involved in the binding of both agonists and antagonists (**Figure 1*a***).

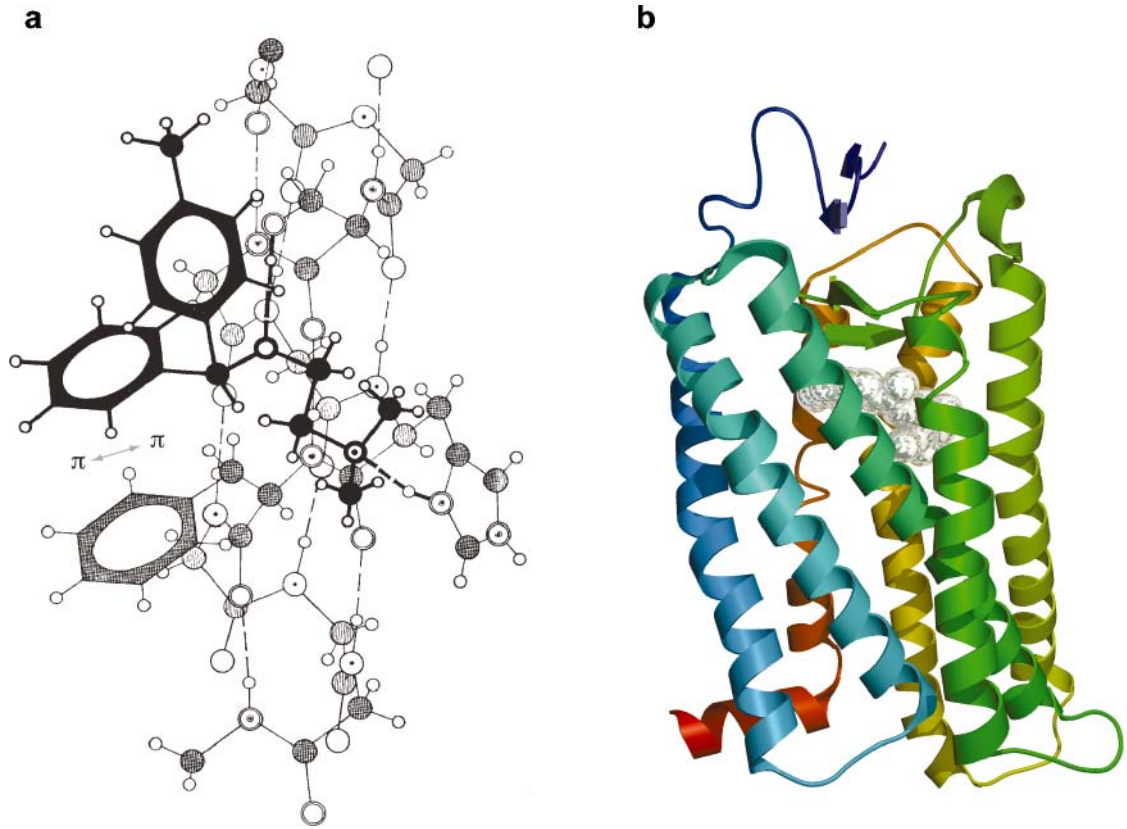

Figure 1

(*a*) Early model of histamine receptor and the binding of 4-methyldiphenhydramine to a phenylalanine residue through a π-π interaction mechanism by Nauta (197). (*b*) Crystal structure of bovine rhodopsin (PDB code 1GZM) (44). Retinal shown using CPK representation.

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1.2 From GPCR Gene Cloning to Constitutive, Agonist-Independent Signaling and Inverse Agonists

With the introduction in 1986 of the molecular biology of GPCRs (12), it became clear that the ideas of Nauta were quite close to reality. We now have available high-resolution X-ray structures of at least one GPCR (**Figure 1*b***), rhodopsin (43, 44) and a wealth of information on the structure-function relationships of various GPCRs (13), including drug binding and GPCR activation (see Section 2). In addition, genome sequencing efforts have led to the classification of human GPCR sequences into five main families: rhodopsin (Class A or family 1), secretin (Class B or family 2), glutamate (Class C or family 3), adhesion, and frizzled/taste2 (14). The rhodopsin family is the largest and is subdivided in four main groups (α, β, γ, δ) with 13 sub-branches (α: prostaglandin, amine, opsin, melatonin, MECA; β: peptides; γ: SOG, MCH, chemokine; δ: MAS, glycoprotein, purine, olfactory). These groups include orphan GPCRs, receptors for which the ligand and the (patho)physiological function remain unknown. Specialized databases of GPCRs can be found at **http://www.gpcr.org/7tm** (15) and **http://www.iuphar-db.org** (16).

Our current insight regarding GPCR activation have been influenced by the evidence that single point mutations can render GPCRs constitutively active, i.e., signal without the presence of the respective agonist (17–19). At the same time, such studies have also led to the general concept that constitutive GPCR signaling is an intrinsic property of most (if not all) GPCR family members, and that either GPCR ligands or single point mutations can change the equilibrium between inactive and active receptor states (18–20). Looking back, it is interesting to note that years before this general acceptance, research with only limited tools had provided convincing experimental evidence for constitutive GPCR signaling. In 1989, Costa & Herz started a shift in the paradigm on drug action by describing antagonists with negative intrinsic activity at wild type delta opioid receptors, endogenously expressed in NG108–15 neuroblastoma cells (21). The authors showed that delta opioid antagonists inhibited basal GTPase activity with differences in negative intrinsic activity; thus, for the first time GPCR pharmacology was faced with intrinsic drug activity that spanned from 1 for agonists to −1 for antagonists with negative efficacy (now also referred to as inverse agonists). Many subsequent studies with either wild-type or mutant GPCRs have confirmed that GPCR proteins can signal in an agonist-independent, constitutive way, as previously reviewed (19, 22). The notion of constitutive GPCR activity and the bidirectional modulation of GPCR activity by ligands has led to the introduction of a simple two-state model of GPCR action. In this model, a GPCR protein can shift spontaneously between an inactive R and an active R* conformation (23–25). GPCR agonists shift the equilibrium to the active R* state, whereas inverse agonists favor the inactive R state. The two-state model also explains the observations that some antagonists do not affect constitutive GPCR signaling, since these neutral antagonists are considered not to affect the thermodynamic equilibrium between the different protein conformations. The two-state model and the concept of inverse agonism are now generally accepted and are included in pharmacology textbooks.

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1.3. Constitutive GPCR Activity of Wild-Type GPCRs

As discussed above, the concept of constitutive activity became accepted following presentation of convincing data obtained with constitutively active mutant (CAM) GPCRs that were artificially generated. Yet, it has become clear that many wild-type GPCRs also show considerable levels of constitutive activity. This has recently been reviewed by Seifert & Wenzel-Seifert (19) and will therefore only be briefly discussed here.

Constitutive activity has been documented for more than 60 wild-type GPCRs from the class A, B, and C families (19). Of note, the GPCRs encoded by herpesviruses exhibit constitutive activity, providing valuable information on this phenomenon that has been linked to the development of disease (see Section 3) (26, 27). The availability of recombinant expression systems has been instrumental in providing such information. The extent of constitutive GPCR activity depends on the expression level of the respective receptor and the cellular context (19). For example, constitutive GPCR activity might be boosted by increased expression of G proteins or downstream effector molecules (19). For several GPCRs, constitutive activity has been observed in native tissue or cells (19). Prominent examples are the histamine H_3 receptor (28, 29) and the melanocortin MCR_1 and MCR_4 receptors, for which endogenous inverse agonists seem to be essential for a proper homeostasis (see also Section 3) (30).

Inverse agonists have been identified for many of the constitutively active GPCRs (19). Most of the compounds that were initially considered competitive antagonists with intrinsic activities of 0 have turned out to be inverse agonists with negative intrinsic activities between −1 and 0. Examples include GPCR antagonists that are important therapeutic agents, such as α_1 and β_1 adrenergic receptor antagonists (e.g., prazosin and metoprolol) (31), angiotensin AT_1 receptor antagonists (e.g., losartan) (32, 33), dopamine D_2 receptor antagonists (e.g., haloperidol) (34), leukotriene receptor antagonists (e.g., montelukast) (35), and histamine H_1 and H_2 receptor antagonists (e.g., cetirizine and cimetidine) (36, 37), which are all recognized to be inverse agonists at their respective targets. At present, it is unclear if the therapeutic success of these drugs is related to their negative intrinsic activity because neutral antagonists have either not yet been identified or have only been tested in a limited number of studies. At the serotonin $5HT_{2C}$ receptor, inverse agonist activity of antagonists did not correlate with their clinical efficacy as antipsychotics (38); however, the clinical efficacy of serotonin $5HT_{2A}$ receptor ligands was reported to depend on inverse agonistic activities (39). Similarly, clinical efficacy of the β−blocker metoprolol in heart failure may be due to its inverse agonist properties as the neutral antagonist bucindolol is not effective (20, 40). These studies indicate that the therapeutic outcome of inverse agonists and neutral antagonists can be different. One also has to consider that long-term exposure to inverse agonists can lead to upregulation of receptors, which may not always be beneficial and may contribute to the development of tolerance to the therapeutic agent (37, 41, 42).

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2. STRUCTURAL ASPECTS OF (CONSTITUTIVE) GPCR ACTIVATION

In contrast to the wealth of available pharmacological data, structural information on GPCRs is still scarce. To date, the only crystal structure available is that of the inactive state of bovine rhodopsin (43, 44). Five structures of rhodopsin are available at the Protein Data Bank (**http://www.rcsb.org/pdb**), at resolutions of 2.8 Å (PDB identifiers 1F88 and 1HZX), 2.65 Å (1GZM), 2.6 Å (1L9H), and 2.2 Å (1U19). Rhodopsin is formed by an extracellular N terminus of four β-strands, seven transmembrane helices (TM1 to TM7) connected by alternating intracellular (I1 to I3) and extracellular (E1 to E3) hydrophilic loops, a disulfide bridge between E2 and TM3, and a cytoplasmic C terminus containing an α-helix (HX 8) parallel to the cell membrane. Statistical analysis of the residues forming the TM helices of the rhodopsin family of GPCRs (Class A) shows a large number of conserved sequence patterns (45). This sequence conservation has been used by Ballesteros & Weinstein (46) to define a general numbering scheme consisting of two numbers: the first (1 through 7) corresponds to the helix in which the amino acid of interest is located; the second indicates its position relative to the most conserved residue in the helix, arbitrarily assigned to 50: $N1.50^{55}$ (the superscript represents the residue number of rhodopsin, 100% conserved in the family), $D2.50^{83}$ (94%), $R3.50^{135}$ (96%), $W4.50^{161}$ (96%), $P5.50^{215}$ (77%), $P6.50^{267}$ (100%), and $P7.50^{303}$ (96%). These patterns are easily identifiable on a multiple sequence alignment and allow comparison among residues in the 7TM segments of different receptors. We employ this generic numbering scheme of amino acid residues in GPCRs throughout the entire manuscript when referring to the GPCRs of the Class A family.

The molecular mechanisms of GPCR activation are still not fully understood. Farrens et al. (47) have shown that extracellular signals trigger rigid-body motions of several, if not all, TMs leading to the active state of the receptor. It was thus proposed that the inactive conformation of the receptor is maintained through restraining intramolecular interactions impeding these TM motions. Release of these constraints is induced by either agonists or constitutive activity-inducing mutations within the receptor. The discovery of CAM GPCRs, together with homology models constructed from the rhodopsin template, has yielded new insights into the mechanism of rhodopsin-like GPCR activation. Importantly, the sequence conservation pattern of GPCRs within this family suggests that this activation mechanism might occur by means of common motifs mainly located in the middle and cytoplasmic ends of the TM helices (45). Below, we describe the different motifs that are involved in GPCR activation. This information is used to explain the properties of naturally occurring GPCR mutants in Section 3.

2.1. The Ionic Lock

The interaction between R at position 3.50 of the highly conserved (D/E)R(Y/W) motif in TM3 with its adjacent D/E residue at position 3.49 and an additional D/E at position 6.30 near the cytoplasmic end of TM6 (**Figure 2*c***) is known as the ionic lock (48). Charge-neutralizing mutation of D/E6.30 in TM6 results in increased constitutive activity (48, 49). Removal of the ionic interaction between D/E6.30 and

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R3.50 in this CAM receptor facilitates the movement of the cytoplasmic end of TM6 away from TM3 by means of the Pro6.50-induced bend angle of TM6 (44, 48). This type of mutation has been described in patients with spontaneous ovarian hyperstimulation syndrome (see Section 3.1.2). Mutation of D/E3.49 in TM3 to either A or N removing the ionic interaction with R3.50 also increases the constitutive activity of rhodopsin (50) and a number of structurally related class A GPCRs (48, 51, 52). Thus, removal of this ionic constraint makes the side chain of R3.50 free to point toward the protein core (the direction of the Cα-Cβ bond).

2.2. The Hydrophobic Arginine Cage

Ballesteros et al. (53) suggested that the highly conserved R3.50 is also restrained in the inactive conformation by a cage shaped by conserved hydrophobic amino acids at positions 3.46 (L:15%, V:8%, I:58%, M:15%) and 6.37 (L:37%, V:24%, I:20%, M:5%) (**Figure 2*c***). Removal of these interfering bulky constraints by A or G replacement leads to constitutive activity in a number of cases (54–56).

2.3. Intracellular Helix 8

The X-ray structure of rhodopsin revealed the presence of a highly conserved helix 8 (Hx8), which is thought to be involved in G protein coupling (43, 57). **Figure 2*b*** shows the interaction of Y7.53 in TM7 with F7.60 in Hx8 and with the side chain and backbone (via water molecule #7) of N2.40 in TM2. Y7.53 and F7.60 are highly conserved in the rhodopsin family of GPCRs (Class A) (92% and 68%, see **Figure 2**) forming the NPxxY$x_{5,6}$F motif (58). Receptor activation was proposed to disrupt this aromatic-aromatic interaction, leading to a realigning of Hx8 (58, 59). It has also been proposed that the conserved charged (K:17%; R:54%) or polar (Q:11%) side chain at position 7.61 helps stabilize the free, helix-ending, carbonyls at positions 7.54 and 7.55 in TM7 through hydrogen bond interactions (**Figure 2*b***). This interaction seems to exert a key role in receptor stabilization, contributing to constitutive receptor activity but also the ligand binding profile of the KSHV-encoded chemokine receptor ORF74 (see Section 3) (60).

2.4. The Asparagine of the NPxxY Motif

The highly conserved N7.49 of the NPxxY motif in TM 7 acts as an on/off switch by adopting two different conformations in the inactive and active states (61, 62). In the inactive state, N7.49 is restrained toward TM 6 either via water molecule #9 in rhodopsin (63) and other family A GPCRs (**Figure 2*d***) or through the interaction with the T6.43D6.44 motif in the glycoprotein hormone receptor family (61, 62) (see Section 3.1.2). Upon receptor activation, N7.49 is proposed to adopt the *trans* conformation to interact with D2.50 of the (N/S)LxxxD motif in TM2 (62). It was hypothesized that this combination of charged and polar side chains leads to a negative electrostatic landscape, which could force relocation of R3.50 from the ionic lock (62; R.A. Bakker, A. Jongejan, K. Sansuk, U. Hacksell, H. Timmerman, manuscript

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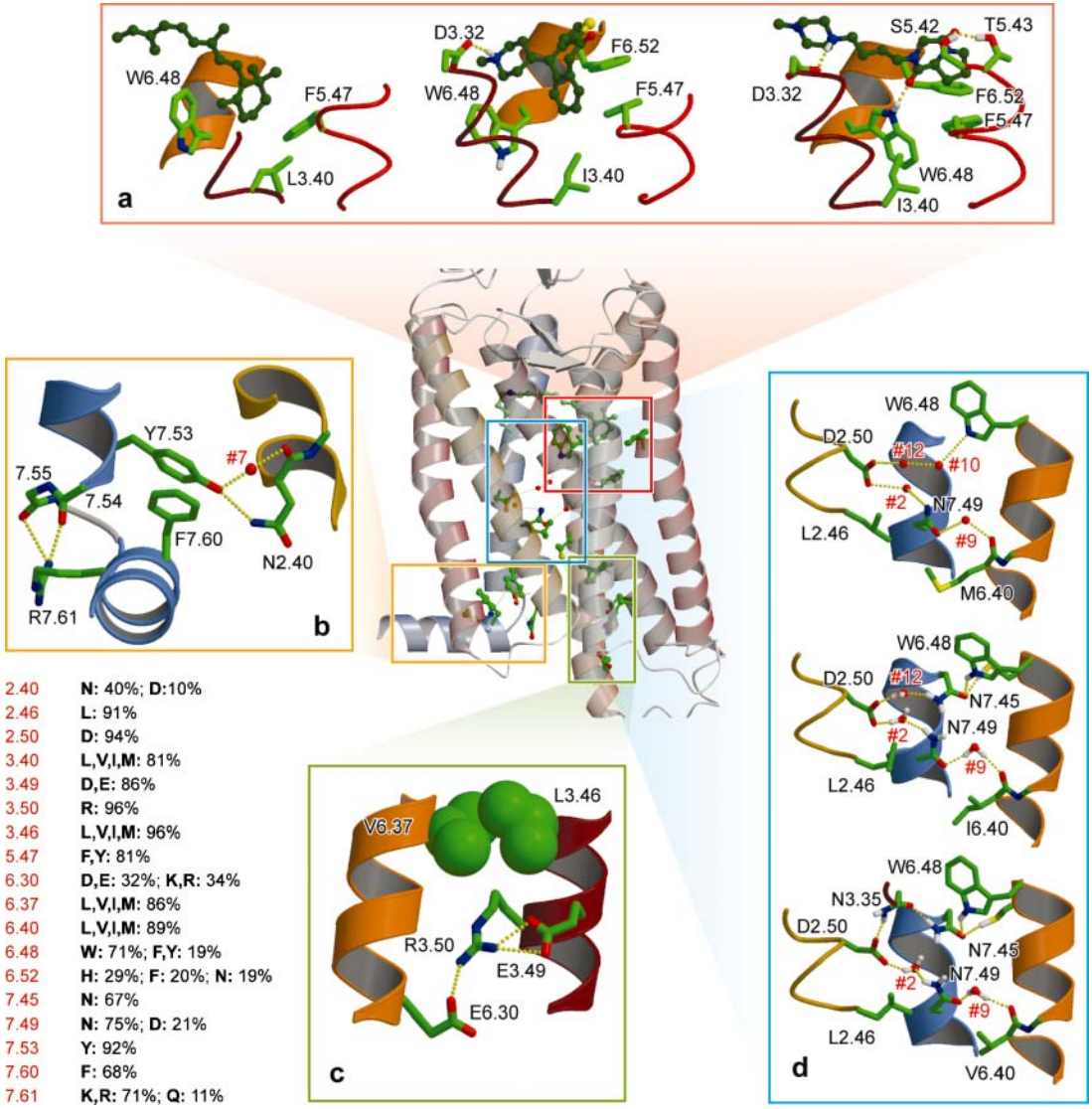

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submitted). Many mutations that modify the N7.49 equilibrium, favoring the inactive or active conformation, would decrease or increase, respectively, the constitutive activity of the receptor.

2.5. The Hydrophobic Asparagine Cage

Similar to the arginine cage, N7.49 is also located in a cage, restraining its conformation toward TM6 in the inactive state, formed by conserved hydrophobic amino

acids at positions 2.46 (L:91%) and 6.40 (L:14%; V:42%; I:28%; M:5%) (**Figure 2*d***). Removal (mutation to A or G) of the bulky and β- or γ-branched amino acids at positions 2.46 in rhodopsin (65) and the TSH receptor (62) and 6.40 in rhodopsin (66), serotonin $5HT_{2A}R$ (67), and histamine H_1 receptors (R.A. Bakker, A. Jongejan, K. Sansuk, U. Hacksell, H. Timmerman, manuscript submitted) induces constitutive activity.

2.6. Extracellular Loop 2

Klco et al. have recently shown that the E2 loop, containing a Cys engaged in a disulfide bridge with TM3, acts as a negative regulator of C5a receptor activation (68). Random saturation mutagenesis of the amino acids forming this E2 loop shows that nearly 80% of the functional receptors have an increase in constitutive activity. The high variability with respect to length [from 4 to more than 50 residues (45)] and amino acid composition in the different GPCR families suggest a nonconserved structure of the E2 loop. The molecular mechanism by which the E2 loop stabilizes the inactive conformation of the C5a receptor is not known.

2.7. The W6.48 Rotamer Toggle Switch

The structure of metarhodopsin I, determined by electron crystallography (69), has shown that W6.48 of the CWxP motif in TM 6 undergoes a conformational transition from pointing toward TM7 in the inactive state to pointing toward TM5 in the active state, as was previously suggested by experimental studies in rhodopsin (70) and computer simulations (71). Rearrangement of W6.48 and the nearby C6.47

Figure 2

Crystal structure of bovine rhodopsin (PDB code 1GZM) (44). The color code of the α-carbon ribbons is TMs 1 (*crimson*), 2 (*goldenrod*), 3 (*dark red*), 4 (*gray*), 5 (*red*), 6 (*orange*), and 7 (*blue*) and Hx8 (*blue*). The positions of the amino acids involved in receptor activation, together with their conservation pattern in the rhodopsin family of GPCRs (45), are shown. The standardized nomenclature of Ballesteros & Weinstein is employed (46). (*a*) Molecular basis of agonism and inverse agonism. Detailed view of the inverse agonists 11-*cis*-retinal (*left structure*) and ketotifen (*middle structure*) in a cavity between TMs 5 and 6 as observed in the crystal structure of rhodopsin (44) and a computational model of the histamine H_1 receptor, respectively. The $5\text{-}HT_{1A}R$ agonist (the naphtyl ring of the ligand is not shown for clarity) triggers the conformational transition of W6.48 towards TM5 by an explicit hydrogen bond (*right structure*) (77). Ligands are shown in dark green. (*b*) Network of interactions involving highly conserved amino acids within TM2, TM7, and Hx8 in rhodopsin (59, 60). (*c*) The ionic lock (48) and the hydrophobic arginine cage (53) between TMs 3 and 6 in rhodopsin. (*d*) Proposed hydrogen-bond network linking D2.50 and W6.48 in the inactive conformation of rhodopsin (*top structure*) (44), the histamine H_1 receptor (*middle structure*) (72), and δ opioid receptor (*bottom structure*) (74). N7.49 of the NPxxY motif is restrained toward TM6 via water molecule #9 in rhodopsin (63) and hydrophobic amino acids at positions 2.46 and 6.40 forming the asparagine cage (62; R.A. Bakker, A. Jongejan, K. Sansuk, U. Hacksell, H. Timmerman, manuscript submitted). These figures were created using MolScript v2.1.1 (198) and Raster3D v2.5 (199).

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decreases the highly conserved Pro6.50-induced bend angle of TM 6 (71), moving the cytoplasmic end of TM 6 away from TM 3, thereby disrupting the proposed ionic lock between D/E6.30 in TM 6 and R3.50 in TM 3 (48). It has also been suggested that the side chain at position 3.36 acts as a rotamer toggle switch simultaneously with W6.48 (72, 73), modulating the constitutive activity of the receptor.

2.8. A Conserved Hydrogen Bond Network Linking D2.50 and W6.48

D2.50 is involved in maintaining W6.48 pointing toward TM7 in the inactive state of the receptor through a conserved hydrogen bond network (44, 69). This conserved network varies among GPCR subfamilies. Rhodopsin forms this network through water molecules #12 and #10 (**Figure 2*d***, top panel) (44). N7.45, present in 67% of the rhodopsin-like sequences but absent in rhodopsin, would be located at the same position as water #10. Thus, N7.45-containing receptors are able to form the D2.50..#12..N7.45..W6.48 network (**Figure 2*d***, middle panel) (72). Similarly, N3.35 (29% of the receptors) would be located at the same position as water molecule #12, thus, N3.35/N7.45-containing receptors would form the D2.50..N3.35..N7.45..W6.48 network of interactions (**Figure 2*d***, bottom panel) (74). Disruption of this network by mutating N7.45 in the H_1 receptor (72) or N3.35 in the AT1 receptor (75) leads to constitutive activity by facilitating the reported conformational transition of W6.48 during receptor activation (69).

2.9. Molecular Basis of (Inverse) Agonism

Many wild-type GPCRs display only moderate constitutive activity under normal conditions and can be significantly activated by addition of agonists. However, GPCRs can in general easily be modified to display enhanced basal activity, and often this constitutive activity can be linked to diseases (17, 19) (see Section 3). In this respect, inverse agonists are potentially important therapeutics in the treatment of diseases caused by constitutive activity-inducing mutations of the wild-type receptor.

The motifs described in Sections 2.1–2.8 appear to be crucial determinants for the molecular basis of both agonism and inverse agonism. The processes initiated by the recognition of the extracellular ligand by the receptor depend to a large extent on the type of receptor because they can be activated by a wide range of extracellular ligands, ranging from small neurotransmitters to large hormones. Each subfamily most likely has developed specific structural motifs that allow the receptor to accommodate and respond to its cognate ligand. However, it seems reasonable to propose that in W6.48-containing GPCRs (71% of the rhodopsin-like sequences), ligand binding modifies the conformation of W6.48. Upon activation, either by agonists or constitutive activity-inducing mutations, a conformational transition of W6.48 toward TM5 occurs (see Section 2.7). Thus, GPCRs possess a small cavity between TMs 5 and 6 to accommodate the side chain of W6.48 in the active conformation. This small cavity is formed by the side chains at positions 3.40 (L:9%; V:25%; I:42%; M:5%), 5.47 (F:70%; Y:11%), and 6.52 (H:29%; F:20%; N:19%). The role of the F/Y5.47 and

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F6.52 aromatic side chains is to further stabilize the active conformation of W6.48 by aromatic-aromatic interactions in the face-to-edge orientation (**Figure 2*a***, right panel). In addition to the known interaction of aminergic ligands with D3.32 in TM 3 and a series of residues at positions 5.42, 5.43, and 5.46 in TM 5 (76), an interaction with W6.48 is found for agonists in the histamine H_1 receptor (A. Jongejan, unpublished results) and the 5-HT_{1A}R. We propose that agonists trigger this conformational transition of W6.48 by hydrogen bonding, an aromatic-aromatic interaction, or both. The right panel of **Figure 2*a*** shows a 5-HT_{1A}R agonist in the binding pocket of the receptor (77).

In contrast to the conformational transition triggered by an agonist or a constitutive activity-inducing mutation, an inverse agonist will stabilize or reinforce the constraints that keep the receptor in its inactive state. The left panel of **Figure 2*a*** shows the inverse agonist 11-*cis*-retinal located in this cavity between TMs 5 and 6 in rhodopsin. We propose that by occupying this small cavity, inverse agonists impede the transition of W6.48 toward TM5, thus decreasing the receptor's constitutive activity. The middle panel of **Figure 2*a*** shows the inverse agonist ketotifen in the binding pocket of the histamine H_1 receptor. The aromatic phenyl moiety of the ligand interacts with the aromatic F/Y5.47, W6.48, and F6.52 side chains, and as a consequence blocks the conformational transition of W6.48; such an action may be an important pharmacophoric element of inverse agonists.

3. PATHOPHYSIOLOGICAL CONSEQUENCES OF NATURALLY OCCURRING CONSTITUTIVELY ACTIVE GPCR VARIANTS

With the recognition of constitutive GPCR activity and the notion that GPCR binding and signaling can be strongly affected by a single point mutation, GPCR pharmacogenomics has recently attracted considerable attention. For a variety of GPCRs, point mutations have been convincingly linked to human disease. In this section, we review the present knowledge on naturally occurring mutant GPCRs involved in human disease and linked to constitutive activity. Moreover, we explain the GPCR phenotype in relation to the structural motifs that are thought to be involved in GPCR activation.

3.1. Class A GPCRs

3.1.1. Rhodopsin. Vision in dim-light occurs by retinal rod photoreceptor cells and is mediated by rhodopsin. Rhodopsin consists of the apoprotein opsin, a class A GPCR, to which an 11-*cis*-retinylidene chromophore is covalently bound through a protonated Schiff-base linkage to the ε-amino group of $K7.43^{296}$ in TM 7 (78), $E3.28^{113}$ in TM3 acting as a counterion for this linkage (79). Bound 11-*cis*-retinal acts as an inverse agonist by constraining rhodopsin in an inactive conformation in the dark (80). Light absorption photoisomerizes 11-*cis*-retinal into the full agonist all-*trans*-retinal, which initiates consecutive conformational changes in the rhodopsin TM domain (TMD), ultimately leading to G protein transducin activation and subsequent

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photoreceptor cell signaling (80). Autocatalyzed hydrolysis of the Schiff-base linkage results in the dissociation of the all-*trans* chromophore, upon which the dark-state (inactive) rhodopsin is regenerated by binding of new 11-*cis*-retinal to the unoccupied binding site.

The apoprotein opsin is constrained in a relatively inactive conformation by a salt bridge interaction between $E3.28^{113}$ and $K7.43^{296}$ (81, 82). Disruption of this salt bridge by mutating $E3.28^{113}$ or $K7.43^{296}$ results in constitutive activation of opsin (81). A $K7.43^{296}E$ mutation was found in a family with a severe form of autosomal dominant retinitis pigmentosa (ADRP) (**Table 1**), which manifests itself clinically by night blindness and a progressive loss of vision owing to the degeneration of both rod and cone photoreceptor cells (83). This CAM opsin is unable to interact with 11-*cis*-retinal and as a result signals continuously (81). Three other mutations in the opsin gene (i.e., $G2.57^{90}D$, $T2.61^{94}I$, and $A7.39^{292}E$) have been identified in families with autosomal-dominant, congenital stationary night blindness (CNSB) (**Table 1**). Expression of these three opsin mutants in heterologous cells results in constitutive signaling in the absence of a bound chromophore by interfering with the salt bridge between $E3.28^{113}$ and $K7.43^{296}$ (84). However, all three CAM opsins are inactive in the dark when reconstituted with 11-*cis*-retinal and can be activated upon light absorption (85). Interestingly, the $G2.57^{90}D$ opsin mutant binds 11-*cis*-retinal with an ~80-fold slower rate than wild-type opsin and the $T2.61^{94}I$ and $A7.39^{292}E$ mutants (86). CNSB, a less severe retinal disorder than ADRP, generally results in impaired vision under dim light and is associated with limited or no degeneration of rods. In contrast, continuous activation of the photosignaling cascade, caused either by an inability to bind 11-*cis*-retinal (i.e., CAM opsin $K7.43^{296}E$) or disrupted biosynthesis of 11-*cis*-retinal (i.e., Rpe65 enzyme mutation), can lead to retinal degeneration (84).

3.1.2. Glycoprotein hormone receptors. The thyroid-stimulating hormone receptor (TSHR), luteinizing hormone/chorionic gonadotropin receptor (LHCGR), and follicle-stimulating hormone receptor (FSHR) form the subfamily of glycoprotein hormone receptors (GpHRs) (87). These receptors distinguish themselves from other class A GPCRs (16) by having a large N-terminal exodomain (NTED), which constitutes the hormone binding site (88–91). Disease-causing CAMs have been found for all three members of this subfamily. The TSHR is more susceptible to natural occurring CAMs than are the LHCGR and FSHR (92).

Thyroid-stimulating hormone receptor. The thyroid regulates overall body metabolism by production of thyroid hormones. Pituitary-derived thyroid-stimulating hormone (TSH) controls growth, differentiation, and function of the thyroid gland by activating the TSHR (93). Autosomal and somatic CAMs in the TSHR gene causes familial nonautoimmune hyperthyroidism and thyroid adenoma, respectively (94). Both pathological conditions are associated with autonomous secretion of thyroid hormones, resulting in accelerated body metabolism. Hitherto, 38 natural occurring CAMs have been identified in the TMD of the TSHR, with the bottom of TM6 being a "hotspot" (**Figure 3**; **Table 1**) (92). An explanation is that the TSHR contains the family-specific T at position 6.43 and D at position 6.44,

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Table 1 Constitutive active GPCR mutants associated with pathophysiological conditions

Family	GPCR	OMIM[a]	Inheritance	Phenotype[b]	CAM[b,c]
A	Rhodopsin	180380	Autosomal dominant	Congenital stationary night blindness	G2.57D, T2.61I, A7.39E
			Autosomal dominant	Retinitis pigmentosa	K7.43E
A	TSHR	603372	Autosomal dominant	Nonautoimmune hyperthyroidism	G1.49S, M2.53V, S3.36R, V3.40A, A6.34V, L6.40F, P6.50S, N650Y [EL3], N7.45Y, C7.47Y
			De novo (germline)	Nonautoimmune hyperthyroidism	S281N [NTED], M2.43T, S3.36N, V5.54L/F, F6.42L
			Somatic	Hyperfunctioning thyroid adenoma	S281T/N/I [NTED], M2.43T, I486M [EL1]
					L3.43R, I568T [EL2], Y5.58N, D6.30G, A6.34V/I/S
					L6.40F, F6.42L/C/I, T6.43A/P/I, D6.44A/E/H/Y
					P6.50S, V656F [EL3]
			Somatic	Hyperfunctioning thyroid carcinoma	M2.43T, I486F [EL1], A6.34V, T6.43A/I, D6.44H, L7.52V
			Autosomal dominant	Euthyroid hyperthyrotropinemia	R310C [NTED], C390W [NTED]
A	LHCGR	152790	Autosomal dominant	Familial male-limited precocious puberty	L1.41P, A1.46V, M2.43T, L3.43R, I5.54L, D6.30G, A6.34V
					M6.37I, A6.38V, I6.41L, T6.43I, D6.44E/G/Y, C6.47R, M6.48G
			Somatic	Leydig cell adenoma	D6.44H
A	FSHR	136435	Autosomal dominant	Spontaneous ovarian hyperstimulation syndrome	T3.32A/I, I.5.54T, D6.30N
				FSH-independent spermatogenesis	D6.30G
B	PTHR1	168468	Autosomal dominant	Jansen's metaphyseal chonfrodysplasia	H223R [TM2], T410R/P [TM6], I458R [TM7]
C	CaSR	601199	Autosomal dominant	Autosomal dominant hypocalcemia	A843E [TM7]

[a]OMIM™: Online Mendelian Inheritance in Man. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), **http://www.ncbi.nlm.nih.gov/omim/**.

[b]Pathophysiological conditions associated with constitutively active mutant (CAM) GPCRs were collected from the OMIM, Glycoprotein-Hormone Receptor Information System (GRIS) (92), and Human Gene Mutation Database (HGMD) (200) databases; see references herein for more specific details.

[c]Amino acid mutations are indicated using Ballesteros & Weinstein numbering if situated in the TM helices of class A GPCRs (see Section 2 for details on this numbering scheme). Amino acid mutations that are situated elsewhere in class A GPCRs, or mutations in class B or C GPCRs are represented by residue number and the location is indicated between brackets.

*Abbreviations: TSHR, thyroid-stimulating hormone receptor; LHCGR, luteinizing hormone/chorionic gonadotropin receptor; FSHR, follicle-stimulating hormone receptor; PTHR1, parathyroid hormone-related peptide type 1 receptor; CaSR, Ca^{2+}-sensing receptor; NTED, N-terminal exodomain; EL1, −2, −3, extracellular loop 1, 2, and 3, respectively; TM1–7, transmembrane helices 1 to 7.

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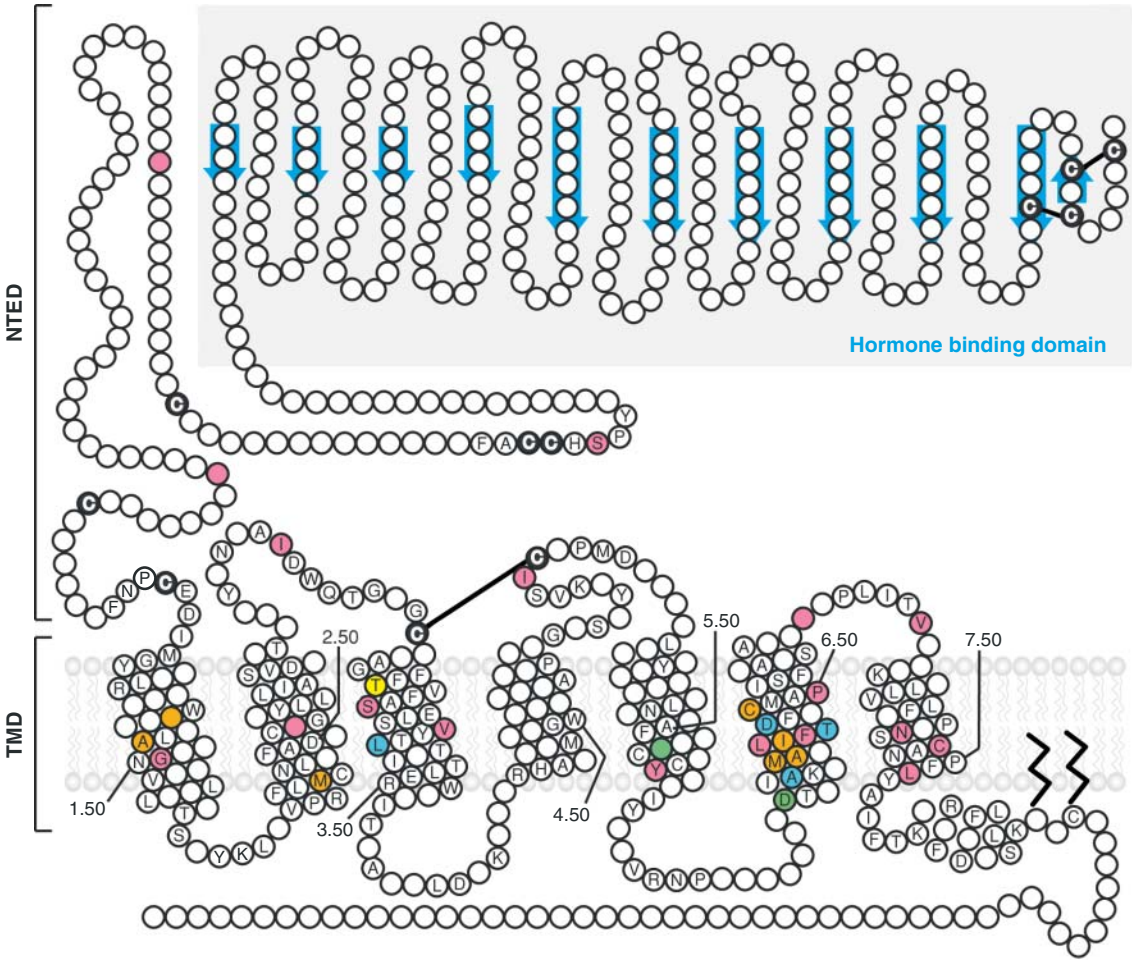

Figure 3

Snake plot of a consensus GpHR showing the NTED, to which glycoprotein hormones bind, and the TMD. Amino acid residues that are conserved in FSHR, LHCGR, and TSHR are indicated. Conserved cysteine residues in the TMD, the N-terminal Cys domain, and C-terminal Cys domain of the NTED that are involved in disulfide bridges are indicated by circles with a black background. The hormone-binding domain in the NTED is boxed and β-strands of the leucine-rich repeats and N-terminal cysteine-rich domain that form the binding surface are represented by arrows (89). The hormone-binding domain is connected to the TMD by the so-called hinge region, which is of variable length between the second and fourth conserved C-terminal Cys residues in the TSHR, LHCGR, and FSHR. The TM helix boundaries correspond to the bovine rhodopsin crystal structure (PDB code 1GZM) (44). The conserved amino acid in each TM helix of class A GPCRs is indicated according to the Ballesteros & Weinstein numbering scheme (see Section 2) (46). Locations of natural occurring GpHR CAMs (see **Table 1**) are indicated: yellow, FSHR; orange, LHCGR; pink, TSHR; blue, LHCGR/TSHR; green, LHCGR/TSHR/FSHR.

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which are the main partners of N7.49 in the inactive state of the receptor (61, 62) (see Section 2.4). Interestingly, five naturally occurring CAMs (e.g., S^{281} of the conserved TYPSHCCAF motif) have been identified in the so-called hinge-region of the NTED. In fact, designed deletion or mild trypsin digestion of the NTED unmasked the constitutive active character of the TSHR TMD (95, 96). Hence, the NTED acts as a tethered inverse agonist to constrain the TMD in a relatively inactive conformation, which is released upon TSH binding to the NTED or can be overruled by point mutations in the hinge region of the NTED (see below).

Luteinizing hormone receptor. The development of the testis and external male genitalia is dependent on testosterone production by Leydig cells (97). During fetal development the proliferation, differentiation, and testosterone production of these cells is induced by LHCGR signaling in response to placental-derived chorionic gonadotropin (CG). After birth, the Leydig cells remain largely inactive until the advent of puberty when pituitary-derived luteinizing hormone (LH) stimulates testosterone production by activating the LHCGR (98). At this stage, testosterone induces the development of male secondary sex characteristics and contributes, together with follicle-stimulating hormone (FSH), to the maturation of reproductive organs and the initiation of spermatogenesis. Hitherto, 17 disease-causing CAMs have been identified in the TMD of the LHCGR (**Figure 3**; **Table 1**) (92, 99). Most CAMs in the LHCGR affect amino acids in TM6, with, for example, D6.30^{564}G releasing the ionic lock (see Section 2.1) and T6.43^{577}I and D6.44^{578}E/G/Y affecting the NPxxY motif-mediated receptor activity constraint (see Section 2.4). The latter constraint is also perturbed in the LHCGR by L3.43H/K/R substitution owing to the formation of a salt bridge between the positive charge at position 3.43 and D6.44 (100, 101). Notably, while the L3.43H/K mutants are able to respond to the hormone, the L3.43R mutant is unresponsive to further hormonal stimulation (101). Mutational analysis revealed that the NTED of LHCGR constrains the TMD in an inactive conformation; however, unlike the TSHR, no natural CAMs have been observed in the LHCGR NTED (102–104). Autosomal CAMs in the LHCGR gene causes familial male-limited precocious puberty (FMPP) or testostoxicosis, whereas somatic CAMs induce sporadic Leydig cell tumors. Males inheriting CAMs in the LHCGR gene show virilization before the age of 4 as a consequence of testosterone hypersecretion by Leydig cells (97). In addition, FMPP is associated with an early growth spurt and accelerated bone maturation resulting in a short stature.

Fetal development of female sexual organs, on the other hand, is independent of gonadotropins and sex steroid hormones (97), whereas pubertal maturation of female reproductive organs and secondary sex characteristics is driven by estrogens that can only be produced by the ovary upon combined stimulation with FSH and LH (see below) (98). This requirement of both FSHR and LHR activity in regulating ovary function explains the absence of apparent pathophysiological phenotypes in females with a CAM LHCGR (97).

Follicle-stimulating hormone receptor. FSH and testosterone regulate the secretion of spermatogenesis-supporting paracrine factors by Sertoli cells in the testis (105). A

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D6.30^{567} to G mutation in TM6 of the FSHR was identified in a hypophysectomized male patient under treatment with testosterone replacement who retained fertility in the absence of FSH and LH (106). This FSHR mutant constitutively elevated cAMP levels in transfected Sertoli cells and mimicked FSH-like activity upon targeted expression on Sertoli cells in transgenic gonadotropin-deficient mice (107–109). The increased constitutive activity caused by this mutation is attributed to disruption of the ionic lock (see Section 2.1, **Figure 2*c***).

In females, the cyclic elevation of plasma FSH levels stimulates recruitment and maturation of advanced follicles in the ovary by activating FSHR on the follicle-surrounding granulosa cells (110). FSHR activity upregulates aromatase expression in these cells, facilitating the conversion of LH-dependent theca cell-derived androgens into estrogens. Estrogens stimulate pubertal development in females and play an essential role in the cyclical preparation of the female reproductive tract for conception (98, 110).

Five different CAMs (i.e., T3.32^{449}I/A, I5.54^{545}T, and D6.30^{567}G/N) in the FSHR gene have been found in the families of women who exhibit familial spontaneous ovarian hyperstimulation syndrome (sOHSS) during pregnancy (49, 111–114). sOHSS is a rare syndrome that may lead to life-threatening complications such as massive ovarian enlargement, multiple ovarian cysts formation, and ascites (115). Mutations at the T3.32 position modify the highly conserved hydrogen bond network linking D2.50 and TM6 (Section 2.8) and the conformational equilibrium of N7.49 (Section 2.4), whereas mutations at the D6.30 position modify the ionic lock between TMs 3 and 6 (Section 2.1, **Figures 2*c*** and **3**) (49). Besides being constitutively active, these sOHSS-causing FSHR mutants display increased responsiveness to chorionic gonadotropic (CG) as compared with the wild-type FSHR (49, 111–114). This apparent promiscuity is quite surprising given the fact that all CAMs were in the TMD and selectivity of glycoprotein hormone receptors for their cognate hormones is defined by their NTED (89–91). The increased sensitivity to CG explains the clinical manifestation of sOHSS during the first trimester of pregnancy when CG plasma levels are highest. Because the increased responsiveness to CG was not associated with an increase in affinity, it was hypothesized that loosening the intramolecular barrier to receptor activation would allow promiscuous receptor activation by low-affinity agonists. In fact, mutational analysis revealed a direct relation between the level of constitutive activity and the responsiveness to promiscuous hormones (49).

Hitherto, patients with nonautoimmune hyperthyroidism receive treatment with antithyroid drugs (e.g., carbimazole), which interferes with thyroid hormone synthesis. Although antithyroid drugs are efficient in controlling hyperthyroidism, they do not prevent thyroid enlargement (116). Consequently, antithyroidal drug therapy is usually followed by thyroidectomy. FMPP is currently controlled by either inhibiting adrenal and testicular androgen biosynthesis using the P450 cytochrome inhibitor ketanazole or by combined administration of an androgen receptor antagonist (spironolactone) and aromatase inhibitor (testolactone) (117, 118). Recently, specific nonpeptide antagonists and agonists have been identified for the FSHR and LHCGR (119–124), with therapeutic potential for anticonception and assisted-reproduction, respectively. Hence, identification of nonpeptide inverse agonists specifically

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inhibiting constitutive signaling of LHCGR, FSHR, or TSHR may therefore be a matter of time.

3.1.3. Growth hormone secretagogue receptor (GHSR) type 1a. The GHSR-1a receptor modulates growth hormone release upon activation by the peptide ghrelin, a signal that stimulates appetite (125). The GHSR-1a has attracted considerable interest for its role in satiety and modulators of its activity or expression (126) are being investigated as potential therapies for the treatment of obesity (127).

The GHSR-1a has been shown to possess a high level of constitutive activity in vitro (128). The recent discovery of a naturally occurring mutant GHSR-1a receptor ($A^{204}E$) that is functional and lacks constitutive activity, but retains agonist-induced signaling, supports a physiological role for the constitutive GHSR-1a activity; this mutation leads to a syndrome characterized by a short stature and might be related to obesity that develops during puberty (129). The $A^{204}E$ mutation in the GHSR-1a receptor, as well as a GHSR-1a $F6.51^{279}L$ mutation, which also shows loss of constitutive activity, can occur in obese individuals (130). Both mutant GHSR-1a receptors maintain the capacity to mediate ghrelin-induced signaling events (128, 129). The $A^{204}E$ mutation occurs in the E2 loop (see Section 2.6), whereas the $F6.51^{279}L$ mutation modifies the conserved aromatic cluster CWxPFF motif in TM 6 (Section 2.7).

The loss of constitutive activity results in the expected phenotype with respect to growth, whereas this is not the case with respect to appetite and energy expenditure (131). These findings indicate the existence of complex interactions and potential pathways that compensate for this loss of function in the GHSR-1a receptor. Perhaps activation of GPR39, recently deorphanized as the receptor for the peptide hormone obestatin, derived from the same gene as ghrelin, accounts for this compensatory pathway because activation of GPR39 has opposite effects on food intake and weight gain (132).

3.1.4. Melanocortin receptors. The family of melanocortin receptors and the existence of endogenous inverse agonists acting at these receptors have greatly strengthened the concept of constitutive receptor activity and have further supported its physiological relevance. The melanocortin system, which controls pigmentation and body weight, encompasses a family of five receptors. Both the melanocortin-1 receptor (MC1R) and −4 receptor (MC4R) illustrate the occurrence and relevance of constitutive receptor activity in vivo. MC1R, originally called the melanocyte stimulating hormone (MSH) receptor, is expressed in cutaneous and hair follicle melanocytes. Stimulation of MC1R by MSH as well as by adrenocorticotrophin (ACTH) promotes melanogenesis through an increased transcription of genes involved in the production of eumelanic dark pigments. In contrast, modulation of the MC1R by the naturally occurring inverse MC1R agonist agouti, a paracrine factor expressed in the skin, induces the production of the yellow pigment pheomelanin (133). The MC1R exhibits a high level of constitutive activity accounting for the phenomenon of inverse agonism. More than 60 naturally occurring MC1R variants, located throughout the receptor, have been described (reviewed in (134, 135)). These mutant receptors are often associated with clearly visible phenotypes, showing

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aberrant cell surface expression, decrease or further increase in constitutive activity. The latter unmasks inverse agonistic properties of endogenous peptides (reviewed in 134, 135).

The MC4R controls body weight, and in the brain constitutive MC4R activity is inhibited by agouti-related protein (AgRP) (136). MC4R knock-out, the presence of inactivating mutations in the NTED ($R^{7}H$, $T^{11}A$, $T^{11}S$, $R^{18}C$, $R^{18}H$, $R^{18}L$), in the I2 loop ($A^{154}D$, $Q^{156}P$) or pharmacological inhibition of the MC4R results in obesity (137, 138). A cluster of naturally occurring mutations in the NTED of the MC4R in obese patients have been identified. The resultant mutant MC4R receptors exhibit a reduced constitutive activity, which led to the suggestion that the NTED in the receptor functions as an intrinsic partial agonist that contributes to the level of constitutive MC4R activity (30, 139). The activity of the MC4R is modulated through the opposing effects of the anorexigenic agonist α-MSH and the orexigenic inverse agonist AgRP (139, 140). Whereas MC4R agonists are of interest for the potential treatment of obesity, inverse MC4R agonists might exhibit favorable characteristics in treating conditions such as cancer-associated cachexia (141).

Although no constitutively activating MC4R mutations have been reported in anorexia nervosa patients (142), one AgRP gene polymorphism has been associated with this disorder (143, 144). In addition, mutations in MC4R are currently thought to be the most common genetic cause of obesity. Besides the inactivating mutations within MCR4, there are also mutant MC4Rs that are retained in the cytoplasm (145), or that respond poorly to MSH (146). Yet, the overall influence of mutations in the MC4R on obesity is not clear. A variety of MC4R mutations found in nonobese individuals also exhibit loss-of-function characteristics, whereas impairment of cell surface expression for some mutant MC4R linked to the occurrence of obesity was not confirmed (142).

3.1.5. Virus-encoded GPCRs. Besides the naturally occurring GPCR variants described above, a relatively novel and intriguing class of GPCRs, encoded by the herpesviruses, exhibit marked constitutive activity. The herpes- and poxviruses encode more than 40 GPCRs, most of them displaying homology to chemokine receptors (147), known to be implicated in the regulation of the immune response (148). Although the roles of these viral-encoded receptors have not been fully defined, they are believed to subvert the immune system and to contribute to virus-induced pathogenesis. Many of these viral GPCRs have acquired additional properties compared with their cellular counterparts, including the ability to bind a broad spectrum of chemokines, couple to a variety of G proteins, and display high constitutive activity (26, 27, 149). In particular, the GPCRs encoded by the Kaposi's sarcoma (KS) associated herpes virus (KSHV), Epstein-Barr virus (EBV), and human cytomegalovirus (HCMV) illustrate the (patho)physiological importance of constitutive receptor activity.

KSHV, implicated in the development of KS, encodes the GPCR ORF74 (150). ORF74 shows highest homology to the human chemokine receptor CXCR2. This viral GPCR binds a broad spectrum of chemokines and unlike CXCR2, is able to constitutively activate a variety of signal transduction cascades linked to proliferation

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(see 151 for references). Expression of ORF74 in vivo within hemopoetic or endothelial cells leads to the development of angioproliferative lesions that morphologically resemble KS lesions (152). Modulation of ORF74 activity occurs by endogenous chemokines but constitutive activity of ORF74 also appears to play an important role in the progression of KS-like lesions in ORF74 transgenic mice. The constitutive activity of ORF74 has been attributed to the absence of residues that normally are involved in GPCR activation (see Section 2) (153). However, reintroduction of these motifs did not result in significant changes in basal activity of the receptor. Mutation of $N2.50^{92}$ to the corresponding D (see Section 2.8) did not lead to inactivation of the ORF74 receptor but substitution of the neighboring $L2.49^{91}$ with an Asp resulted in loss of constitutive activity (153). Expression of this constitutively inactive mutant in vivo ($L2.49^{91}D$) completely prevented development of a KS-like disease in transgenic mice (154), emphasizing the relevance of constitutive receptor activity. Of further interest are the mutations within helix 8 that not only influence constitutive activity but also affect ligand binding properties of KSHV-ORF74 (Section 2.3, **Figure 2*b***) (60). Constitutive activation of Akt by ORF74, for example, plays a crucial role in ORF74-mediated sarcomagenesis (155, 156). Moreover, ORF74-induced upregulation and release of proangiogenic factors, including proinflammatory cytokines and chemokines, appear sufficient to drive angioproliferative tumor formation by autocrine or paracrine stimulation (156–159). Hence, the constitutively active viral chemokine receptor ORF74 seems to be implicated in the pathology of KS.

In addition, EBV, known to be associated with many lymphoproliferative diseases, such as infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma, encodes a GPCR referred to as BILF1 (160, 161). Like ORF74, BILF1 constitutively activates signaling to NFκB and CRE, both implicated in proliferative signaling (161). The increased activation of signaling pathways was also apparent in EBV-positive lymphoblastoid B cell lines (161), suggesting a role for BILF1 in EBV-related proliferative diseases.

The human cytomegalovirus (HCMV) encodes four GPCRs (US27, US28, UL33, and UL78), which show highest homology to chemokine receptors. Both US28 and UL33 alter cellular signaling in a constitutively active manner when ectopically expressed and, more importantly, after HCMV infection, as shown using HCMV US28 and UL33 deletion strains (162–165). Through promiscuous G protein coupling, US28 and UL33 activate multiple signaling pathways, including effectors and transcription factors within infected cells. In contrast to other GPCRs, US28 does not possess the ionic lock, offering a possible explanation for its constitutive activity. Substitution of $R3.50^{129}$ of the conserved DRY-motif with an Ala removes an important determinant for G protein coupling, resulting in the loss of constitutive activation of G protein-mediated signaling pathways (165a). Moreover, nonpeptidergic ligands have been identified that can act as inverse agonists (e.g., VUF2274), inhibiting basal US28 signaling; VUF2274 has also been shown to partially inhibit HIV-1 entry into US28-expressing cells (166).

HCMV has been associated with chronic diseases, including vascular diseases (167) and malignancies (168, 169). Because the CMV-encoded receptors US28 and UL33 constitutively activate transcription factors implicated in inflammatory events

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associated with atherosclerosis and tumorigenesis (170), these receptors may play a role in onset or progression of these HCMV-related pathologies. Recently, we have observed that expression of US28 induces transformation and tumorigenesis in vivo, suggesting that US28 might act in a concerted manner with other oncogenic HCMV-encoded proteins (171) to enhance tumorigenesis (172). The use of constitutively inactive mutants, the development of disease models, and testing of recently identified inverse agonists that target HCMV-encoded receptors (173) will serve as important tools to determine the (patho)physiological relevance of constitutive receptor activity of these receptors.

3.2. Class B/C and Frizzled Family GPCRs

In contrast to GPCRs belonging to the rhodopsin family of GPCRs (class A), little information is available on structural determinants involved in GPCR activation and inverse agonism of the class B/C and Frizzled GPCR families. Nonetheless, natural occurring CAMs in these receptor families have been identified, examples of which are discussed below.

3.2.1 Parathyroid hormone (PTH)-related peptide (PTHrP) type 1 receptor (PTHR1). The PTHR1 for PTH and PTHrP belongs to the class B GPCRs. This receptor is highly expressed in bone and kidney and mediates the PTH-dependent regulation of mineral ion homeostasis, including the circulating concentrations of calcium and phosphorous (174). Mutation of a histidine at the bottom of TM2 ($H^{223}R$), of a threonine in TM6 ($T^{410}R$ and $T^{410}P$) and of an isoleucine in TM7 ($I^{458}R$) of the human PTH-receptor have been reported to be associated with constitutive PTH receptor activation in Jansen-type metaphyseal chondrodysplasia, a rare disorder that is typically characterized by severe growth plate abnormalities that lead to short-limbed dwarfism (175). The high level of constitutive activity of the mutant receptor is thought to result in hypercalcemia, hypophosphatemia, and the abnormal formation of endochondral bone (175).

3.2.2 Ca^{2+}-sensing receptor (CaSR). Extracellular Ca^{2+} ($Ca^{2+}{}_o$) homeostasis is regulated by the parathyroid hormone (PTH), which is produced by parathyroid glands. PTH stimulates the (re)absorption of Ca^{2+} by the kidney and intestine and Ca^{2+} mobilization from bone. Fluctuation in $Ca^{2+}{}_o$ levels is detected by the Ca^{2+}-sensing receptor (CaSR), which is abundantly expressed on PTH-producing chief cells in the parathyroid gland and tubular cells in the kidney (176). As with most other members of the class C GPCR family (16), the CaSR contains a Venus-flytrap-like ligand-binding domain within its large NTED (177, 178). Activation of the CaSR by elevated $Ca^{2+}{}_o$ levels inhibits PTH secretion by the parathyroid chief cells and stimulates urinary Ca^{2+} excretion, as such restoring the homeostatic $Ca^{2+}{}_o$ concentration (176). Hence, the CaSR is crucial for the negative feedback regulation of $Ca^{2+}{}_o$ homeostasis. Activating mutations in the CaSR results in hypoparathyroidism, which is clinically manifested as autosomal dominant hypocalcemia (ADH). To date, 35 sporadic or familial ADH-causing mutations have been identified in the CaSR

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(179). Only one of these mutations (i.e., $A^{843}E$ in TM7) appeared to be a true CAM, inducing ligand-independent basal signaling (180, 181). Other activating mutations involve increased receptor sensitivity to $Ca^{2+}{}_o$ without affecting basal signaling (178).

3.2.3. Smoothened. Smoothened, a GPCR thought to signal via heterotrimeric G proteins and possibly via non-G protein signaling pathways (182), together with frizzled receptors, forms a distinct group within the family of GPCRs. Smoothened is kept in an inactive state through its interaction with the transmembrane protein Patched (Ptc), the receptor for lipid-modified secreted glycoproteins of the Hedgehog (Hh) family. Binding of Hh to Ptc activates Ptc to release the catalytic repression of Ptc on Smoothened activity to unleash the constitutive activity of Smoothened (183). Whereas the Hh pathway plays fundamental roles during pattern formation in animal development, dysfunction of Hh-pathway components is frequently associated with congenital disorders and cancer (184–187). Cyclopamide, a teratogen found in the *Veratrum californicum* plant that blocks cholesterol synthesis, is an Hh/smoothened inhibitor and induces regression of skin tumors (188). The effects of oncogenic mutations in TM7 ($S^{537}N$ and $W^{539}L$) and constitutively activating mutations in TMs 6 ($G^{460}Y$) and 7 ($S^{537}T$, $G^{533}L$, $G^{533}H$, $G^{533}T$, $G^{533}Y$, $G^{533}S$, and $G^{533}A$) in Smoothened and Ptc can be reversed by cyclopamide treatment (189). Hence, modulators of smoothened activity appear promising for the treatment of certain cancers as well as psoriasis (188–191). Several Smoothened modulators have recently been described (192–194).

4. CONCLUDING REMARKS

Not only mutational analysis of wild-type GPCRs but also those natural occurring CAMs that are associated with disease have contributed to our knowledge of constitutive receptor activity. Specific, conserved domains within the receptors appear essential in the regulation of (spontaneous) signaling. In particular, mutational changes near the interface at the bottom of TM3 or top of TM6 often result in increases of constitutive activity (19). Analogous to these observations, agonists are believed to relieve the receptor from these intrinsic constraints, inducing a movement of the bottom of TM6 (47, 195). Mutations in these regions are found in a number of GPCRs, e.g., of the glycoprotein family, that are associated with human diseases. Disruption of the constraint within those receptors, leads to constitutive receptor activity and to the development of disease. In these cases, the use of inverse agonists is a particularly rational treatment.

For some GPCRs, autoantibodies recognize epitopes on the second, most variable, extracellular loop, causing pathologies (see 20, 22 for references). The second extracellular loop has been implicated in constitutive activity of some receptors (68), perhaps explaining the observed effects of autoantibodies. These antibodies can influence receptor activity, some even displaying agonistic activity (196) that can be counteracted by inverse agonists.

Numerous of the clinically used "antagonist" drugs acting on GPCRs, in particular those of the bioaminergic families, are inverse agonists. Their inverse agonistic

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properties have in particular been shown in recombinant systems, but also in native systems (21, 29). Inverse agonists inhibit constitutive signaling initially, yet chronic use might lead to receptor upregulation and sensitization. Taken together, the importance of inverse agonism for the clinical efficacy of drugs targeting receptors with low or high constitutive activity is just beginning to emerge and requires further study.

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LITERATURE CITED

1. Langley JN. 1878. On the physiology of the salivary secretion. Part II. On the mutual antagonism of atropin and pilocarpin, having especial reference to their relations in the submaxillary gland of the cat. *J. Physiol.* 1:339–60
2. Ehrlich P. 1909. Über den jetzigen Stand der Chemotherapie. *Ber. Dtsch. Chem. Ges.* 42:17–47
3. Fischer E. 1894. Einfluss der Configuration auf die Wirkung der Enzyme. *Ber. Dtsch. Chem. Ges.* 27:2985–33
4. Clark AJ. 1933. *The Mode of Action of Drugs on Cells*. London: Edward Arnold
5. Clark AJ. 1937. *General Pharmacology: Heffter's Handbuch der Experimentellen Pharmacology*. Berlin: Ergband 4, Springer
6. Ariens EJ. 1964. *Molecular Pharmacology*. New York: Academic
7. Stephenson RP. 1956. A modification of receptor theory. *Br. J. Pharmacol.* 11:379–93
8. Furchgott RF. 1966. The use of b-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. In *Advances in Drug Research*, ed. NJ Harper, AB Simmonds, pp. 21–55. New York: Academic
9. Furchgott RF. 1972. The classification of adrenoreceptors (adrenergic receptors): an evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, ed. H Blaschko, E Muscholl, pp. 283–335. Berlin: Springer-Verlag
10. Ariens EJ, Simonis AM. 1967. Cholinergic and anticholinergic drugs, do they act on common receptors? *Ann. N.Y. Acad. Sci.* 144:842–69
11. Nauta WT, Rekker RF, Harms AF. 1968. Diarylcarbinol ethers: structure activity relationships. A physico-chemical approach. In *Physico-Chemical Aspects of Drug Action*, ed. EJ Ariens, pp. 305–25. Oxford: Pergamon
12. Dixon RAF, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, et al. 1986. Cloning of the gene and cDNA for mammalian b-adrenergic receptor and homology with rhodopsin. *Nature* 321:75–79
13. Kristiansen K. 2004. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol. Ther.* 103:21–80

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

14. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. 2003. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharmacol.* 63:1256–72
15. Horn F, Bettler E, Oliveira L, Campagne F, Cohen FE, Vriend G. 2003. GPCRDB information system for G protein-coupled receptors. *Nucleic. Acids Res.* 31:294–97
16. Foord SM, Bonner TI, Neubig RR, Rosser EM, Pin JP, et al. 2005. International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharmacol. Rev.* 57:279–88
17. Milligan G. 2003. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol. Pharmacol.* 64:1271–76
18. Costa T, Cotecchia S. 2005. Historical review: negative efficacy and the constitutive activity of G-protein-coupled receptors. *Trends Pharmacol. Sci.* 26:618–24
19. Seifert R, Wenzel-Seifert K. 2002. Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* 366:381–416
20. Bond RA, Ijzerman AP. 2006. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. *Trends Pharmacol. Sci.* 27:92–96
21. Costa T, Herz A. 1989. Antagonists with negative intrinsic activity at d opioid receptors coupled to GTP-binding proteins. *Proc. Natl. Acad. Sci. USA* 86:7321–25
22. de Ligt RA, Kourounakis I, IJzerman AP. 2000. Inverse agonism at G protein-coupled receptors: (patho)physiological relevance and implications for drug discovery. *Br. J. Pharmacol.* 130:1–12
23. Kenakin T. 2003. Ligand-selective receptor conformations revisited: the promise and the problem. *Trends Pharmacol. Sci.* 24:346–54
24. Kenakin T. 2001. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J.* 15:598–611
25. Samama P, Cotecchia S, Costa T, Lefkowitz RJ. 1993. A mutation-induced activated state of the b_2-adrenergic receptor. *J. Biol. Chem.* 268:4625–36
26. Vischer HF, Leurs R, Smit MJ. 2006. HCMV-encoded G-protein-coupled receptors as constitutively active modulators of cellular signaling networks. *Trends Pharmacol. Sci.* 27:56–63
27. Sodhi A, Montaner S, Gutkind JS. 2004. Viral hijacking of G-protein-coupled-receptor signaling networks. *Nat. Rev. Mol. Cell Biol.* 5:998–1012
28. Wieland K, Bongers G, Yamamoto Y, Hashimoto T, Yamatodani A, et al. 2001. Constitutive activity of histamine H(3) receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H(3) antagonists. *J. Pharmacol. Exp. Ther.* 299:908–14
29. Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, et al. 2000. High constitutive activity of native H3 receptors regulates histamine neurons in brain. *Nature* 408:860–64

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

30. Adan RA. 2006. Constitutive receptor activity series: endogenous inverse agonists and constitutive receptor activity in the melanocortin system. *Trends Pharmacol. Sci*. 27:183–86
31. Rossier O, Abuin L, Fanelli F, Leonardi A, Cotecchia S. 1999. Inverse agonism and neutral antagonism at alpha(1a)- and alpha(1b)-adrenergic receptor subtypes. *Mol. Pharmacol*. 56:858–66
32. Takezako T, Gogonea C, Saad Y, Noda K, Karnik SS. 2004. "Network leaning" as a mechanism of insurmountable antagonism of the angiotensin II type 1 receptor by nonpeptide antagonists. *J. Biol. Chem*. 279:15248–57
33. Miserey-Lenkei S, Parnot C, Bardin S, Corvol P, Clauser E. 2002. Constitutive internalization of constitutively active angiotensin II AT(1A) receptor mutants is blocked by inverse agonists. *J. Biol. Chem*. 277:5891–901
34. Hall DA, Strange PG. 1997. Evidence that antipsychotic drugs are inverse agonists at D-2 dopamine receptors. *Br. J. Pharmacol*. 121:731–36
35. Dupre DJ, Le Gouill C, Gingras D, Rola-Pleszczynski M, Stankova J. 2004. Inverse agonist activity of selected ligands of the cysteinyl-leukotriene receptor 1. *J. Pharmacol. Exp. Ther*. 309:102–8
36. Bakker RA, Wieland K, Timmerman H, Leurs R. 2000. Constitutive activity of the histamine H(1) receptor reveals inverse agonism of histamine H(1) receptor antagonists. *Eur. J. Pharmacol*. 387:R5–7
37. Smit MJ, Leurs R, Alewijnse AE, Blauw J, Amerongen GPV, et al. 1996. Inverse agonism of histamine H_2 antagonists accounts for upregulation of spontaneously active histamine H_2 receptors. *Proc. Natl. Acad. Sci. USA* 93:6802–7
38. Rauser L, Savage JE, Meltzer HY, Roth BL. 2001. Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. *J. Pharmacol. Exp. Ther*. 299:83–89
39. Weiner DM, Burstein ES, Nash N, Croston GE, Currier EA, et al. 2001. 5-hydroxytryptamine2A receptor inverse agonists as antipsychotics. *J. Pharmacol. Exp. Ther*. 299:268–76
40. Maack C, Cremers B, Flesch M, Hoper A, Sudkamp M, Bohm M. 2000. Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium. *Br. J. Pharmacol*. 130:1131–39
41. Milligan G, Bond RA. 1997. Inverse agonism and the regulation of receptor number. *Trends Pharmacol. Sci*. 18:468–74
42. Leurs R, Smit MJ, Alewijnse AE, Timmerman H. 1998. Agonist-independent regulation of constitutively active G-protein-coupled receptors. *Trends Biochem. Sci*. 23:418–22
43. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, et al. 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 289:739–45
44. Li J, Edwards PC, Burghammer M, Villa C, Schertler GF. 2004. Structure of bovine rhodopsin in a trigonal crystal form. *J. Mol. Biol*. 343:1409–38
45. Mirzadegan T, Benko G, Filipek S, Palczewski K. 2003. Sequence analyses of G-protein-coupled receptors: similarities to rhodopsin. *Biochemistry* 42:2759–67

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

46. 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
47. 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* 274:768–70
48. Ballesteros JA, Jensen AD, Liapakis G, Rasmussen SG, Shi L, et al. 2001. Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *J. Biol. Chem*. 276:29171–77
49. Montanelli L, van Durme JJ, Smits G, Bonomi M, Rodien P, et al. 2004. Modulation of ligand selectivity associated with activation of the transmembrane region of the human follitropin receptor. *Mol. Endocrinol*. 18:2061–73
50. 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* 94:14273–78
51. Alewijnse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, et al. 2000. The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. *Mol. Pharmacol*. 57:890–98
52. Scheer A, Fanelli F, Costa T, Debenedetti PG, Cotecchia S. 1996. Constitutively active mutants of the a_{1B}-adrenergic receptor: role of highly conserved polar amino acids in receptor activation. *EMBO J*. 15:3566–78
53. Ballesteros J, Kitanovic S, Guarnieri F, Davies P, Fromme BJ, et al. 1998. Functional microdomains in G-protein-coupled receptors. The conserved arginine-cage motif in the gonadotropin-releasing hormone receptor. *J. Biol. Chem*. 273:10445–53
54. Laue L, Chan WY, Hsueh AJ, Kudo M, Hsu SY, et al. 1995. Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. *Proc. Natl. Acad. Sci. USA* 92:1906–10
55. Baranski TJ, Herzmark P, Lichtarge O, Gerber BO, Trueheart J, et al. 1999. C5a receptor activation. Genetic identification of critical residues in four transmembrane helices. *J. Biol. Chem*. 274:15757–65
56. Ringkananont U, Van Durme J, Montanelli L, Ugrasbul F, Yu YM, et al. 2006. Repulsive separation of the cytoplasmic ends of transmembrane helices 3 and 6 is linked to receptor activation in a novel thyrotropin receptor mutant (M626I). *Mol. Endocrinol*. 20:893–903
57. Lu ZL, Saldanha JW, Hulme EC. 2002. Seven-transmembrane receptors: crystals clarify. *Trends Pharmacol. Sci*. 23:140–46
58. Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP. 2003. Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. *Proc. Natl. Acad. Sci. USA* 100:2290–95

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

59. Prioleau C, Visiers I, Ebersole BJ, Weinstein H, Sealfon SC. 2002. Conserved helix 7 tyrosine acts as a multistate conformational switch in the 5HT2C receptor. Identification of a novel "locked-on" phenotype and double revertant mutations. *J. Biol. Chem*. 277:36577–84
60. Verzijl D, Pardo L, Van Dijk M, Gruijthuijsen YK, Jongejan A, et al. 2006. Helix 8 of the viral chemokine receptor ORF74 directs chemokine binding. *J. Biol. Chem*. doi:10.1074/jbc.M606877200
61. Govaerts C, Lefort A, Costagliola S, Wodak SJ, Ballesteros JA, et al. 2001. A conserved Asn in transmembrane helix 7 is an on/off switch in the activation of the thyrotropin receptor. *J. Biol. Chem*. 276:22991–99
62. Urizar E, Claeysen S, Deupi X, Govaerts C, Costagliola S, et al. 2005. An activation switch in the rhodopsin family of G protein-coupled receptors: the thyrotropin receptor. *J. Biol. Chem*. 280:17135–41
63. Okada T, Fujiyoshi Y, Silow M, Navarro J, Landau EM, Shichida Y. 2002. Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. *Proc. Natl. Acad. Sci. USA* 99:5982–87
64. Deleted in proof
65. Madabushi S, Gross AK, Philippi A, Meng EC, Wensel TG, Lichtarge O. 2004. Evolutionary trace of G protein-coupled receptors reveals clusters of residues that determine global and class-specific functions. *J. Biol. Chem*. 279:8126–32
66. 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*. 271:32337–42
67. Shapiro DA, Kristiansen K, Weiner DM, Kroeze WK, Roth BL. 2002. Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. *J. Biol. Chem*. 277:11441–49
68. Klco JM, Wiegand CB, Narzinski K, Baranski TJ. 2005. Essential role for the second extracellular loop in C5a receptor activation. *Nat. Struct. Mol. Biol*. 12:320–26
69. Ruprecht JJ, Mielke T, Vogel R, Villa C, Schertler GF. 2004. Electron crystallography reveals the structure of metarhodopsin I. *EMBO J*. 23:3609–20
70. Lin SW, Sakmar TP. 1996. Specific tryptophan UV-absorbance changes are probes of the transition of rhodopsin to its active state. *Biochemistry* 35:11149–59
71. Shi L, Liapakis G, Xu R, Guarnieri F, Ballesteros JA, Javitch JA. 2002. Beta2 adrenergic receptor activation. Modulation of the proline kink in transmembrane 6 by a rotamer toggle switch. *J. Biol. Chem*. 277:40989–96
72. Jongejan A, Bruysters M, Ballesteros JA, Haaksma E, Bakker RA, et al. 2005. Linking agonist binding to histamine H1 receptor activation. *Nat. Chem. Biol*. 1:98–103
73. McAllister SD, Hurst DP, Barnett-Norris J, Lynch D, Reggio PH, Abood ME. 2004. Structural mimicry in class A G protein-coupled receptor rotamer toggle switches: the importance of the F3.36(201)/W6.48(357) interaction in cannabinoid CB1 receptor activation. *J. Biol. Chem*. 279:48024–37

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

74. Xu W, Campillo M, Pardo L, Kim de Riel J, Liu-Chen LY. 2005. The seventh transmembrane domains of the delta and kappa opioid receptors have different accessibility patterns and interhelical interactions. *Biochemistry* 44:16014–25
75. Auger-Messier M, Clement M, Lanctot PM, Leclerc PC, Leduc R, et al. 2003. The constitutively active N111G-AT1 receptor for angiotensin II maintains a high affinity conformation despite being uncoupled from its cognate G protein Gq/11alpha. *Endocrinology* 144:5277–84
76. Shi L, Javitch JA. 2002. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.* 42:437–67
77. Lopez-Rodriguez ML, Morcillo MJ, Fernandez E, Benhamu B, Tejada I, et al. 2005. Synthesis and structure-activity relationships of a new model of arylpiperazines. 8. Computational simulation of ligand-receptor interaction of 5-HT(1A)R agonists with selectivity over alpha1-adrenoceptors. *J. Med. Chem.* 48:2548–58
78. Bownds D. 1967. Site of attachment of retinal in rhodopsin. *Nature* 216:1178–81
79. Sakmar TP, Franke RR, Khorana HG. 1989. Glutamic acid-113 serves as the retinylidene Schiff base counterion in bovine rhodopsin. *Proc. Natl. Acad. Sci. USA* 86:8309–13
80. Okada T, Ernst OP, Palczewski K, Hofmann KP. 2001. Activation of rhodopsin: new insights from structural and biochemical studies. *Trends Biochem. Sci.* 26:318–24
81. Robinson PR, Cohen GB, Zhukovsky EA, Oprian DD. 1992. Constitutively active mutants of rhodopsin. *Neuron* 9:719–25
82. Kim JM, Altenbach C, Kono M, Oprian DD, Hubbell WL, Khorana HG. 2004. Structural origins of constitutive activation in rhodopsin: role of the K296/E113 salt bridge. *Proc. Natl. Acad. Sci. USA* 101:12508–13
83. Keen TJ, Inglehearn CF, Lester DH, Bashir R, Jay M, et al. 1991. Autosomal dominant retinitis pigmentosa: four new mutations in rhodopsin, one of them in the retinal attachment site. *Genomics* 11:199–205
84. Lem J, Fain GL. 2004. Constitutive opsin signaling: night blindness or retinal degeneration? *Trends Mol. Med.* 10:150–57
85. Gross AK, Rao VR, Oprian DD. 2003. Characterization of rhodopsin congenital night blindness mutant T94I. *Biochemistry* 42:2009–15
86. Gross AK, Xie G, Oprian DD. 2003. Slow binding of retinal to rhodopsin mutants G90D and T94D. *Biochemistry* 42:2002–8
87. Vassart G, Pardo L, Costagliola S. 2004. A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem. Sci.* 29:119–26
88. 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.* 11:1659–68
89. Fan QR, Hendrickson WA. 2005. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 433:269–77

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

90. Vischer HF, Granneman JC, Noordam MJ, Mosselman S, Bogerd J. 2003. Ligand selectivity of gonadotropin receptors. Role of the beta-strands of extracellular leucine-rich repeats 3 and 6 of the human luteinizing hormone receptor. *J. Biol. Chem*. 278:15505–13
91. Smits G, Campillo M, Govaerts C, Janssens V, Richter C, et al. 2003. Glycoprotein hormone receptors: determinants in leucine-rich repeats responsible for ligand specificity. *EMBO J*. 22:2692–703
92. Van Durme J, Horn F, Costagliola S, Vriend G, Vassart G. 2006. GRIS: glycoprotein-hormone receptor information system. *Mol. Endocrinol*. 20:2247–55
93. De Felice M, Postiglione MP, Di Lauro R. 2004. Minireview: thyrotropin receptor signaling in development and differentiation of the thyroid gland: insights from mouse models and human diseases. *Endocrinology* 145:4062–67
94. Corvilain B, Van Sande J, Dumont JE, Vassart G. 2001. Somatic and germline mutations of the TSH receptor and thyroid diseases. *Clin. Endocrinol*. 55:143–58
95. Zhang M, Tong KP, Fremont V, Chen J, Narayan P, et al. 2000. The extracellular domain suppresses constitutive activity of the transmembrane domain of the human TSH receptor: implications for hormone-receptor interaction and antagonist design. *Endocrinology* 141:3514–17
96. Vlaeminck-Guillem V, Ho SC, Rodien P, Vassart G, Costagliola S. 2002. Activation of the cAMP pathway by the TSH receptor involves switching of the ectodomain from a tethered inverse agonist to an agonist. *Mol. Endocrinol*. 16:736–46
97. Themmen APN, Huhtaniemi IT. 2000. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr. Rev*. 21:551–83
98. Delemarre-van de Waal HA. 2002. Regulation of puberty. *Best Pract. Res. Clin. Endocrinol. Metab*. 16:1–12
99. Themmen AP. 2005. An update of the pathophysiology of human gonadotrophin subunit and receptor gene mutations and polymorphisms. *Reproduction* 130:263–74
100. Shinozaki H, Fanelli F, Liu X, Jaquette J, Nakamura K, Segaloff DL. 2001. Pleiotropic effects of substitutions of a highly conserved leucine in transmembrane helix III of the human lutropin/choriogonadotropin receptor with respect to constitutive activation and hormone responsiveness. *Mol. Endocrinol*. 15:972–84
101. Zhang M, Mizrachi D, Fanelli F, Segaloff DL. 2005. The formation of a salt bridge between helices 3 and 6 is responsible for the constitutive activity and lack of hormone responsiveness of the naturally occurring L457R mutation of the human lutropin receptor. *J. Biol. Chem*. 280:26169–76
102. Nakabayashi K, Kudo M, Hsueh AJ, Maruo T. 2003. Activation of the luteinizing hormone receptor in the extracellular domain. *Mol. Cell Endocrinol*. 202:139–44

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

103. Nishi S, Nakabayashi K, Kobilka B, Hsueh AJ. 2002. The ectodomain of the luteinizing hormone receptor interacts with exoloop 2 to constrain the transmembrane region: studies using chimeric human and fly receptors. *J. Biol. Chem*. 277:3958–64
104. Nakabayashi K, Kudo M, Kobilka B, Hsueh AJ. 2000. Activation of the luteinizing hormone receptor following substitution of Ser-277 with selective hydrophobic residues in the ectodomain hinge region. *J. Biol. Chem*. 275:30264–71
105. Plant TM, Marshall GR. 2001. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocr. Rev*. 22:764–86
106. Gromoll J, Simoni M, Nieschlag E. 1996. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J. Clin. Endocrinol. Metab*. 81:1367–70
107. Haywood M, Tymchenko N, Spaliviero J, Koch A, Jimenez M, et al. 2002. An activated human follicle-stimulating hormone (FSH) receptor stimulates FSH-like activity in gonadotropin-deficient transgenic mice. *Mol. Endocrinol*. 16:2582–91
108. Simoni M, Gromoll J, Nieschlag E. 1997. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr. Rev*. 18:739–73
109. Allan CM, Garcia A, Spaliviero J, Jimenez M. 2006. Maintenance of spermatogenesis by the activated human (Asp567Gly) FSH receptor during testicular regression due to hormonal withdrawal. *Biol. Reprod*. 74:938–44
110. Macklon NS, Stouffer RL, Giudice LC, Fauser BC. 2006. The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocr. Rev*. 27:170–207
111. Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. 2003. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N. Engl. J. Med*. 349:760–66
112. Montanelli L, Delbaere A, Di Carlo C, Nappi C, Smits G, et al. 2004. A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. *J. Clin. Endocrinol. Metab*. 89:1255–58
113. Vasseur C, Rodien P, Beau I, Desroches A, Gerard C, et al. 2003. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N. Engl. J. Med*. 349:753–59
114. De Leener A, Montanelli L, Van Durme J, Chae H, Smits G, et al. 2006. Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J. Clin. Endocrinol. Metab*. 91:555–62
115. Kaiser UB. 2003. The pathogenesis of the ovarian hyperstimulation syndrome. *N. Engl. J. Med*. 349:729–32
116. Borgel K, Pohlenz J, Koch HG, Bramswig JH. 2005. Long-term carbimazole treatment of neonatal nonautoimmune hyperthyroidism due to a new activating TSH receptor gene mutation (Ala428Val). *Horm. Res*. 64:203–8

117. Soriano-Guillen L, Lahlou N, Chauvet G, Roger M, Chaussain JL, Carel JC. 2005. Adult height after ketoconazole treatment in patients with familial male-limited precocious puberty. *J. Clin. Endocrinol. Metab*. 90:147–51
118. Reiter EO, Norjavaara E. 2005. Testotoxicosis: current viewpoint. *Pediatr. Endocrinol. Rev*. 3:77–86
119. Manivannan E, Prasanna S. 2005. First QSAR report on FSH receptor antagonistic activity: quantitative investigations on physico-chemical and structural features among 6-amino-4-phenyltetrahydroquinoline derivatives. *Bioorg. Med. Chem. Lett*. 15:4496–501
120. Wrobel J, Green D, Jetter J, Kao W, Rogers J, et al. 2002. Synthesis of (bis)sulfonic acid, (bis)benzamides as follicle-stimulating hormone (FSH) antagonists. *Bioorg. Med. Chem*. 10:639–56
121. van Straten NC, Schoonus-Gerritsma GG, van Someren RG, Draaijer J, Adang AE, et al. 2002. The first orally active low molecular weight agonists for the LH receptor: thienopyr(im)idines with therapeutic potential for ovulation induction. *Chembiochem* 3:1023–26
122. van Straten NC, van Berkel TH, Demont DR, Karstens WJ, Merkx R, et al. 2005. Identification of substituted 6-amino-4-phenyltetrahydroquinoline derivatives: potent antagonists for the follicle-stimulating hormone receptor. *J. Med. Chem*. 48:1697–700
123. Guo T, Adang AE, Dolle RE, Dong G, Fitzpatrick D, et al. 2004. Small molecule biaryl FSH receptor agonists. Part 1: lead discovery via encoded combinatorial synthesis. *Bioorg. Med. Chem. Lett*. 14:1713–16
124. Arey BJ, Deecher DC, Shen ES, Stevis PE, Meade EHJ, et al. 2002. Identification and characterization of a selective, nonpeptide follicle-stimulating hormone receptor antagonist. *Endocrinology* 143:3822–29
125. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402:656–60
126. Hornby PJ. 2006. Designing spiegelmers to antagonise ghrelin. *Gut* 55:754–55
127. Grove KL, Cowley MA. 2005. Is ghrelin a signal for the development of metabolic systems? *J. Clin. Invest*. 115:3393–97
128. Holst B, Holliday ND, Bach A, Elling CE, Cox HM, Schwartz TW. 2004. Common structural basis for constitutive activity of the ghrelin receptor family. *J. Biol. Chem*. 279:53806–17
129. Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, et al. 2006. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J. Clin. Invest*. 116:760–68
130. Wang HJ, Geller F, Dempfle A, Schauble N, Friedel S, et al. 2004. Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J. Clin. Endocrinol. Metab*. 89:157–62
131. Holst B, Schwartz TW. 2006. Ghrelin receptor mutations—too little height and too much hunger. *J. Clin. Invest*. 116:637–41

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

132. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, et al. 2005. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 310:996–99
133. Graham A, Wakamatsu K, Hunt G, Ito S, Thody AJ. 1997. Agouti protein inhibits the production of eumelanin and phaeomelanin in the presence and absence of alpha-melanocyte stimulating hormone. *Pigment. Cell Res.* 10:298–303
134. Tao YX. 2006. Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. *Pharmacol. Ther*. 111:949–73
135. Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C. 2005. Melanocortin-1 receptor structure and functional regulation. *Pigment. Cell Res.* 18:393–410
136. Siegrist W, Drozdz R, Cotti R, Willard DH, Wilkison WO, Eberle AN. 1997. Interactions of alpha-melanotropin and agouti on B16 melanoma cells: evidence for inverse agonism of agouti. *J. Recept. Signal. Transduct. Res*. 17:75–98
137. Vergoni AV, Bertolini A, Guidetti G, Karefilakis V, Filaferro M, et al. 2000. Chronic melanocortin 4 receptor blockage causes obesity without influencing sexual behavior in male rats. *J. Endocrinol*. 166:419–26
138. Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, et al. 2000. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J. Clin. Invest*. 106:271–79
139. Srinivasan S, Lubrano-Berthelier C, Govaerts C, Picard F, Santiago P, et al. 2004. Constitutive activity of the melanocortin-4 receptor is maintained by its N-terminal domain and plays a role in energy homeostasis in humans. *J. Clin. Invest*. 114:1158–64
140. Adan RA, Kas MJ. 2003. Inverse agonism gains weight. *Trends Pharmacol. Sci*. 24:315–21
141. Nicholson JR, Kohler G, Schaerer F, Senn C, Weyermann P, Hofbauer KG. 2006. Peripheral administration of a melanocortin 4-receptor inverse agonist prevents loss of lean body mass in tumor-bearing mice. *J. Pharmacol. Exp. Ther*. 317:771–77
142. Tao YX, Segaloff DL. 2005. Functional analyses of melanocortin-4 receptor mutations identified from patients with binge eating disorder and nonobese or obese subjects. *J. Clin. Endocrinol. Metab*. 90:5632–38
143. Adan RA, Hillebrand JJ, De Rijke C, Nijenhuis W, Vink T, et al. 2003. Melanocortin system and eating disorders. *Ann. N.Y. Acad. Sci*. 994:267–74
144. Adan RA, Vink T. 2001. Drug target discovery by pharmacogenetics: mutations in the melanocortin system and eating disorders. *Eur. Neuropsychopharmacol*. 11:483–90
145. Nijenhuis WA, Garner KM, van Rozen RJ, Adan RA. 2003. Poor cell surface expression of human melanocortin-4 receptor mutations associated with obesity. *J. Biol. Chem*. 278:22939–45
146. Govaerts C, Srinivasan S, Shapiro A, Zhang S, Picard F, et al. 2005. Obesity-associated mutations in the melanocortin 4 receptor provide novel insights into its function. *Peptides* 26:1909–19

147. Finlay BB, McFadden G. 2006. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. *Cell* 124:767–82
148. Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, et al. 2000. International Union of Pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* 52:145–76
149. Vischer HF, Vink C, Smit MJ. 2006. A viral conspiracy: hijacking the chemokine system through virally encoded pirated chemokine receptors. *Curr. Top. Microbiol. Immunol.* 303:121–54
150. Cesarman E, Nador RG, Bai F, Bohenzky RA, Russo JJ, et al. 1996. Kaposi's sarcoma-associated herpesvirus contains G protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. *J. Virol.* 70:8218–23
151. Smit MJ, Vink C, Verzijl D, Casarosa P, Bruggeman CA, Leurs R. 2003. Virally encoded G protein-coupled receptors: targets for potentially innovative antiviral drug development. *Curr. Drug. Targets* 4:431–41
152. Yang TY, Chen SC, Leach MW, Manfra D, Homey B, et al. 2000. Transgenic expression of the chemokine receptor encoded by human herpesvirus 8 induces an angioproliferative disease resembling Kaposi's sarcoma. *J. Exp. Med.* 191:445–54
153. Rosenkilde MM, Kledal TN, Holst PJ, Schwartz TW. 2000. Selective elimination of high constitutive activity or chemokine binding in the human herpesvirus 8 encoded seven transmembrane oncogene ORF74. *J. Biol. Chem.* 275:26309–15
154. Holst PJ, Rosenkilde MM, Manfra D, Chen SC, Wiekowski MT, et al. 2001. Tumorigenesis induced by the HHV8-encoded chemokine receptor requires ligand modulation of high constitutive activity. *J. Clin. Invest.* 108:1789–96
155. Sodhi A, Montaner S, Patel V, Gomez-Roman JJ, Li Y, et al. 2004. Akt plays a central role in sarcomagenesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. *Proc. Natl. Acad. Sci. USA* 101:4821–26
156. Jensen KK, Manfra DJ, Grisotto MG, Martin AP, Vassileva G, et al. 2005. The human herpes virus 8-encoded chemokine receptor is required for angioproliferation in a murine model of Kaposi's sarcoma. *J. Immunol.* 174:3686–94
157. Grisotto MG, Garin A, Martin AP, Jensen KK, Chan P, et al. 2006. The human herpesvirus 8 chemokine receptor vGPCR triggers autonomous proliferation of endothelial cells. *J. Clin. Invest.* 116:1264–73
158. Montaner S, Sodhi A, Molinolo A, Bugge TH, Sawai ET, et al. 2003. Endothelial infection with KSHV genes in vivo reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. *Cancer Cell* 3:23–36
159. Montaner S, Sodhi A, Ramsdell AK, Martin D, Hu J, et al. 2006. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor as a therapeutic target for the treatment of Kaposi's sarcoma. *Cancer Res.* 66:168–74
160. Paulsen SJ, Rosenkilde MM, Eugen-Olsen J, Kledal TN. 2005. Epstein-Barr virus-encoded BILF1 is a constitutively active G protein-coupled receptor. *J. Virol.* 79:536–46

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

161. Beisser PS, Verzijl D, Gruijthuijsen YK, Beuken E, Smit MJ, et al. 2005. The Epstein-Barr virus BILF1 gene encodes a G protein-coupled receptor that inhibits phosphorylation of RNA-dependent protein kinase. *J. Virol.* 79:441–49
162. Casarosa P, Bakker RA, Verzijl D, Navis M, Timmerman H, et al. 2001. Constitutive signaling of the human cytomegalovirus-encoded chemokine receptor US28. *J. Biol. Chem.* 276:1133–37
163. Casarosa P, Gruijthuijsen YK, Michel D, Beisser PS, Holl J, et al. 2003. Constitutive signaling of the human cytomegalovirus-encoded receptor UL33 differs from that of its rat cytomegalovirus homolog R33 by promiscuous activation of G proteins of the Gq, Gi, and Gs classes. *J. Biol. Chem.* 278:50010–23
164. Minisini R, Tulone C, Luske A, Michel D, Mertens T, et al. 2003. Constitutive inositol phosphate formation in cytomegalovirus-infected human fibroblasts is due to expression of the chemokine receptor homologue pUS28. *J. Virol.* 77:4489–501
165. Streblow DN, Soderberg-Naucler C, Vieira J, Smith P, Wakabayashi E, et al. 1999. The human cytomegalovirus chemokine receptor US28 mediates vascular smooth muscle cell migration. *Cell* 99:511–20
165a. Waldhoer M, Casarosa P, Rosenkilde MM, Smit MJ, Leurs R, et al. 2003. The carboxyl terminus of human cytomegalovirus-encoded 7 transmembrane receptor US28 camouflages agonism by mediating constitutive endocytosis. *J. Biol. Chem.* 278:19473–82
166. Casarosa P, Menge WM, Minisini R, Otto C, van Heteren J, et al. 2003. Identification of the first nonpeptidergic inverse agonist for a constitutively active viral-encoded G protein-coupled receptor. *J. Biol. Chem.* 278:5172–78
167. Stassen FR, Vega-Cordova X, Vliegen I, Bruggeman CA. 2006. Immune activation following cytomegalovirus infection: more important than direct viral effects in cardiovascular disease? *J. Clin. Virol.* 35:349–53
168. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, et al. 2002. Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res.* 62:3347–50
169. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, et al. 2002. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet* 360:1557–63
170. Chen F, Castranova V, Shi X, Demers LM. 1999. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. *Clin. Chem.* 45:7–17
171. Cinatl JJ., Vogel JU, Kotchetkov R, Wilhelm Doerr H. 2004. Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. *FEMS Microbiol. Rev.* 28:59–77
172. Maussang D, Verzijl D, van Walsum M, Leurs R, Holl J, et al. 2006. Human cytomegalovirus-encoded chemokine receptor US28 promotes tumorigenesis. *Proc. Natl. Acad. Sci. USA* 103:13068–73
173. Hulshof JW, Casarosa P, Menge WM, Kuusisto LM, van der Goot H, et al. 2005. Synthesis and structure-activity relationship of the first nonpeptidergic

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

inverse agonists for the human cytomegalovirus encoded chemokine receptor US28. *J. Med. Chem*. 48:6461–71

174. Mannstadt M, Juppner H, Gardella TJ. 1999. Receptors for PTH and PTHrP: their biological importance and functional properties. *Am. J. Physiol. Renal Physiol*. 277:F665–75
175. Schipani E, Kruse K, Juppner H. 1995. A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* 268:98–100
176. Brown EM, MacLeod RJ. 2001. Extracellular calcium sensing and extracellular calcium signaling. *Physiol. Rev*. 81:239–97
177. Pin JP, Galvez T, Prezeau L. 2003. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol. Ther*. 98:325–54
178. Hu J, Spiegel AM. 2003. Naturally occurring mutations of the extracellular Ca2+-sensing receptor: implications for its structure and function. *Trends Endocrinol. Metab*. 14:282–88
179. Pidasheva S, D'Souza-Li L, Canaff L, Cole DE, Hendy GN. 2004. CASRdb: calcium-sensing receptor locus-specific database for mutations causing familial (benign) hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum. Mutat*. 24:107–11
180. Zhao XM, Hauache O, Goldsmith PK, Collins R, Spiegel AM. 1999. A missense mutation in the seventh transmembrane domain constitutively activates the human Ca2+ receptor. *FEBS Lett*. 448:180–84
181. Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, et al. 2002. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet* 360:692–94
182. DeCamp DL, Thompson TM, de Sauvage FJ, Lerner MR. 2000. Smoothened activates Galphai-mediated signaling in frog melanophores. *J. Biol. Chem*. 275:26322–27
183. Taipale J, Cooper MK, Maiti T, Beachy PA. 2002. Patched acts catalytically to suppress the activity of Smoothened. *Nature* 418:892–97
184. Huang S, He J, Zhang X, Bian Y, Yang L, et al. 2006. Activation of the hedgehog pathway in human hepatocellular carcinomas. *Carcinogenesis*. 27:1334–40
185. Kayed H, Kleeff J, Keleg S, Guo J, Ketterer K, et al. 2004. Indian hedgehog signaling pathway: expression and regulation in pancreatic cancer. *Int. J. Cancer* 110:668–76
186. Ma X, Sheng T, Zhang Y, Zhang X, He J, et al. 2006. Hedgehog signaling is activated in subsets of esophageal cancers. *Int. J. Cancer* 118:139–48
187. Sheng T, Li C, Zhang X, Chi S, He N, et al. 2004. Activation of the hedgehog pathway in advanced prostate cancer. *Mol. Cancer* 3:29
188. Tabs S, Avci O. 2004. Induction of the differentiation and apoptosis of tumor cells in vivo with efficiency and selectivity. *Eur. J. Dermatol*. 14:96–102
189. Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, et al. 2000. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. *Nature* 406:1005–9

Annu. Rev. Pharmacol. Toxicol. 2007.47:53-87.

190. Athar M, Li C, Tang X, Chi S, Zhang X, et al. 2004. Inhibition of Smoothened signaling prevents UV B-induced basal cell carcinomas through regulation of Fas expression and apoptosis. *Cancer Res.* 64:7545–52
191. Tas S, Avci O. 2004. Rapid clearance of psoriatic skin lesions induced by topical cyclopamine. A preliminary proof of concept study. *Dermatology* 209:126–31
192. Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. 2002. Small molecule modulation of Smoothened activity. *Proc. Natl. Acad. Sci. USA* 99:14071–76
193. Frank-Kamenetsky M, Zhang XM, Bottega S, Guicherit O, Wichterle H, et al. 2002. Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. *J. Biol.* 1:10
194. Sinha S, Chen JK. 2006. Purmorphamine activates the Hedgehog pathway by targeting Smoothened. *Nat. Chem. Biol.* 2:29–30
195. Hubbell WL, Altenbach C, Hubbell CM, Khorana HG. 2003. Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. *Adv. Protein. Chem.* 63:243–90
196. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, et al. 2005. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N. Engl. J. Med.* 352:558–69
197. Nauta WT, Rekker RF, Harms AF. 1968. *Proc. Int. Pharmacol. Meet., 3rd, 1966* 7:305
198. Kraulis J. 1991. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structure. *J. Appl. Cryst.* 24:946–50
199. Merritt EA, Bacon DJ. 1997. Raster3D: photorealistic molecular graphics. *Methods Enzymol.* 277:505–24
200. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, et al. 2003. Human gene mutation database (HGMD): 2003 update. *Hum. Mutat.* 21:577–81

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