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Review: Amino Acid Domains Involved in Constitutive Activation of G-Protein-Coupled Receptors

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Absract

Guanine nucleotide-binding protein-coupled receptors may attain an active conformation in the absence of agonist by spontaneous isomerization and thus yield constitutive, agonist-independent, activity. This has mainly been demonstrated for isolated membranes and recombinant wild-type receptors, and mutant receptors. They generally show remarkable increases in the sensitivity of a biological response. The location of activating mutations both within a single receptor and across receptors is widespread, with changes reported in the seven-transmembrane domains, the second and third intracellular loop. For most of these receptors, examples of ligands defined as inverse agonists have been documented. Regulation of these receptors by inverse agonists opposite to that observed by agonists, and the therapeutic potential of inverse agonists is underlined.

Index Entries: G-protein-coupled receptor; mutant receptor; constitutive receptor activation; negative efficacy; inverse agonist.

Introduction

Classical concepts of drug-receptor interactions define two major classes of ligands: agonists, which at maximally effective concentrations elicit a full or partial response, and antagonists, which have no intrinsic activity but block the response to agonists. Pharmacological models generally envisage a system that displays no or very little activity in the absence of agonist. Recently, several lines of research have revealed that a number of guanine nucleotide-binding protein-coupled receptors (GPCR) demonstrate activity in the absence of agonist in their native state as well as in recombinant heterologous receptor-expression systems. This has led to the concept that GPCR can change conformation spontaneously or by mutation and oscillate between an active and inactive receptor conformation (Leff, 1995). Receptors exist in an equilibrium between both conformations: an inactive conformation that is structurally constrained and unable to bind a G-protein subunit and an active conformation

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that can interact productively with a GTP-bound G!iga-subunit. It is now appreciated that receptors can attain the active conformation in the absence of agonist by spontaneous isomerization and display constitutive, that is agonist-independent, activity (Lefkowitz et al., 1993). Certain ligands, termed "inverse agonists" or "negative antagonists," appear capable of driving receptors to the inactive state (Samama et al., 1993). Negative antagonism is demonstrated when a ligand binds to a receptor that exhibits constitutive activity and reduces this activity. Although first described for the actions of β-carbolines at the ionotropic γ-aminobutyric acid A/benzodiazepine-receptor complex (Braestrup et al., 1982; Schütz and Freissmuth, 1992), negative antagonism has been shown to occur with GPCR, for example δ-opioid (Costa and Herz, 1989; Costa et al., 1992), β_2-adrenergic (Chidiac et al., 1994; Pei et al., 1994; Samama et al., 1994), serotonin 5-HT_{2C} (Barker et al., 1994), bradykinin B_2 (Leeb-Lundberg et al., 1994), and dopamine D_5 receptors (Tiberi and Caron, 1994). Deletional mutations in receptors, construction of receptor point mutations and receptor chimera highlight portions of the third and second intracellular loop (ICL) and proximal portions of the carboxyl terminus as being important for interaction of GPCR with G!iga-subunits (Strader et al., 1987; Dixon et al., 1988; O'Dowd et al., 1988; Kobilka et al., 1988; Wong et al., 1990; Dalman et al., 1991). The ability to detect negative intrinsic activity of GPCR depends mainly on a measurable constitutive or agonist-independent activity. Constitutive activity of GPCR is a well-established phenomenon in phospholipid vesicles, isolated membranes, and reconstituted wild-type receptor systems (Cerione et al., 1984; Costa et al., 1992; Schutz and Freissmuth, 1992; Samama et al., 1993; Adie and Milligan, 1994a; Adie and Milligan, 1994b; Barker et al., 1994; Tiberi and Caron, 1994; Westphal and Sanders-Bush, 1994; Kim et al., 1995). It has also recently been described for mutant GPCR (Cotecchia et al., 1990; Lefkowitz et al., 1993; Kjelsberg et al., 1992; Samama et al., 1993; Ren et al., 1993) and transgenic mice with myocardial overexpression of the β_2-adrenergic receptor (Milano et al., 1994a; Bond et al., 1995). In these various models, the respective GPCR exhibit spontaneous agonist-independent receptor activity. This enhanced basal-receptor activity has been shown to be inhibited by inverse agonists, but not by neutral antagonists. The reader is referred to the reviews of Schütz and Freissmuth (1992), Lefkowitz et al. (1993), Parma et al. (1994), Kenakin (1995), and Milligan et al. (1995) for previous reported aspects on constitutive activition of GPCR and reverse-intrinsic activity of antagonists. In the present review, attention will be given to the following aspects of GPCR: the amino acid domains that appear to be involved in constitutive activation, regulation of these receptors by inverse agonists, and the therapeutic potential of inverse agonists at these receptors.

Constitutive Activation of Wild-Type G-Protein-Coupled Receptors

The pharmacological properties of inverse agonists tend to be more apparent in systems that express relatively high receptor levels with an associated high-basal-effector activity. It is probably for this reason that the majority of recent reports examining inverse agonism have used transfected cell lines that express defined wild-type GPCR (Milligan et al., 1995). An early report on functional reconstitution of β_2-adrenergic receptors in phospholipid vesicles, containing purified β_2-adrenergic receptor and G_s protein, describes an increased basal GTPase activity for vesicles containing both β_2-adrenergic receptor and G_s compared to vesicles containing only the receptor or the G_s protein (Cerione et al., 1984). Spontaneous coupling of β-adrenergic receptors to a stimulatory G_s protein has been postulated by Nerme et al. (1986) based on iodopindolol-binding experiments performed with cardiac membranes of cat, rat, and guinea pig. Neubig et al. (1988) reported later on evidence for a precoupled α_2-adrenergic receptor-guanine nucleotide-binding pro-

tein complex. Early indications for inverse-agonist activity were based on guanine nucleotide-dependent alterations in antagonist binding, such as for muscarinic acetylcholine (mAchR), β_2-adrenergic, dopamine D_2, adenosine A_1 and μ-opioid receptors integrated in the plasma membrane or reconstituted with G proteins (*see* Schütz and Freissmuth, 1992). Analysis of inverse agonism on GPCR was derived from the ability of the δ-opioid-receptor ligand ICI 174864 to inhibit basal GTPase activity in membranes of NG108-15 cells (Costa and Herz, 1989; Costa et al., 1990). Recently, ICI 174864 has also been shown to act as an inverse agonist in intact human embryonic kidney HEK 293 cells expressing a cloned murine δ-opioid receptor (Chiu et al., 1996). The inverse agonistic effect seems to require inhibitory G_i proteins and is clearly manifested when adenylyl cyclase is activated by forskolin. Interestingly, ICI 174864 fails to elicit negative-intrinsic activity in intact NG108-15 cells (Chiu et al., 1996). Therefore, it is likely that the ability of ICI 174864 to act as an inverse agonist in intact cells is cell-type specific. This may illustrate how important a cell type may be for detection of inverse-agonist activity at a receptor subtype.

Table 1 summarizes a series of wild-type GPCR yielding constitutive activation in either recombinant-expression systems or by native expression. For most of these receptors, examples of ligands defined as inverse agonists have been demonstrated. Milligan et al. (1995) suggested that inverse-agonist activity should be observable at all GPCRs on condition that suitable ligands are available. In general, the following parameters have been measured in either membrane preparations or intact cells to monitor constitutive receptor activation: ligand binding properties, GTPase activity, [^{35}S] GTPγS-binding responses, cAMP formation, phosphoinositide hydrolysis, electrophysiological recording, or mitogenic activity. 5-HT_{2C}-receptor antagonists (e.g., mianserin, spiperone, mesulergine, ketanserin, and clozapine) possess negative-intrinsic activity in NIH 3T3 fibroblasts expressing recombinant rat 5-HT_{2C} receptors (Barker et al., 1994; Westphal and Sanders-Bush, 1994; Westphal and Sanders-Bush, 1996). This action reflects the consequence of binding to unoccupied constitutively active receptors, rather than an action mediated by blockade of binding of an endogenous agonist. The inverse agonists (e.g., clozapine) show higher affinity for the G-protein-uncoupled form of the 5-HT_{2C} receptor as they decrease basal-receptor activation, thereby shifting receptor equilibrium away from the precoupled state. Agonists (e.g., (-)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane) yield higher affinity for the G-protein-coupled form inasmuch as they increase receptor activation by binding with higher affinity to the receptor in a conformation that interacts with G proteins. Neutral antagonists (e.g., (+)-2-bromolysergic acid) bind both forms of the receptor with equal affinity and therefore have no effect on basal-receptor activity but block the effects of both agonists and inverse agonists. These binding results are consistent with the ternary complex model proposed for β_2-adrenergic-receptor activation (Costa et al., 1992; Samama et al., 1993). Inverse-agonist activity has also been observed in Sf_9 insect cells expressing the rat 5-HT_{2C} receptor using a recombinant baculovirus-expression system (Labrecque et al., 1995; Hartman and Northup, 1996). Furthermore, studies with recombinant 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors in stably transfected C6-glial, HeLa and CHO cell lines have shown that spiperone, WAY100635, methiothepin and/or ketanserin yield inverse agonist properties (Thomas et al., 1995; Newman-Tancredi et al., 1997; Pauwels et al., 1997a; Pauwels et al., 1997b; Stanton and Beer, 1997).

(+)-butaclamol and flupentixol act as inverse agonists at dopamine D_1 and D_5 receptors expressed in HEK 293 cells (Tiberi and Caron, 1994). Similar ligands have also been shown to act as inverse agonists at dopamine D_3 receptors expressed in NG108-15 cells (Griffon et al., 1996). Hall and Strange (1997) put forward that dopamine D_2-receptor antagonists, many of which are used clinically as antipsychotic drugs, are in fact inverse agonists at human recombinant dopamine D_2 receptors in CHO

Table 1
Examples of Wild-Type G-Protein-Coupled Receptors that Yield Constitutive Activation

Receptor Subtype	Cell Type	Investigated Parameters	Inverse Agonists	References
Recombinant receptors				
δ-opioid	HEK 293	cAMP	ICI 174864	Chiu et al. 1996
5-HT_{2C}	NIH 3T3	PI hydrolysis	mianserin, spiperone, mesulergine, ketanserin	Barker et al. 1994
	NIH 3T3	ligand binding, PI hydrolysis	clozapine, cyproheptadine, ketanserin, mesulergine	Westphal and Sanders-Bush 1994
	NIH 3T3	thymidine incorporation	clozapine, ketanserin, spiperone, mianserin	Westphal and Sanders-Bush 1996
	Sf_9, insect	PI hydrolysis	spiperone, ketanserin, clozapine, methysergide, metergoline, mesulergine, ritanserin, mianserin	Labrecque et al. 1995
	Sf_9, insect	[^{35}S]GTPγS binding	mianserin, ketanserin	Hartman and Northup 1996
h 5-HT_{1A}	CHO	"	spiperone,	Newman-Tancredi et al. 1997
	C6-glial	"	spiperone, WAY100635	Pauwels et al. 1997b
	HeLa	"	methiothepin	Stanton and Beer 1997
h 5-HT_{1B}	CHO	"	methiothepin	Thomas et al. 1995
	C6-glial	"	methiothepin	Pauwels et al. 1997a
h 5-HT_{1D}	CHO	"	methiothepin, ketanserin	Thomas et al. 1995
	C6-glial	"	methiothepin, ketanserin	Pauwels et al. 1997a
Dopamine D	HEK 293	cAMP	(+)-butaclamol, flupenthixol	Tiberi and Caron 1994
Dopamine D_5 (D_{1B})	HEK 293	"	"	"
Dopamine D_2	CHO	cAMP	(+)-butaclamol, flupenthixol, haloperidol	Hall and Strange, 1997
Dopamine D_3	NG108-15	thymidine incorporation	flupenthixol, fluphenazine, haloperidol	Griffon et al. 1996
α_{2B}-adrenergic	PC12	ligand binding		Shi and Deth 1994
α_{2D}-adrenergic	PC12	[^{35}S]GTPγS binding	rauwolscine, yohimbine, WB 4101, idazoxan, phentolamine	Tian et al. 1994
β_2-adrenergic	NG108-15	cAMP	propranolol	Adie and Milligan 1994a, b
	Sf_9, insect	"	dichloroisoproterenol, pindolol, labetamol, timolol, propranolol, alprenolol	Chidiac et al. 1994
	myocardium transgenic mice	left atrial tension	ICI 118551	Milano et al. 1994a Bond et al. 1995
Muscarinic m_1, m_3, m_5	NIH 3T3 (Gαq over-expression)	proliferative response	atropine, pirenzepine, N-methylscopolamine, QNB, trihexy-phenidyl, 4-di-phenylacetyoxy-N-methylpiperidine	Burstein et al. 1997
Histamine H_2	CHO	cAMP	cimetidine, ranitidine	Smit et al. 1996a
Calcitonin	HEK 293	ligand binding, cAMP	SDZ212-769, SDZ 219-379 AC512, sCT (8-32)	Pozvek et al., 1997

(continued)

Table 1
Continued

Receptor Subtype	Cell Type	Investigated Parameters	Inverse Agonists	References
Native Receptors				
δ-opioid	NG108-15	GTPase	ICI 174864	Costa and Herz 1989, Costa et al. 1990
Bradykinin B_2	rat myocardial cells	ligand binding, PI hydrolysis	HOF 140, NPC 17731, NPC 567	Leeb-Lundberg et al. 1994
β-adrenergic	turkey erythrocytes	cAMP, GDP release, ligand binding	propranolol	Murray and Keenan, 1989
β_1-adrenergic	cardiomyocytes	electrophysiological	atenolol, propranolol	Mewes et al. 1993
β_2-adrenergic	turkey erythrocytes	cAMP	propranolol, pindolol	Götze and Jacobs 1994
Muscarinic acetylcholine	atrial myocytes	electrophysiological recording	atropine, AF-DX116	Hanf et al. 1993
	atrial membranes	[^{35}S]GTPγS binding	atropine	Hilf and Jakobs, 1992
	ventricular myocardium	cAMP, PTX treatment	atropine, AF-DX116	Akaishi et al., 1997
Adenosine A_1	chicken myocytes	cAMP	BW-A8444, CPX	Ma and Green, 1992

cells. Constitutive receptor activation has also been demonstrated for α_{2B}-and α_{2D}-adrenergic receptors in PC12 cells (Shi and Deth, 1994; Tian et al., 1994), and β_2-adrenergic receptors in NG108-15 cells (Adie and Milligan, 1994a; Adie and Milligan, 1994b). Chidiac et al. (1994) investigated a series of antagonists at β_2-adrenergic receptors in Sf_9 insect cells; they observed that most of the ligands were inverse agonists with a varied range of efficacy. Muscarinic m_1, m_3, and m_5 receptors coexpressed with a G protein α-subunit G_q in NIH 3T3 cells exhibit constitutive activity (Burstein et al., 1997), suggesting that local cellular G protein concentrations may also regulate constitutive receptor activity. Inverse agonism of the histamine H_2 antagonists cimetidine and ranitidine has been demonstrated at histamine H_2 receptors in transfected CHO cells (Smit et al., 1996a). Similarly, stable expression of a calcitonin receptor gene in HEK 293 cells yields constitutive receptor activity (Pozvek et al., 1997).

Currently, a debate on the pharmacological phenomenon of inverse agonism or negative antagonism of GPCR antagonists is going on (*see* Baxter and Tilford, 1995; Kenakin, 1995; Milligan et al., 1995). Major criticism is often directed against the fact that spontaneous, agonist-independent GPCR activity has been observed in transfected cell lines with receptor densities exceeding expression levels of 1 pmol/mg protein (Adie and Milligan, 1994a; Adie and Milligan, 1994b; Samama et al., 1993; Barker et al., 1994; Kim et al., 1995; Tiberi and Caron, 1994; Westphal and Sanders-Bush, 1994). Yet, inverse agonism at bradykinin B_2 receptors has also been observed in myometrial cells (Leeb-Lundberg et al., 1994), β-adrenergic receptors in erythrocytes (Murray and Keenan, 1989; Götze and Jakobs, 1994), and β_1-adrenergic receptors and mAchR in cardiomyocytes (Hanf et al., 1993; Mewes et al., 1993), suggesting that inverse agonism is of physiological relevance. Furthermore, atropine has also been shown to be an inverse agonist at mAchR in porcine cardiac membranes using a [^{35}S]GTPγS binding response (Hilf and Jakobs, 1992). Akaishi et al. (1997) put forward that a G_i protein of rabbit ventricular myocytes is tonically activated by mAchR in the absence of

agonist. Adenosine A_1 receptors precoupled to inhibitory G_i proteins also exert in embryonic chicken cardiomyocytes a tonic influence on adenylyl cyclase activity (Ma and Green, 1992).

Transgenic mice have been created with cardiac-specific overexpression of the β_2-adrenergic receptor (Milano et al., 1994a). This resulted in increased basal myocardial adenylyl cyclase activity, enhanced atrial contractility, and increased left ventricular function in vivo; these parameters at baseline in the transgenic animals were equal to those observed in control animals maximally stimulated with isoproterenol. Bond et al. (1995) showed that the β_2-adrenergic ligand ICI 118551 functions as an inverse agonist in these transgenic mice, providing evidence supporting the activity of inverse agonism in vivo. In a similar transgenic mouse model, overexpression of a constitutively active α_{1B}-adrenergic receptor results in myocardial hypertrophy at adult age (Milano et al., 1994b).

Constitutive Activation Arising from G-Protein-Coupled Receptor Mutations

Analysis of the alignment of GPCR amino acid sequences provides clues to the activating mechanism of GPCR by emphasizing highly conserved, presumably critical residues, such as Pro, Trp, and Tyr in transmembrane domains (TM) IV, V, VI, and VII (Baldwin, 1993). The location of activating mutations both within a single receptor and across receptors is widespread (*see* Lefkowitz et al., 1993), with mutations reported in each of the seven TM with the exception of TM IV, the second and third intracellular loop (ICL). Table 2 summarizes a series of receptor mutations inducing constitutively activated GPCR. Figure 1 illustrates the critical amino acid domains apparently involved in constitutive receptor activation. This diversity suggests that specific intramolecular amino acid interactions maintain GPCR in their off-state(s) and that these interactions can be disrupted in a variety of ways although a common three-dimensional structure exists for all GPCR (Nanevicz et al., 1996). Whereas there are no general rules for prediction of the magnitude of constitutive activation by the mutant receptors, this activity can vary considerably compared to the activity driven by wild-type receptors occupied by a full agonist. In some cases, constitutive activity of the mutant receptor (e.g., α_{2A} Thr373Cys) can virtually attain the agonist-driven activity of the wild-type receptor (Ren et al., 1993). Different constitutively activating mutations may yield different pharmacological properties, the mechanisms that produce these changes are also most likely different. The following two paragraphs will focus on mutations of GPCR in the second and third ICL, and the seven TM; they generally cause remarkable increases in the sensitivity of a biological response.

Mutations of GPCR Located Within Intracellular Loops

BBXXB Protein Motif

Molecular changes that are involved in the activation process of GPCR have been identified in the C-terminus of the third ICL. Conservative substitutions of two residues in this region (Lys290His, Ala293Leu) of the α_{1B}-adrenergic receptor increase the potency of agonists for stimulating phosphatidylinositol metabolism (Cotecchia et al., 1990). Remarkably, all 19 possible amino acid substitutions at this single Ala293 site confer constitutive receptor activity with an agonist-independent increase in phosphatidylinositol hydrolysis ranging from 21 to 211% above basal levels compared to the wild-type α_{1B}-adrenergic receptor. The Ala293Glu mutant, with an elevated basal IP_3 level of 211%, is 2.7-fold stimulated by epinephrine compared to a 200-fold stimulation of the wild-type α_{1B}-adrenergic receptor vs its basal condition (Kjelsberg et al., 1992). The 19 mutated receptors exhibit a graded range of elevated affinities and potencies for agonists, apparently representing a

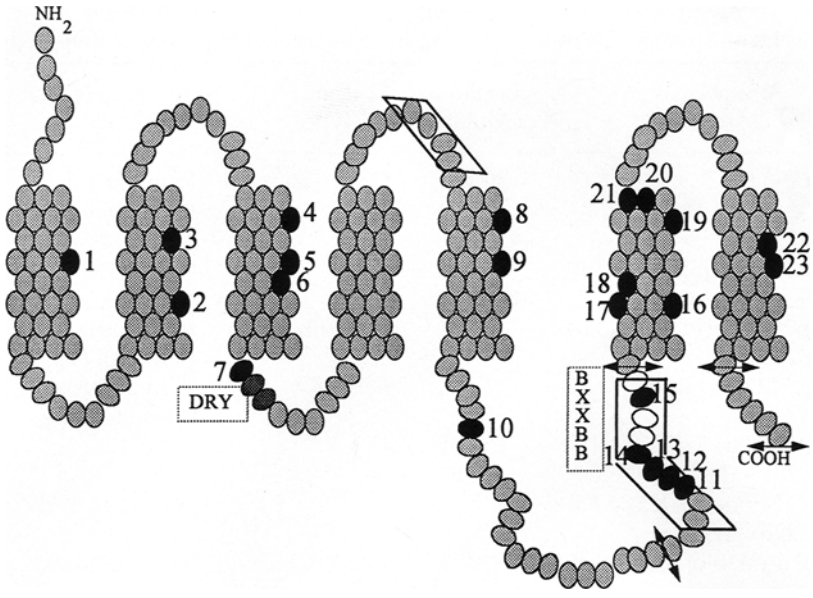

Fig. 1. Mutated G-protein-coupled receptors that yield constitutive activation. A schematic representation of a GPCR is shown with 7 TM and connecting loops of arbitrary length. The numbers indicate the position of specific mutations in the following receptors, α_{1B}-adrenergic: **1** Asn63Ala; **5** Cys128Phe; **7** Asp142all amino acids (AA); **8** Ala204Val; **12** Arg288Lys; **14** Lys290His; **15** Ala293allAA. α_{2A}-adrenergic: **15** Thr373Phe,Ala,Cys, Glu,Lys. 5-HT_{2A}: **15** Cys322Lys,Ala,Glu. Yeast mating-pheromone α-factor: **10** Leu194Gln; **16** Pro258Leu; **17** Ser259Leu. Platelet-activating factor: **6** Asn100Ala; **11** Leu231Arg. Muscarinic m_1: **13** Glu360Ala. Rhodopsin: **2** Gly90Asp; **4** Glu113Gln; **7** Asp134Asn; **22** Ala292Glu; **23** Lys296Glu,Met,Gly. Dopamine D_1: **13** Phe264Ile; **18** Leu286Ala. Luteinizing hormone: **9** Ile542Leu; **19** Asp578Gly. Melanocyte-stimulating hormone: **3** Glu92Lys. Angiotensin AT1: **6** Asn111Ala. Muscarinic m_5: **20** Ser465Tyr, Lys, Arg, Phe; **21** Thr466Pro. [open-square] represents substitution of human thrombin receptor residues (259–268) in the second ECL by *Xenopus* thrombin-receptor equivalent, and substitution of β_2-adrenergic receptor residues 266–272 in the third ICL by α_{1B}-adrenergic-receptor equivalent; ↔ indicates partial deletion of the third ICL and truncation of C-terminal part of yeast mating-pheromone α-factor receptor, and truncation of C-terminal part of prostaglandin E receptor. DRY (Asp-Arg-Tyr) and BBXXB (B for basic and X for a nonbasic amino acid) represent two protein motifs involved in G-protein interaction.

spectrum of receptor conformations that mimic more or less the active state of the wild-type α_{1B}-adrenergic receptor (Kjelsberg et al., 1992). Surprisingly, discrete modifications like Ala293 to Gly or Ala293 to Val, which probably result in subtle structural changes of the α_{1B}-adrenergic receptor, increase basal phosphatidylinositol hydrolysis by 80% (Kjelsberg et al., 1992). The fact that each possible mutation at this particular site results in an increased basal-receptor activity suggests this region may function to constrain the G protein coupling of this receptor, a constraint that is normally relieved by agonist occupancy. Indeed, Lys290 and Ala293 of the α_{1B}-adrenergic receptor are part of a "BBXXB" protein motif (where B is a basic amino acid residue: Arg, Lys, or His, and X: a nonbasic residue), postulated to act as a universal G-protein-activation domain (Okamoto and Nishimoto, 1992). This protein motif is located for many GPCR in the C-terminus of the third ICL, and for α_2- and β_3-adrenergic receptors and 5-HT_{1B} receptors in the C-terminus of the second ICL. It has been extensively mutated in α_{1B}-, β_2- and α_{2A}-adrenergic receptors, 5-HT_{2A} and platelet-activating factor (PAF) receptors;

Table 2
Examples of Mutated G-Protein-Coupled Receptors that Yield Constitutive Activation

Receptor Subtype	Amino Acid Substitution	Location of Mutation	Cell Type	Investigated Parameters	References
α_{1B}-adrenergic	Lys 290 His	3rd ICL	Cos-7	PI hydrolysis, ligand binding	Cotecchia et al. 1990
	Ala 293 Leu	3rd ICL	Cos-7	"	Cotecchia et al. 1990
	Ala 293 Glu (all A.A.)	3rd ICL	"	"	Scheer et al. 1996, Kjelsberg et al. 1992
	Arg 288 Lys + Lys 290 His + Ala 293 Leu	3rd ICL	Rat-1 fibroblast	focus formation, PI hydrolysis	Allen et al. 1991
	Asp 142 Glu (all A.A)	2nd ICL	Cos-7	PI hydrolysis,	Scheer et al. 1997
	Asn 63 Ala	TM I	"	ligand binding	Scheer et al. 1996
	Cys 128 Phe	TM III	Cos-1	"	Perez et al. 1996
	Ala 204 Val	TM V	"	"	Hwa et al. 1996
	Cys 128 Phe + Ala 204 Val + Ala 293 Glu	"	"	Hwa et al. 1997	
β_2-adrenergic	Substitution of residues (266	3rd ICL	Cos-7/CHO	ligand binding, cAMP,	Samama et al. 1993
	to 272) by α_{1B} AR equivalent		Sf_9, insect	GTPase	Pei et al., 1994
α_{2A}-adrenergic	Thr 373 Phe,Ala,Cys,Glu,Lys	3rd ICL	HEK 293/Cos-7	ligand binding, cAMP,	Ren et al. 1993
5-HT_{2A}	Cys 322 Lys,Ala,Glu	3rd ICL	Cos-1	PI hydrolysis	Casey et al. 1996
Yeast mating pheromone α-factor	Partial deletions	3rd ICL	Yeast	β-galactosidase-based mating test	Boone et al. 1993
	Leu 194 Gln	3rd ICL	"	"	"
	Truncation C-terminal part	"	"	"	
	Leu 194 Gln plus C-terminal part	"	"	"	
	Pro 258 Leu	TM VI	"	ligand binding, β-galactosidase-based mating test	Konopka et al. 1996
	Pro 258 Leu + Ser 259 Leu	TM VI	"	"	"
Prostaglandin E	Truncation C-terminal part		CHO	cAMP	Jin et al. 1997
Platelet activating factor	Leu 231 Arg	3rd ICL	Cos-7 CHO	ligand binding, PI hydrolysis	Parent et al. 1996
	Asn 100 Ala	TM III	'	arachidonic acid release, PI hydrolysis	Ishii et al. 1997
Muscarinic m_1	Glu 360 Ala	3rd ICL	HEK 293	ligand binding,	Högger et al. 1995
Muscarinic m_5	Ser 465 Tyr + Thr 466 Pro	TM VI	Cos 7, NIH 3T3	ligand binding proliferative response	Spalding et al. 1995
	Ser 465 Lys, Arg, Phe	TM VI	NIH 3T3	proliferative response	Spalding et al. 1997
Dopamine D_1	Phe 264 Ile	3rd ICL	HEK 293	ligand binding,	Charpentier et al. 1996
	Leu 286 Ala	TM VI	Cos-7	cAMP formation	Cho et al. 1996

(continued)

Table 2
Continued

Receptor Subtype	Amino Acid Substitution	Location of Mutation	Cell Type	Investigated Parameters	References
Rhodopsin	Asp 134 Asn	2nd ICL	Cos-7	[35S]GTPγS binding	Cohen et al. 1993
	Gly 90 Asp	TM II	"	pH-rate profile	Rim and Oprian 1995
	Glu 113 Gln	TM III	"	[35S]GTPγS binding	Robinson et al. 1992
	Ala 292 Glu	TM VII	"	pH-rate profile	Rim and Oprian 1995
	Lys 296 Glu, Met, Gly	TM VII	"	[35S]GTPγS binding	Robinson et al. 1992
	Glu 113 Gln + Lys 296 Gly	"	pH-rate profile	Cohen et al. 1993	
Luteinizing hormone	Asp 578 Gly	TM VI	Cos-7	cAMP	Shenker et al. 1993
Melanocyte-stimulating hormone	Glu 92 Lys	TM II	HEK 293	cAMP	Robbins et al. 1993
Angiotensin AT_1	Asn 111 Ala	TM III	Cos-7	PI hydrolysis	Groblewski et al. 1997
Thrombin	Substitution of residues (259 to 268) by *Xenopus* thrombin receptor equivalent	2nd ECL	Cos-7, oocytes	PI hydrolysis, Ca^{2+} release, luciferase	Nanevicz et al. 1996
Follicle-stimulating hormone	Substitution of residues (548 to 570) by LH receptor equivalent	TM V and VI + 3rd ICL	HEK 293	cAMP	Kudo et al. 1996

A.A: amino acids

each of these mutations show constitutive receptor properties (Cotecchia et al., 1990; Allen et al.,1991; Kjelsberg et al., 1992; Samama et al., 1993; Ren et al., 1993; Casey et al., 1996; Parent et al., 1996).

Replacement of the C-terminal portion of the third ICL (including the BBXXB motif) of the β_2-adrenergic receptor (residues 266–272) with the homologous region of the α_{1B}-adrenergic receptor leads to agonist-independent activation of adenylyl cyclase (Samama et al., 1993) in a fashion analogous to the reciprocal amino acid exchange in the α_{1B}-adrenergic receptor (Cotecchia et al., 1990). This substitution exhibits an increased affinity for agonists; an increased potency of agonists for stimulation of adenylyl cyclase; and an increased intrinsic activity of partial agonists. In a similar way, mutation of a single residue Thr373 in the BBXXB motif of the third ICL of the α_{2A}-adrenergic receptor into five different amino acids (Phe, Ala, Cys, Glu, Lys) results in various levels (31–90% of agonist-driven wt α_{2A}-adrenergic receptor activity) of constitutive receptor activation (Ren et al., 1993). Constitutive α_{2A}-adrenergic receptor activity also leads to agonist-independent inhibition of adenylyl cyclase and increased affinity of the receptor for the binding of the agonist UK14304. Coexpression of the α_{2A}-adrenergic-receptor mutants with the β-adrenergic receptor-specific kinase 1 (βARK1) indicated that the constitutively active receptors are substrates for βARK-mediated phosphorylation even in the absence of agonist (Ren et al., 1993). This interaction that results in a constitutive phosphorylation of this mutant receptor is similar to that described for a mutant β_2-adrenergic receptor (Pei et al., 1994). Therefore, constitutively active mutant adrenergic receptors are partially desensitized

even in the absence of agonist occupancy (Pei et al., 1994). These findings strengthen the idea that constitutively active adrenergic receptors mimic the active state of a GPCR, adopting conformations similar to those induced by agonist when it binds to wild-type receptors. Hwa et al. (1997) suggested that each individual mutation, because of its critical location in receptor-G-protein interaction domains, alters the conformation of the receptor such that mimicry occurs that partially conforms to the activated state. Otherwise, Kudo et al. (1996) proposed that mutations may not alter the tertiary structure of the receptor, but exert a direct effect on the respective G-protein.

Interestingly, a BBXXB motif is also found in the N-terminal end of the third ICL of the yeast mating-pheromone α-factor receptor. Deletion of the BBXXB motif yields an increased sensitivity of the mutated receptor, although a Leu194Gln mutation, located outside this BBXXB motif in the third ICL, also causes a partial constitutive activation of the pheromone response (Boone et al., 1993). Furthermore, partial deletions of the third ICL as well as truncation of the C-terminal part of this receptor leads to constitutive activity. A prostaglandin E receptor EP3 truncated of its 31 last amino acids (T-359) is also fully active in CHO-K1 cells, but unable to respond to the agonist sulprostone (Jin et al., 1997). The degree of constitutive activity of this modified receptor correlates with the inverse of the length of the C-terminal tail. Otherwise, a C-terminal truncated form of the prostaglandin E2 receptor EP4 does not show constitutive activity in CHO cells (Bastepe and Ashby, 1997). Both receptors are differently coupled to adenylate cyclase (EP3: negative and EP4: positive) and have respectively a short and long C-terminal end. Whether the length of the C-terminal is involved in constitutive receptor activity is still an open question. Combination of a Leu194Gln mutation of the mating-pheromone α-factor receptor with truncation of the C-terminus appears to effect receptor activity independently; the double mutant is associated with a 400-fold increase in pheromone sensitivity compared to the wild-type receptor (Boone et al., 1993). Mutation of Leu231 to Arg of the PAF receptor, a nonbasic amino acid of the BBXXB motif, yields an increased affinity for binding of PAF, but not for the antagonist WEB2086, and agonist-independent accumulation of inositol phosphates (Parent et al., 1996). The implication of the BBXXB motif of the muscarinic-receptor subtypes in constitutive activity seems controversial: random saturation mutagenesis of a portion including the 22 last amino acids of the third ICL of the muscarinic m_5 receptor does not result in constitutively active mutants (Burstein et al., 1995). Deletion or point mutations in the muscarinic m_1 receptor BBXXB motif does not result in constitutively active mutants (Arden et al., 1992 ; Shapiro et al., 1993). Otherwise, mutation of the Glu360 to Ala in the muscarinic m_1 receptor, one amino acid upstream the BBXXB motif, induces an elevated phosphatidylinositol turnover in the absence of agonist and sensitization towards carbachol activation (Högger et al., 1995). A similar situation is found for the dopamine D_1 receptor: mutation of Phe264, one amino acid upstream the BBXXB motif, to Ile yields a 40% increased basal adenylate cyclase activity compared to the wild-type dopamine D_1 receptor (Charpentier et al., 1996).

E/DRY sequence

Scheer et al. (1997) investigated the role of Asp142, which belongs to a highly conserved E/DRY (Glu/Asp-Arg-Tyr) sequence located at the junction of TM III and the second ICL, in the activation process of the α_{1B}-adrenergic receptor. A series of receptor mutants containing each of the possible amino acid substitutions show various levels of constitutive activity dependent on the substituted amino acid. In a similar way, Cohen et al. (1993) mutated the Asp134 residue of the E/DRY motif of rhodopsin into Asn; this also promotes constitutive receptor activation. A similar mutation (Asp to Asn) in the α_{1B}-adrenergic receptor increases basal phosphatidylinositol hydrolysis by 23%, whereas an Asp to Thr mutation increases basal phosphatitidylinositol accumulation up to 700% (Scheer et al., 1997). In this latter case, norepinephrine stimu-

lates the mutant receptor only by 10% compared to a 375% agonist-driven stimulus of the wt receptor. Although the exact function of the E/DRY motif remains unknown, it is a characteristic motif of GPCR and apparently critical for constitutive receptor activation. Whereas amino acid variations may occur for the E/D and Y positions of the E/DRY sequence (Probst et al., 1992), the Arg (R) residue is completely conserved within the GPCR family. The maintenance of a positive charge at this position may be critical for G-protein activation (Jones et al., 1995).

Mutations of GPCR Located Within Transmembrane Domains

The residue Asn63, located in the central part of TM I of the α_{1B}-adrenergic receptor, is involved in induction of constitutive receptor activity (Scheer et al., 1996). The activity of the mutant Asn63Ala is dependent on receptor density and sensitive to blockade by the inverse agonists prazosin and phentolamine. Although this Asn residue is highly conserved in a homologous position in TM I of other GPCR, no similar active receptor mutants have been reported. The mutations Cys128Phe and Ala204Val, respectively in the third and fifth TM of the α_{1B}-adrenergic receptor, also induce constitutive activation (Perez et al., 1996; Hwa et al., 1996). Both mutations display higher binding affinity and potency for agonists as well as higher basal signal transduction levels. Hwa et al. (1997) reported on synergism obtained with a triple mutation (Cys128Phe/Ala204Val/Ala293Glu) in the α_{1B}-adrenergic receptor. Since these mutations, in combination, produce a greater basal activity than the fully agonist-stimulated wild-type receptor, this reveals apparently a possibility of a super-activated state of the α_{1B}-adrenergic receptor, likely to be a result of a significant conformational change. A Cys residue is also conserved at the same position in TM III in the three β-adrenergic receptor subtypes as well as the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors. On the other hand, a Phe residue is present in TM III of wild-type α_2-adrenergic and 5-HT_{2C} receptors. Since these receptor subtypes are not constitutively active, a Phe residue at this particular position of TM III is apparently not sufficient by itself to cause constitutive activation. Moreover, the Cys 128 residue is located only three residues upstream of the Asp 125 residue being a major agonist-binding site of the α_{1B}-adrenergic receptor (Hibert et al., 1991). In case of the α_{1B}-adrenergic-receptor mutant, the aromatic ring of the Phe residue may mimic the amino acid interactions generated by an agonist and thereby contribute to an activated-receptor conformation. A similar observation can be put forward for the Ala204 to Val mutation in TM V of the α_{1B}-adrenergic receptor: two Ser residues in TM V of the α_{1B}-adrenergic receptor, interacting with the hydroxyl groups of noradrenaline (Strader et al., 1989), are located near the Ala204Val mutation site, although this amino acid modification induces only minor changes in the three-dimensional structure of this receptor.

The search for conserved residues amongst GPCR reveals several highly conserved Pro residues within the TM V, VI, and VII. Each Pro residue produces a "kink" in the helical backbone of the TM and is involved in crucial structural motifs of GPCR (Von Heijne et al., 1991). Peculiarities of Pro and the X-Pro peptide bond (X = an amino acid) have led to the speculation that highly conserved Pro may not only be important for the structure but also for protein function (Brandl and Deber, 1986). Substitution of Pro323 in TM VII of the β-adrenergic receptor appears to result in an incorrectly or incompletely processed protein (Strader et al., 1987). The authors speculated that this Pro conserved amongst GPCR, must be critical for proper receptor-folding events. The Pro258 to Leu mutation in TM VI of the yeast mating-pheromone α-factor receptor causes constitutive receptor signaling (Konopka et al., 1996). The nature of the variable residue adjacent to membrane-embedded Pro is important for ligand binding and intrinsic activity. Substitution of a Leu with an Ala in TM VI (Leu286Ala) adjacent to a transmem-

brane Pro confers constitutive activity to the dopamine D_1 receptor, which is characterized by an enhanced basal and agonist-stimulated accumulation of cAMP (Cho et al., 1996). This constitutive activating substitution induces R(+)-SCH23390, a classical antagonist at the wild-type dopamine D_1 receptor, to behave surprisingly as a partial agonist (Cho et al., 1996). This aspect of receptor activation may be conserved in other members of the GPCR family since more than 90% of the GPCR contain a Pro in the central portion of TM VI (Baldwin, 1993). When the same Leu286 residue was replaced by an aromatic Tyr residue, disruption of both ligand binding and signal-transduction properties are produced (Cho et al., 1996). A similar blunted efficacy for cAMP stimulation has been obtained for the Ile205Tyr mutation, adjacent to a Pro in TM V of the D_1 dopamine receptor (Cho et al., 1996). Thus, similar modifications may result in different receptor responses yielding either constitutively active or inactive receptors. A double mutation of both Pro258 and Ser259 to Leu of the yeast mating-pheromone α-factor receptor increases constitutive signaling to > 90% of its maximal level. This increase in constitutive receptor activity caused by the additional mutation of Ser259Leu suggests that disruption of intramolecular contacts also promotes receptor activation (Konopka et al., 1996). The hydroxyl group side chain on Ser is predicted to form intramolecular contacts instead of facing the nonpolar membrane environment. Consequently, this Ser-to-Leu mutation will prevent the formation of this intramolecular contact. Failure to form proper intramolecular contacts may also explain why an Asn578 to Gly mutation in TM VI of the luteinizing-hormone receptor (Shenker et al., 1993) and the Glu92-to-Lys mutation in TM II of the melanocyte-stimulating-hormone receptor cause constitutive receptor activity (Robbins et al., 1993). However, disruption of intramolecular contacts does not always lead to constitutive receptor activation. For example, disruption of an intramolecular contact (Asp120, TM II, and Asn376, TM VII) in the 5-HT_{2A} receptor prevents receptor activation (Sealfon et al., 1995).

Mutations have also been introduced in various TM of the visual pigment rhodopsin: Gly90Asp in TM II, Glu113Gln in TM III, Ala292Glu and Lys296Glu, Met and Gly in TM VII. Each of them are constitutively active by coupling to the G protein transducin (Robinson et al., 1992; Rim and Oprian, 1995). They also result in constitutive receptor phosphorylation by rhodopsin kinase and binding of arrestin to inhibit the receptor activity (Rim and Oprian, 1995). Studies on rhodopsin have identified a specific intramolecular contact between Glu113 in TM III and Lys296 in TM VII. This interaction is thought to be perturbed during photoactivation, and mutations that disrupt this interaction cause apparently constitutive activation of rhodopsin (Robinson et al., 1992).

Mutation of Asn111Ala in TM III of the angiotensin type I (AT1) receptor induces constitutive activation (Groblewski et al., 1997). Besides inverse agonism behavior of the nonpeptide ligands DuP 753, LF 7-0156, and LF 8-0129, agonist behavior was observed with the peptide antagonists [Sar^1, Ile^8]AII and [Sar^1, Ala^8]AII at the mutant receptor. This latter observation is similar to that of R(+)-SCH23390 at the Leu286Ala dopamine D_1-receptor mutant (Cho et al., 1996). Therefore, it appears that classical antagonists may behave as inverse agonists or as partial agonists at constitutively active mutant receptors. The Asn111 residue of the angiotensin AT1 receptor is found at homologous positions in other peptide hormone receptors: AT2 and *Xenopus* angiotensin receptors, bradykinin, opioid, interleukin 8, somatostatin, and PAF receptors for instance (Groblewski et al., 1997). Mutation of Asn100Ala in TM III of the PAF receptor induces constitutive activation. It shows a high affinity for PAF and is responsive to lyso-PAF, an inactive derivative of PAF (Ishii et al., 1997). It is noticeable that mutation of Cys128 to Phe in TM III of the α_{1B}-adrenergic receptor, which occupies a position homologous to that of Asn111 in the AT1 receptor, also induces constitutive receptor activation (Perez et al., 1996).

Spalding et al. (1995) have isolated a mutant m_5 muscarinic receptor that mediates robust functional responses in the absence of agonists. This constitutively active receptor was isolated from a library of receptors containing randomly introduced mutations in TM VI. It contains the substitutions Ser465Tyr and Thr466 Pro and yields a 90% increased atropine-inhibitable response in the absence of agonist. Although these individual residues are not conserved in other GPCR, they are predicted to be at the junction between TM VI and the third extracellular loop (ECL). Mutations causing the activation of muscarinic and adrenergic receptors occur at opposite ends of TM VI, suggesting that alterations in the packing of TM VI may be important in the activation mechanism of these receptors (Spalding et al., 1995). The importance of Ser 465 in the muscarinic m_5 constitutive receptor activation has been further investigated (Spalding et al., 1997). Replacement of this Ser residue by either a large (Phe or Val) or basic (Arg or Lys) residue increases the constitutive activity of the receptor between 55 and 110%, whereas small and acidic amino acids (Gly, Asp, Glu) were apparently without effect (Spalding et al., 1997).

Other Receptor Domains Involved in Constitutive Receptor Activity

Substitution of eight amino acids from the *Xenopus laevis* thrombin receptor's second ECL (XECL2B) for the equivalent sequence in the human thrombin receptor was sufficient to confer constitutive activity, probably by altering interactions among the human receptor's first and second ECL (Nanevicz et al., 1996). A change in an ECL of a GPCR may transmit information across the cell membrane to cause cell signaling, perhaps via a conformational change similar to that caused by thrombin binding. In case with the mutation of the second ECL in the *Xenopus* thrombin receptor, it coincides with a putative peptide-binding site, supporting the hypothesis that agonist interactions within the thrombin receptor's ECLs contribute to receptor activation (Nanevicz et al., 1996). Replacement of the third ICL, TM V and VI of the follicle-stimulating-hormone receptor by the equivalent regions of the luteinizing-hormone receptor yields constitutive activation of the mutant receptor only if a point mutation (Asn564Gly) was introduced in the C-terminus of the third ICL (Kudo et al., 1996). No constitutive activation was generated by mutating alone the Asn564Gly of the follicle-stimulating-hormone receptor (Kudo et al., 1996), suggesting that interactions between TM and extramembrane regions may be essential for maintaining the mutated receptor in a constrained state.

Constitutive Activation of G-Protein-Coupled Receptors in Pathology

Mutations in GPCR have been reported to underlie a variety of diseases yielding phenotypes characterized by an apparent gain-of-function (e.g., hyperthyroidism) or loss-of-function (e.g., blindness, *see* Coughlin, 1994). Several examples of constitutively activating mutations of GPCR in human and animal pathologies are summarized in Table 3. The locations of these mutations in GPCR are represented in Fig. 2. The mutations of the luteinizing hormone Ile542Leu (L1) and Asp 578Gly/Tyr (L8), melanoyte-stimulating hormone Glu92Lys (M), rhodopsin receptor Gly 90Asp (R1), Ala292Glu (R2), and Lys296Glu (R3) are identical to those shown in Fig. 1. The Ala623Ile mutation in the third ICL of the thyrotropin/thyroid-stimulating-hormone receptor (T6) is equivalent to the Ala293 mutation in the third ICL of the α_{1B}-adrenergic receptor (Fig. 1, 15). The other presented receptor mutants are not correlated with a given mutation in Fig. 1.

Mutations in the rhodopsin gene have been found in families in which autosomal dominant retinitis pigmentosa and stationary night blindness occur. Mutation of Lys296 to Glu in TM VII of rhodopsin has been described in families showing an early onset of retinitis pigmentosa (Keen et al., 1991). Expression of the

Table 3
Examples of Constitutively Activating Receptor Mutants in Pathology

Disease	Receptor Subtype	Amino Acid Substitution	Location of Mutation	References
Autosomal dominant retinitis pigmentosa	rhodopsin	Lys 296 Glu	TM VII	Keen et al. 1991
Stationary night blindness	rhodopsin	Gly 90 Asp	TM II	Rao et al. 1994
	rhodopsin	Ala 292 Glu	TM VII	Dryja et al. 1993
Different color coats in mice	melanocyte-stimulating hormone	Glu 92 Lys	TM II	Robbins et al. 1993
Jansen-type metaphyseal chondrodysplasia	parathyroid hormone-PTH related protein	His 223 Arg	1st ICL	Schipani et al. 1995
Hyperfunctioning thyroid adenoma and congenital hyperthyroidism	thyrotropin	Ser 505 Arg	TM III	Van Sande et al. 1995
		Val 509 Ala	TM III	"
		Phe 631 Leu/Cys	TM VI	Kopp et al. 1995
		Thr 632 Ile	TM VI	Kopp et al. 1995
		Asp 633 Glu/Tyr	TM VI	Paschke et al. 1994
		Asn 670 Ser	TM VII	Porcellini et al. 1994
		Cys 672 Tyr	TM VII	'
		Ile 486 Phe/Met	1st ECL	Van Sande et al. 1995
		Ile 568 Thr	2nd ECL	"
		Asn 650 Tyr	3rd ECL	"
		Asp 619 Gly	3rd ICL	Parma et al. 1993
	Ala 623 Ile/Val		3rd ICL	"
Familial male precocious puberty (FMPP)	luteinizing hormone	Ile 542 Leu	TM V	Laue et al. 1995
		Met 571 Ile	TM VI	Kosugi et al. 1995
		Ala 572 Val	TM VI	Yano et al. 1995
		Ile 575 Leu	TM VI	Laue et al. 1996
		Thr 577 Ile	TM VI	Kosugi et al. 1995
		Asp 578 Gly/Tyr	TM VI	Shenker et al. 1993
		Cys 581 Arg	TM VI	Laue et al. 1995
		Asp 564 Gly	3rd ICL	"
		Ala 568 Val	3rd ICL	Latronico et al. 1995

Lys296Glu rhodopsin mutant in Cos cells demonstrated constitutive activation of the mutated photoreceptor (Robinson et al., 1992). The Lys296 residue of rhodopsin constitutes the retinal (agonist)-binding site by covalent linkage via a Schiff-base reaction. The activated photoreceptor has been suggested as a cause of rod-cell death and retinal degeneration (Sung et al., 1991b). On the other hand, mutations of Gly90 to Asp (Rao et al., 1994) in TM II and Ala292 to Glu (Dryja et al., 1993) in TM VII of rhodopsin may cause stationary night blindness. Similarly, in vitro studies of these mutated photoreceptors demonstrated constitutive activation (Rim and Oprian, 1995). These activating rhodopsin mutations have been postulated to give rise to a signal causing the photoreceptor cells to desensitize in a process resembling normal light adaptation (Dryja et al., 1993; Rao et al., 1994). It is very likely that the constitutively activated state of these mutated photoreceptors is responsible

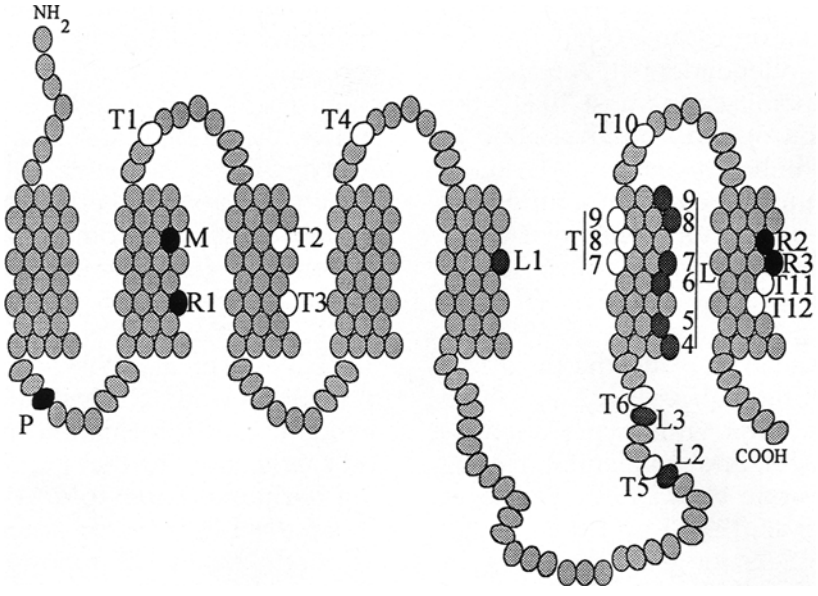

Fig. 2. Constitutively activating G-protein-coupled receptor mutants in pathology. A schematic representation of a GPCR is shown as in Fig. 1. Luteinizing-hormone receptor: **L1** Ile542Leu; **L2** Asp564Gly; **L3** Ala568Val; **L4** Met571Ile; **L5** Ala572Val; **L6** Ile575Leu; **L7** Thr577Ile; **L8** Asp578Gly/Tyr; **L9** Cys581Arg. Melanocyte-stimulating hormone receptor: **M** Glu92Lys. Parathyroid-hormone receptor: **P** His223Arg. Rhodopsin receptor: **R1** Gly90Asp; **R2** Ala292Glu; **R3** Lys296Glu. Thyrotropin receptor: **T1** Ile486Phe/Met; **T2** Ser505Arg; **T3** Val509 Ala; **T4** Ile568Thr; **T5** Asp619Gly; **T6** Ala623Ile/Val; **T7** Phe631Leu/Cys; **T8** Thr632Ile; **T9** Asp633Glu/Tyr; **T10** Asn650Tyr; **T11** Asn670Ser; **T12** Cys672Tyr. The following 6 mutations are identical to those shown in Fig. 1: L1=9, L8=19, M=3, R1=2, R2=22, and R3=23. The T6 mutation is equivalent to mutation 15 in Fig. 1. The other mutations in this figure cannot be superimposed to those shown in Fig. 1.

for the mentioned visual diseases. Nevertheless, more than 20 other mutations in the rhodopsin gene, which are linked to vision pathologies, have been identified. They do not cause constitutive photoreceptor activation, but are likely to induce improper protein folding (Dryja et al., 1990a,b; Sung et al., 1991a,b).

Mutations in the melanocyte-stimulating-hormone (MSH) receptor lead to different color coats in mice (Robbins et al., 1993). One of these naturally occurring mutants (Glu92Lys) in TM II of the MSH receptor, corresponding to the $E^{so\text{-}3J}$ allele responsible for a dominant black phenotype, yields a constitutively active mutant MSH receptor (50% activity compared to the MSH-stimulated wild-type receptor) in a stable HEK 293 cell line (Robbins et al., 1993). Strikingly, this mutant receptor fails to respond to MSH stimulation (Robbins et al., 1993). A second MSH-receptor mutation (Ser69Leu) in the first ICL, responsible for the tobacco darkening phenotype, has been shown to encode a hyperactive receptor with a threefold greater maximal response to MSH than the wild-type receptor. Otherwise, basal-receptor activity of this mutant was not affected (Robbins et al., 1993).

A single heterozygous nucleotide exchange in exon M2 of the gene encoding the parathyroid hormone (PTH)-PTH-related-peptide receptor has been identified in a patient with Jansen-type metaphyseal chondrodysplasia, a rare form of short-limbed dwarfism (Schipani et al., 1995). This mutation changes a conserved His223 residue in the first ICL to Arg. Constitutive, ligand-independent cAMP accumulation is observed in Cos-7 cells expressing

this mutant PTH receptor. Schipani et al. (1995) proposed this mutation as an explanation for the severe ligand-independent hypercalcemia and hypophosphatemia, and most likely the abnormal formation of endochondral bone in this form of short-limbed dwarfism.

In the thyrotropin/thyroid-stimulating hormone (TSH) receptor, mutations in TM III (Ser505Arg, Val509Ala), TM VI (Phe631Leu/Cys, Thr632Ile, Asp633Glu/Tyr) TM VII (Asn 670Ser, Cys672Tyr), the three ECL (Ile486Phe/Met, Ile568Thr, Asn650Tyr), and the third ICL (Asp619Gly, Ala623Ile/Val; *see* Fig. 2 and Table 3) have been associated with hyperfunctioning-thyroid adenoma and congenital hyperthyroidism (Van Sande et al., 1995; Parma et al., 1993; Paschke et al., 1994; Kopp et al., 1995). Interestingly, Ala623 is the exact homolog in the TSH receptor of Ala293 in the BBXXB motif in the third ICL of the α_{1B}-adrenergic receptor yielding constitutive activity (Cotecchia et al., 1990). The Asp619Gly mutation, located near the BBXXB motif can be compared to the constitutively activated mutation Glu360Ala of the muscarinic m_1 receptor (Högger et al., 1995). All of the mutant TSH receptors confer constitutive activation of adenylyl cyclase when tested by transfection in Cos cells; the most pronounced effect has been observed for the Ile486Phe mutation in the first ECL (a 30-fold increase in cAMP formation compared to the wild-type TSH receptor, Van Sande et al., 1995). All of these mutations are restricted to tumor tissue and affect a single allele in each tumor (Van Sande et al., 1995; Parma et al., 1993; Paschke et al., 1994). Constitutive activation of the Ala293Leu α_{1B}-adrenergic-mutant receptor also leads to an enhanced ability of norepinephrine to induce focus formation in Rat-1 fibroblasts and nude mice, compared to cells expressing the wild-type α_{1B}-adrenergic receptor (Allen et al., 1991). These observations for the TSH and the α_{1B}-adrenergic-receptor mutants may suggest an association of constitutively active GPCR acting as protooncogenes, with uncontrolled cell proliferation (Allen et al., 1991).

The region spanning TM V to VI including the third ICL of the luteinizing-hormone (LH) receptor appears as a hotspot for point mutations (Ile542Leu, Asp564Gly, Ala568Val, Met 571Ile, Ala572Val, Ile575Leu, Thr577Ile, Asp 578Gly/Tyr, Cys581Arg) leading to familial male precocious puberty (FMPP), which is inherited in an autosomal-dominant, male-limited manner (Shenker et al., 1993; Kremer et al., 1993; Laue et al., 1995; Kosugi et al., 1995; Yano et al., 1995; Yano et al., 1994; Latronico et al., 1995; Laue et al., 1996). These heterozygous mutations lead to constitutive, agonist independent cAMP formation in Cos or HEK 293 cells, and show further stimulation to luteinizing hormone. The Asp578Gly mutation in TM VI of the LH receptor accounts currently for approx 80% of all reported FMPP families (Laue et al., 1995) as demonstrated by *Msp1* restriction-fragment analysis. The existence of other undiscovered sporadic mutations in the LH-receptor gene cannot be excluded (Laue et al., 1995).

Several cases of disease states have been exemplified in the above section; some of them implicate one or more mutations leading to constitutive activation of a given GPCR. Mutations at the level of heterotrimeric G proteins have as well been described; they may induce constitutive activation of the effector. One example is a mutation in the GTPase domain of the stimulatory G_{as}-subunit that leads to an elevated activity of adenylyl cyclase. This has been found in tumor cells (*see* Spiegel et al., 1992). The diversity of the GPCR family and the widely distributed mutations causing constitutive GPCR activation make it plausible that more pathologies will be found with an origin in constitutive receptor activity (Van Sande et al., 1995). The ability of inverse agonists to reduce this constitutive receptor activation may open new applications for this family of drugs. Moreover, polymorphism in GPCR may be responsible not only for disease states, but also for individual subject differences. This has been demonstrated for how color is perceived by different individuals: a single amino

acid change (Ser/Ala180 polymorphism in rhodopsin) alters the spectral sensitivity of rhodopsin (Winderickx et al., 1992).

Regulation of G-Protein-Coupled Receptors by Inverse Agonists

The C-terminal cytoplasmic domain of GPCR serves as a major target for desensitization that negatively regulates the receptor activity. Cells expressing C-terminally truncated β_2-adrenergic, mating-pheromone α-factor, and histamine H_2 receptors show a delayed onset of desensitization without constitutive activity (Bouvier et al., 1988; Konopka et al., 1988; Reneke et al., 1988; Smit et al., 1996b). Otherwise, the constitutively active TRH receptor, carrying a deletion of the C-terminal last 59 amino acids, is chronically downregulated in AtT-20 pituitary cells in the absence of agonist (Heinflink et al., 1995). The inverse agonist chlordiazepoxide has been shown to cause upregulation of this mutant receptor in contrast to AtT-20 cells expressing wild-type TRH receptors (Heinflink et al., 1995). The third ICL may also function to negatively regulate receptor activity. This region as well as the C-terminal end serve as targets of a cAMP-dependent protein kinase and specific receptor kinases that desensitize the receptor, possibly by direct uncoupling from its G protein because of receptor phosphorylation (Okamoto et al., 1991). Deletions that remove part of the third ICL and an amino acid substitution at a single site (Leu194Gln) within this loop create yeast mating-pheromone α-factor receptors with both a constitutive and hypersensitive phenotype (Boone et al., 1993). This suggests that the third ICL functions as a negative regulatory domain that ensures a nonsignaling resting state of the receptor in the absence of ligand. Analysis of the α_{1B} and β-adrenergic receptors has suggested two mechanisms by which the third ICL could participate in negative regulation of receptor activity (Boone et al., 1993). First, phosphorylation of Ser/Thr residues in the third ICL, and the C-terminal end of the β_2-adrenergic receptor leads to desensitization (Okamoto et al., 1991; Hausdorff et al., 1990). β_2-adrenergic-receptor mutants in which an Ala residue replaces two Ser of the protein kinase A phosphorylation sequence are defective in desensitization to agonist and do not exhibit a constitutive phenotype (Hausdorff et al., 1989). Second, a region of the third ICL of both the β_2-adrenergic and the α_{1B}-adrenergic receptors appears to constrain wild-type-receptor activation and maintain the receptor in a nonsignaling mode unless bound by an agonist (Samama et al., 1993). This conclusion follows from the identification of constitutively active forms of adrenergic receptors (Cotecchia et al., 1990; Kjelsberg et al., 1992; Samama et al., 1993). Thus, the properties associated with the loss of normal structure of the third ICL of mutant-adrenergic receptors mimic those of the agonist-activated wild-type receptor. Computer simulations imply that these mutant receptors isomerize to an active state more readily than wild-type receptors do (Samama et al., 1993). That is, increased G-protein coupling is a direct consequence of the change in the isomerization properties of the mutant receptor. Pei et al. (1994) have shown that not only is the mutant β_2-adrenergic receptor constitutively active, it is also constitutively desensitized and downregulated. The purified- mutant receptor in the absence of agonist is phosphorylated in vitro by a recombinant βARK in a manner comparable to the agonist-occupied wild-type receptor. Thus, the constitutively activated mutant receptor in its native state is both tonically downregulated and partially uncoupled from its G protein. A similar conclusion has been put forward for constitutively active α_{2A}-adrenergic receptors (Ren et al., 1993). A wild-type 5-HT_{2C} receptor stably expressed in NIH 3T3 fibroblasts exhibits constitutive activation and phosphorylation under basal condition (Westphal et al., 1995). Furthermore, phosphorylation of the receptor was increased by the agonist 5-HT but was unaffected by the inverse-agonist mianserin. This

contrasts with the inhibition of agonist-independent βARK-mediated phosphorylation of a constitutively active, mutant β_2-adrenergic receptor by an inverse agonist ICI 118551 in a reconstituted system (Samama et al., 1994). This discrepancy in inverse-agonist effects on receptor phosphorylation could be because of differences in assay conditions or in the mechanism of constitutive receptor activation (Westphal et al., 1995). The increase in basal-receptor activity and magnitude of agonist-induced responses are likely to be consistent with upregulation of signaling components downstream of the receptors, such as G proteins or enzymes involved in the signal-transduction pathway (Pozvek et al., 1997).

Milligan et al. (1995) extended the concept of inverse agonism to GPCR regulation. Whereas GPCR expression is reduced upon prolonged agonist exposure, GPCR upregulation has been hypothesized for inverse agonists. According to classical models for drug–GPCR interaction, GPCR antagonists are believed to prevent the binding of agonists to the receptor (De Lean et al., 1980). Consequently, their physiological effects, including receptor upregulation, is ascribed to their ability to prevent activation/downregulation of receptors by endogenous hormones or neurotransmitters. However, there is now substantial evidence that contradicts this concept. For a series of GPCR, some antagonists have been shown to induce effects opposite to those observed by agonists, thereby displaying negative-intrinsic activity (Schütz and Freissmuth, 1992; Lefkowitz et al., 1993; Kenakin, 1995; Milligan et al., 1995). Smit et al. (1996b) hypothesized that the observed inverse agonism displayed by cimetidine and ranitidine could be a mechanistic basis for the observed wild-type histamine H_2-receptor upregulation. In a fashion similar to that reported for constitutively active β_2-adrenergic receptors (Pei et al., 1994), negative antagonists upregulated and increased the sensitivity of constitutively activated histamine H_2 receptors. Similarly, a constitutively active β_2-adrenergic receptor mutant shows betaxolol-mediated upregulation in a time and concentration-dependent manner (Mac Ewan and Milligan, 1996). The inverse agonist betaxolol did neither modify the mutant β_2-adrenergic receptor nor the G_{as}-subunit mRNA level, suggesting that the inverse agonist is likely to increase translational efficiency of receptor mRNA and/or stabilize the receptor polypeptide (Mac Ewan and Milligan, 1996). This latter hypothesis has been confirmed by Samama et al. (1997) in transgenic mice expressing a constitutively active β_2-adrenergic receptor. It is tempting to speculate that the inverse agonism displayed by the H_2 antagonists cimetidine and ranitidine, consequently resulting in an upregulation of H_2 receptors, contributes to the development of tolerance after chronic treatment (Bertaccini and Coruzzi, 1992; Deakin and Williams, 1992; Merki and Wilder-Smith, 1994; Wilder-Smith et al., 1990; Nwokolo et al., 1990; Lundell et al., 1991). In view of this hypothesis, the chronic use of neutral H_2 antagonists might offer advantages, as inverse agonists are more likely to cause H_2 receptor upregulation and thus development of tolerance. Otherwise, temporary therapy with an inverse agonist may potentially be used to provide a therapeutic benefit.

Recently, Samama et al. (1997) reported on overexpression of a constitutively active β_2-adrenergic receptor in transgenic mice induced by the inverse agonist ICI 118151 compared to nontreated transgenic mice. They suggested ligand-induced stabilization of a constitutively active mutant receptor as a novel mechanism for regulating cellular signaling in vivo. The constitutively active mutant receptor is considered as a molecular switch that is permanently on but expressed at such low levels because of its inherent instability, that it functions minimally. When expression levels are increased by administration of receptor ligands that stabilize the protein, cognate physiological pathways become activated. Signaling is induced by ligands that do not conformationally activate the receptors, but rather increase their receptor number in cell membranes. This provides an alternative means for regulating gene expression that is often focused at the level of gene expression.

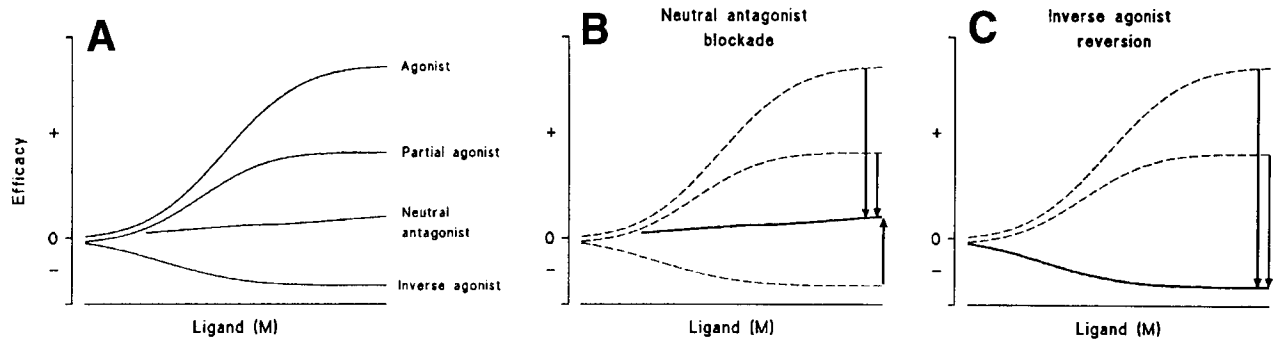

Fig. 3. Intrinsic activity of 5-HT receptor ligands at 5-$HT_{1B/D}$ receptors and their differential blockade by neutral antagonists and inverse agonists. The diagrams are constructed on observations made in a [^{35}S]GTPγS binding assay to membrane preparations of stably transfected C6-glial cells containing recombinant h 5-HT_{1B} and h 5-HT_{1D} receptors. **(A)** Positive efficacy is obtained with agonists and partial agonists, whereas inverse agonists display negative efficacy. Neutral antagonists do not yield intrinsic activity. **(B)** Neutral antagonists block agonist-, partial agonist- and inverse agonist-mediated activity. **(C)** Inverse agonist-reverse agonist- and partial agonist-mediated activity to lower levels than neutral antagonists, supposedly the level of negative efficacy of inverse agonists. Reprinted with permission from *Gen. Pharmacol.* **29, (3),** Pauwels P. J., 5-$HT_{1B/D}$ antagonists, 293–303, 1997, Elsevier Science Inc.

Therapeutic Potential of Inverse Agonists

If ligand efficacy is the differential affinity of a ligand for the active and/or inactive conformation of the receptor, then an expectation of zero efficacy, which would be required for a pure neutral antagonist, may be relatively uncommon, because this would require identical drug affinities for both receptor states (Bond et al., 1995). Numerous drugs previously classified as neutral antagonists may actually function as inverse agonists (Bond et al., 1995), and such a reclassification may have pharmacological and therapeutic implications. Although in case of the antipsychotic drugs, many of which are used clinically, they are in fact inverse agonists and not antagonists of dopamine D_2 receptors in recombinant CHO cells (Hall and Strange, 1997). Inverse agonists should not be considered as a single entity but, as for partial agonists, they may cover a range of negative efficacies (Milligan et al., 1995). This has been demonstrated for the negative efficacy of β_2-adrenergic-receptor inverse agonists in Sf_9 insect cells; they show the following rank order of efficacy: timolol > propranolol > alprenolol > pindolol (Chidiac et al., 1994).

Despite the lack of in vivo supportive data at the present time, it may be more appropriate to develop inverse agonists to enhance neurotransmitter/hormone release rather than neutral antagonists because the latter would only operate in the presence of a sufficient tone on the receptor (Pauwels, 1997). In the absence of such a tone, an inverse agonist may still increase neurotransmitter/hormone release, whereas a neutral antagonist supposedly will be inactive. This is illustrated in Fig. 3 for the intrinsic activity of 5-HT ligands at 5-$HT_{1B/D}$ receptors and their differential blockade by neutral antagonists and inverse agonists. Maximal-positive efficacy is obtained with the native agonist 5-HT. Partial agonists display less positive efficacy than 5-HT does. Neutral antagonists do not show intrinsic activity, because they neither stimulate nor inhibit the receptor. An inverse agonist, in the absence of agonist, shows negative-intrinsic activity because it inhibits basal-receptor activity if any. Neutral antagonists block agonist-, partial agonist- and inverse ago-

nist-mediated activity. The blocked response will be close to the `0' level. Inverse agonists reverse agonist- and partial agonist-mediated activity as well as the basal-receptor activity to levels below `0', supposedly the level of negative efficacy of an inverse agonist in the absence of agonist. Hence, the magnitude of the blockade will be larger with an inverse agonist than with a neutral antagonist. The difference between the `0' level and negative-intrinsic activity of an inverse agonist will vary between various model systems. This will mainly depend on the degree of constitutive receptor activity and determine whether there is a yes or no difference between the effect of a neutral antagonist and an inverse agonist.

Inverse agonists can also negate constitutive receptor activity; this may be particularly important in disease states that result from constitutively activating receptor mutations. The development of thyroid adenomas and male precocious puberty are two examples of such human diseases in which receptor mutants play a role in pathogenesis by their constitutive activity. Several studies have implicated constitutively active GPCR as having latent oncogenic potential (e.g., α_{1B}-adrenergic [Allen et al., 1991] and TRH receptors [Parma et al., 1993]). The transforming effects of high cAMP levels have been observed both in vivo and in vitro, with a constitutively active TRH receptor as the causative agent in producing thyroid adenomas by constitutive stimulation of a G_{as}-subunit (Parma et al., 1993). However, whether inverse agonists developed for constitutively active hormone receptors are of possible therapeutic benefit as anticancer drugs requires further investigation.

Furthermore, besides the application of inverse agonists, the study of mutant receptors may lead to new insights of receptor activation. Hwa et al. (1997) suggested that if a higher activation potential can eventually be induced by ligands compared to an endogenous agonist, there is a possibility to design super agonists. They would have intrinsic activities greater than a natural agonist. An example being isoproterenol which has a higher intrinsic activity at the β-adrenergic receptor than the natural hormones. The design of a super agonist may simply enhance the known interactions of the agonist-receptor complex (i.e., Asp125 in TM III and Ser207 in TM V of the α_{1B}-adrenergic receptor, interact respectively with the protonated amine and the m-hydroxyl of the catechol moiety of catecholamines) or take advantage of yet undiscovered interactions between receptor amino acid residues and an agonist (Hwa et al., 1997).

Conclusion

It appears that a large number of different amino acids are implicated in constitutive activation of GPCR. Most mutated residues are located in TM, the second and third ICL. Site-directed mutagenesis and molecular dynamics analysis will be further essential in elucidating the structural/dynamic properties of different GPCR. The functionally distinct properties of inverse agonists and neutral antagonists may elucidate the mechanisms controlling basal-receptor activity states and lead to novel approaches in the development of therapeutic agents.

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References

Adie E. J. and Milligan G. (1994a) Agonist regulation of cellular G_s α-subunit levels in neuroblastoma x glioma hybrid NG108-15 cells transfected to express different levels of the human β2 adrenoreceptor. *Biochem. J.* **300,** 709–715.

Adie E. J. and Milligan G. (1994b) Regulation of basal adenylate cyclase activity in neuroblastoma x glioma hybrid, NG108-15, cells transfected to express the human β2 adrenoreceptor: evidence

for empty receptor stimulation of the adenylate cyclase cascade. *Biochem. J.* **303,** 803–808.

Akaishi Y., Hattori Y., Kanno M., Sakuma I., and Kitabatake A. (1997) Agonist-independent tonic inhibitory influence of G_i on adenylate cyclase activity in rabbit ventricular myocardium and its removal by Pertussis toxin: a role of empty receptor-mediated G_i activation. *J. Mol. Cell. Cardiol.* **29,** 765–775.

Allen L. F., Lefkowitz R., Caron M. G., and Cotecchia, S. (1991) G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the α_{1B}-adrenergic receptor enhances mitogenesis and tumorigenicity. *Proc. Natl. Acad. Sci. USA* **88,** 11,354–11,358.

Arden J. R., Nagata O., Shockley M. S., Philip M., Lameh J., and Sadée W. (1992) Mutational analysis of third cytoplasmic loop domains in G-protein coupling of the HM1 muscarinic receptor. *Biochem. Biophys. Res. Comm.* **188,** 1111–1115.

Baldwin J., (1993) The probable arrangement of the helices in G protein-coupled receptors. *EMBO J.* **12,** 1693–1703.

Barker E. L., Westphal R. S., Schmidt D., and Sanders-Bush E. (1994) Constitutively active 5-hydroxytryptamine 2C (5-HT_{2C}) receptors reveal novel inverse agonist activity of receptor ligands. *J. Biol. Chem.* **269,** 11,687–11,690.

Bastepe M. and Ashby B. (1997) The long cytoplasmic carboxyl terminus of the prostaglandin E2 receptor EP4 subtype is essential for agonist-induced desensitization. *Mol. Pharmacol.* **51,** 343–349.

Baxter G. S. and Tilford N. S. (1995) Endogenous ligands and inverse agonism. *Trends Pharmacol. Sci.* **16,** 258–259.

Bertaccini G. and Coruzzi G. (1992) in *Histamine Receptors in the Digestive System.* (Schwartz J-C., and Haas H. L., eds.) Wiley-Liss, New York, pp. 193–231.

Bond R. A., Leff P., Johnson T. D, Milano C. A., Rockman H. A., McMinn T. R., Apparsundaram S., Hyek M. F., Kenakin T. P., Allen L. F., and Lefkowitz R. J. (1995) Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β2-adrenoreceptor. *Nature* (London) **374,** 272–276.

Boone C., Davis N. G., and Sprague G. F. (1993) Mutations that alter the third cytoplasmic loop of the α-factor receptor lead to a constitutive and hypersensitive phenotype. *Proc. Natl. Acad. Sci. USA* **90,** 9921–9925.

Bouvier M., Hausdorff W. P., De Blasi A., O'Dowd B. F., Kobilka B. K., Caron M. G., and Lefkowitz R. J. (1988) Removal of phosphorylation sites from the β_2-adrenergic receptor delays onset of agonist-promoted desensitization. *Nature* (London) **333,** 370–373.

Braestrup C., Schmiechen R., Neef G., Nielsen M., and Petersen E. N. (1982) Interaction of convulsive ligands with benzodiazepine receptors. *Science* **216,** 1241–1243.

Brandl C. J. and Deber C. M. (1986) Hypothesis about the function of membrane-buried proline residues in transport proteins. *Proc. Natl. Acad. Sci. USA* **83,** 917–921.

Burstein E. S., Spalding T. A., Hill-Eubanks D., and Brann M. R. (1995) Structure-function of muscarinic receptor coupling to G proteins. Random saturation mutagenesis identifies a critical determinant of receptor affinity for G proteins. *J. Biol. Chem.* **270,** 3141–3146.

Burstein E. S., Spalding T. A., and Brann M. R. (1997) Pharmacology of muscarinic receptor subtypes constitutively activated by G proteins. *Mol. Pharmacol.* **51,** 312–319.

Casey C., Herrick-Davis K., and Teitler M. (1996) $5HT_{2A}$ receptor mutagenesis reveals a receptor which has radioligand binding properties indicative of onstitutive activity. Experimental Biology 96™ Washington, D.C., April 14–17, 1996.

Cerione R. A., Codina J., Benovic J. L., Lefkowitz R. J., Birnbaumer L., and Caron M. G. (1984) The mammalian β_2-adrenergic receptor: Reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. *Biochemistry* **23,** 4519–4525.

Charpentier S., Jarvie K. R., Severynse D. M., Caron M. G., and Tiberi M. (1996) Silencing of the constitutive activity of the dopamine D1B receptor. Reciprocal mutations between D1 receptor subtypes delineate residues underlying activation properties. *J. Biol. Chem.* **271,** 28,071–28,076.

Chidiac P., Hebert T. E., Valiquette M., Dennis M., and Bouvier M. (1994) Inverse agonist activity of β-adrenergic antagonists. *Mol. Pharmacol.* **45,** 490–499.

Chiu T. T., Yung L. Y., and Wong Y. H. (1996) Inverse agonistic effect of ICI-174,864 on the cloned δ-opioid receptor: role of G protein and adenylyl cyclase activation. *Mol. Pharmacol.* **50,** 1651–1657.

Cho W., Taylor L. P., and Akil H. (1996) Mutagenesis of residues adjacent to transmembrane prolines alters D_1 dopamine receptor binding

and signal transduction. *Mol. Pharmacol.* **50,** 1338–1345.

Cohen G. B., Yang T., Robinson P. R., and Oprian D. D. (1993) Constitutive activation of opsin: influence of charge at position 134 and size at position 296. *Biochemistry* **32,** 6111–6115.

Costa T. and Herz A. (1989) Antagonists with negative intrinsic activity at delta opioid receptors coupled to GTP-binding proteins. *Proc. Natl. Acad. Sci. USA* **86,** 7321–7325.

Costa T., Lang J., Gless C., and Herz A. (1990) Spontaneous association between opioid receptors and GTP-binding regulatory proteins in native membranes: specific regulation by antagonist and sodium ions. *Mol. Pharmacol.* **37,** 383–394.

Costa T., Ogino Y., Munson P. J., Onaran H. O., and Rodbard D. (1992) Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. *Mol. Pharmacol.* **41,** 549–560.

Cotecchia S., Exum S., Caron M. G., and Lefkowitz R. J. (1990) Regions of the α_1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. *Proc Natl. Acad. Sci. USA* **87,** 2896–2990.

Coughlin S. R. (1994) Expanding horizons for receptors coupled to G proteins: diversity and disease. *Curr. Opin. Cell. Biol.* **6,** 191–197.

Dalman H. M. and Neubig R. R. (1991) Two peptides from the α_{2A}-adrenergic receptor alter receptor G-protein coupling by distinct mechanisms. *J. Biol. Chem.* **266,** 11,025–11,029.

Deakin M. and Williams J. G. (1992) Histamine H_2-receptor antagonists in peptic ulcer disease. Efficacy in healding peptic ulcers. *Drugs* **44,** 709–719.

De Lean A., Stadel J. M., and Lefkowitz R. J. (1980) A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J. Biol. Chem.* **255,** 7108–7117.

Dixon R. A. F., Signal I. S., and Strader C. D. (1988) Structure-function analysis of the β-adrenergic receptor. *Cold Spring Harbor Symp. Quant. Biol.* **53,** 487–497.

Dryja T. P., Mcgee T. L., Reichel E., Hahn L. B., Cowley G. S., Yandell D. W., Olsson J. E., Sandberg M. A., and Berson E. L. (1990a) A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* **343,** 364–366.

Dryja T. P., Mcgee T. L., Hahn L. B., Cowley G. S., Olsson J. E., Reichel E., Sandberg M. A., and Berson E. L. (1990b) Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N. Engl. J. Med.* **232,** 1302–1307.

Dryja T. P., Berson E. L., Rao V. H., and Oprian D. D. (1993) Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. *Nature Genet.* **4,** 280–283.

Götze K. and Jakobs K. H. (1994) Unoccupied β-adrenoceptor-induced adenylyl cyclase stimulation in turkey erythrocyte membranes. *Eur. J. Pharmacol.* **268,** 151–158.

Groblewski T., Maigret B., Larguier R., Lombard C., Bonnafous J-C., and Marie J. (1997) Mutation of Asn^{111} in the third transmembrane domain of the AT_{1A} angiotensin II receptor induces its constitutive activation. *J. Biol. Chem.* **272,** 1822–1826.

Griffon N., Pilon C., Sautel F., Schwartz J-C., and Sokoloff P. (1996) Antipsychotics with inverse agonists activity at the dopamine D_3 receptor. *J. Neural Transm.* **103,** 1163–1175.

Hall D. A. and Strange P. G. (1997) Evidence that antipsychotic drugs are inverse agonists at D_2 dopamine receptors. *Br. J. Pharmacol.* **121,** 731–736.

Hanf R., Li Y., Szabo G., and Fischmeister R. (1993) Agonist-independent effects of muscarinic antagonists on Ca^{2+} and K^+ currents in frog and rat cardiac cells. *J. Physiol.* (London) **461,** 743–765.

Hartman J. L. and Northup J. K. (1996) Functional reconstitution *in situ* of 5-hydroxytryptamine$_{2C}$ ($5HT_{2C}$) receptors with α_q and inverse agonism of $5HT_{2C}$ receptor antagonists. *J. Biol. Chem.* **271,** 22591–22597.

Hausdorff W. P., Bouvier M., O'Dowd B. F., Irons G. P., Caron M. G., and Lefkowitz R. J. (1989) Phosphorylation sites on two domains of the beta 2-adrenergic receptor are involved in distinct pathways of receptor desensitization. *J. Biol. Chem.* **264,** 12,657–12,665.

Hausdorff W. P., Caron M. G., and Lefkowitz R. J. (1990) Turning off the signal: desensitization of β-adrenergic receptor function. *FASEB J.* **4,** 2881–2889.

Heinflink M., Nussenzveig D. R., Grimberg H., Lupu-Meiri M., Oron Y., and Gershengorn M. C. (1995) A constitutively active mutant thyrotropin-releasing hormone receptor is chronically down-regulated in pituitary cells: evidence using chlordiazepoxide as a negative antagonist. *Mol. Endocrinol.* **9,** 1455–1460.

Hibert M. F., Trumpp-Kallmeyer S., Bruinvels A., and Hoflack J. (1991) Three-dimensional models

of neurotransmitter G-binding protein-coupled receptors. *Mol. Pharmacol.* **40,** 8–15.

Hilf G. and Jakobs, K. H. (1992) Agonist-independent inhibition of G protein activation by muscarinic acetylcholine receptor antagonists in cardiac membranes. *Eur. J. Pharmacol. Mol. Pharmacol. Section* **225,** 245–252.

Högger P., Shockley M. S., Lameh J., and Sadée W. (1995) Activating and inactivating mutations in N- and C-terminal i3 loop junctions of muscarinic acetylcholine Hm1 receptors. *J. Biol. Chem.* **270,** 7405–7410.

Hwa J., Graham R. M., and Perez D. M. (1996) Chimeras of α1-adrenergic receptor subtypes identify critical residues that modulate active state isomerization. *J. Biol. Chem.* **271,** 7956–7964.

Hwa J., Gaivin R., Porter J. E., and Perez D. M. (1997) Synergism of constitutive activity in α1em1-adrenergic receptor activation. *Biochemistry* **36,** 633–639.

Ishii I., Izumi T., Tsukamoto H., Umeyama H., Ui M., and Shimizu T. (1997) Alanine exchanges of polar amino acids in the transmembrane domains of a platelet-activating factor receptor generate both constitutively active and inactive mutants. *J. Biol. Chem.* **272,** 1846–1854.

Jin J., Mao G. F., and Ashby B. (1997) Constitutive activity of human prostaglandin E receptor EP_3 isoforms. *Br. J. Pharmacol.* **121,** 317–323.

Jones P. G., Curtis C. A. M., and Hulme E. C. (1995) The function of a highly-conserved arginine residue in activation of the muscarinic m1 receptor. *Eur. J. Pharmacol.* **288,** 251–257.

Keen T. J., Inglehearn C. F., Lester D. H., Bashir R., Jay M., Bird A. C., Jay B., and Bhattacharya S. S. (1991) Autosomal dominant retinitis pigmentosa: four new mutations in rhodopsin, one of them in the retinal attachment site. *Genomics* **11,** 199–205.

Kenakin T. (1995) Pharmacological Proteus? *Trends Pharmacol. Sci.* **16,** 256–258.

Kim G. D., Carr I. C., and Milligan G. (1995) Detection and analysis of agonist-induced formation of the complex of the stimulatory guanine nucleotide-binding protein with adenylate cyclase in intact wild-type and beta 2-adrenoreceptor-expressing NG108-15 cells. *Biochem. J.* **308,** 275–281.

Kjelsberg M. A., Cotecchia S., Ostrowski J., Caron M. G., and Lefkowitz R. J. (1992) Constitutive activation of the α_{1B}-adrenergic receptor by all amino acid substitutions at a single site: evidence for a region which constrains receptor activation. *J. Biol. Chem.* **267,** 1430–1433.

Kobilka B. K., Kobilka T. S., Daniel K., Regan J. W., Caron M. G., and Lefkowitz R. J. (1988) Chimeric α_2-β_2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specifity. *Science* **240,** 1310–1316.

Konopka J. B., Jenness D. D., and Hartwell L. H. (1988) The C-terminus of the *S. cerevisiae* α-pheromone receptor mediates and adaptative response to pheromone. *Cell* **54,** 609–620.

Konopka J. B., Margarit S. M., and Dube P. (1996) Mutation of Pro-258 in transmembrane domain 6 constitutively activates the G protein-coupled α-factor receptor. *Proc. Natl. Acad. Sci. USA* **93,** 6764–6769.

Kopp P., Van Sande J., Parma J., Duprez L., Gerber H., Joss E., Jameson J. L., Dumont J. E., and Vassart G. (1995) Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N. Engl. J. Med.* **332,** 150–154.

Kosugi S., Van Dop C., Geffner M. E., Rabl W., Carel J-C., Chaussain J-L., Mori T., Merendino J. J. Jr., and Shenker A. (1995) Characterization of heterogenous mutations causing constitutive activation of the luteinizing hormone receptor in familial male precocious puberty. *Human Mol. Genet.* **4,** 183–188.

Kremer H., Mariman E., Otten B. J., Moll G. W., Stoelinga G. B. A., Wit J. M., Jansen M., Drop S. L., Faas B., Ropers H-H., and Brunner H. G. (1993) Cosegregation of missense mutations of the luteinizing hormone receptor gene with familial male-limited precocious puberty. *Human Mol. Genet.* **2,** 1779–1783.

Kudo M., Osuga Y., Kobilka B. K., and Hsueh A. J. W. (1996) Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. *J. Biol. Chem.* **271,** 22,470–22,478.

Labrecque J., Fargin A., Bouvier M., Chidiac P., and Dennis M. (1995) Serotoninergic antagonists differentially inhibit spontaneous activity and decrease ligand binding capacity of the rat 5-hydroxytryptamine type 2C receptor in Sf9 cells. *Mol. Pharmacol.* **48,** 150–159.

Latronico A. C., Anasti J., Arnhold I. J. P., Mendonca B. B., Domenice S., Albano M. C., Zachman K., Wajchenberg B. L., and Tsigos C. (1995) A novel mutation of the luteinizing hormone receptor gene causing male gonadotropin-independent precocious puberty. *J. Clin. Endocrinol. Metab.* **80,** 2490–2494.

Laue L., Chan W-Y., Hsueh A. J. W., Kudo M., Hsu S. Y., Wu S-M., Blomberg L., and Cutler G. B. Jr. (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,** 1906–1910.

Laue L., Wu S-M., Kudo M., Hsueh A. J. W., Cutler G. B. Jr., Jelly D. H., Diamond F. B., and Chan W-Y. (1996) Heterogeneity of activating mutations of the human luteinizing receptor in male-limited precocious puberty. *Biochem. Mol. Medicine.* **7,** 192–198.

Leeb-Lundberg F. L. M., Mathis S. A., and Herzig M. C. S. (1994) Antagonists of bradykinin that stabilize a G-protein-uncoupled state of the B2 receptor act as inverse agonists in rat myometrial cells. *J. Biol. Chem.* **269,** 25,970–25,973.

Leff P. (1995) The two-state model of receptor activation. *Trends Pharmacol. Sci.* **16,** 89–97.

Lefkowitz R. J., Cotecchia S., Samama P., and Costa T. (1993) Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol. Sci.* **14,** 303–307.

Lundell L., Backman L., Eström P., Enander L. K., Falkmer S., Fausa O., Grimelius L., Havu N., Lind T., Lonroth H., Sandmark S., Sandzen B., Unge P., and Westin I. H. (1991) *Scand. J. Gastroenterol.* **26,** 248–256.

Ma H. and Green R. D. (1992) Modulation of cardiac cyclic AMP metabolism by adenosine receptor agonists and antagonists. *Mol. Pharmacol.* **12,** 831–837.

Mac Ewan D. J. and Milligan G. (1996) Inverse agonist-induced up-regulation of the human β_2-adrenoreceptor in transfected neuroblastoma X glioma hybrid cells. *Mol. Pharmacol.* **50,** 1479–1486.

Merki H. S. and Wilder-Smith C. H. (1994) Do continuous infusions of omeprazole and ranitidine retain their effect with prolonged dosing? *Gastroenterology* **106,** 60–64.

Mewes T., Dutz S., Ravens U., and Jakobs K. H. (1993) Activation of calcium currents in cardiac myocytes by empty β-adrenoceptors. *Circulation* **88,** 2916–2922.

Milano C. A., Allen L. F., Rockman H. A., Dolber P. C., McMinn T. R., Chien K. R., Johnson T. D., Bond R. A., and Lefkowitz R. J. (1994a) Enhanced myocardial function in transgenic mice overexpressing the β_2-adrenergic receptor. *Science* **264,** 582–586.

Milano C. A., Dolber P. C., Rockman H. A., Bond R. A., Venable M. E., Allen L. F., and Lefkowitz R. J. (1994b) Myocardial expression of a constitutively active α_{1B}-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* **91,** 10109–10113.

Milligan G., Bond R. A., and Lee M. (1995) Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol. Sci.* **16,** 10–13.

Murray R. and Keenan A. K. (1989) The β-adrenoceptor is precoupled to Gs in chicken erythrocyte membranes. *Cell. Signal.* **1,** 173–179.

Nanevicz T., Wang L., Chen M., Ishii M., and Coughlin S. R. (1996) Thrombin receptor activating mutations. Alteration of an extracellular agonist recognition domain causes constitutive signaling. *J. Biol. Chem.* **271,** 702–706.

Nerme V., Severne Y., Abrahamsson T., and Vauquelin G. (1986) Spontaneous coupling of the β-adrenergic receptor to Ns in mammalian cardiac membranes. *Mol. Pharmacol.* **30,** 1–5.

Neubig, R. N., Gantzos, R. D., and Thomsen W. J. (1988) Mechanism of agonist and antagonist binding to α_2 adrenergic receptors: evidence for a precoupled receptor-guanine nucleotide protein complex. *Biochemistry* **27,** 2374–2384.

Newman-Tancredi A., Conte C., Chaput C., Spedding M., and Millan M. J. (1997) Inhibition of the constitutive activity of human 5-HT_{1A} receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635. *Br. J. Pharmacol.* **120,** 737–739.

Nwokolo C. U., Mistry P., and Pounder R. E. (1990) The absorption of bismuth and salicylate from oral doses of Pepto-Bismol (bismuth salicylate). *Aliment. Pharmacol. Ther.* **4,** 163–169.

O'Dowd B. F., Hnatowich M., Regan J. W., Leader W. M., Caron M. G., and Lefkowitz R. J. (1988) Site-directed mutagenesis of the cytoplasmic domains of the human β_2-adrenergic receptor. Localization of regions involved in G protein-receptor coupling. *J. Biol. Chem.* **263,** 15985–15992.

Okamoto T., Murayama Y., Hayashi Y., Inagaki M., Ogata E., and Nishimoto Y. (1991) Identification of a Gs activator region of the β_2-adrenergic receptor that is autoregulated via protein kinase A-dependent phosphorylation. *Cell* **67,** 723–730.

Okamoto T. and Nishimoto I. (1992) Detection of G protein-activator regions in M4 subtype muscarinic, cholinergic, and α_2-adrenergic receptors based upon characteristics in primary structure. *J. Biol. Chem.* **267,** 8342–8346.

Parent J-L., Le Gouill C., de Brum-Fernandes A. J., Rola-Pleszczynski M., and Stankova J. (1996)

Mutations of two adjacent amino acids generate inactive and constitutively active forms of the human platelet-activating factor receptor. *J. Biol. Chem.* **271,** 7949–7955.

Parma J., Duprez L., Van Sande J., Cochaux P., Gervy C., Mockel J., Dumont J., and Vassart G. (1993) Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* **365,** 649–651.

Parma J., Duprez L. Van Sande J., Paschke R., Tonacchera M., Dumont J., and Vassart G. (1994) Constitutively active receptors as a disease-causing mechanism. *Mol. Cell. Endocrinol.* **100,** 159–162.

Paschke R., Tonacchera M., Van Sande J., Parma J., and Vassart G. (1994) Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. *J. Clin. Endocrinol. Metab.* **79,** 1785–1789.

Pauwels P. J. (1997) 5-$HT_{1B/D}$ receptor antagonists. *Gen. Pharmacol.* **29,** 293–303.

Pauwels P. J., Tardif S., Palmier C., Wurch T., and Colpaert F. C. (1997a) How efficacious are 5-$HT_{1B/D}$ receptor ligands: an answer from GTPγS binding studies with stably transfected C6-glial cell lines. *Neuropharmacology* **36,** 499–512.

Pauwels P. J., Tardif S., Wurch T., and Colpaert F. C. (1997b) Stimulated [^{35}S]GTPγS binding by 5-HT_{1A} receptor antagonists in recombinant cell lines: modulation of apparent efficacy by G-protein activation state. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **356,** 551–561.

Pei G., Samama P., Lohse M., Wang M., Codina J., and Lefkowitz R. J. (1994) A constitutively active mutant β_2-adrenergic receptor is constitutively desensitized and phosphorylated. *Proc. Natl. Acad. Sci. USA* **91,** 2699–2702.

Perez D. M., Hwa J., Gaivin R., Mathur M., Brown F., and Graham R. M. (1996) Constitutive activation of a single effector pathway: evidence for multiple activation states of a G protein-coupled receptor. *Mol. Pharmacol.* **49,** 112–122.

Porcellini A., Ciullo I., Laviola L., Amabile G., Fenzi G., and Avvedimento V. E. (1994) Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas. *J. Clin. Endocrinol. Metab.* **79,** 657–661.

Pozvek G., Hilton J. M., Quiza M., Houssami S., and Sexton P. M. (1997) Structure/function relationships of calcitonin analogues as agonists, antagonists, or inverse agonists, in a constitutively activated receptor cell system. *Mol. Pharmacol.* **51,** 658–665.

Probst W. C., Snyder L. A., Schuster D. I., Brosius J., and Sealfon S. C. (1992) Sequence aligment of the G protein coupled receptor superfamily. *DNA Cell. Biol.* **11,** 1–20.

Rao V. R., Cohen G. B., and Oprian D. D. (1994) Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. *Nature* **367,** 639–642.

Ren Q., Kurose H., Lefkowitz R. J., and Cotecchia S. (1993) Constitutively active mutants of the α_2-adrenergic receptor. *J. Biol. Chem.* **268,** 16483–16487.

Reneke J. E., Blumer K. J., Courchesne W. E., and Thorner J. (1988) The carboxy-terminal segment of the yeast a-factor receptor is a regulatory domain. *Cell* **55,** 221–234.

Rim J. and Oprian D. (1995) Constitutive activation of opsin: interaction of mutants with rhodopsin kinase and arrestin. *Biochemistry* **34,** 11938–11945.

Robinson P. R., Cohen G. B., Zhukovsky E. A., and Oprian D. D. (1992) Constitutively active mutants of rhodopsin. *Neuron* **9,** 719–725.

Robbins L. S., Nadeau J. H., Johnson K. R., Kelly M. A., Roselli-Rehfuss L. R., Baack E., Mountjoy K. G., and Cone R. D. (1993) Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* **72,** 827–834.

Samama P., Cotecchia S., Costa T., and Lefkowitz R. J. (1993) A mutation-induced activated state of the β_2-adrenergic receptor: extending the ternary complex model. *J. Biol. Chem.* **268,** 4625–4636.

Samama P., Pei G., Costa T., Cotecchia S., and Lefkowitz R. J. (1994) Negative antagonists promote an inactive conformation of the β_2-adrenergic receptor. *Mol. Pharmacol.* **45,** 390–394.

Samama P., Bond R. A., Rockman H. A., Milano C. A., and Lefkowitz R. J. (1997) Ligand-induced overexpression of a constitutively active β2-adrenergic receptor: pharmacological creation of a phenotype in transgenic mice. *Proc. Natl. Acad. Sci. USA* **94,** 137–141.

Scheer A., Fanelli F., Costa T., De Benedetti P. G., and Cotecchia S. (1996) Constitutively active mutants of the α_{1B}-adrenergic receptor: role of highly conserved polar amino acids in receptor activation. *EMBO J.* **15,** 3566–3578.

Scheer A., Fanelli F., Costa T., De Benedetti P. G., and Cotecchia S. (1997) The activation process of the α_{1B}-adrenergic receptor: potential role of protonation and hydrophobicity of a highly con-

served aspartate. *Proc. Natl. Acad. Sci. USA* **94,** 808–813.

Schipani E., Kruse K., and Jüppner H. (1995) A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* **268,** 98–100.

Schütz W. and Freissmuth M. (1992) Reverse intrinsic activity of antagonists on G protein-coupled receptors. *Trends Pharmacol. Sci.* **13,** 376–380.

Sealfon S., Chi L., Ebersole B. J., Rodic V., Zhang D., Ballesteros J. A., and Weinstein H. (1995) Related contribution of specific helix 2 and 7 residues to conformational activation of the serotonin 5-HT_{2A} receptor. *J. Biol. Chem.* **270,** 16,683–16,688.

Shapiro R. A., Palmer D., and Cislo T. (1993) A deletion mutation in the third cytoplasmic loop of the mouse m1 muscarinic acetylcholine receptor unmasks cryptic G-protein binding sites. *J. Biol. Chem.* **15,** 21,734–21,738.

Shenker A., Laue L. Kosugi S., Merendino J. J., Minegishi T., and Cutler G. B. (1993) A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature.* **365,** 652–654.

Shi A-G. and Deth R. C. (1994) Precoupling of *alpha*-2B adrenergic receptors and G-proteins in transfected PC-12 cell membranes: influence of pertussis toxin and a lysine directed cross-linker. *JPET* **271,** 1520–1527.

Smit M. J., Leurs R., Alewijnse A. E., Blauw J., Van Nieuw Amerongen G. P., Van De Vrede Y., Roovers E., and Timmerman H. (1996a) Inverse agonism of histamine H2 antagonists accounts for upregulation of spontaneously active histamine H2 receptors. *Proc. Natl. Acad. Sci. USA* **93,** 6802–6807.

Smit M. J., Timmerman H., Blauw J., Beukers M. W., Roovers E., Jacobs E. H., Hoffman M., and Leurs R. (1996b) The C terminal tail of the histamine H_2 receptor contains positive and negative signals important for signal transduction and receptor down-regulation. *J. Neurochem.* **67,** 1791–1800.

Spalding T. A., Burnstein E. S., Brauner-Osborne H., Hill-Eubanks D., and Brann M. R. (1995) Pharmacology of a constitutively active muscarinic receptor generated by random mutagenesis. *J. Pharmacol. Exp. Ther.* **275,** 1274–1279.

Spalding T. A., Burstein E. S., Wells J. W., and Brann M. R. (1997) Constitutive activation of the m5 muscarinic receptor by a series of mutations at the extracellular end of transmembrane 6. *Biochemistry* **36,** 10109–10116.

Spiegel A. M., Shenker A., and Weinstein L. S. (1992) Receptor effector coupling by G proteins: implications for normal and abnormal signal transduction. *Endocr. Rev.* **13,** 536–565.

Stanton J. A. and Beer M. S. (1997) Characterisation of a cloned human 5-HT_{1A} receptor cell line using [^{35}S]GTPγS binding. *Eur. J. Pharmacol.* **320,** 267–275.

Strader C. D., Dixon R. A., Cheung A. H., Candelore M. R., Blacke A. D., and Signal I. S. (1987) Mutations that uncouple the β-adrenergic receptor from G_S and increase agonist affinity. *J. Biol. Chem.* **262,** 16,439–16,443.

Strader C. D., Candelore M. R., Hill W. S., Sigal I. S., and Dixon R. A. F. (1989) Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. *J. Biol. Chem.* **264,** 13,572–13,578.

Sung C. H., Davenport C. M., Hennessey J. C., Maumenee I. H., Jacobson S. G., Heckenlively J. R., Nowakowski R., Fishman G., Gouras P., and Nathans J. (1991a) Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* **88,** 6481–6485.

Sung C. H., Schneider B. G., Agarwal N., Papermaster D. S., and Nathans J. (1991b) Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* **88,** 8840–8844.

Thomas D. R., Faruq S. A., Balcarek J. M., and Brown A. M. (1995) Pharmacological characterisation of [^{35}S]GTPγS binding to Chinese hamster ovary cell membranes stably expressing cloned human 5-HT_{1D} receptor subtypes. *J. Receptor Res.* **15,** 199–211.

Tian W. N., Duzic E., Lanier S. M., and Deth R. C. (1994) Determinants of α_2-adrenergic receptor activation of G proteins: evidence for a precoupled receptor/G protein state. *Mol. Pharmacol.* **45,** 524–531.

Tiberi M. and Caron M. G. (1994) High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. *J. Biol. Chem.* **269,** 27,925–27,931.

Van Sande J., Parma J., Tonacchera M., Swillens S., Dumont J., and Vassart G. (1995) Genetic basis of endocrine disease. Somatic and germline mutations of the TSH receptor gene in thyroid diseases. *J. Clin. Endocrinol. Metab.* **80,** 2577–2585.

Von Heijne G. (1991) Proline kinks in transmembrane alpha-helices. *J. Mol. Biol.* **218,** 499–503.

Westphal R. S. and Sanders-Bush E. (1994) Reciprocal binding properties of 5-hydroxytryptamine

type 2C receptor agonists and inverse agonists. *Mol. Pharmacol.* **46,** 937–942.

Westphal R. S., Backstrom J. R., and Sanders-Bush E. (1995) Increased basal phosphorylation of the constitutively active serotonin 2C receptor accompanies agonist-mediated desensitization. *Mol. Pharmacol.* **48,** 200–205.

Westphal R. S. and Sanders-Bush E. (1996) Differences in agonist-independent and-dependent 5-Hydroxytryptamine2C receptor-mediated cell division. *Mol. Pharmacol.* **49,** 474–480.

Wilder-Smith C. H., Ernst T., Gennoni M., Zeyen B., Halter F., and Merki H. S. (1990) Tolerance to oral H_2-receptor antagonists. *Dig. Dis. Sci.* **35,** 976–983.

Winderickx J., Lindsey D. T., Sanochi E., Teller D. Y., Motulsky A. G., and Deeb S. S. (1992) Polymorphism in red photopigment underlies variation in colour matching. *Nature* **356,** 431–433.

Wong S. E., Parker E. M., and Ross E. (1990) Chimeric muscarinic cholinergic/β-adrenergic receptors that activate Gs in response to muscarinic agonists. *J. Biol. Chem.* **265,** 6219–6224.

Yano K., Hidaka A., Saji M., Polymeropoulos M. H., Okuno A., Kohn L. D., and Cutler G. B. Jr. (1994) A sporadic case of male-limited precocious puberty has the same constitutively activating point mutation in luteinizing hormone/choriogonadotropin receptor gene as familial cases. *J. Clin. Endocrinol. Metab.* **79,** 1818–1823.

Yano K., Saji M., Hidaka A., Moriya N., Okuno A., Kohn L. D., and Cutler G. B. Jr. (1995) A new constitutively activating point mutation in the luteinizing hormone/choriogonadotropin receptor gene in cases of male-limited precocious puberty. *J. Clin. Endocrinol. Metab.* **80,** 1162–1168.