Published in final edited form as:
Drug Dev Res. 1996 January 1; 37(1): 1–38. doi:10.1002/(SICI)1098-2299(199601)37:1<1::AID-DDR1>3.0.CO;2-S.

Molecular Architecture of G Protein-Coupled Receptors

A. Michiel van Rhee and **Kenneth A. Jacobson**
Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

Abstract

This review of the current literature on mutations in G protein-coupled receptors (GPCRs) of the rhodopsin-related family intends to draw inferences from amino acid sequences for single receptors and multiple sequence alignments with regard to the molecular architecture of this class of receptors. For this purpose a comprehensive list of mutations within the transmembrane helical regions (TMs; over 390 mutations from 38 different receptor subtypes) and their effects on function was compiled, and an alignment of known GPCR sequences (over 150 separate sequences) was made. Regions most prominently involved in ligand binding are located in TMs 3, 5, 6, and 7. Position 3.32 in TM3 is occupied by a D in all biogenic amine receptors (sequence conservation) but may be occupied by uncharged residues in other receptors while its role in ligand binding is analogous (function conservation). TMs 5, 6, and 7 display considerable sequence conservation throughout the majority of GPCRs investigated, but not necessarily at those positions involved in ligand binding. However, considerable function conservation is observed for positions 5.42 (frequently hydrophilic), 5.46 (small amino acids required for agonist binding to "small ligand" receptors), 6.52 and 7.39 (high variability), and 7.43 (frequently aromatic). A general conclusion of this review is that there is overwhelming conservation of structure-function correlates among GPCRs. Thus, it is now possible to cross-correlate the results of mutagenesis studies between GPCRs of different subfamilies, and to use those results to predict the function of specific residues in new GPCR sequences.

Keywords

G protein-coupled receptors; GPCRs; transmembrane domains; mutagenesis; sequence alignment; identifiers; molecular architecture; molecular structure

INTRODUCTION

The aim of this study was to analyze the effects of amino acid substitutions in G protein-coupled receptors (GPCRs) on ligand binding. In an effort to identify functions of particular sites among various receptors, we have focused on mutations within the helical transmembrane regions, since sequence homology is clearly discernible in those regions. The result is a comprehensive, but probably not exhaustive, list of these substitutions. We have tried to correlate substitutions of specific amino acids with the effect(s) exerted on ligand binding, to discriminate between agonist and antagonist binding, coupling to G proteins, and receptor activation. The literature is abundant with reports of structure-activity/

Address reprint requests to Dr. K.A. Jacobson, NIH/NIDDK/LBC/Molecular Recognition Section, Building 8A Room B1A19, Bethesda, MD 20892-0810.

Dedicated to Prof. Ephraim Katzir on the occasion of his 80th birthday.

affinity relationships (SAR) based on modelling ligands; however, very few (if any) SAR studies are based on the insights obtained from modelling the receptor-ligand interaction at the molecular level of the receptor. Other aspects of mutagenesis techniques applied to GPCRs have been reviewed extensively [Houslay, 1992; Baldwin, 1994; Coughlin, 1994; Donnelly and Findlay, 1994; Schwartz, 1994]. Structure and function of one subfamily of GPCRs, i.e., the biogenic amine receptors, have been analyzed in more detail [Ostrowski et al., 1992; Strader et al., 1994; Burstein et al., 1995].

From Isolation to Cloning of G Protein-Coupled Receptors

Isolation, purification, sequencing

The first biophysical data on GPCRs were obtained in the early seventies, when Lefkowitz et al. [1972] isolated the cardiac β-adrenoceptor. It was a very laborious process that required large amounts of receptor protein and perseverance. It took 14 years before the next large step in the elucidation of the structure of this GPCR was completed. In 1986, Dixon et al. [1986] reported the isolation and cloning of a mammalian β-adrenoceptor. In the years since, hundreds of sequences for GPCRs have been identified, and their function attributed to a specific neurotransmitter/humoral regulator system. The receptor isolation techniques developed over the years, however, have not been totally abandoned. They have been demonstrated to be useful in, for instance, determining the binding site for a specific antagonist in muscarinic acetylcholine receptors [Kurtenbach et al., 1990].

The polymerase chain reaction

Considerable progress in the identification of GPCR sequences was made with the advent of a novel molecular biology technique currently known as the polymerase chain reaction (PCR). Its immediate predecessor was described as early as 1985 [Saiki et al.], but the method gained wide popularity [Vosberg, 1989; White et al., 1989] only after the introduction of a thermostable DNA polymerase in the procedure [Saiki et al., 1988]. The method was successfully applied to the cloning of proteins in general [Bonner et al., 1987; Intres and Crabb, 1992], and to GPCRs more specifically [Libert et al., 1989; Parmentier et al., 1993]. The PCR method has since seen the development of a large number of specialized and/or more sophisticated applications [Bockstahler, 1994; Edwards and Gibbs, 1994; Rashtchian, 1995].

Cloning methodology

Cloning procedures for specific receptors have been described extensively [Allard et al., 1987; Arai et al., 1990; Arakawa et al., 1990; Barberis et al., 1993; Birdsall, 1991; Bunzow et al., 1988; Cone et al., 1993; Corjay et al., 1991; D'Angelo et al., 1994; Gantz et al., 1991; Gershengorn, 1993; Inagami et al., 1992; Ishihara et al., 1992; Jüppner et al., 1991; Kieffer et al., 1992; Kimura et al., 1993; Klein et al., 1988; Kubo et al., 1986; Lubbert et al., 1987; Lustig et al., 1993; Marsh and Herskowitz, 1988; Masu et al., 1991; McEachern et al., 1991; Miyajima et al., 1987; Morel et al., 1993; Murphy et al., 1992; Ruat et al., 1991; Salvatore et al., 1993; Straub et al., 1990; Sugimoto et al., 1992; Wank et al., 1992; Webb et al., 1993; Yamashita et al., 1991; Ye et al., 1991; Yokota et al., 1989], and an overview of such procedures was published by Parmentier et al. [1993]. Explicit procedures for cloning and expression of the serotonin $5HT_{1A}$ receptor [Albert, 1992], or site-directed mutagenesis in the serotonin $5HT_2$ receptor [Shih et al., 1992] were described, and could serve as a reference for receptors yet to be cloned.

Targeted searching

The first GPCR to be cloned was rhodopsin [Nathans and Hogness, 1983]. Rhodopsin is a special case within the superfamily of GPCRs; the receptor couples only to the specific G-

protein homologue transducin, and its "endogenous agonist" is a steric and electronic transition of the covalently linked retinal chromophore by photoinduction rather than a circulating/topically released chemical substance. Its sequence and apparent functional homology to other GPCRs, however, qualify it as a GPCR par excellence. It has proven to be a well-behaved model and test system for analyzing process kinetics and thermodynamics, and has served especially well in mutagenesis studies [Kaushal and Khorana, 1994, and references therein]. After the initial cloning of the β-adrenoceptor [Dixon et al., 1986], many other members of the GPCR superfamily have been identified through targeted cloning techniques (Table 1). In Table 1, receptors were classified according to their "ligand family" which is not necessarily the best option. For instance, even though adenosine (4 subtypes) and adenosine 5′-triphosphate (ATP; at least 8 subtypes) receptors are all considered members of the purinergic receptor subfamily, there seems to be minimal crossover in ligand, agonist, or antagonist, affinity between either receptors for adenosine (P_1 or receptors for ATP (P_2) [Fredholm et al., 1994; Dalziel and Westfall, 1994; Fredholm, 1995]. A better way to classify receptor subfamilies is probably through amino acid sequence homology and genealogy, as was proposed by Kolakowski [1994]. Another case in point is the interleukin 8 (IL-8) receptor. Initially classified by its supposed ligand, it was deemed the receptor for formyl-methionyl-leucyl-phenylalanyl (fMLP) peptide [Thomas et al., 1990]; it currently is more properly defined as an IL-8 receptor [Beckmann et al., 1991].

Orphan receptors

It was not long until the first GPCRs were cloned where the endogenous ligand was unknown. These clones were dubbed "orphan receptors" (Table 1) [see Mills and Duggan, 1994, and references therein]. The serotonin $5HT_{1A}$ receptor started out as an orphan receptor [Kobilka et al., 1987], but its function was rapidly elucidated [Fargin et al., 1988]. The same was the case for the somatostatin receptors [Meyerhof et al., 1991; Li et al., 1992]. However, of the four orphan receptors originally identified by Libert et al. [1989], one sequence (RDC4) was assigned to the serotonin $5HT_{1D}$ receptor [Maenhaut et al., 1991], and two others were assigned to the adenosine A_1 (RDC7) [Libert et al., 1991], and adenosine A_{2a} (RDC8) [Maenhaut et al., 1990] receptors, respectively. Still unresolved is the functionality of the clone RDC1. Other orphan receptors are listed in Table 1.

Detection of GPCRs

Use of receptor-antibodies

GPCRs can be detected in various ways. Probably the oldest way is the registration of a functional effect certain agents exert in vitro/ex vivo/in vivo on tissues, organs, or whole organisms. The results of such experiments can be expressed in consistent and predictive structure-activity relationships. The translation of the signal to the final effect, however, requires any number of transduction steps that may be modulated/moderated by other factors. Not the least of these steps is the receptor-to-G protein coupling itself, and the concomitant coupling to effector systems, such as adenylyl cyclase and phospholipases. A more direct way of detecting GPCRs is through the use of radioligand binding assays. Data derived from such assays have been used extensively to define structure-affinity relationships to aid the development of newer ligands. In cases where radioligand binding assays were either not available, or not useable, employing (auto)antibodies raised against GPCR sequences was demonstrated to be helpful. For example, immunological detection proved to be instrumental in the isolation and identification of the β-adrenoceptor [Venter and Fraser, 1983]. More recently, this method of tracking GPCRs has been shown to be effective in determining (splice variant) subtypes of D_5 dopamine receptors [Bergson et al., 1995], monitoring the expression of chimerical D_2 dopamine [Fishburn et al., 1994], and

NKl/NK2 neurokinin receptors [Vigna et al., 1994], and identifying an $A_{p4}A$ dinucleotide receptor [Walker and Hilderman, 1993].

Use of epitope-tags

Another advantage of applying the PCK technique to the field of GPCR research, is that primer sequences are readily extended at either the 3′ or the 5′ end. Translated from the nucleotide sequence to the protein, this means that proteins can be supplied with a “flag” or “tag” sequence at either the N- or C-terminal side. Such a tag can be used to monitor expression levels of the tagged protein by means of radio-immunoassays (RIAs) or enzyme-linked immunosorbent assays (ELISAs) directed against the tag sequence. This method has successfully been applied to quantitate cell surface expression levels of transfected platelet-activating factor (PAF) receptors [Kunz et al., 1992] and dopamine D_2 receptors [Sanderson and Strange, 1995], to monitor the internalization of β_2-adrenoceptors [von Zastrow and Kobilka, 1994], and to establish the expression levels of mutant adenosine A_{2a} receptors that were pharmacologically undetectable [Kim et al., 1995].

Peptide and cytokine receptors

A very arbitrary, but useful, distinction can be made between two classes of GPCRs that is based solely on the size of the endogenous ligand. Peptide and cytokine receptors are activated by (macro)molecules that, due to their size, interact with the receptor protein over a much larger surface area than the “small ligand” receptors. The interactions vary from relatively small polypeptides, such as angiotensin II, that interact partly with the helical bundle and partly with the extracellular extensions [Yamano et al., 1995], through thrombin receptors, where the N-terminal part of the receptor sequence is a precursor for the agonist [Vu et al., 1991; Vouret-Craviari et al., 1995], to receptors such as the LH/CG receptor, where the agonist is thought to interact entirely with the N-terminal part of the receptor sequence [Kakar et al., 1992]. The binding of large ligands leads to participation of a larger portion of the helical bundle in the process of binding agonists, but not usually (non-peptide) antagonists [Beinborn et al., 1993; Bihoreau et al., 1993; Bhogal et al., 1994; Breu el al., 1995; Yamano et al., 1995]. The advantage of working with such receptors in understanding ligand receptor interactions is that a larger part of the receptor is “covered.” A disadvantage is that interactions between agonists and their receptors tend to be much harder to assign to a specific region of the receptor. In the case of the thrombin receptor, studying agonist-receptor interactions is even more difficult compared to other peptide receptors, because of the required enzymatic conversion of the N-terminus in receptor activation [Vouret-Craviari et al., 1995]. Enzymatic activation of GPCRs is, however, not limited to peptide receptors: a yet uncloned AP_4A dinucleotide receptor requires activation by a serine protease [Walker and Hilderman, 1993].

Small ligand receptors

The analysis of mutagenesis data for small ligand receptors can yield valuable information concerning the relative height of the ligand binding site in the helical bundle, and the mode of binding of agonists and antagonists to the receptor [e.g., Fong et al., 1993b; Javitch et al., 1995; Kim et al., 1995]. A recent review by Ballesteros and Weinstein [1995] indicates that the binding sites for the class of smaller ligands in GPCRs are allocated to a rather narrow region. A special case, in which more data are available than for any other receptor subgroup, is the biogenic amine receptors. Several models have been generated, and all indicate similar binding domains around the upper third to upper half of the helical transmembrane bundle towards the extracellular surface [Hibert et al., 1991; Trumpp-Kallmeyer et al., 1992]. For both agonists and antagonists for small ligand receptors, and for antagonists for peptide/cytokine receptors there is a remarkable coincidence of the ligand

binding site with the position of the retinal chromophore in either bacteriorhodopsin or rhodopsin [Henderson et al., 1990; IJzerman et al., 1992; Baldwin, 1993]. The possibilities of using the smallest possible ligand, a one-atom ligand, for probing the receptor domains was demonstrated for the "zinc-receptor" that was engineered from a neurokinin receptor [Elling et al., 1995]. Data on the naturally occurring one-atom ligand receptor, the extracellular Ca^{2+}-sensitive GPCR, are sparse [Garrett et al., 1995].

Agonists and antagonists

Even in such cases where ligand size is not the limiting factor for activity/affinity analysis, the existence of multiple "affinity states" for ligands, especially if not exclusively agonists, makes the deduction of cause-effect relationships very difficult. A ternary complex model has been proposed for the binding of ligands to GPCRs to explain these multiple "affinity states." In this model the ternary complex of agonist-receptor-G protein is energetically favoured over the binary agonist-receptor complex. Agonists therefore experience two "affinity states" (affinity being the inverse of the equilibrium dissociation constant) [De Lean et al., 1980; Lefkowitz et al., 1993; Samama et al., 1993]. The effects of binary and ternary complex formation on ligand binding characteristics are tentatively mediated by conformational changes of the receptor architecture. The allosteric interactions between agonists and guanine nucleotides influencing such conformational changes have been described for, e.g., the serotonin $5HT_{1A}$ and the adenosine A_1 receptor [Mahle et al., 1992]. Recently, random saturation mutagenesis was used to demonstrate similar effects in the muscarinic m1 and m2 receptors [Page et al., 1995]. If the number of intermediary states in rhodopsin [Zvyaga et al., 1993; Fahmy et al., 1995] is any measure for the number of possible states in other G protein-coupled receptors, the interpretation of mutagenesis results will become even more complex.

Aligning and Interpreting G Protein-Coupled Receptor Sequences

Structure prediction

It is now generally accepted that GPCRs consist of a single polypeptide featuring seven α-helical transmembrane domains (TMs), an extracellular N-terminus, and an intracellular C-terminus (Fig. 1). GPCRs are therefore sometimes referred to as "7TM receptors." The prediction of GPCR structure from its nucleotide or amino acid sequence is, however, less than straightforward [von Heijne and Manoil, 1990]. Most sequence analysis software packages, such as GCG (Sequence Analysis Software Package of the Genetics Computer Group; University of Wisconsin, Madison, WI; in our laboratory version 7.3.1-UNIX of this program was run on an SGI Challenge XL; Silicon Graphics Inc., Mountain View, CA, MIPS R4400 CPU), are tuned towards structure prediction for (soluble) globular proteins. Because GPCRs are not only membrane bound proteins, but also have to traverse the lipid membrane, their structure and properties are inherently different from the datasets used for structure predictions. The α-helical segments of the GPCRs that actually span the lipid bilayer, designated transmembrane domains (TMs) or transmembrane helical domain (TMHs), are more lipophilic than the solvent (water) exposed loops connecting them. Residues with a higher hydrophobic (or lipophilic) index tend to form β-strands/sheets in globular proteins, and GCG will invariably assign a β-strand structure to these TMs, whereas they are presumed to be α-helical in GPCRs [Schertler et al., 1993; Baldwin, 1994; Ballesteros and Weinstein, 1995]. A better indicator for locating TMs, although it does not assign any structure, is the Kyte-Doolittle (KD) hydrophobicity parameter [Kyte and Doolittle, 1982]. A caveat in such predictions is that GPCRs are thought to be composed of multiple TMs within a single contiguous sequence that are grouped together in a seven-membered helical bundle. Transmembrane domains in GPCRs therefore, often display an amphipatic character (lipid exposed on one surface, and protein/solvent exposed on the other

surface), which greatly reduces the feasibility of predicting TMs by analysis of the Kyte-Doolittle hydrophobicity indices alone. This is especially pertinent when the TMs are part of the ligand binding domain and contain one or more charged residues [Oliveira et al., 1993]. In the case of the biogenic amine receptors, the conserved aspartate residue in TM3 (D3.32; sec below) is sufficient to cause a "dip" in the KD profile. The KD profile of the P_{2U} purinergic receptor, expressing multiple basic residues in the ligand binding domain, is greatly offset by the presence of these residues and makes accurate assignment of TMs virtually impossible [Erb et al., 1995]. More sophisticated methods using, e.g., Emini surface probability analysis [Emini et al., 1985], or amino acid preferences for specific locations near the ends of α-helices [Richardson and Richardson, 1988; White and Jacobs, 1990; Zhang and Weinstein, 1994; van Rhee et al., 1995], have been used to better define the TMs. Several such methods, including the use of α-helical periodicity, were reviewed recently by Ballesteros and Weinstein [1995].

Automated methods

Various algorithms, with a varying degree of accuracy, have been implemented in automated (computer) analysis and prediction methods. Automated methods for sequence retrieval and pattern searching are available from many sources on the internet [e.g., URL: http://www.nih.gov/ or http://www.ncbi.nlm.nih.gov/], as are possibilities to obtain secondary structure predictions [URL: http://www.embl-heidelberg.de/], or even complete models of receptors [URL:http://expasy.hcuge.ch/].

Alignment strategies

To compare whole GPCR sequences (retrieved from either the SwissProtein or Genbank databases maintained at the National Center for Biotechnology Information; NCBI, Bethesda, Maryland), or the effects of residue substitutions between two or more GPCRs, with each other, it is useful to construct a sequence alignment. Sequence analysis packages like GCG will generate such an alignment based on residue identity (conserved residues: the arginine near the C-terminus of TM3 is "always" conserved as an arginine) or homology parameters (e.g., "L may substitute for M, but not for K."). To optimize the alignment, gaps are introduced in the sequence to maximize the number of identical residue pairs, and pairs with the highest degree of homology. Since these gaps in the sequence would ultimately translate into "gaps" in the structure, which is not tolerable in the structurally explicit α-helical segments, the introduction of gaps in the sequence may be penalized by 2 parameters. The first one used by GCG is the "GapWeight" ("where can a gap be introduced?"), and the second one is the "GapLength Weight" ("how many gaps, if any, can be introduced sequentially?"). Setting GapWeight to 5, and GapLengthWeight to 0.2, is usually sufficient to align closely related sequences to a satisfactory level. For more divergent sequences, however, the sequence alignment needs to be optimized by other means [Probst et al., 1992; Cserzö et al., 1994]. For the family of rhodopsin-related GPCRs (family A), the use of "conservation patterns" in optimization of alignments has been proven useful [Oliveira et al., 1993; Ballesteros and Weinstein, 1995]. The alignment of the TMs of seven divergent GPCRs is presented in Table 2, along with the "conservation patterns" used in its construction. (A more comprehensive alignment will be made available electronically through URL: http://mgddk1.niddk.nih.gov:8000/GPCR.html).

Sequence identifiers

The use of sequence identifiers, a term coined by Ballesteros and Weinstein [1995], in sequence alignments could aid in the understanding of similarities in, and differences between, GPCR sequences. Although Hibert et al. [1991] and Oliveira et al. [1993] do not explicitly claim a need for an integrative method of numbering corresponding residues in different receptor sequences, they apparently feel compelled to offer such a scheme.

Baldwin [1993], on the other hand, clearly states that "an integrated numbering scheme for positions in the structure is necessary." Each of the aforementioned authors offered their own solution to this problem. Hibert et al. [1991] use a system in which the start of the helix is 1 plus 100 times the assigned number of the helix. Each residue is numbered sequentially from thereon. Example: P215 in human rhodopsin becomes P511. This scheme is very simple to apply, eminently suitable for building models of receptors, and facilitating comparisons between receptor models within the same subset. Oliveira et al. [1993] recognize that helical TMs can be characterized by a conservation pattern, and propose that: "In each helix the number of the most conserved residue is a multiple of 10 while the start of the helix is at the same time as close as possible to 100 times the number of the helix." Example: P215 in human rhodopsin becomes P520. This system has as an advantage over the method used by Hibert et al. [1991], because, not only are helices easily identified, but the alignment of sequences with a lower homology is also taken into consideration. Baldwin's approach differs significantly from these two, and encompasses a system where: Each helix is numbered in roman numerals with the number assigned to it. Each residue is then numbered sequentially from the (estimated) onset of the helix. "The numbering scheme allows for each helix to include 26 residues." [Baldwin, 1993] Example: P215 in human rhodopsin becomes V:14P. This method greatly resembles the scheme proposed by Oliveira et al. [1993], but is not very suitable for the purpose of building molecular models, since these have to adhere to a format consistent with the Arabic numbering used in all Protein Data Bank (PDB) files, or program-dependent internal coordinate files derived thereof. The one drawback to all three proposals is that the numbering is dependent on the assignment of the apparent/estimated start of the TMs. The aforementioned authors disagree on the assignment of this particular point, considering the range of identifiers assigned to P215 in human rhodopsin. The start of TM5 is thought to originate either 11 [Hibert et al., 1991], 13 [Oliveira et al., 1993], or 14 [Baldwin, 1993] residues upstream, i.e., towards the N-terminal side of the TM, of this conserved proline residue. Ballesteros and Weinstein [1995] use a novel approach with a dual numbering scheme: "Every amino acid identifier starts with the transmembrane helix number, e.g., 4 for TMH4, and is followed by the position relative to a reference residue among the most conserved amino acid in that TMH. That reference residue is arbitrarily assigned the number 50." Example: P215 in human rhodopsin becomes $P_{5.50(215)}$. In effect, the numbering scheme becomes independent of the perceived (absolute) start of the TM, and is therefore more generally applicable. This is advantageous, not only for GPCR molecular modellers, but perhaps even more so for molecular biologists, who have no mathematical tools to establish such criteria. Yet another proposition was put forward, where the residue numbering of bacteriorhodopsin is used throughout a model [Teeter et al., 1994]. Given the low similarity between GPCR sequences and the bacteriorhodopsin sequence (15% homology at best, with virtually no identity), and the existing disagreement on the alignment of GPCRs and bacteriorhodopsin, the method is not very well suited for comparing larger numbers of GPCR sequences. In this paper we use a slightly modified version of the Ballesteros and Weinstein [1995] proposal that is in accordance with the numbering used in protein sequence databases and constitutes common practice in molecular biology. Although we adhere to the reasoning of Ballesteros and Weinstein [1995], we propose that the residue maintains its original sequence residue number, and is supplemented with a new extension containing the residue identifier. Example: P215 in human rhodopsin becomes P215(5.50). This will circumvent the foreseeable problems arising from drastically renumbering all residues (P511 [Hibert et al., 1991], P520 [Oliveira et al., 1993], V:14P [Baldwin, 1993], and $P_{5.50}$ [Ballesteros and Weinstein, 1995] all point to the proline residue at sequence position 215 of human rhodopsin). Moreover, it keeps the numbering schemes backward- and forward-compatible, and adds functionality to the notation rather than obscuring it. An example of this "extended notation," used throughout this paper, is presented in Table 2. To aid in the interconversion

of residue designators, we have added notations for the most conserved residues in each helix as footnotes in the table.

Genetic Heterogeneity

Receptor subtypes

Considerable heterogeneity exists in the expression levels of GPCRs in various tissues [Peralta et al., 1987]. The adenosine receptors were initially divided in A_1 and A_2 receptors based on their pharmacological profile, but only later was it confirmed that the A_2 receptors could be subdivided into A_{2a} and A_{2b} receptor subtypes based on their respective sequences [Rollins et al., 1994].

Allelic variations

Another form of genetic heterogeneity is allelic variation. This form of heterogeneity is largely neglected in depositing and retrieving amino acid or nucleotide sequences in databases. Allelic variation is responsible for phenotypic differences within a single species, and for the species differences themselves. Natural variability by allelic variation accounts for, e.g., the pigmentation phenotypes regulated by the MSH receptor within one species [Robbins et al., 1993]. Species differences resulting from (minor) genetic variability have been documented for the human and hamster β_2 adrenoceptors [Caron et al., 1988], the rat and human serotonin $5HT_{1B}$ receptors [Parker et al., 1993], the human and rat neurokinin NK1 receptors [Jensen et al., 1994], adenosine A_3 receptors [Linden, 1994], mammalian adenosine A_1 receptors [Meng et al., 1994; Tucker et al., 1994], and primate dopamine D_4 receptors [Livak et al., 1995]. Moreover, allelic variation can result in an abundance of pathological conditions [Raymond, 1994]. Mutations in the vasopressin V2 receptor resulting in decreased sensitivity to vasopressin, with nephrogenic diabetes insipidus as the somatic characteristic, have been well documented [Pan et al., 1992; Bichet et al., 1993; Rosenthal et al., 1993; Tsukaguchi et al., 1993; Birnbaumer et al., 1994; Faa et al., 1994; Knoers et al., 1994; Rosenthal et al., 1994; Wildin et al., 1994]. Constitutive activation of rhodopsin in some allelic mutants leads to a decrease in the signal-to-noise ratio from light-sensitive rods in the retina. Under conditions of low light intensity this results in a condition known as retinitis pigmentosa, hence “night-blindness” [Robinson et al., 1992; Zvyaga et al., 1993; Kaushal and Khorana, 1994; Li et al., 1995; Macke et al., 1995; Millán et al., 1995; Richards et al., 1995]. Cases of naturally occurring mutations leading to constitutively active GPCRs have been described for the TSH receptor in hyperthyroidism [Parma et al., 1993; Paschke et al., 1994a; Ohno et al., 1995], and the LH/CG receptor in male precocious puberty [Shenker et al., 1993; Yano et al., 1995]. Nocturnal asthma [Turki et al., 1995], dominantly inherited bleeding disorder [Hirata et al., 1994], and an induced eating disorder in mice [Tecott et al., 1995] can be traced to hyporesponsiveness of the β-adrenoceptor, the TXA_2, and the serotonin $5HT_{2C}$ receptor, respectively.

Alternative splicing

The third form of genetic heterogeneity is alternative splicing, although strictly speaking the heterogeneity is not expressed at the genomic, but at the protein level. Alternative splicing has been demonstrated for dopamine D_2 receptors [Dal’ Toso et al., 1989; Monsma et al., 1989; Guiramand et al., 1995], where the D_{2L} splice variant contains a 29 amino acid insert in the third intracellular loop compared to the D_{2S} splice variant, resulting in differential coupling to adenylyl cyclase. Divergence caused by alternative splicing, was also demonstrated for the C-terminus of the human endothelial TXA_2 receptor [Raychowdhury et al., 1994]. In the rat, alternative splicing of the vasopressin V2 receptor accounted for the existence of two protein products: the active V_{2L} receptor, or complete sequence, comprising 85% of the receptor population, and the inactive V_{2S} receptor, consisting of TMs

1 through 6 only, comprising 15% of the receptor population [Firsov et al., 1994]. Exon-shuffling in the human adenosine A_1 receptor, without resulting protein heterogeneity, was demonstrated to regulate the level of expression of these receptors [Ren and Stiles, 1994; Deckert et al., 1995].

Structural Segmentation

GPCRs can be divided into 15 discreet structural segments (Fig. 1), although the assignment of specific residues at the interface between two such segments to either segment is highly contentious. Also, due to low sequence homology in the non-helical segments, even at low stringency, it becomes very difficult to assign residue identifiers to particular residues outside the more conserved regions.

The structural information on the intracellular and extracellular regions of GPCRs is very limited. A high flexibility in these regions is implied by electron diffraction studies of the GPCR rhodopsin [Schertler et al., 1993], and the proton pump protein bacteriorhodopsin [Henderson et al., 1990]. The motility in these regions is too high to contribute sufficiently to the electron density of the protein to allow structure elucidation.

As indicated in Figure 1, several structural features have been posited for GPCRs, such as a disulfide bond between cysteines located near the N-termini of TM3 and TM5, respectively, a β-pleated sheet in I2, an α-helical fragment in I3, glycosylation sites in NT and E2, and an acylation site in the C-terminal tail [van Galen et al., 1992], that will not be discussed within the limits of this review.

N-terminus (NT)

As was to be expected, the involvement of the NT in agonist binding has been demonstrated for GPCRs of the peptide/cytokine class [La Rosa et al., 1992; Baumgartner et al., 1994; Hjorth et al., 1994; Paschke et al., 1994b; Chini et al., 1995].

First intracellular loop (I1)

Very few data have been published on the significance of the first intracellular loop in either G protein-coupling or receptor activation, but this segment was indicated to be, at least, involved in the coupling of the human TXA_2 receptor to its effector [Hirata et al., 1994].

First extracellular loop (E1)

Not surprisingly, the first extracellular loop, like the N-terminus, was shown to be important in agonist binding for GPCRs of the cytokine/peptide class [Haraguchi et al., 1994b; Hjorth et al., 1994; Chini et al., 1995].

Second intracellular loop (I2)

The second intracellular loop has been implicated in the coupling of rat m3 acetylcholine receptors to phospholipase C [Blüml et al., 1994a]. If some degree of structural homology among all GPCRs, whether they are members of the rhodopsin-like subfamily or not, may be inferred, recent experiments with metabotropic glutamate receptors regarding G protein-coupling suggest that the second intracellular loop is a major determinant in deciding G protein-coupling specificity [Pin et al., 1995].

Second extracellular loop (E2)

Even though in the cartoon representation of Figure 1 there is considerable distance between the N-terminus and the second extracellular loop, in a 3D representation (model) of GPCRs these segments are in proximity. It is therefore only logical that this segment is also involved

in binding of agonists to GPCRs of the peptide/cytokine class [Hjorth et al., 1994; Kosugi and Mori, 1994]. However, there is mounting evidence that this particular segment is also involved in the binding of agonists to small ligand GPCRs, such as adenosine receptors [Olah et al., 1994b].

Third intracellular loop (I3)

There is ample evidence for the involvement of the third intracellular loop in the coupling of GPCRs to G proteins. Variations in the sequence can result in several different functional effects. The best documented effect is probably the effect of mutations on coupling selectivity [Strader et al., 1987b; Kosagi et al., 1992; Blount and Krause, 1993; McAllister et al., 1993; Samama et al., 1993; Shapiro et al., 1993; Tsukaguchi et al., 1993], but coupling efficiency is also affected by these variations [Monsma et al., 1989; Guiramand et al., 1995]. Modulation of coupling specificity and efficiency is ultimately reflected in the levels of homologous [Moro et al., 1993; Samana et al., 1993], and heterologous regulation observed [Lee and Fraser, 1993]. Direct physical evidence for the interaction of the third intracellular loop and a G protein (homologue) was provided by cross-linking experiments between bovine rhodopsin and the α-subunit of transducin through the use of cysteine mutants [Resek et al., 1994; Ridge et al., 1995].

Third extracellular loop (E3)

The third extracellular loop is relatively short when compared to the second extracellular loop. It varies in length between at most 20 (e.g., prostaglandin EP_3 receptor), and possibly as few as 5 amino acid residues (e.g., adenosine A_1 receptor). The peptide backbone is subject to space/size constraints: individual helices may not be too close to each other to avoid van der Waals repulsion, but must be close enough to satisfy the “contiguous chain” criterion. It is therefore likely that this loop serves a general structural function, rather than direct involvement in ligand binding. The effects observed for mutations in rat and human AT_1 receptors [Hjorth et al., 1994] could reflect either possibility, and more data are needed before any definitive conclusions can be drawn.

C-terminus (CT)

As is the case for the third intracellular loop, there is ample evidence for the involvement of the C-terminus in various aspects of GPCR-to-G protein coupling [Blount and Krause, 1993; Goujon et al., 1994; Haraguchi et al., 1994a; Sasakawa et al., 1994]. In addition, there is evidence that specific residues are palmitoylated [Karnik et al., 1993], a feature involved in anchoring protein sequences to the lipid membrane bilayer, or phosphorylated [Lattion et al., 1994; Wang et al., 1995], which is thought to be important in “post-activation processing.”

Transmembrane domains

This area comprises the most extensive and rigorous part of mutational analyses performed to date. Because of the rigidity (both in requirements, and in physical coordinates) imposed by the organization of the TMs in a helical bundle, point mutations in the TMs may affect changes in affinity of agonists, antagonists, or activity exclusively, or affect more than one parameter concurrently. To facilitate comparison of residue positions between multiple GPCR sequences, we constructed alignments of over 150 sequences, and annotated it with the “extended notation.” Table 2 represents a selection of 7 sequences out of the complete alignment. A comprehensive, although most likely incomplete, list was compiled of point mutations reported in the literature (Table 3). (It is the intent of the authors to make this list electronically available at the URL above, and to update it at regular intervals after publication of this paper. The cooperation of all authors, present and future, in maintaining the database would be greatly appreciated). This list will be discussed below, in detail.

Figure 3 is an inventory of the number of mutants reported in the literature before August 1995, and their effect(s), sorted by sequence identifier. This effectively yielded a geographical map of already investigated, and yet to discover areas in the architecture of GPCRs. The map could then be used to draw inferences for sequences where mutations at a specific identifier have not yet been performed [e.g., van Rhee et al., 1995], or to pinpoint areas of interest for new mutagenesis studies [e.g., Kim et al., 1995].

Chimeric Receptors

Before focusing entirely on single-point mutations, it should be pointed out that the use of chimeric receptors has contributed considerably to the understanding of receptor structure and function.

Constructs for “homo-chimeric” receptors (mixed constructs of receptors belonging to the same subfamily) have been reported for adrenergic [Kobilka et al., 1988; Liggett et al., 1993; Samama et al., 1993], muscarinic [Kubo et al., 1988; Wess et al., 1992a; Ellis et al., 1993; Pittel and Wess, 1994; Liu et al., 1995], dopaminergic [MacKenzie et al., 1993; McAllister et al., 1993; Fishburn et al., 1994; Kozell et al., 1994; Robinson et al., 1994], serotoninergic [Choudhary et al., 1992], neurokinin [Blount and Krause, 1993; Gether et al., 1993a, b; Vigna et al., 1994], adenosine [Olah et al., 1994a, b], and cholecystokinin receptors [Mantamadiotis and Baldwin, 1994]. One such chimeric construct offers the first evidence in favour of the clockwise arrangement of TMs in the helical bundle (when viewed from the intracellular surface), as opposed to a possible anti-clockwise orientation [Liu et al., 1995]. A clockwise arrangement had been deducted earlier, based on physicochemical parameters, but experimental evidence had been lacking so far [Baldwin, 1993; Baldwin, 1994]. Liu et al. [1995] constructed hybrids containing m5 sequence in TM1, and m2 sequence in TM7. This construct is incompatible with ligand binding and receptor function. However, single point mutations into either TM1 (m5:T37(1.39) → m2:A30(1.39)) or TM7 (m2:T423(7.36) → m5:H478(7.36)) were able to rescue this hybrid. It was concluded that the sites on TM1 (1.39) and TM7 (7.36) are located at the interhelical interface, and therefore the helical bundle must be organized in a clockwise fashion (as viewed from the intracellular membrane surface).

“Hetero-chimeric” constructs have been proven useful in determining ligand specificity for a TRH/β_2 chimeric receptor [Kosugi and Mori, 1994], and in investigating G protein selectivity for a chimeric receptor containing a β_1 sequence insertion in either an m1 or an m2 receptor [Wong and Ross, 1994].

Insertion/Deletion Mutagenesis

When aligning amino acid sequences, particular care has to be taken to avoid random introduction of gaps. Gaps in one sequence signify the absence of structure in that sequence, when compared to another sequence not containing that particular gap. By the same reasoning, insertion and deletion mutagenesis can be applied to intentionally introduce structural aberration in GPCRs. Consequently, because the structure of the receptor is targeted, effects observed resulting from this approach are not easily attributed to specific interactions. Genetically engineered [Blount and Krause, 1993; Lee and Fraser, 1993; Maggio et al., 1993; Shapiro et al., 1993; Blüml et al., 1994a, c; Lattion et al., 1994; Sasakawa et al., 1994; van Koppen et al., 1994], or naturally occurring [Monden et al., 1992; Haraguchi et al., 1994a; Wang et al., 1995] insertion/deletion mutagenesis products, most notably affect the activity of these receptors.

Site-Directed Mutagenesis (Point Mutations)

Recently, we published a systematic investigation of single amino acid residue replacements within the TMs of the human adenosine A_{2a} receptor, and correlated the findings with ligand SAR data accumulated over the years [Kim et al., 1995; Jiang et al., 1995]. Many similar studies for other receptor subtypes (predominantly those of the biogenic amine subfamily) exist [e.g., Strader et al., 1994]. Another approach is systematic probing of the receptor surface by measuring the substituted-cysteine accessibility [Javitch et al., 1995]. The ultimate probe, would be the use of a ligand existing of a single atom. The feasibility of this approach was demonstrated by converting a NK1 receptor into a receptor for zinc ions [Elling et al., 1995].

Before venturing into the analysis of the mutations described in Table 3, one should consider which amino acids are replaced, and even more so with what other residue it is replaced. This leads, inevitably, to a different effect for homologous amino acid substitutions, than for techniques using substitutions with non-homologous residues as used in, e.g., the “alanine-scanning” procedure. The “alanine-scanning” procedure targets specific residues in the sequence, and replaces them with an alanine regardless of the nature of the wild type residue. This contrasts with the “fine-tuning” approach of replacing, e.g., an aspartate residue with either an asparagine or a glutamate, which has been applied extensively in characterizing the conserved aspartate in TM2 (Table 3: identifier 2.50). In Table 3 (and Fig. 3), correlating mutation position with mutation effect, no distinction between those two approaches was (or can be) made. Furthermore, no distinction is made between receptor (sub)types or ligands in the summation of Figure 3. Reports marked “ineffective” or “no effect” should therefore not necessarily be considered contradictory to other reports.

Moreover, the incidence (the frequency at which a particular amino acid residue appears in the sequence) of amino acids varies, not only between the 20 types of naturally occurring amino acids, but also between the segments of GPCRs. To illustrate this principle we calculated the incidence of each amino acid in either the whole sequence or only the TMs for 40 GPCRs chosen randomly from the alignment of over 100 GPCRs. To illustrate this principle we calculated the incidence of each amino acid in either the whole sequence or only the TMs for 40 GPCRs chosen randomly from the alignment of over 150 GCPRs (Fig. 2). The incidence of an amino acid in the TMs was deemed significantly different from the incidence of the same amino acid in the complete sequence (= reference value) if its value deviated by more than 10% from the reference value. If all amino acids were distributed randomly, the incidence for each amino acid would be 5%. The most abundant amino acid (more than twice the expected value if randomly distributed), in either the whole sequence or the TMs was L, with an incidence of 11.4%, and 14.6%, respectively. The lowest incidence in TMs was recorded for Q (1.0%), H (1.1%), and E (1.3%), whereas the lowest incidence in the whole sequence was observed for H (1.8%), and W (1.8%). The presence of residues that ordinarily occur with a low incidence in a particular sequence cannot be regarded as a random occurrence and begs for an explanation.

Perhaps more significant than the incidence of particular residues in the whole sequence is the incidence of the residues in the TMs. More abundant in TMs than in the whole sequence are A (8.7 vs. 7.6%), F (7.9 vs. 5.4%), I (10.3 vs. 6.6%), L (14.6 vs. 11.4%), M (3.5 vs. 2.6%), V (10.8 vs. 7.3%), W (2.3 vs. 1.8%), and Y (4.9 vs. 3.7%). This is to be expected considering the increase in the (KD) hydrophobicity profile, necessary to span the length of the lipid bilayer. Less abundant in TMs than in the whole sequence are: D (1.9 vs. 3.2%), E (1.3 vs. 3.8%), G (3.8 vs. 5.6%), H (1.1 vs. 1.8%), K (2.4 vs. 4.4%), P (3.5 vs. 5.3%), Q (1.0 vs. 2.9%), R (3.1 vs. 5.1%), and S (6.2 vs. 7.8%). Although for some residues (D, E, K, Q, R, and S) this decreased incidence could equally well be attributed to the requirements of the

(KD) hydrophobicity profile, the profound structural implications of G and P residues interfere with the conditions of α-helical periodicity of the TMs [von Heijne, 1991; Sankararamakrishnan and Vishveshwara, 1992]. The low incidence of H in GPCR sequences as a whole, and its even lower incidence in the TMs therefore, signify an important role for it in the function of these receptors. Indeed, the two histidines present in the human adenosine A_{2a} receptor have been shown to be crucial for its function [Kim et al., 1995]. About equally distributed between TMs and whole sequences are: C (3.0 vs. 3.2%), N (4.0 vs. 4.3%), and T (5.8 vs. 6.1%). Apart from the apparent lack of correlation between position and special functionality represented by the cysteine, i.e., the ability to form disulfide bonds with adjacent cysteines [Ridge et al., 1995], no conclusions can be drawn from this particular observation.

Transmembrane domain 1 (TM1)

Fewer data are available for this TM. In accordance with its presumed structural role, rather than involvement in ligand binding [Baldwin, 1993, 1994], there is one report indicating the presence of position 1.39 at the interhelical contact surface with TM7 [Liu et al., 1995]. Other reports indicate that, mainly, the activity of the system is affected, further supporting a structural role [Min et al., 1993; Knoers et al., 1994; Wu et al., 1994].

An incidence of 21% for P at position 1.36 indicates that this position frequently functions as a helix initiator, and this coincides with an increased incidence for residues more prevalent in TMs following this position. The helical surface containing both positions 1.36, and 1.39, continues with helical periodicity along positions 1.43, 1.46, 1.50, 1.51, 1.55, and 1.58. This includes the most conserved residue (N at 1.50) in this TM. It also suggests that the S found at 1.43 in m1, m2, m4, and m5 receptors could play an important role in receptor structure. The most abundant residue at position 1.46 is G (38%), allowing for flexibility in the helix, rather than breaking the tertiary structure, whereas T (26%) and S (16%) are very likely to be involved in hydrogen bonding networks maintaining intrahelical or interhelical structure. G (10%) and S (15%) also occur at position 1.51. Position 1.55 shows an increase in W (11%) and Y (9%) residues, well above the average incidence rates of 2.3% and 4.9%, respectively. The first basic residues occur at position 1.58, indicating the protrusion of the TM into the negatively charged surface of the intracellular membrane environment, and ensuing helical periodicity seems absent until the initiation of TM2.

Transmembrane domain 2 (TM2)

It comes as no surprise that the most heavily targeted residue in this TM is the conserved aspartate at position 2.50 (Table 3). Most abundant are the reports on agonist affinity and activity of its mutants. Even the most conservative substitution, i.e., D → N or D → E, profoundly affect these parameters. It was postulated by Horstman et al [1990], that this residue in the α_2-adrenoceptor acted as an accessory site modulated by sodium ions. This hypothesis was corroborated by similar experiments on the D_2 dopamine [Neve et al., 1991], and the LH/CC: receptor [Ji and Ji, 1991; Quintana et al., 1993]. Although most reports indicate a loss of affinity for agonists, at least 2 reports to the contrary, i.e., an increase in agonist affinity, exist [Suprenant et al., 1992; Fraser et al., 1989b]. Antagonist binding to this residue has been implicated in only three cases: D(2.50)N [Wang et al., 1993a; Bihoreau et al., 1993] and D(2.50)A [Perlman et al., 1992], whereas 13 studies that explicitly investigated antagonist binding to mutant receptors found no difference between wild type and mutant behaviour. The majority of the reports suggest that the activity of the mutant receptor-ligand complexes is attenuated. It is therefore more likely that this particular position is involved in signal transduction, in casu signal propagation, than directly involved in agonist binding.

The periodicity implied by Figure 3B suggests that more residues on TM2, especially those towards the extracellular surface that are facing the interior of the helical bundle, are involved in signal transduction if not in ligand binding. Most receptor models indicate that TM2 is too far removed from other residues deemed essential, to be involved in direct ligand contact [Hibert et al., 1991; Hoflack et al., 1994; Trumpp-Kallmeyer et al., 1992; Zhou et al., 1994; Ballesteros and Weinstein, 1995]. This is substantiated by the effects observed at positions 2.53, 2.57, 2.60, and 2.64, provided the GPCR under investigation contains a "non-standard" amino acid at that position. The cumulative incidence of A, V, I, L, and M at position 2.53 is 69% vs. a total cumulative incidence of 47.7% over all positions in all TMs. "Non-standard" residues at this position occur in the GRH, green and red opsin (E), SST5, P_{2Y}, P_{2U}, and ET-A (Y), and ET-B (H) receptors. For position 2.57 the cumulative incidence is 71%, with "nonstandard residues" present in, e.g., the ET-A, and ET-B (D), all muscarinic (S), and all neurokinin (N) receptors. On the other hand, the cumulative incidence for positions 2.60, and 2.64 is 42%, and 33%, respectively, whereas the incidence for the relatively rare W (2.3% on all TMs) increases to 18% at position 2.60, and the equally rare Y (4.9% on all TMs) increases to 26%. More significant, probably, is the occurrence of P residues at positions 2.58 (28% of GPCRs), and 2.59 (43% of GPCRs) because of the structural implications of this particular residue. This P is not only not absolutely conserved (it is lacking in, e.g., all muscarinic receptors), but also not "position conserved." Whereas in some receptors the helical angle between the conserved aspartate and the proline is only 80° (2.58), in other receptors it is 180° (2.59).

Transmembrane domain 3 (TM3)

The most heavily targeted residue in TM3 is the D that is conserved among all biogenic amine GPCRs at position 3.32 (Table 3). Aspartate (D) residues also occur at the equivalent position in opioid and somatostatin receptors, and the octopamine receptor, whereas a lysine (K) may be found in the GRH and IL-8 receptors, a glutamine (Q) in the TRH, vasopressin, endothelin, and neuromedin-B receptors, and a proline (P) in the neurokinin receptors. Site-directed mutagenesis of the conserved D in biogenic nitrogen receptors, usually led to decreased affinity of agonists and/or antagonists, although decreased activity also has been reported (Table 3). This loss of affinity is probably attributable to the binding requirements imposed by the quaternary amine present in all biogenic amine agonists, and in most antagonists. Replacing the P in the NK1 receptor with an A, apparently was without effect [Fong et al., 1994b]. Targeted mutagenesis of the Q in the TRH receptor led to a mainly size-dependent decrease in agonist affinity, but activity was not affected [Perlman et al., 1994b]. Even if this particular position is occupied by residues not generally involved in specific interactions, such as A in rhodopsin [Ridge et al., 1992], and V in the A_{2a} receptor [unpublished data], substitution lead to significant changes in receptor functionality.

Following the rules of strict helical periodicity, the next position up on the same helical face (i.e., towards the N-terminal end of the TM) as 3.32, is position 3.28 (or 3.29). The involvement of 3.28 in agonist binding (NK2) [Bhogal et al., 1994], antagonist binding (D_2) [Javitch et al., 1995], or coordination of the Schiff-base in rhodopsin has been demonstrated [Lin et al., 1992], whereas a (3.29)C mutant showed reactivity towards a thiol reagent (meaning it is solvent accessible), but no effect on ligand binding was observed [Javitch et al., 1995].

If the low-resolution structure of rhodopsin [Schertler et al., 1993] represents a general template for all (rhodopsin-related) GPCRs, then the fraction of the solvent- and protein-exposed surface of TM3 exceeds 180° due to the central position of this TM in the helical bundle. In this respect, this structure differs from the high resolution structure determined for bacteriorhodopsin [Henderson et al., 1990]. Support for a more central position of TM3 than suggested by the bacteriorhodopsin model, is provided by mutants at position 3.32 (see

above), 3.33, 3.34, 3.35, and 3.36. Charged residues occur at position 3.33 in the endothelin receptors (K), IL-8 receptors (E), and formyl peptide receptors (D). Cysteine-replacement mutagenesis of 3.33 revealed involvement in agonist binding, and reactivity towards a thiol reagent, indicating that at least a 100° fraction of the helix is exposed [Javitch et al., 1995]. A similar mutation at position 3.34 was not efficacious [Mansour et al., 1992]. A (3.34)C mutant showed no reactivity towards a thiol reagent [Javitch et al., 1995], and mutations at 3.35 showed a similar profile [Dixon et al., 1987; Mansour et al., 1992; Javitch et al., 1995; Perlman et al., 1995b]. Residues at 3.36 were involved in ligand binding in several GPCR subfamilies, including among others, the neurokinin receptors [Fong et al., 1992b; Bhogal et al., 1994; Jensen et al., 1994], and were solvent accessible. Mutations at 3.37 also affected ligand binding, but was apparently not solvent accessible, and this site is therefore, likely located at a helix-helix interface [Javitch et al., 1995; Jiang et al., 1995]. Remarkable at position 3.37, is the presence of a glutamate (E) residue in rhodopsin [Lin et al., 1992; Ridge et al., 1992; Zvyaga et al., 1993] and the LH/CG receptor only, whereas the most prevalent residue is a threonine (T; 30% of GPCRs).

The first residue following this series, adhering to the helical periodicity requirements, is 3.39. This residue is solvent accessible [Javitch et al., 1995], and apparently influences the post-translational processing of the receptor protein [Dixon et al., 1987; Strader et al., 1989a]. In 85% of GPCRs this position is occupied by a either a serine (S) or a threonine (T), in 10% by a glycine (G), and in 4% by a proline (P) residue. Most subsequent residues seem to affect predominantly the activity of the ligand-receptor-effector system.

The conserved ‘DRY’ pattern near the C-terminus of the helix, seems mainly involved in receptor activity or G protein coupling (Table 3, Fig. 3). The D occurs in 83% of GPCRs, and is substituted with the highly homologous E in another 14% of GPCRs, totaling an acidic residue in 97% of GPCRs. In contrast, a H is found in the P_{2Y} and P_{2U} receptors, and an N in the PAF receptor. It is unclear whether this reflects a differential G protein-coupling mechanism/efficiency, and the inconsistencies reported for mutants at this position leave this issue unresolved (Table 3, Fig. 3). The R at position 3.50, on the other hand, is fully conserved, and mutants reported in certain pathological conditions invariably lead to a decrease in activity [Bichet et al., 1993; Rosenthal et al., 1993; Zvyaga et al., 1993]. Although a vast majority of GPCRs contain a Y at position 3.51 (84%), some degree of variability is allowed considering the occurrence of F (5%), C (4%), W (3%), H (2%), A (l %), and S (1%) at this position.

Transmembrane domain 4 (TM4)

The incidence of basic residues near the N-terminus (up till and including 4.43) indicates the proximity of phospholipids in the inner layer of the membrane [Richardson and Richardson, 1988]. The most conserved residue in this TM is W (96% of GPCRs) at 4.50, and only some prostaglandin receptors deviate from this rule. Mutation of W(4.50) to the somewhat similar F in the m3 receptor led to a decrease in both agonist and antagonist affinity, but the activity of the system was not affected [Wess et al., 1993]. Mutations at positions 4.53 [Strader et al., 1989a; Chan et al., 1992; Knoers et al., 1994; Lin et al., 1994] and 4.54 [Bhogal et al., 1994] are inconclusive, whereas mutations at positions 4.59 [Wess et al., 1993; Fong et al., 1994b] and 4.60 [Fong et al., 1994b] seem to affect both agonist and antagonist affinity, but not activity. Activity, or more accurately post-translational processing, is affected in a β_2 S(4.57)A mutant [Strader et al., 1989a], and considering the high incidence (57% S, and 27% A) of certain residues it is very likely involved in helix structure (intrahelical contacts) or packing of the helical bundle (interhelical contacts). Positions 4.59 and 4.60 have a high incidence of P residues (66% and 34%, respectively), indicating that this position is important for the formation of the ligand binding domain, although no obvious differentiation between GPCR subfamilies (either by ligand size, or ligand class) seems to

exist. Reports of substitution effects on residues closer to the C-terminus of TM4 exist (Table 3, Fig. 3), but seem to be restricted to GPCRs of the peptide/cytokine class, which is consistent with prior observations and considerations.

Transmembrane domain 5 (TM5)

This is the first TM in which a proline residue is both present throughout the vast majority (84%) of GPCRs investigated (notable exceptions are the LH/CG receptor, all lipid mediator, and all melanocortin receptors), and its position in the sequence is conserved (without introducing gaps in the helical region). This reflects a probable role of its position in overall GPCR structure, rather than individual ligand binding domains. Since only one mutant has been reported, P(5.50)A [Wess et al., 1993], which affected only agonist affinity, but not antagonist affinity or receptor activity, such a structural role can neither be confirmed nor rejected.

Position 5.35 is the most N-terminal residue in TM5 for which efficacious mutants have been reported [Bichet et al., 1993; Gether et al., 1994]. This is consistent with the initiation of a helical periodicity pattern proceeding along the face of the helix composed of residues located at positions 5.38/5.39, 5.42/5.43, 5.46/5.47, 5.50, 5.53/554, 5.58, and 5.62/5.63, Positions 5.38 and 5.41 have a relatively high incidence of aromatic residues (10% F and 30% Y, and 13% F and 17% Y, respectively), whereas position 5.42 marks an increase in hydrophilic residues (27% S, 15% T, 3% N, 3% K, 2% D, 2% H, 1% Q, and 1% E). Substitutions mostly affect agonist affinity, with few reported effects on affinity of antagonists (Table 3, 5.42). There are also indications that point mutations at this position (5.42) may impair post-translational processing of the receptor protein [Strader et al., 1989a; Pollock et al., 1992]. The same phenomena have been observed for position 5.43 (Table 3), although the amino acid distribution is considerably different with 23% F, 17% S, 14% T, 11% L, and 10% A. This may reflect a different ligand binding environment between positions 5.42 and 5.43, where position 5.42 defines a mainly hydrophilic interaction surface, and position 5.43 defines either a hydrophobic (44%) or a hydrophilic (33%) surface. Since none of the amino acids is conserved in more than 50% of GPCRs, substantial heterogeneity exists at this position and may therefore reflect an essential role in ligand recognition and ligand specificity.

In accordance with the helical periodicity, position 5.46 has been proven essential for most agonist binding [Strader et al., 1989a; Wang et al., 1991; Kao et al., 1992; Mansour et al., 1992; Pollock et al., 1992; Leurs et al., 1994; Ohta et al., 1994; Moguilevsky et al., 1995], and less frequently for antagonist binding [Gantz et al., 1992; Kao et al., 1992; Mansour et al., 1992; Leurs et al., 1994]. This position is predominantly occupied by small amino acid residues (21% G, 20% S, and 19% A), but larger residues occur in several GPCRs (e.g., H in rhodopsin, Y in endothelin, and W in adenosine A_1 and A_3 receptors). There is preliminary evidence that the point mutation H(5.46)C, F in rhodopsin results in a conformational change [Weitz and Nathans, 1992], but this experiment was not confirmed by others [Cohen et al., 1992]. However, the high incidence of G at this position (21%) is consistent with a structural role of this position in the formation of the ligand binding site.

Few mutants have been reported C-terminal to this position, but there is some evidence that positions as far down as 5.58 may affect agonist binding [Hunyady et al., 1995]. Furthermore, residues ranging from 5.56 (not on the same helical face as positions mentioned earlier; this is reflected in the incidence of residues V 24%, L 17%, F 16%, and I 15%) to 5.62 (23% Y, 15% F, and 14% I) influence receptor activity [Laue et al., 1995].

Although not strictly belonging to TM5, residue C240 in rhodopsin, which could be designated C(5.75) (not counting gaps), was shown to be involved in coupling to transducin by direct cross-linking experiments [Resek et al., 1994].

Transmembrane domain 6 (TM6)

The hydrophilic helical periodicity seems to evolve from position 6.52, such that the absolutely conserved P(6.50) is located at the opposite surface of 6.52, facing the lipid membrane environment. The hydrophilic surface propagates from 6.52 towards the C-terminus into the extracellular environment, but is hardly detectable in the N-terminal sequence near the intracellular surface. The water-lipid interface at the N-terminus is marked by an increase in basic residues at position 6.35 (30% K, and 24% R). The low incidence of residues located between positions 6.35 and 6.47 that are capable of hydrogen bond formation, suggests that the N-terminal part of TM6 is not involved in direct ligand contact or ligand specificity. Mutations in this part of TM6, generally, lead to constitutively active receptors [Kjelsberg et al., 1992; Parma et al., 1993; Ren et al., 1993; Shenker et al., 1993; Kosugi et al., 1994; Paschke et al., 1994a; Laue et al., 1995; Yano et al., 1995], although impaired responses have been reported in several cases [Fraser et al., 1989a; Kosugi et al., 1992; Tsukaguchi et al., 1993; Faa et al., 1994; Laue et al., 1995]. The deletion mutation ΔV(6.43) reported in cases of diabetes insipidus effectively causes a rotational perturbation by about 100° within the helix. Whether this results in a changed orientation of only one face of the helix or completely destroys the helical packing is not yet clear. Although phenotyping for this condition has been performed [Tsukaguchi et al., 1993; Faa et al., 1994], no detailed data are available regarding ligand binding, and it is thought that the occurrence of diabetes insipidus results from decreased activity of the receptor, instead of constitutive activation. This further supports a more general role of the N-terminal part of TM6 in GPCR structure and activation, rather than strictly ligand binding.

Since residue 6.35 is presumably located at the water-lipid interface, it is very likely that residue 6.34, immediately preceding it, and other residues in its vicinity are involved in G protein coupling. Site-directed mutagenesis of residues at position 6.34 indicates that this supposition is viable, considering that mutations in this region invariably result in constitutive activity of the receptor-effector system [Kjelsberg et al., 1992; Kosugi et al., 1992; Parma et al., 1993; Ren et al., 1993; Paschke et al., 1994a]. In some cases, an increase in agonist affinity was also observed [Kjelsberg et al., 1992; Kosugi et al., 1992; Ren et al., 1993].

The residue at position 6.44 is most frequently F (91% of GPCRs), but this residue is replaced with a D in the LH/CG and the TSH receptors. This position must therefore be pointing into the central cavity of the helical bundle, or be involved in a specific interhelical contact. D(6.44)E, G, Y mutants were all constitutively active, but agonist affinity was apparently not affected [Shenker et al., 1993; Kosugi et al., 1994; Laue et al., 1995]. The homologous substitution D(6.44)N was well tolerated, and behaved like the wild type receptor [Ji and Ji, 1991]. The reciprocal substitutions F(6.44)Y (mammalian to fish) [Chan et al., 1992] and Y(6.44)F (fish to mammalian) [Yokoyama et al., 1995] in rhodopsin resulted in changed absorption spectra, which suggests the proximity of the retinal chromophore, and a position within the central cavity.

Cysteines have the unique ability to form dimers by establishing a covalent disulfide bond. The presence of cysteine residues, therefore, may indicate additional structural constraints within a protein. Although the incidence of C at position 6.47 is 80%, the presence of 6% T, 6% S, and residues other than these, indicate that this unique ability of C is subordinate to its role at this position. In site-directed mutagenesis studies with amino acids of similar size no effects on agonist or antagonist binding were observed, but the C(6.47)R mutant in the LH/

CC, receptor caused constitutive activity of the system [Laue et al., 1995]. It would be interesting to measure the basal activity of GPCRs that contain larger residues at this position. Unfortunately, the only GPCRs reported to possess a large side chain substituent (F), are orphan receptors (EBI1, RBS1, and RTA) [Birkenbach et al., 1993; Harrison et al., 1994; Ross et al., 1990] and olfactory receptors. The $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ receptors contain the larger than cysteine, but also sulfur-containing, methionine, and apparently do not display disparate behaviour. Moreover, since 92% of all GPCRs exhibit a residue at position 6.47 with hydrogen bonding capacity, it is very likely that this particular position takes over the peptide-backbone hydrogen-bonding network disrupted by P(6.50), which is supposedly facing the lipid phase of the membrane bilayer. A survey of the function of C, S, and T residues in protein crystal structures deposited in the PDB reveals that these amino acids are excellently suitable for reinforcing the peptide backbone [Ballesteros and Weinstein, 1995].

Adding to stabilization of the backbone, following destabilization by P(6.50), are most likely the residues located at positions 6.48 and 6.51. The occurrence of aromatic residues at position 6.48 (89% W, 5% F, and 2% Y), and the presence of aromatic residues at position 6.51 (49% F, and 28% Y), could be providing rigidity through non-covalent aromatic stacking (often, mistakenly, referred to as "π-π stacking"). Such a system would allow for a receptor-activation mechanism that requires conformational changes [Ballesteros and Weinstein, 1995]. Mutational analysis of either of these positions is inconclusive, bearing in mind the wide variety of effects observed [Wess et al., 1991, 1992b, 1993; Ridge et al., 1992; Beinborn et al., 1993; Choudhary et al., 1993; Yamano et al., 1995] or even the absence of effects [Ridge et al., 1992; Bhogal et al., 1994; Perlman, 1995a; Yamano et al., 1995]. Such observations, however, are consistent with a subtle balance between stabilizing and destabilizing effects.

Residues at position 6.52 are apparently involved in ligand recognition, more specifically ligand selectivity, considering the incidence of amino acids on the one hand; and the effects observed upon introduction of mutations on the other hand (Table 3, Fig. 3). This position is predominantly occupied by aromatic residues (38% F, and 22% H), although a wide selection of other residues is allowed (12% N, 8% Y, 4% T, 3% Q, 2% S, and 1% R). At the same position some GPCRs carry A, L, or V residues, but this seems restricted to GPCRs that have endogenous ligands consisting of (repeated) isoprene units, such as the opsins, rhodopsin, and the prostaglandin and thromboxane receptors. Site-directed mutagenesis of this position results in a remarkable number of reports citing effects on agonist or antagonist selectivity [Choudhary et al., 1993; Blüml et al., 1994b; Fong et al., 1994a; Kim et al., 1995; Ozenberger and Hadcock, 1995], instead of straightforward decreased affinities [Olah et al., 1992; Zoffmann et al., 1993; Perlman et al., 1995a].

One helical turn separated from position 6.52, position 6.55 offers an exceptional variety of amino acids (17 out of 20 of the the naturally occurring), with an incidence that differs extensively from the average distribution for TMs. In order of decreasing incidence, they are 18% N, 14% V, 10% L, 6% A, 6% F, 6% Y, 5% Q, 5% S, 5% E, 4% T, 4% M, 3% H, 3% R, 2% K, and 1% each of C, G, and I. Absent are P, D, and W. This offers the possibility to differentiate between ligands; an effect that was observed by mutational analysis of the N(6.55) residue in the adenosine A_{2a} receptor [Kim et al., 1995]. Such effects are even more pronounced in the case of the R(6.55) residue in the P_{2U} receptor [Erb et al., 1995], but could, seemingly, not be demonstrated for T(6.55) in the AT_{1a} receptor [Yamano et al., 1995].

The incidence of rare amino acids at position 6.58 is even more unusual: 11% N (vs. 4% in all TMs), 10% D (vs. 1.9% in all TMs), 6% R (vs. 3.1% in all TMs), 4% K (vs. 2.4% in all

TMs), 3% Q (vs. 1.0% in all TMs), and 2% E (vs. 1.3% in all TMs). Site-directed mutagenesis of the AT_{1a} and NK2 receptors indicates that this position distinctly affects agonist and antagonist affinities [Bhogal et al., 1994; Yamano et al., 1995].

Residues occupying positions 6.59 through 6.62 are located within the last, distinguishable, full turn of the α-helix of TM6. Site-directed mutagenesis in this region has resulted in decreased affinity of agonists and decreased activity (A_{2a}) [Kim et al., 1995], or decreased affinity for antagonists (NK1) [Gether et al., 1994], but is not entirely consistent (NK2) [Bhogal et al., 1994].

Transmembrane domain 7 (TM7)

The helical periodicity for TM7 is initiated at position 7.29; propagates along residues 7.32, 7.35/7.36, 7.39/7.40, 7.43, 7.45/7.46, 7.49/7.50, 7.53; and terminates at position 7.57. The first helical turn is relatively rich in residues that are charged under physiological conditions, i.e., D, E, K, and R, indicating that this fragment may form an extension of the TM into the extracellular medium beyond the lipid membrane. The presence of P(7.31) in, e.g., muscarinic receptors, and P(7.32) in, e.g., the opsins, is consistent with initiation of an α-helix in this region of the sequence. The importance of this first helical turn in ligand binding, was extensively documented for the AT_1 receptor [Yamano et al., 1992; Hjorth et al., 1994; Perlman et al., 1995b].

The incidence of aromatic residues at position 7.35 is fairly high (23% F, 18% Y, 6% W), possibly signifying the entry point of the helix into the lipid environment of the membrane. The orientation of position 7.36, with regard to the arrangement of the TM in the helical bundle, is not entirely clear. Two reports suggest that this residue may be involved in interhelical contacts [Liu et al., 1995; Kim et al., 1995]. One report is explicit in this, citing a mutation that is incompatible with interhelical contacts with TM1, and several “rescue mutations” [Liu et al., 1995], whereas the other report merely mentions improper receptor processing [Kim et al., 1995]. Alternately, two reports suggest involvement of this position in ligand binding [Funk et al., 1993; Erb et al., 1995]. This position is therefore critical in more than one way, and may reflect minor differences in receptor structure between various classes of GPCRs. The high incidence of acidic (12% vs. 3.2% on all TMs) and basic (8% vs. 5.5% on all TMs) amino acids favours an orientation where this position is exposed to the central cavity of the helical bundle, but amino acids capable of hydrogen bond formation (11% S, 9% N, 7% Q, 7% H, 3% T, and 3% Y) could be accommodated in both a solvent-exposed orientation, and in an interhelical contact region. It is, therefore, likely that the size of the side chain plays a role in the decision process. Smaller residues, such as occur in the muscarinic receptor, would be important for interhelical contacts, while on the contrary larger residues extend into the central cavity.

The presence of lipophilic residues in 91% of all GPCRs rightfully marks the orientation of position 7.37 towards the lipid environment. The next position, 7.38, differing by 100° from 7.37, and 200° from 7.36, is probably involved in interhelical contacts with TM6. This is consistent with the presence of the structurally important T (22%), F (19%), G (8%), and P (4%) residues at this position. However, site-directed mutagenesis of the NK1 and NK2 receptors suggests that this position is important in determining species and subtype selectivity of agonists and antagonists [Fong et al., 1992a; Sachais et al., 1993; Bhogal et al., 1994; Jensen et al., 1994]. Although it is quite possible that changes in affinity result from changes in overall receptor structure, it can not be excluded that position 7.38 is solvent-exposed based on the current dataset [Fong et al., 1992a; Sachais et al., 1993; Bhogal et al., 1994; Jensen et al., 1994].

There is ample evidence that position 7.39 is exposed to the central cavity of the helical bundle. The presence of D, E, H, N, Q, and R residues renders it unlikely that this position is not solvent-exposed. Mutational analysis of several GPCRs from divergent subfamilies recorded profound effects on agonist and antagonist affinity (Table 3, Fig. 3). In addition, improper receptor processing and constitutive activity were observed. Especially noteworthy is the observation that an A(7.39)D mutation in bovine rhodopsin was tolerated and behaved like the wild type rhodopsin [Ridge et al., 1992], but that the A(7.39)E mutation in human rhodopsin lead to constitutive activity [Dryja et al., 1993]. The E residue, which differs from the D residue by only one methylene group in the side chain, is apparently sufficient to shift the receptor from the inactive to the (pre-) active state. In the TRH receptor the, slightly smaller, homologous mutation R(7.39)K was tolerated, but substitution with other residues greatly decreased the affinity for an agonist ligand [Perlman et al., 1995a].

Some controversy exists on the orientation of position 7.40. Despite the presence of a large majority (81%) of lipophilic residues (A, F, I, L, M, V, and W, with W in 40% of all cases) this position is also occupied by R residues in the arachidonate-derived lipid mediator receptors. Following the rules of helical periodicity, this ligand should be exposed to the lipid membrane environment, which is consistent with the high incidence of lipophilic residues, but clearly R residues can not easily be accommodated by such an environment. Moreover, it was demonstrated that it is possible to photochemically label the W(7.40) residue present in the β_2 adrenoceptor [Wong et al., 1988]. However, there is one interpretation that might afford an explanation for this apparent paradox. When the Orwellian notion that “all animals are equal, but some animals are more equal than others” [Orwell, 1945] is applied to GPCRs, it follows that the extent and positioning of TM7 may vary amongst GPCRs, regardless of homology and conservation pattern. Whereas some GPCRs may have a longer TM7 segment initiated at or around position 7.31, other receptors may consist of a smaller TM7 segment that is located with position 7.40 at about 1 helical turn from the solvent-lipid interface. Considering the length of the side chain of R, a “depth” of 1 helical turn into the membrane is sufficient for solvent-exposure of the guanidinium group. Modification of the side chain would thus affect ligand binding through optimization of the positioning of the TM with regard to the solvent-lipid interface. The R(7.40)K mutant, with a somewhat shorter but isoelectric side chain, exposes the ligand binding domain more (bringing it closer to the surface), resulting in increased affinity [Huang and Tai, 1995], than mutations abolishing the positive charge of the side chain. The R(7.40)E, V mutants in the PGE_2-EP3 receptor and the R(7.40)Q mutant TXA_2 receptor, indeed, exhibit a lower binding affinity [Funk et al., 1993; Huang and Tai, 1995]. Since this position is proximal to the solvent-lipid interface, it would also account for the photoaffinity labeling in the β_2-adrenoceptor [Wong et al., 1988], and the lack of effect observed in the m3 muscarinic receptor [Wess et al., 1993].

The location of positions 7.42 and 7.43 facing the central cavity of the helical bundle is consistent with the effects observed in site-directed mutagenesis studies. Position 7.43 is occupied by a K in the (rhod)opsins that serves as the anchoring point of the retinal chromophore by means of Schiff base formation. Destabilization of the rhodopsin protein by mutating K(7.43) to either A, E, G, or H results in constitutive activity of the receptor [Cohen et al., 1992; Robinson et al., 1992; Li et al., 1995]. Experimental data from targeted mutagenesis of the H conserved at this position among all adenosine receptors [Olah et al., 1992; Kim et al., 1995], or the Y conserved (48% of all GPCRs) among most biogenic amine receptors [Strader et al., 1989a; Wess et al., 1991; Wess et al., 1992b], demonstrate that this position is also a prominent anchoring site for ligands that are not covalently bound to the protein.

W(7.44) occurs only in some lipid mediator receptors, and according to the requirements of helical periodicity, is located at the same side of the helix as R(7.40) in those receptors. The interaction between a positively charged residue and aromatic residues is thought to be essential for ligand binding to the biogenic amine receptors [e.g., Hibert et al., 1991], and may play a role in these receptors as well. Replacing W(7.44) with an aliphatic residue such as L, or a charged residue such as R, proved detrimental for ligand affinity [Funk et al., 1993].

Residue 7.45 (76% N, 10% S, and 8% H) is located at the same face of the helix as position 7.38, but two helical turns closer to the intracellular surface. It thus exhibits a similar profile as position 7.38. The two site-directed mutagenesis studies available, suggest that this position distinguishes between agonist and antagonist binding, and a direct role in ligand binding is therefore plausible [Strader et al., 1987a; Perlman 1995b]. The abundance of hydrophilic residues at position 7.46 (94% of all GPCRs) clearly locates this position in the central cavity of the helical bundle. Considering that 70% of all GPCRs contain a S at this position, 11% a C, and another 7% a T, it is very likely that this position assists in maintaining the backbone structure of the TM. Furthermore, it is located 1 helical turn above the conserved P at position 7.50, and could take over the hydrogen bonding network disturbed by the presence of this proline. Mutation studies of this position reveal intricate effects on agonist and antagonist binding, that might be explained either by direct interaction with the ligand or by more subtle regulation of the shape of the ligand binding site through intrahelical contacts [Strader et al., 1989a; Jiang et al., 1995; Kim et al., 1995].

N(7.49) in the NPXXY conservation motif has been considered one of the better conserved residues in TM7. Indeed, 82% of all GPCRs, including all biogenic amine receptors, maintain an N at this position, but another 17% of all GPCRs express an D at the equivalent position. Furthermore, at least 1 GPCR has a K(7.49) residue, although in this latter case it concerns the uncharacterized orphan receptor RTA [Ross et al., 1990]. It has been hypothesized that in the case of the GRH receptor this constitutes a reciprocal mutation with N(2.50) [Zhou et al., 1994], but all other GPCRs express an D(2.50). The position is apparently solvent-exposed, but its function is not clear. Moreover, the one mutation reported D(7.49)N in the GRH receptor was without effect [Davidson et al., 1994b].

Position 7.50 is characterized by an incidence of 100% P. It therefore serves a function in receptor structure, that not only affects ligand binding to the receptor, but also modulates receptor activity. The effect on receptor structure of a P(7.50)S mutation in the β_2-adrenoceptor is so drastic that the receptor cannot attain proper folding [Strader et al., 1987a].

Beyond the conserved P(7.50) very few experimental data are available. One study employing site-directed mutagenesis of C(7.54) in the β_2-adrenoceptor indicates the involvement, either directly or indirectly, of this residue in agonist binding and receptor activity [O'Dowd et al., 1988], but this finding is disputed by others [Fraser et al., 1989a].

Receptor Models

Many GPCR models [e.g., IJzerman et al., 1994; Teeter et al., 1994; Prusis et al., 1995; ter Laak et al., 1995] have been published founded on the work initiated by Hibert et al. [1991], and based on a bacteriorhodopsin template [Henderson et al., 1990]. More recently, the rhodopsin template [Schertler et al., 1993] has been used for the same purpose. The use of such models has greatly aided in the understanding of receptor structure and the interpretation of mutation analyses [Donnelly and Findlay, 1994; Perlman et al., 1994b; Zhou et al., 1994; Kim et al., 1995]. The construction and application of such models was recently discussed by Ballesteros and Weinstein [1995]. We also took it upon ourselves to

devise a model for the recently cloned P_{2Y} purinergic receptor, and are now preparing to investigate the implications of this model, and the survey presented above, by means of site-directed mutagenesis [van Rhee et al., 1995].

Acknowledgments

A.M.v.R. thanks the Cystic Fibrosis Foundation for financial support.

REFERENCES

Albert PR. Molecular biology of the $5HT_{1A}$ receptor: low-stringency cloning and eukaryotic expression. J Chem Neuroanat. 1992; 5:283–288. [PubMed: 1524715]

Allard JW, Sigal IS, Dixon RA. Sequence of the gene encoding the human M1 muscarinic acetylcholine receptor. Nucleic Acids Res. 1987; 15:10604. [PubMed: 3697105]

Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature. 1990; 348:730–732. [PubMed: 2175396]

Arakawa S, Gocayne JD, McCombie WR, Urquhart DA, Hall LM, Fraser CM, Venter JC. Cloning, localization, and permanent expression of a Drosophila octopamine receptor. Neuron. 1990; 4:343–354. [PubMed: 2156539]

Baldwin JM. The probable arrangement of the helices in G protein-coupled receptors. EMBO J. 1993; 12:1693–1703. [PubMed: 8385611]

Baldwin JM. Structure and function of receptors coupled to G proteins. Curr Opinion Cell Biol. 1994; 6:180–190. [PubMed: 8024808]

Ballesteros JA, Weinstein H. Integrated methods for the construction of three dimensional models and computational probing of structure-function relations in G-protein coupled receptors. Methods Neurosci. 1995; 25:366–428.

Barberis C, Seibold A, Ishido M, Rosenthal W, Birnbaumer M. Expression cloning of the human V2 vasopressin receptor. Regul Pept. 1993; 45:61–66. [PubMed: 8511368]

Baumgartner JW, Wells CA, Chen CM, Waters MJ. The role of the WSXWS equivalent motif in growth hormone receptor function. J Biol Chem. 1994; 269:29094–29101. [PubMed: 7961876]

Beckmann MP, Munger WE, Kozlosky C, van den Bos T, Price V, Lyman S, Gerard NP, Gerard C, Cerretti DP. Molecular characterization of the interleukin-8 receptor. Biochem Biophys Res Commun. 1991; 179:784–789. [PubMed: 1898400]

Beinborn M, Lee YM, McBride EW, Quinn SM, Kopin AS. A single amino acid of the cholecystokinin-B/gastrin receptor determines specificity for non-peptide antagonists. Nature. 1993; 362:348–350. [PubMed: 8455720]

Bergson C, Mrzljak L, Lidow MS, Goldman RPS, Levenson R. Characterization of subtype-specific antibodies to the human D_5, dopamine receptor: studies in primate brain and transfected mammalian cells. Proc Natl Acad Sci USA. 1995; 92:3468–3472. [PubMed: 7536933]

Bhogal N, Donnelly D, Findlay JB. The ligand binding site of the neurokinin 2 receptor. Site-directed mutagenesis and identification of neurokinin A binding residues in the human neurokinin 2 receptor. J Biol Chem. 1994; 269:27269–27274. [PubMed: 7961636]

Bichet DG, Arthus MF, Lonergan M, Hendy GN, Paradis AJ, Fujiwara TM, Morgan K, Gregory MC, Rosenthal W, Didwania A, Antaramian A, Birnbaumer M. X-linked nephrogenic diabetes insipidus mutations in North America and the Hopewell hypothesis. J Clin Invest. 1993; 92:1262–1268. [PubMed: 8104196]

Bihoreau C, Monnot C, Davies E, Teutsch B, Bernstein KE, Corvol P, Clauser E. Mutation of Asp74 of the rat angiotensin II receptor confers changes in antagonist affinities and abolishes G-protein coupling. Proc Natl Acad Sci USA. 1993; 90:5133–5137. [PubMed: 8506360]

Birdsall NJ. Cloning and structure-function of the H_2 histamine receptor. Trends Pharmacol Sci. 1991; 12:9–10. [PubMed: 2006542]

Birkenbach M, Josefsen K, Yalamanchili R, Lenoir G, Kieff E. Epstein-Barr virus-induced genes: first lymphocyte-specific G protein-coupled peptide receptors. J Virol. 1993; 67:2209–2220. [PubMed: 8383238]

Birnbaumer M, Gilbert S, Rosenthal W. An extracellular congenital nephrogenic diabetes insipidus mutation of the vasopressin receptor reduces cell surface expression, affinity for ligand, and coupling to the G_s/adenylyl cyclase system. Mol Endocrinol. 1994; 8:886–894. [PubMed: 7984150]

Blount P, Krause JE. The roles of the putative third cytoplasmic loop and cytoplasmic carboxyl tail of NK-1 and NK-2 receptors in agonist stimulated second messenger responses in stably transfected CHO cells. Regul Pept. 1993; 46:447–449. [PubMed: 7692563]

Blüml K, Mutschler E, Wess J. Identification of an intracellular tyrosine residue critical for muscarinic receptor-mediated stimulation of phosphatidylinositol hydrolysis. J Biol Chem. 1994a; 269:402–405.

Blüml K, Mutschler E, Wess J. Functional role in ligand binding and receptor activation of an asparagine residue present in the sixth transmembrane domain of all muscarinic acetylcholine receptors. J Biol Chem. 1994b; 269:18870–18876.

Blüml K, Mutschler E, Wess J. Insertion mutagenesis as a tool to predict the secondary structure of a muscarinic receptor domain determining specificity of G-protein coupling. Proc Natl Acad Sci USA. 1994c; 91:7980–7984.

Blüml K, Mutschler E, Wess J. Functional role of a cytoplasmic aromatic amino acid in muscarinic receptor-mediated activation of phospholipase C. J Biol Chem. 1994d; 269:11537–11541.

Bockstahler LE. Overview of international PCR standardization efforts. PCR Methods Appl. 1994; 3:263–267. [PubMed: 8038694]

Bonner TI, Buckley NJ, Young AC, Brann MR. Identification of a family of muscarinic acetylcholine receptor genes. Science. 1987; 237:527–532. [PubMed: 3037705]

Breu V, Hashido K, Broger C, Miyamoto C, Furuichi Y, Hayes A, Kalina B, Löffler BM, Ramuz H, Clozel M. Separable binding sites for the natural agonist endothelin-1 and the non-peptide antagonist bosentan on human enfothelin-A receptors. Eur J Biochem. 1995; 231:266–270. [PubMed: 7628480]

Bunzow JR, van Tol HH, Grandy DK, Albert P, Salon J, Christie M, Machida CA, Neve KA, Civelli O. Cloning and expression of a rat D_2, dopamine receptor cDNA. Nature. 1988; 336:783–787. [PubMed: 2974511]

Burstein ES, Spalding TA, Hill-Eubanks D, Brann MR. Structure-function of muscarinic receptor coupling to G proteins. J Biol Chem. 1995; 270:3141–3146. [PubMed: 7852396]

Caron MG, Kobilka BK, Frielle T, Bolanowski MA, Benovic JL, Lefkowitz RJ. Cloning of the cDNA and genes for the hamster and human beta 2-adrenergic receptors. J Recept Res. 1988; 8:7–21. [PubMed: 2838630]

Chan T, Lee M, Sakmar TP. Introduction of hydroxyl-bearing amino acids causes bathochromic spectral shifts in rhodopsin. Amino acid substitutions responsible for red-green color pigment spectral tuning. J Biol Chem. 1992; 267:9478–9480. [PubMed: 1577792]

Chini B, Mouillac B, Ala Y, Blaestre MN, Trumpp-Kallmeyer S, Hoflack J, Elands J, Hibert M, Manning M, Jard S, Barberis C. Tyr115 is the key residue for determining agonist selectivity in the V1a vasopressin receptor. EMBO J. 1995; 14:2176–2182. [PubMed: 7774575]

Choudhary MS, Craigo S, Roth BL. Identification of receptor domains that modify ligand binding to 5-hydroxytryptamine 2 and 5-hydroxytryptamine 1c serotonin receptors. Mol Pharmacol. 1992; 42:627–633. [PubMed: 1435740]

Choudhary MS, Craigo S, Roth BL. A single point mutation (Phe340→Leu340) of a conserved phenylalanine abolishes 4-[^{125}I]iodo-(2,5-dimethoxy)phenylisopropyl-amine and [^{3}H]me-sulergine but not [^{3}H]ketanserin binding to 5-hydroxytryptamine 2 receptors. Mol Pharmacol. 1993; 43:755–761. [PubMed: 8388989]

Chung FZ, Wang CD, Potter PC, Venter JC, Fraser CM. Site-directed mutagenesis and continuous expression of human beta-adrenergic receptors. Identification of a conserved aspartate residue involved in agonist binding and receptor activation. J Biol Chem. 1988; 263:4052–4055. [PubMed: 2831218]

Cohen GB, Oprian DD, Robinson PR. Mechanism of activation and inactivation of opsin: role of Glu113 and Lys296. Biochemistry. 1992; 31:12592–12601. [PubMed: 1472495]

Cone RD, Mountjoy KG, Robbins LS, Nadeau JH, Johnson KR, Roselli RL, Mortrud MT. Cloning and functional characterization of a family of receptors for the melanotropic peptides. Ann NY Acad Sci. 1993; 680:342–363. [PubMed: 8390157]

Corjay MH, Dobrzanski DJ, Way JM, Viallet J, Shapira II, Worland P, Sausville EA, Battey JF. Two distinct bombesin receptor subtypes are expressed and functional in human lung carcinoma cells. J Biol Chem. 1991; 266:18771–18779. [PubMed: 1655761]

Coughlin SR. Expanding horizons for receptors coupled to G proteins: diversity and disease. Curr Opinion Cell Biol. 1994; 6:191–197. [PubMed: 8024809]

Cserzö M, Bernassau JM, Simon I, Maigret B. New alignment strategy for transmembrane proteins. J Mol Biol. 1994; 243:388–396. [PubMed: 7966267]

Dal'Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, Seeburg PH. The dopamine D_2, receptor: two molecular forms generated by alternative splicing. EMBO J. 1989; 8:4025–4034. [PubMed: 2531656]

Dalziel HH, Westfall DP. Receptors for adenine nucleotides and nucleosides: Subclassification, Distribution, and Molecular Characterization. Pharmacol Rev. 1994; 46:449–466. [PubMed: 7899473]

D'Angelo DD, Davis MG, Ali S, Dorn GW. Cloning and pharmacologic characterization of a thromboxane A_2 receptor from K562 (human chronic myelogenous leukemia) cells. J Pharmacol Exp Ther. 1994; 271:1034–1041. [PubMed: 7965765]

Davidson FF, Loewen PC, Khorana HG. Structure and function in rhodopsin: replacement by alanine of cysteine residues 110 and 187, components of a conserved disulfide bond in rhodopsin, affects the light-activated metarhodopsin II state. Proc Natl Acad Sci USA. 1994a; 91:4029–4033. [PubMed: 8171030]

Davidson JS, Flanagan CA, Becker II, Illing N, Scalfon SC, Millar RP. Molecular function of the gonadotropin-releasing hormone receptor: insights from site-directed mutagenesis. Mol Cell Endocrinol. 1994b; 100:9–14. [PubMed: 8056165]

Deckert J, Nothen MM, Bryant SP, Ren HZ, Wolf HK, Stiles GL, Spurr NK, Propping P. Human adenosine A_1 receptor gene—systematic screening for DNA-sequence variation and linkage mapping on chromosome lq31-32.1 using a silent polymorphism in the coding region. Biochem Biophys Res Commun. 1995; 214:614–621. [PubMed: 7677773]

De Lean A, Stadel JM, Lefkowitz RJ. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. J Biol Chem. 1980; 255:7108–7117. [PubMed: 6248546]

Dixon RAF, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ, Strader CD. Cloning of the gene: and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature. 1986; 321:75–79. [PubMed: 3010132]

Dixon RA, Sigal IS, Candelore MR, Register RB, Scattergood W, Rands E, Strader CD. Structural features required for ligand binding to the beta-adrenergic receptor. EMBO J. 1987; 6:3269–3275. [PubMed: 2828022]

Dohlman HG, Caron MG, DeBlasi A, Frielle T, Lefkowitz RJ. Role of extracellular disulfide-bonded cysteines in the ligand binding function of the beta 2-adrenergic receptor. Biochemistry. 1990; 29:2335–2342. [PubMed: 2159799]

Donnelly D, Findlay JBC. Seven-helix receptors: structure and modelling. Curr Opinion Struct Biol. 1994; 4:582–589.

Donnelly D, Findlay JBC, Blundell TL. The evolution and structure of aminergic G protein-coupled receptors. Receptors Channels. 1994; 2:61–78. [PubMed: 8081733]

Drubbisch V, Lameh J, Philip M, Sharma YK, Sadee W. Mapping the ligand binding pocket of the human muscarinic cholinergic receptor Hml: contribution of tyrosine-82. Pharm Res. 1992; 9:1644–1647. [PubMed: 1488411]

Dryja TP, Berson EL, Rao VR, Oprian DD. Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. Nat Genet. 1993; 4:280–283. [PubMed: 8358437]

Edwards MC, Gibbs RA. Multiplex PCR: advantages, development, and applications. PCR Methods Appl. 1994; 3:S65–S75. [PubMed: 8173510]

Elling CE, Møller Nielsen S, Schwartz TW. Conversion of antagonist binding site to metal-ion site in the tachykinin NK-1 receptor. Nature. 1995; 374:74–77. [PubMed: 7532789]

Ellis J, Seidenberg M, Brann MR. Use of chimeric muscarinic receptors to investigate epitopes involved in allosteric interactions. Mol Pharmacol. 1993; 44:583–588. [PubMed: 7690450]

Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol. 1985; 55:836–839. [PubMed: 2991600]

Erb L, Garrad K, Wang Y, Quinn T, Turner JT, Weisman GA. Site-directed mutagenesis of P_{2U} purinoceptors. Positively charged amino acids in transmembrane helices 6 and 7 affect agonist potency and specificity. J Biol Chem. 1995; 270:4185–4188. [PubMed: 7876172]

Faa V, Ventruto ML, Loche S, Bozzola M, Podda R, Cao A, Rosatelli MC. Mutations in the vasopressin V2-receptor gene in three families of Italian descent with nephrogenic diabetes insipidus. Hum Mol Genet. 1994; 3:1685–1686. [PubMed: 7833930]

Fahmy K, Siebert F, Sakmar TP. Photoactivated state of rhodopsin and how it can form. Biophys Chem. 1995; 56:171–181. [PubMed: 7662864]

Fargin A, Kaymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ. The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. Nature. 1988; 335:358–360. [PubMed: 3138543]

Fathi Z, Benya RV, Shapira H, Jensen RT, Battey JF. The fifth transmembrane segment of the neuromedin B receptor is critical for high affinity neuromedin B binding. J Biol Chem. 1993; 268:14622–14626. [PubMed: 8392057]

Firsov D, Mandon B, Morel A, Merot J, Le Maout S, Bellanger AC, de Rouffignac C, Elalouf JM, Buhler JM. Molecular analysis of vasopressin receptors in the rat nephron. Evidence for alternative splicing of the V2 receptor. Pflügers Arch Eur J Physiol. 1994; 429:79–89.

Fishburn CS, David C, Carmon S, Wein C, Fuchs S. In vitro translation of D_2 dopamine receptors and their chimaeras: analysis by subtype-specific antibodies. Biochem Biophys Res Commun. 1994; 205:1460–1466. [PubMed: 7802682]

Fong TM, Huang RR, Strader CD. Localization of agonist and antagonist binding domains of the human neurokinin-1 receptor. J Biol Chem. 1992a; 267:25664–25667. [PubMed: 1281469]

Fong TM, Yu H, Strader CD. Molecular basis for the species selectivity of the neurokinin-1 receptor antagonists CP-96,345 and RP67580. J Biol Chem. 1992b; 267:25668–25671. [PubMed: 1281470]

Fong TM, Cascieri MA, Yu H, Bansal A, Swain C, Strader CD. Amino-aromatic interaction between histidine 197 of the neurokinin-1 receptor and CP 96345. Nature. 1993a; 362:350–353. [PubMed: 8384323]

Fong TM, Huang RR, Yu H, Strader CD. Mapping the ligand binding site of the NK-1 receptor. Regul Pept. 1993b; 46:43–48. [PubMed: 8210503]

Fong TM, Yu H, Cascieri MA, Underwood D, Swain CJ, Strader CD. The role of histidine 265 in antagonist binding to the neurokinin-1 receptor. J Biol Chem. 1994a; 269:2728–2732. [PubMed: 8300604]

Fong TM, Yu H, Cascieri MA, Underwood D, Swain CJ, Strader CD. Interaction of glutamine 165 in the fourth transmembrane segment of the human neurokinin-1 receptor with quinuclidine antagonists. J Biol Chem. 1994b; 269:14957–14961. [PubMed: 8195129]

Franke RR, Sakmar TP, Graham RM, Khorana HG. Structure and function in rhodopsin. Studies of the interaction between the rhodopsin cytoplasmic domain and transducin. J Biol Chem. 1992; 267:14767–14774. [PubMed: 1634520]

Fraser CM, Chung FZ, Wang CD, Venter JC. Site-directed mutagenesis of human beta-adrenergic receptors: substitution of aspartic acid-130 by asparagine produces a receptor with high-affinity agonist binding that is uncoupled from adenylate cyclase. Proc Natl Acad Sci USA. 1988; 85:5478–5482. [PubMed: 2840663]

Fraser CM. Site-directed mutagenesis of beta-adrenergic receptors. Identification of conserved cysteine: residues that independently affect ligand binding and receptor activation. J Biol Chem. 1989a; 264:9266–9270. [PubMed: 2542304]

Fraser CM, Wang CD, Robinson DA, Gocayne JD, Venter JC. Site-directed mutagenesis of m1 muscarinic acetylcholine receptors: conserved aspartic acids play important roles in receptor function. Mol Pharmacol. 1989b; 36:840–847. [PubMed: 2557534]

Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P, Williams M. Pharmacol Rev. 1994; 46:143–156.

Fredholm BB. Purinoceptors in the nervous system. Pharmacol Toxicol. 1995; 76:228–239. [PubMed: 7617551]

Funk CD, Furci L, Moran N, Fitzgerald GA. Point mutation in the seventh hydrophobic domain of the human thromboxane A_2 receptor allows discrimination between agonist and antagonist binding sites. Mol Pharmacol. 1993; 44:934–939. [PubMed: 8246916]

Gantz I, Schaffer M, DelValle J, Logsdon C, Campbell V, Uhler M, Yamada T. Molecular cloning of a gene encoding the histamine H_2 receptor. Proc Natl Acad Sci USA. 1991; 88:429–433. [PubMed: 1703298]

Gantz I, DelValle J, Wang LD, Tashiro T, Munzert G, Guo YJ, Konda Y, Yamada T. Molecular basis for the interaction of histamine with the histamine H_2 receptor. J Biol Chem. 1992; 267:20840–20843. [PubMed: 1356984]

Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, Hebert SC, Nemeth EF, Fuller F. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. J Biol Chem. 1995; 270:12919–12925. [PubMed: 7759551]

Gershengorn MC. Thyrotropin-releasing hormone receptor: cloning and regulation of its expression. Recent Prog Horm Res. 1993; 48:341–363. [PubMed: 8382829]

Gether U, Johansen TE, Schwartz TW. Chimeric NK1 (substance P)/NK3 (neurokinin B) receptors. Identification of domains determining the binding specificity of tachykinin agonists. J Biol Chem. 1993a; 268:7893–7898. [PubMed: 7681831]

Gether U, Johansen TE, Snider RM, Lowe JA, Emonds-Alt X, Yokota Y, Nakanishi S, Schwartz TW. Binding epitopes for peptide and non-peptide ligands on the NK1 (substance P) receptor. Regul Pept. 1993b; 46:49–58. [PubMed: 7692567]

Gether U, Nilsson L, Lowe JA, Schwartz TW. Specific residues at the top of transmembrane segment V and VI of the neurokinin-1 receptor involved in binding of the nonpeptide antagonist CP 96,345. J Biol Chem. 1994; 269:23959–23964. [PubMed: 7929043]

Goujon L, Allevato G, Simonin G, Paquereau L, Le Cam A, Clark J, Nielsen JH, Djiane J, Postel-Vinay MC, Edery M, Kelly PA. Cytoplasmic sequences of the growth hormone receptor necessary for signal transduction. Proc Natl Acad Sci USA. 1994; 91:957–961. [PubMed: 8302873]

Guan XM, Peroutka JS, Kobilka BK. Identification of a single amino acid residue responsible for the binding of a class of beta-adrenergic receptor antagonists to 5-hydroxytryptamine 1A receptors. Mol Pharmacol. 1992; 41:695–698. [PubMed: 1349154]

Cuiramand J, Montmayeur JP, Ceraline J, Bhatia M, Borrelli E. Alternative splicing of the dopamine D_2 receptor directs specificity of coupling to G-proteins. J Biol Chem. 1995; 270:7354–7358. [PubMed: 7706278]

Haraguchi K, Saito T, Kaneshige M, Endo T, Onaya T. Desensitization and internalization of a thyrotrophin receptor lacking the cytoplasmic carboxy-terminal region. J Mol Endocrinol. 1994a; 13:283–288. [PubMed: 7893346]

Haraguchi K, Saito T, Endo T, Onaya T. Disruption of the first extracellular loop of thyrotropin receptor prevents ligand binding. Life Sci. 1994b; 55:961–968. [PubMed: 7914657]

Harrison JK, Barber CM, Lynch KR. cDNA cloning of a G-protein-coupled receptor expressed in rat spinal cord and brain related to chemokine receptors. Neurosci Lett. 1994; 169:85–89. [PubMed: 8047298]

Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH. Model for the structure of bacteriorhodopsin based on high-resolution electron cry-microscopy. J Mol Biol. 1990; 213:899–929. [PubMed: 2359127]

Hibert MF, Trumpp-Kallmeyer S, Bruinvels A, Hoflack J. Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. Mol Pharmacol. 1991; 40:8–15. [PubMed: 1649965]

Hirata T, Kakizuka A, Ushikubi F, Fuse I, Okuma M, Narumiya S. Arg60 to Leu mutation of the human thromboxane A_2 receptor in a dominantly inherited bleeding disorder. J Clin Invest. 1994; 94:1662–1667. [PubMed: 7929844]

Hjorth SA, Schambye HT, Greenlee WJ, Schwartz TW. Identification of peptide binding residues in the extracellular domains of the AT_1 receptor. J Biol Chem. 1994; 269:30953–30959. [PubMed: 7983030]

Ho BY, Karschin A, Branchek T, Davidson N, Lester HA. The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT_{1A} receptor: a site-directed mutation study. FEBS Lett. 1992; 312:259–262. [PubMed: 1426261]

Hoflack J, Trumpp-Kallmeyer S, Hibert M. Re-evaluation of bacteriorhodopsin as a model for G protein-coupled receptors. Trends Pharmacol Sci. 1994; 43:348–350.

Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, Okado H, Toh H, Ito K, Miyamoto T, Shimizu T. Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature. 1991; 349:342–346. [PubMed: 1846231]

Horstman DA, Brandon S, Wilson AL, Guyer CA, Cragoe EJ, Limbird LE. An aspartate conserved among G-protein receptors confers allosteric regulation of α_2-adrenergic receptors by sodium. J Biol Chem. 1990; 265:21590–21595. [PubMed: 2174879]

Houslay MD. G-protein linked receptors: a family probed by molecular cloning and mutagenesis procedures. Clin Endocrinol. 1992; 36:525–534.

Huang C, Tai HH. Expression and site-directed mutagenesis of mouse prostaglandin E_2 receptor EP_3 subtype in insect cells. Biochem J. 1995; 307:493–498. [PubMed: 7733888]

Hunyady L, Bor M, Balla T, Catt KJ. Critical role of a conserved intramembrane tyrosine residue in angiotensin II receptor activation. J Biol Chem. 1995; 270:9702–9705. [PubMed: 7730346]

IJzerman AP, van Galen PJM, Jacobson KA. Molecular modeling of adenosine receptors. I. The ligand binding site on the A_1 receptor. Drug Des Discov. 1992; 9:49–67. [PubMed: 1457698]

Inagami T, Iwai N, Sasaki K, Yamamo Y, Bardhan S, Chaki S, Guo DF, Furuta H. Cloning, expression and regulation of angiotensin II receptors. J Hypertens. 1992; 10:713–716. [PubMed: 1325501]

Intres R, Crabb JW. Cloning strategies. Targeted Diagn Ther. 1992; 7:43–53. [PubMed: 1633304]

Ishihara T, Shigemoto R, Mori K, Takahashi K, Nagata S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron. 1992; 8:811–819. [PubMed: 1314625]

Javitch JA, Fu D, Chen J, Karlin A. Mapping the binding-site crevice of the dopamine D_2 receptor by the substituted-cysteine accessibility method. Neuron. 1995; 14:825–831. [PubMed: 7718244]

Jensen CJ, Gerard NP, Schwartz TW, Gether U. The species selectivity of chemically distinct tachykinin nonpeptide antagonists is dependent on common divergent residues of the rat and human neurokinin-1 receptors. Mol Pharmacol. 1994; 45:294–299. [PubMed: 7509441]

Ji I, Ji TH. Asp383 in the second transmembrane domain of the lutropin receptor is important for high affinity hormone binding and CAMP production. J Biol Chem. 1991; 266:14953–14957. [PubMed: 1714448]

Jiang Q, van Rhee AM, Kim J, Wess J, Jacobson KA. Hydrophilic side chains in the third and seventh transmembrane helical domains of human A_{2a} adenosine receptors are required for ligand recognition. 1995 submitted.

Jüppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E, Richards J, Kolakowski LF, Hock J, Potts JT, Kronenberg HM, Segre GV. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science. 1991; 254:1024–1026. [PubMed: 1658941]

Kakar SS, Musgrove LC, Devor DC, Sellers JC, Neill JD. Cloning, sequencing, and expression of human gonadotropin releasing hormone (GnRH) receptor. Biochem Biophys Res Commun. 1992; 189:289–295. [PubMed: 1333190]

Kao HT, Adham N, Olsen MA, Weinshank RL, Branchek TA, Hartig PR. Site-directed mutagenesis of a single residue changes the binding properties of the serotonin 5-HT_2 receptor from a human to a rat pharmacology. FEBS Lett. 1992; 307:324–328. [PubMed: 1644189]

Karnik SS, Sakmar TP, Chen HB, Khorana HG. Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. Proc Natl Acad Sci USA. 1988; 85:8459–8463. [PubMed: 3186735]

Karnik SS, Ridge KD, Bhattacharya S, Khorana HG. Palmitoylation of bovine opsin and its cysteine mutants in COS cells. Proc Natl Acad Sci USA. 1993; 90:40–44. [PubMed: 8419942]

Kaushal S, Khorana HG. Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa. Biochemistry. 1994; 33:6121–6128. [PubMed: 8193125]

Kieffer BL, Befort K, Gaveriaux RC, Hirth CG. The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci USA. 1992; 89:12048–12052. [PubMed: 1334555]

Kim J, Wess J, van Rhee AM, Schöneberg T, Jacobson KA. Site-directed mutagenesis identifies residues involved in ligand recognition in the human A_{2a} adenosine receptor. J Biol Chem. 1995; 270:13987–13997. [PubMed: 7775460]

Kimura T, Azuma C, Takemura M, Inoue T, Kikuchi T, Kubota Y, Ogita K, Saji F, Tanizawa O. Molecular cloning of a human oxytocin receptor. Regul Pept. 1993; 45:73–77. [PubMed: 8390082]

Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG, Lefkowitz RJ. Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. J Biol Chem. 1992; 267:1430–1433. [PubMed: 1346134]

Klein PS, Sun TJ, Saxe CL, Kimmel AR, Johnson RL, Devreotes PN. A chemoattractant receptor controls development in Dictyostelium discoideum. Science. 1988; 241:1467–1472. [PubMed: 3047871]

Knoers NV, van den Ouweland AM, Verdijk M, Monnens LA, van Oost BA. Inheritance of mutations in the V2 receptor gene in thirteen families with nephrogenic diabetes insipidus. Kidney Int. 1994; 46:170–176. [PubMed: 7933835]

Kobilka BK, Frielle T, Collins S, Yang FT, Kobilka TS, Francke U, Lefkowitz RJ, Caron MG. An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. Nature. 1987; 329:75–79. [PubMed: 3041227]

Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ. Chimeric alpha 2-, beta-2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. Science. 1988; 240:1310–1316. [PubMed: 2836950]

Kolakowski LF. GCRDb: a G-protein-coupled receptor database. Receptors Channels. 1994; 2:l–7.

Kong H, Raynor K, Yasuda K, Bell GI, Reisine T. Mutation of an aspartate at residue 89 in somatostatin receptor subtype 2 prevents Na^+ regulation of agonist binding but does not alter receptor-G protein association. Mol Pharmacol. 1993; 44:380–384. [PubMed: 8102784]

Kosugi S, Okajima F, Ban T, Hidaka A, Shenker A, Kohn LD. Mutation of alanine 623 in the third cytoplasmic loop of the rat thyrotropin (TSH) receptor results in a loss in the phos-phoinositide but not cAMP signal induced by TSH and receptor autoantibodies. J Biol Chem. 1992; 267:24153–24156. [PubMed: 1332945]

Kosugi S, Shenker A, Mori T. Constitutive activation of cyclic AMP but not phosphatidylinositol signaling caused by four mutations in the 6th transmembrane helix of the human thyrotropin receptor. FEBS Lett. 1994; 356:291–294. [PubMed: 7805857]

Kosugi S, Mori T. The third exoplasmic loop of the thyrotropin receptor is partially involved in signal transduction. FEBS Lett. 1994; 349:89–92. [PubMed: 8045308]

Kozell LB, Machida CA, Neve RL, Neve KA. Chimeric D_1/D_2 dopamine receptors. Distinct determinants of selective efficacy, potency, and signal transduction. J Bid Chem. 1994; 269:30299–30306.

Krystek JS, Patel PS, Rose PM, Fisher SM, Kienzle BK, Lach DA, Liu EC, Lynch JS, Novotny J, Webb ML. Mutation of peptide binding site in transmembrane region of a G protein-coupled receptor accounts for endothelin receptor subtype selectivity. J Biol Chem. 1994; 269:12383–12386. [PubMed: 8175640]

Kubo T, Fukuda K, Mikama A, Maeda A, Takahashi H, Mishina M, Haga Y, Ichiyama A, Kangawa K, Kajima M, Matsuo H, Hirose T, Numa T. Cloning, sequencing and expressing of complementary DNA coding the muscarinic acetylcholine receptor. Nature. 1986; 323:411–416. [PubMed: 3762692]

Kubo T, Bujo H, Akiba I, Nakai J, Mishina M, Numa S. Location of a region of the muscarinic acetylcholine receptor involved in selective effector coupling. FEBS Lett. 1988; 241:119–125. [PubMed: 3197827]

Kunz D, Gerard NP, Gerard C. The human leukocyte platelet-activating factor receptor. cDNA cloning, cell surface expression, and construction of a novel epitope-bearing analog. J Biol Chem. 1992; 267:9101–9106. [PubMed: 1374385]

Kurtenbach E, Curtis CA, Pedder EK, Aitken A, Harris AC, Hulme EC. Muscarinic acetylcholine receptors. Peptide sequencing identifies residues involved in antagonist binding and disulfide bond formation. J Biol Chem. 1990; 265:13702–13708. [PubMed: 2380182]

Kyte J, Doolittle RF. A simple method for displaying the hydrophobic character of a protein. J Mol Biol. 1982; 157:105–132. [PubMed: 7108955]

La Rosa GJ, Thomas KM, Kaufmann ME, Mark R, White M, Taylor L, Gray G, Witt D, Navarro J. Amino terminus of the interleukin-8 receptor is a major determinant of receptor subtype specificity. J Biol Chem. 1992; 267:25402–25406. [PubMed: 1281158]

Lattion AL, Diviani D, Cotecchia S. Truncation of the receptor carboxyl terminus impairs agonist-dependent phosphorylation and desensitization of the alpha 1B-adrenergic receptor. J Biol Chem. 1994; 269:22887–22893. [PubMed: 8077240]

Laue L, Chan WY, Hsueh AJ, Kudo M, Hsu SY, Wu SM, Blomberg L, Cutler GJ. Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. Proc Natl Acad Sci USA. 1995; 92:1906–1910. [PubMed: 7892197]

Lee NH, Fraser CM. Cross-talk between m1 muscarinic acetylcholine and beta 2-adrenergic receptors. cAMP and the third intracellular loop of m1 muscarinic receptors confer heterologous regulation. J Biol Chem. 1993; 268:7949–7957. [PubMed: 8385129]

Lefkowitz RJ, Haber E, O'Hara D. Identification of the cardiac beta-adrenergic receptor protein: solubilization and purification by affinity chromatography. Proc Natl Acad Sci USA. 1972; 69:2828–2832. [PubMed: 4507606]

Lefkowitz RJ, Cotecchia S, Samama P, Costa T. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. Trends Pharmacol Sci. 1993; 14:303–307. [PubMed: 8249148]

Leurs R, Smit MJ, Tensen CP, Ter Laak AM, Timmerman H. Site-directed mutagenesis of the histamine H_1-receptor reveals a selective interaction of asparagine 207 with subclasses of H_1-receptor agonists. Biochem Biophys Res Commun. 1994; 201:295–301. [PubMed: 8198587]

Li XJ, Forte M, North K4, Ross CA, Snyder SH. Cloning and expression of a rat somatostatin receptor enriched in brain. J Biol Chem. 1992; 267:21307–21312. [PubMed: 1400442]

Li T, Franson WK, Gordon JW, Berson EL, Dryja TP. Constitutive activation of phototransduction by K296E opsin is not a cause of photoreceptor degeneration. Proc Natl Acad Sci USA. 1995; 92:3551–3555. [PubMed: 7724596]

Libert F, Parmentier M, Lefort A, Dinsart C, Van Sande J, Maenhaut C, Simons MJ, Dumont JE, Vassart G. Selective amplification and cloning of four new members of the G protein-coupled receptor family. Science. 1989; 244:569–572. [PubMed: 2541503]

Libert F, Schiffmann SN, Lefort A, Parmentier M, Gerard C, Dumont JE, Vanderhaeghen JJ, Vassart G. The orphan receptor cDNA RDC7 encodes an A_1 adenosine receptor. EMBO J. 1991; 10:1677–1682. [PubMed: 1646713]

Liggett SB, Freedman NJ, Schwinn DA, Lefkowitz RJ. Structural basis for receptor subtype-specific regulation revealed by a chimeric beta 3/beta 2-adrenergic receptor. Proc Natl Acad Sci USA. 1993; 90:3665–3669. [PubMed: 8386380]

Lin SW, Sakmar TP, Franke RR, Khorana HG, Mathies RA. Resonance: Raman microprobe spectroscopy of rhodopsin mutants: effect of substitutions in the third transmembrane helix. Biochemistry. 1992; 31:5105–5111. [PubMed: 1351402]

Lin SW, Imamoto Y, Fukada Y, Shichida Y, Yoshizawa T, Mathies RA. What makes red visual pigments red? A resonance Raman microprobe study of retinal chromophore structure in iodopsin. Biochemistry. 1994; 33:2151–2160. [PubMed: 8117671]

Linden J. Cloned adenosine A_3 receptors: pharmacological properties, species differences and receptor functions. Trends Pharmacol Sci. 1994; 15:298–306. [PubMed: 7940998]

Link R, Daunt D, Barsh G, Chruscinski A, Kobilka B. Cloning of two mouse genes encoding alpha 2-adrenergic receptor subtypes and identification of a single amino acid in the mouse alpha 2-C10 homolog responsible for an interspecies variation in antagonist binding. Mol Pharmacol. 1992; 42:16–27. [PubMed: 1353249]

Liu J, Schöneberg T, van Rhee AM, Wess J. Mutational analysis of the relative orientation of transmembrane helices I and VII in G protein-coupled receptors. J Biol Chem. 1995; 13:133–154.

Livak KJ, Rogers J, Lichter JB. Variability of dopamine D_4 receptor (DRD4) gene sequence within and among nonhuman primate species. Proc Natl Acad Sci USA. 1995; 92:427–431. [PubMed: 7831304]

Lubbert H, Hoffman BJ, Snutch TP, van Dijke T, Levine AJ, Hartig PR, Lester HA, Davidson N. cDNA cloning of a serotonin 5-HT_{1C} receptor by electrophysiological assays of mRNA-injected Xenopus oocytes. Proc Natl Acad Sci USA. 1987; 84:4332–4336. [PubMed: 3473504]

Lustig KD, Shiau AK, Brake AJ, Julius D. Expression cloning of an ATP receptor from mouse neuroblastoma cells. Proc Natl Acad Sci USA. 1993; 90:5113–5117. [PubMed: 7685114]

Macke JP, Hennessey JC, Nathans J. Rhodopsin mutation proline^{347}-to-alanine in a family with autosoma1 dominant retinitis pigmentosa indicates an important role for proline at position 347. Hum Mol Genetics. 1995; 4:775–776.

MacKenzie RG, Steffey ME, Manelli AM, Pollock NJ, Frail UE. A D_1/D_2 chimeric dopamine receptor mediates a D_1 response to a D_2-selective agonist. FEBS Lett. 1993; 323:59–62. [PubMed: 8098694]

Maenhaut C, van Sande J, Libert F, Abramowicz M, Parmentier M, Vanderhaegen JJ, Dumont JE, Vassart G, Schiffmann S. RCD8 codes for an adenosine A_2 receptor with physiological constitutive activity. Biochem Biophys Res Commun. 1990; 173:1169–1178. [PubMed: 2125216]

Maenhaut C, van Sande J, Massart C, Dinsart C, Libert F, Monferini E, Giraldo E, Ladinsky H, Vassart G, Dumont JE. The orphan receptor cDNA RDC4 encodes a 5-HT_{1D} serotonin receptor. Biochem Biophys Res Commun. 1991; 180:1460–1468. [PubMed: 1659418]

Maggio R, Vogel Z, Wess J. Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for inter-molecular cross-talk between G-protein-linked receptors. Proc Natl Acad Sci USA. 1993; 90:3103–3107. [PubMed: 8385357]

Mahle CD, Wiener HL, Yocca FD, Maayani S. Allosteric interactions between the binding sites of receptor agonists and guanine nucleotides: a comparative study of the hydroxytryptamine$_{1A}$ and adenosine A_1 receptor systems in rat hippocampal membranes. J Pharmacol Exp Therap. 1992; 263:1275–1284. [PubMed: 1469633]

Mansour A, Meng F, Meador WJ, Taylor LP, Civelli O, Akil H. Site-directed mutagenesis of the human dopamine D_2 receptor. Eur J Pharmacol. 1992; 227:205–214. [PubMed: 1358663]

Mantamadiotis T, Baldwin GS. The seventh transmembrane domain of gastrin/CCK receptors contributes to non-peptide antagonist binding. Biochem Biophys Res Commun. 1994; 201:1382–1389. [PubMed: 8024583]

Marsh L, Herskowitz I. STE2 protein of Saccharomyces kluyveri is a member of the rhodopsin/beta-adrenergic receptor family and is responsible for recognition of the peptide ligand alpha factor. Proc Natl Acad Sci USA. 1988; 85:3855–3859. [PubMed: 2836861]

Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S. Sequence and expression of a metabotropic glutamate receptor. Nature. 1991; 349:760–765. [PubMed: 1847995]

McAllister G, Knowles MR, Patel S, Marwood R, Emms F, Seabrook GR, Graziano M, Borkowski D, Hey PJ, Freedman SB. Characterisation of a chimeric hD_3/D_2 dopamine receptor expressed in CHO cells. FEBS Lett. 1993; 324:81–86. [PubMed: 8099332]

McEachern AE, Shelton ER, Bhakta S, Obernolte R, Bach C, Zuppan P, Fujisaki J, Aldrich RW, Jarnagin K. Expression cloning of a rat B2 bradykinin receptor. Proc Natl Acad Sci USA. 1991; 88:7724–7728. [PubMed: 1715575]

Meng F, Xie GX, Chalmers D, Morgan C, Watson SJ, Akil H. Cloning and characterization of a pharmacologically distinct A_1 adenosine receptor from guinea pig brain. Brain Res Mol Brain Res. 1994; 26:143–155. [PubMed: 7854041]

Meyerhof W, Paust HJ, Schönrock C, Richter D. Cloning of a cDNA encoding a novel putative G-protein-coupled receptor expressed in specific rat brain regions. DNA Cell Biol. 1991; 10:689–694. [PubMed: 1661599]

Menziani MC, Cocchi M, Fanelli D, De Benedetti PG. Quantitative structure-affinity/selectivity relationship analysis of three-dimensional models of the complexes between the ET_A and ET_B receptors and C-terminal endothelin hexapeptide antagonists. J Mol Struct. 1995; 333:243–248.

Millán JM, Fuchs S, Paricio N, Wedemann H, Gal A, Nájera C, Prieto F. Gly114Asp mutation of rhodopsin in antosomal dominant retinitis pigmentosa. Mol Cell Probes. 1995; 9:67–69. [PubMed: 7760863]

Mills A, Duggan MJ. Orphan seven transmembrane domain receptors: reversing pharmacology. Trends Biotechnol. 1994; 12:47–49. [PubMed: 7764535]

Min KC, Zvyaga TA, Cypess AM, Sakmar TP. Characterization of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. Mutations on the cytoplasmic surface affect transducin activation. J Biol Chem. 1993; 268:9400–9404. [PubMed: 8486634]

Miyajima I, Nakafuku M, Nakayama N, Brenner C, Miyajima A, Kaibuchi K, Arai K, Kaziro Y, Matsumoto K. GPA1, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may he involved in mating factor signal transduction. Cell. 1987; 50:1011–1019. [PubMed: 3113739]

Moguilevsky N, Varsalona F, Guillaume JP, Noyer M, Gillard M, Daliers J, Henichart JP, Bollen A. Pharmacological and Functional Characterisation of the Wild-Type and Site-Directed Mutants of the Human H_1 Histamine Receptor Stably Expressed in CHO Cells. J Receptor & Signal Transduction Res. 1995; 15:91–102.

Monden T, Yamada M, Satoh T, Iizuka M, Mori M. Analysis of the TSH receptor gene structure in various thyroid disorders: DNA from thyroid adenomas can have large insertions or deletions. Thyroid. 1992; 2:189–192. [PubMed: 1358304]

Monsma FJ, McVittie LD, Gerfen CR, Mahan LC, Sibley DR. Multiple D_2 dopamine receptors produced by alternative RNA splicing. Nature. 1989; 342:926–929. [PubMed: 2480527]

Morel A, Lolait SJ, Brownstein MJ. Molecular cloning and expression of rat V1a and V2 arginine vasopressin receptors. Regul Pept. 1993; 45:53–59. [PubMed: 8511367]

Moro 0, Lameh J, Sadee W. Serine- and threonine-rich domain regulates internalization of muscarinic cholinergic receptors. J Biol Chem. 1993; 268:6862–6865. [PubMed: 8463213]

Murphy TJ, Takeuchi K, Alexander RW. Molecular cloning of AT_1 angiotensin receptors. Am J Hypertens. 1992; 5:236S–242S. [PubMed: 1290618]

Nathans J, Hogness DS. Isolation, sequence analysis, and intron-exon arrangement of the gene encoding bovine rhodopsin. Cell. 1983; 34:807–814. [PubMed: 6194890]

Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL. Pivotal role for aspartate-80 in the regulation of dopamine D_2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol. 1991; 39:733–739. [PubMed: 1828858]

O'Dowd BF, Hnatowich M, Regan JW, Leader WM, Caron MG, Lefkowitz RJ. Site-directed mutagenesis of the cytoplasmic domains of the human beta 2-adrenergic receptor. Localization of regions involved in G protein-receptor coupling. J Biol Chem. 1988; 263:15985–15992. [PubMed: 2846532]

Ohno M, Endo T, Ohta K, Gunji K, Onaya T. Point mutations in the thyrotropin receptor in human thyroid tumors. Thyroid. 1995; 5:97–100. [PubMed: 7647578]

Ohta K, Hayashi H, Mizuguchi H, Kagamiyama H, Fujimoto K, Fukui H. Site-directed mutagenesis of the histamine H_I receptor: roles of aspartic acid 107, asparagine 198 and threonine 194. Biochem Biophys Res Commun. 1994; 203:1096–1101. [PubMed: 8093027]

Oksenberg D, Marsters SA, O'Dowd BF, Jin H, Havlik S, Peroutka SJ, Ashkenazi A. A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT_{1B}, receptors. Nature. 1992; 360:161–163. [PubMed: 1436092]

Olah ME, Ren H, Ostrowski J, Jacobson KA, Stiles CL. Cloning, expression, and characterization of the unique bovine A_1 adenosine receptor. Studies on the ligand binding site by site-directed mutagenesis. J Biol Chem. 1992; 267:10764–10770. [PubMed: 1587851]

Olah ME, Jacobson KA, Stiles GL. Identification of an adenosine receptor domain specifically involved in binding of 5′-substituted adenosine agonists. J Biol Chem. 1994a; 269:18016–18020. [PubMed: 8027060]

Olah ME, Jacobson KA, Stiles GL. Role of the second extracellular loop of adenosine receptors in agonist and antagonist binding. Analysis of chimeric A_1/A_3 adenosine receptors. J Biol Chem. 1994b; 269:24692–24698. [PubMed: 7929142]

Oliveira L, Paiva ACM, Vriend C. A common motif in G-protein-coupled seven transmembrane helix receptors. J Comp-Aided Mol Design. 1993; 7:649–658.

Orwell, G. Animal Farm: a fairy story. London: Secker & Warburg; 1945.

Ostrowski J, Kjelsberg MA, Caron MG, Lefkowitz RJ. Mutagenesis of the beta 2-adrenergic receptor: how structure elucidates function. Ann Rev Pharmacol Toxicol. 1992; 32:167–183. [PubMed: 1318669]

Ozenberger BA, Hadcock JR. A single amino acid substitution in somatostatin receptor subtype 5 increases affinity for somatostatin-14. Mol Pharmacol. 1995; 47:82–87. [PubMed: 7838136]

Page KM, Curtis CAM, Jones PG, Hulme EC. The functional role of the binding-site aspartate in muscarinic acetylcholine-receptors probed by site-directed mutagenesis. Eur J Pharmacol-Mol Pharmacol Sect. 1995; 289:429–437.

Pan Y, Metzenberg A, Das S, Jing B, Gitschier J. Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. Nat Genet. 1992; 2:103–106. [PubMed: 1303257]

Parker EM, Grisel DA, Iben LG, Shapiro RA. A single amino acid difference accounts for the pharmacological distinctions between the rat and human 5-hydroxytryptamine 1B receptors. J Neurochem. 1993; 60:380–383. [PubMed: 8417162]

Parma J, Duprez L, van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature. 1993; 365:649–651. [PubMed: 8413627]

Parmentier M, Libert F, Perret J, Eggerickx D, Ledent C, Schurmans S, Raspe E, Dumont JE, Vassart G. Cloning and characterization of G protein-coupled receptors. Adv Second Messenger Phosphoprotein Res. 1993; 28:11–18. [PubMed: 8398392]

Paschke R, Tonacchera M, van Sande J, Parma J, Vassart G. 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. 1994a; 79:1785–1789. [PubMed: 7989485]

Paschke R, Parmentier M, Vassart G. Importance of the extracellular domain of the human thyrotrophin receptor for activation of cyclic AMP production. J Mol Endocrinol. 1994b; 13:199–207. [PubMed: 7848531]

Peralta EG, Ashkenazi A, Winslow JW, Smith DH, Ramachandran J, Capon DJ. Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. EMBO J. 1987; 6:3923–3929. [PubMed: 3443095]

Perlman JH, Nussenzveig DR, Osman R, Gershengorn MC. Thyrotropin-releasing hormone binding to the mouse pituitary receptor does not involve ionic interactions. A model for neutral peptide binding to G protein-coupled receptors. J Biol Chem. 1992; 267:24413–24417. [PubMed: 1332958]

Perlman JH, Thaw CN, Laakkonen L, Bowers CY, Osman R, Gershengorn MC. Hydrogen bonding interaction of thyrotropin-releasing hormone (TRH) with transmembrane tyrosine 106 of the TRH receptor. J Biol Chem. 1994a; 269:1610–1613. [PubMed: 8294406]

Perlman JH, Laakkonen L, Osman R, Gershengorn MC. A model of the thyrotropin-releasing hormone (TRH) receptor binding pocket. Evidence for a second direct interaction between transmembrane helix 3 and TRH. J Biol Chem. 1994b; 269:23383–23386. [PubMed: 8089099]

Perlman JH, Laakkonen L, Osman R, Gershengorn MC. Distinct roles for arginines in transmembrane helices 6 and 7 of the thyrotropin-releasing hormone receptor. Mol Pharmacol. 1995a; 47:480–484. [PubMed: 7700246]

Perlman S, Schambye HT, Rivero RA, Greenlee WJ, Hjorth SA, Schwartz TW. Non-peptide angiotensin agonist. Functional and molecular interaction with the AT_1 receptor. J Biol Chem. 1995b; 270:1493–1496. [PubMed: 7829475]

Pin JP, Gomeza J, Joly C, Bockaert J. The metabotropic glutamate receptors: their second intracellular loop plays a critical role in the G-protein coupling specificity. Biochem Soc Trans. 1995; 23:91–96. [PubMed: 7758811]

Pittel Z, Wess J. Intramolecular interactions in muscarinic acetylcholine receptors studied with chimeric m2/m5 receptors. Mol Pharmacol. 1994; 45:61–64. [PubMed: 8302281]

Pollock NJ, Manelli AM, Hutchins CW, Steffey ME, MacKenzie RG, Frail DE. Serine mutations in transmembrane V of the dopamine D_1 receptor affect ligand interactions and receptor activation. J Biol Chem. 1992; 267:17780–17786. [PubMed: 1355478]

Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon SC. Sequence alignment of the G-protein coupled receptor super-family. DNA Cell Biol. 1992; 11:1–20. [PubMed: 1310857]

Prusis P, Frändberg PA, Muceniece R, Kalvinsh I, Wikberg JES. A three dimensional model for the interaction of MSH with the melanocortin-I receptor. Biochem Biophys Res Commun. 1995; 210:205–210. [PubMed: 7741742]

Quintana J, Wang H, Ascoli M. The regulation of the binding affinity of the luteinizing hormone/choriogonadotropin receptor by sodium ions is mediated by a highly conserved aspartate located in the second transmembrane domain of G protein-coupled receptors. Mol Endocrinol. 1993; 7:767–775. [PubMed: 8395653]

Rashtchian A. Novel methods for cloning and engineering genes using the polymerase chain reaction. Curr Opin Biotechnol. 1995; 6:30–36. [PubMed: 7894080]

Raychowdhury MK, Yukawa M, Collins LJ, McGrail SH, Kent KC, Ware JA. Alternative splicing produces a divergent cytoplasmic tail in the human endothelial thromboxane A_2 receptor. J Biol Chem. 1994; 269:19256–19261. [PubMed: 8034687]

Raymond JR. Hereditary and acquired defects in signaling through the hormone-receptor-G protein complex. Am J Physiol. 1994; 226:F163–F174. [PubMed: 8141317]

Ren Q, Kurose H, Lefkowitz RJ, Cotecchia s. Constitutively active mutants of the alpha 2-adrenergic receptor. J Biol Chem. 1993; 268:16483–16487. [PubMed: 8393865]

Ren H, Stiles GL. Posttranscriptional mRNA processing as a mechanism for regulation of human A_1 adenosine receptor expression. Proc Natl Acad Sci USA. 1994; 91:4864–4866. [PubMed: 8197148]

Resek JF, Farrens D, Khorana HG. Structure and function in rhodopsin: covalent crosslinking of the rhodopsin (metarhodopsin II)-transducin complex—the rhodopsin cytoplasmic face links to the transducin alpha subunit. Proc Natl Acad Sci USA. 1994; 91:7643–7647. [PubMed: 8052635]

Richards JE, Scott KM, Sieving PA. Disruption of conserved rhodopsin disulfide bond by Cys187Tyr mutation causes early and severe antosomal dominant retinitis pigmentosa. Ophthalmol. 1995; 102:669–677.

Richardson JS, Richardson DC. Amino acid preferences for specific locations at the ends of α helices. Science. 1988; 240:1648–1652. [PubMed: 3381086]

Ridge KD, Bhattacharya S, Nakayama TA, Khorana HG. Light-stable rhodopsin. II. An opsin mutant (Trp265→Phe) and a retinal analog with a nonisomerizable II-cis configuration form a photostable chromophore. J Biol Chem. 1992; 267:6770–6775. [PubMed: 1532391]

Ridge KD, Lu Z, Liu X, Khorana HG. Structure and function in rhodopsin. Separation and characterization of the correctly folded and misfolded opsins produced on expression of an opsin mutant gene containing only the native intradiscal cysteine codons. Biochemistry. 1995; 34:3261–3267. [PubMed: 7880821]

Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli RL, Baack E, Mountjoy KG, Cone RD. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell. 1993; 72:827–834. [PubMed: 8458079]

Robinson PR, Cohen GB, Zhukovsky EA, Oprian DD. Constitutively Active Mutants of Rhodopsin. Neuron. 1992; 9:719–725. [PubMed: 1356370]

Robinson SW, Jarvie KR, Caron MG. High affinity agonist binding to the dopamine D_3 receptor: chimeric receptors delineate a role for intracellular domains. Mol Pharmacol. 1994; 46:352–356. [PubMed: 7915820]

Rollins PJ, Turner SJ, Akpoguma CI, Ray KP. Characterisation of human adenosine type 1, 2a and 2b receptors expressed in CHO cells. Biochem Soc Trans. 1994; 22:195S. [PubMed: 7958258]

Rose PM, Krystek SJ, Patel PS, Liu EC, Lynch JS, Lach DA, Fisher SM, Webb ML. Aspartate mutation distinguishes ET_A but not ET_B receptor subtype-selective ligand binding while abolishing phospholipase C activation in both receptors. FEBS Lett. 1995; 361:243–249. [PubMed: 7698331]

Rosenkilde MM, Cahir M, Gether U, Hjorth SA, Schwartz TW. Mutations along transmembrane segment II of the NK-1 receptor affect substance P competition with non-peptide antagonists but not substance P binding. J Biol Chem. 1994; 269:28160–28164. [PubMed: 7525569]

Rosenthal W, Antaramian A, Gilbert S, Birnbaumer M. Nephrogenic diabetes insipidus. A V2 vasopressin receptor unable to stimulate adenylyl cyclase. J Biol Chem. 1993; 268:13030–13033. [PubMed: 8514744]

Rosenthal W, Seibold A, Antaramian A, Gilberts, Birnbaumer M, Bichet DG, Arthus MF, Lonergan M. Mutations in the vasopressin V_2 receptor gene in families with naphrogenic diabetes insipidus and functional expression of the Q-2 mutant. Cell Mol Biol. 1994; 40:429–436. [PubMed: 7920187]

Ross PC, Figler RA, Corjay MH, Barber CM, Adam N, Harcus DR, Lynch KR. RTA, a candidate G protein-coupled receptor: cloning, sequencing, and tissue distribution. Proc Natl Acad Sci USA. 1990; 87:3052–3056. [PubMed: 2109324]

Ruat M, Traiffort E, Arrang JM, Leurs R, Schwartz JC. Cloning and tissue expression of a rat histamine H_2-receptor gene. Biochem Biophys Res Commun. 1991; 179:1470–1478. [PubMed: 1930188]

Sachais BS, Snider RM, Lowe JA, Krause JE. Molecular basis for the species selectivity of the substance P antagonist CP-96,345. J Biol Chem. 1993; 268:2319–2323. [PubMed: 7679096]

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 1985; 230:1350–1354. [PubMed: 2999980]

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988; 239:487–491. [PubMed: 2448875]

Salvatore CA, Jacobson MA, Taylor HE, Linden J, Johnson RG. Molecular cloning and characterization of the human A_3 adenosine receptor. Proc Natl Acad Sci USA. 1993; 90:10365–10369. [PubMed: 8234299]

Samama P, Cotecchia S, Costa T, Lefkowitz RJ. A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. J Biol Chem. 1993; 268:4625–4636. [PubMed: 8095262]

Sanderson EM, Strange PG. Expression of epitope-tagged D_2 dopamine receptors in Sf21 cells. Biochem Soc Trans. 1995; 23:89S. [PubMed: 7538953]

Sankararamakrishnan R, Vishveshwara S. Geometry of proline-containing alpha-helices in proteins. Int J Peptide Protein Res. 1992; 39:356–363. [PubMed: 1428525]

Sasakawa N, Sharif M, Hanley MR. Attenuation of agonist-induced desensitization of the rat substance P receptor by progressive truncation of the C-terminus. FEBS Lett. 1994; 347:181–184. [PubMed: 7518397]

Savarese TM, Wang CD, Fraser CM. Site-directed mutagenesis of the rat m1 muscarinic acetylcholine receptor. Role of conserved cysteines in receptor function. J Biol Chem. 1992; 267:11439–11448. [PubMed: 1317867]

Schertler GF, Villa C, Henderson R. Projection structure of rhodopsin. Nature. 1993; 362:770–772. [PubMed: 8469290]

Schwartz TW. Locating ligand-binding sites in 7TM receptors by protein engineering. Curr Opinion Biotechnol. 1994; 5:434–444.

Shapiro RA, Palmer D, Cislo T. A deletion mutation in the third cytoplasmic loop of the mouse m1 muscarinic acetylcholine receptor unmasks cryptic G-protein binding sites. J Biol Chem. 1993; 268:21734–21738. [PubMed: 8408028]

Shenker A, Laue L, Kosugi S, Merendino JJ, Minegishi T, Cutler GJ. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature. 1993; 365:652–654. [PubMed: 7692306]

Shih JC, Gallaher T, Wang CD, Chen K. Site-directed mutagenesis of serotonin 5-HT_2 receptors. J Chem Neuroanat. 1992; 5:281–282. [PubMed: 1524714]

Strader CD, Sigal IS, Register RB, Candelore MR, Rands E, Dixon RA. Identification of residues required for ligand binding to the beta-adrenergic receptor. Proc Natl Acad Sci USA. 1987a; 84:4384–4388. [PubMed: 2885836]

Strader CD, Dixon RA, Cheung AH, Candelore MR, Blake AD, Sigal IS. Mutations that uncouple the beta-adrenergic receptor from G_s and increase agonist affinity. J Biol Chem. 1987b; 262:16439–16443. [PubMed: 2890637]

Strader CD, Sigal IS, Candelore MR, Rands E, Hill WS, Dixon RA. Conserved aspartic acid residues 79 and 113 of the beta-adrenergic receptor have different roles in receptor function. J Biol Chem. 1988; 263:10267–10271. [PubMed: 2899076]

Strader CD, Candelore MR, Hill WS, Dixon RA, Sigal IS. A single amino acid substitution in the beta-adrenergic receptor promotes partial agonist activity from antagonists. J Biol Chem. 1989a; 264:16470–16477. [PubMed: 2570781]

Strader CD, Candelore MR, Hill WS, Sigal IS, Dixon RA. Identification of two serine residues involved in agonist activation of the beta-adrenergic receptor. J Biol Chem. 1989b; 264:13572–13578. [PubMed: 2547766]

Strader CD, Gaffney T, Sugg EE, Candelore MR, Keys R, Patchett AA, Dixon RA. Allele-specific activation of genetically engineered receptors. J Biol Chem. 1991; 266:5–8. [PubMed: 1670767]

Strader CD, Fong TM, Tota MR, Underwood D, Dixon RAF. Structure and function of G protein-coupled receptors. Ann Rev Biochem. 1994; 63:101–132. [PubMed: 7979235]

Straub RE, Freeh GC, Joho RH, Gershengorn MC. Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc Natl Acad Sci USA. 1990; 87:9514–9518. [PubMed: 2175902]

Sugimoto Y, Namba T, Honda A, Hayashi Y, Negishi M, Ichikawa A, Narumiya S. Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype. J Biol Chem. 1992; 267:6463–6466. [PubMed: 1372606]

Suprenant A, Horstman DA, Akbarali II, Limbird LE. A point mutation of the α_2-adrenoceptor that blocks coupling to potassium but not calcium currents. Science. 1992; 257:977–980. [PubMed: 1354394]

Suryanarayana S, Daunt DA, von Zastrow M, Kobilka BK. A point mutation in the seventh hydrophobic domain of the alpha 2 adrenergic receptor increases its affinity for a family of beta receptor antagonists. J Biol Chem. 1991; 266:15488–15492. [PubMed: 1678390]

Suryanarayana S, Kobilka BK. Amino acid substitutions at position 312 in the seventh hydrophobic segment of the beta 2-adrenergic receptor modify ligand-binding specificity. Mol Pharmacol. 1993; 44:111–114. [PubMed: 8101966]

Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. Nature. 1995; 374:542–546. [PubMed: 7700379]

Teeter MM, Froimowitz M, Stec B, DuRand CJ. Homology modeling of the dopamine D_2 receptor and its testing by docking of agonists and tricyclic antagonists. J Med Chem. 1994; 37:2874–2888. [PubMed: 7915325]

ter Laak AM, Timmerman H, Leurs R, Nederkoorn PHJ, Smit M, Donné-Op den Kelder GM. Modelling and mutation studies on the histamine H_1-receptor agonist binding site reveal different binding modes for H_1-agonists: Asp^{116} (TM3) has a constitutive role in receptor stimulation. J Computer-Aided Mol Design. 1995; 9:319–330.

Thomas KM, Pyun HY, Navarro J. Molecular cloning of the fMet-Leu-Phe receptor from neutrophils. J Biol Chem. 1990; 265:20061–20064. [PubMed: 1700779]

Townsend-Nicholson A, Schofield PR. A threonine residue in the seventh transmembrane domain of the human A_1 adenosine receptor mediates specific agonist binding. J Biol Chem. 1994; 269:2373–2376. [PubMed: 8300561]

Trumpp-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M. Modeling of G-Protein-Coupled Receptors—Application to Dopamine, Adrenaline, Serotonin, Acetylcholine, and Mammalian Opsin Receptors. J Med Chem. 1992; 35:3448–3462. [PubMed: 1328638]

Tsukaguchi H, Matsubara H, Aritaki S, Kimura T, Abe S, Inada M. Two novel mutations in the vasopressin V2 receptor gene in unrelated Japanese kindreds with nephrogenic diabetes insipidus. Biochem Biophys Res Commun. 1993; 197:1000–1010. [PubMed: 8267567]

Tucker AL, Robeva AS, Taylor HE, Holeton D, Bockner M, Lynch KR, Linden J. A_1 adenosine receptors. Two amino acids are responsible for species differences in ligand recognition. J Biol Chem. 1994; 269:27900–27906. [PubMed: 7961722]

Turki J, Pak J, Green SA, Martin RJ, Liggett SB. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. J Clin Invest. 1995; 95:1635–1641. [PubMed: 7706471]

van der Wenden EM, Price SL, Apaya RP, IJzerman AP, Soudijn W. Relative binding orientations of adenosine-A_l receptor ligands—a test-case for distributed multipole analysis in medicinal chemistry. J Comp Aided Mol Design. 1995; 9:44–54.

van Galen PJM, Stiles GL, Michaels G, Jacobson KA. Adenosine A_1 and A_2 receptors: structure-function relationships. Med Res Rev. 1992; 12:423–471. [PubMed: 1513184]

van Koppen CJ, Sell A, Lenz W, Jakobs KH. Deletion analysis of the m4 muscarinic acetylcholine receptor. Molecular determinants for activation of but not coupling to the G_i guanine-nucleotide-binding regulatory protein regulate receptor internalization. Eur J Biochem. 1994; 222:525–531. [PubMed: 8020490]

van Rhee AM, Fischer B, van Galen PJM, Jacobson KA. Modelling the P_{2Y} purinoceptor using rhodopsin as template. Drug Design Discovery. 1995 In press.

Venter JC, Fraser CM. beta-Adrenergic receptor isolation and characterization with immobilized drugs and monoclonal antibodies. Fed Proc. 1983; 42:273–278. [PubMed: 6295826]

Vigna SR, Bowden JJ, McDonald DM, Fisher J, Okamoto A, McVey DC, Payan DG, Bunnett NW. Characterization of antibodies to the rat substance P (NK-1) receptor and to a chimeric substance P receptor expressed in mammalian cells. J Neurosci. 1994; 14:834–845. [PubMed: 7507985]

von Heijne G, Manoil C. Membrane proteins: from sequence to structure. Protein Eng. 1990; 4:109–112. [PubMed: 2075184]

von Heijne G. Proline kinks in transmembrane α-helices. J Mol Biol. 1991; 218:499–503. [PubMed: 2016741]

von Zastrow M, Kobilka RK. Antagonist-dependent and -independent steps in the mechanism of adrenergic receptor internalization. J Biol Chem. 1994; 269:18448–18452. [PubMed: 7518433]

Vosberg HP. The polymerase chain reaction: an improved method for the analysis of nucleic acids. Hum Genet. 1989; 83:1–15. [PubMed: 2475423]

Vouret-Craviari V, Grall D, Chambard JC, Rasmussen UB, Pouyssegur J, van Obberghen-Schilling E. Post-translational and activation-dependent modifications of the G protein-coupled thrombin receptor. J Biol Chem. 1995; 270:8367–8372. [PubMed: 7713946]

Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell. 1991; 64:1057–1068. [PubMed: 1672265]

Walker J, Hilderman RH. Identification of a serine protease which activates the mouse heart adenosine 5′,5″,P_1,P_4-tetra-phosphate receptor. Biochemistry. 1993; 32:3119–3123. [PubMed: 8384488]

Wang H, Lipfert L, Malbon CC, Bahouth S. Site-directed anti-peptide antibodies define the topography of the beta-adrenergic receptor. J Biol Chem. 1989; 264:14424–14431. [PubMed: 2474546]

Wang CD, Buck MA, Fraser CM. Site-directed mutagenesis of alpha 2A-adrenergic receptors: identification of amino acids involved in ligand binding and receptor activation by agonists. Mol Pharmacol. 1991; 40:168–179. [PubMed: 1678850]

Wang CD, Gallaher TK, Shih JC. Site-directed mutagenesis of the serotonin 5-hydroxytrypamine 2 receptor: Identification of amino acids necessary for ligand binding and receptor activation. Mol Pharmacol. 1993a; 43:931–940. [PubMed: 8316224]

Wang Z, Wang H, Ascoli M. Mutation of a highly conserved acidic residue present in the second intracellular loop of G-protein-coupled receptors does not impair hormone binding or signal transduction of the luteinizing hormone/chorionic gonadotropin receptor. Mol Endocrinol. 1993b; 7:85–93. [PubMed: 8383288]

Wang X, Souza SC, Kelder B, Cioffi JA, Kopchick JJ. A 40-amino acid segment of the growth hormone receptor cytoplasmic domain is essential for GH-induced tyrosine-phosphorylated cytosolic proteins. J Biol Chem. 1995; 270:6261–6266. [PubMed: 7534308]

Wank SA, Harkins R, Jensen RT, Shapira H, de Weerth A, Slattery T. Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas. Proc Natl Acad Sci USA. 1992; 89:3125–3129. [PubMed: 1313582]

Webb TE, Simon J, Krishek BJ, Bateson AN, Smart TG, King BF, Burnstock G, Barnard EA. Cloning and functional expression of a brain G-protein-coupled ATP receptor. FEBS Lett. 1993; 324:219–225. [PubMed: 8508924]

Weitz CJ, Nathans J. Histidine residues regulate the transition of photoexcited rhodopsin to its active conformation, metarhodopsin II. Neuron. 1992; 8:465–472. [PubMed: 1532320]

Wess J, Brann MR, Bonner TI. Identification of a small intracellular region of the muscarinic m3 receptor as a determinant of selective coupling to PI turnover. FERS Lett. 1989; 258:133–136.

Wess J, Gdula D, Brann MR. Site-directed mutagenesis of the m3 muscarinic receptor: Identification of a series of threonine and tyrosine residues involved in agonist but not antagonist binding. EMBO J. 1991; 10:3729–3734. [PubMed: 1657592]

Wess J, Gdula D, Brann MR. Structural basis of the subtype selectivity of muscarinic antagonists: A study with chimeric m2/m5 muscarinic receptors. Mol Pharmacol. 1992a; 41:369–374. [PubMed: 1538713]

Wess J, Maggio R, Palmer JR, Vogel Z. Role of conserved threonine and tyrosine residues in acetylcholine binding and muscarinic receptor activation. A study with m3 muscarinic receptor point mutants. J Biol Chem. 1992b; 267:19313–19319. [PubMed: 1527051]

Wess J, Nanavati S, Vogel Z, Maggio R. Functional role of proline and tryptophan residues highly conserved among G protein-coupled receptors studied by mutational analysis of the m3 muscarinic receptor. EMBO J. 1993; 12:331–338. [PubMed: 7679072]

White TJ, Arnheim N, Erlich HA. The polymerase chain reaction. Trends Genet. 1989; 5:185–189. [PubMed: 2672459]

White SH, Jacobs RE. Observations concerning topology and locations of helix ends of membrane proteins of known structure. J Membrane Biol. 1990; 115:145–158. [PubMed: 2192066]

Wildin RS, Antush MJ, Bennett RL, Schoof JM, Scott CR. Heterogeneous AVPR2 gene mutations in congenital nephrogenic diabetes insipidus. Am J Hum Genet. 1994; 55:266–277. [PubMed: 7913579]

Wong SK, Slaughter C, Ruoho AE, Ross EM. The catecholamine binding site of the beta-adrenergic receptor is formed by juxtaposed membrane-spanning domains. J Biol Chem. 1988; 263:7925–7928. [PubMed: 2836401]

Wong SK, Ross EM. Chimeric muscarinic cholinergic: beta-adrenergic receptors that are functionally promiscuous among G proteins. J Biol Chem. 1994; 269:18968–18976. [PubMed: 8034654]

Wu LH, Vartanian MA, Oxender DL, Chung FZ. Identification of methionine134 and alanine146 in the second transmembrane segment of the human tachykinin NK3 receptor as reduces involved in species-selective binding to SR 48968. Biochem Biophys Res Commun. 1994; 198:961–966. [PubMed: 8117303]

Yamano Y, Ohyama K, Chaki S, Guo DF, Inagami T. Identification of amino acid residues of rat angiotensin II receptor for ligand binding by site directed mutagenesis. Biochem Biophys Res Commun. 1992; 187:1426–1431. [PubMed: 1417818]

Yamano Y, Ohyama K, Kikyo M, Sano T, Nakagomi Y, Inoue Y, Nakamura N, Morishima I, Guo DF, Hamakubo T, Inagami T. Mutagenesis and the molecular modeling of the rat angiotensin II receptor (AT_1). J Biol Chem. 1995; 270:14024–14030. [PubMed: 7775462]

Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizuguchi H, Wada H. Expression cloning of a cDNA encoding the bovine histamine H_1 receptor. Proc Natl Acad Sci USA. 1991; 88:11515–11519. [PubMed: 1722337]

Yano K, Saji M, Hidaka A, Moriya N, Okuno A, Kohn LD, Cutler GJ. A new constitutively activating point mutation in the luteinizing hormone/choriogonadotropin receptor gene in cases of male-limited precocious puberty. J Clin Endocrinol Metab. 1995; 80:1162–1168. [PubMed: 7714085]

Ye RD, Prossnitz ER, Zou AH, Cochrane CG. Characterization of a human cDNA that encodes a functional receptor for platelet activating factor. Biochem Biophys Res Commun. 1991; 180:105–111. [PubMed: 1656963]

Yokota Y, Sasai Y, Tanaka K, Fujiwara T, Tsuchida K, Shigemoto R, Kakizuka A, Ohkubo H, Nakanishi S. Molecular characterization of a functional cDNA for rat substance P receptor. J Biol Chem. 1989; 264:17649–17652. [PubMed: 2478537]

Yokoyama R, Knox BE, Yokoyama S. Rhodopsin from the fish, Astyanax: Role of tyrosine 261 in the red shift. Invest Ophthalmol Vis Sci. 1995; 36:939–945. [PubMed: 7706043]

Zhang D, Weinstein H. Polarity conserved positions in transmembrane domains of G-protein coupled receptors and bacteriorhodopsin. FEBS Lett. 1994; 337:207–212. [PubMed: 8287978]

Zhou W, Flanagan C, Ballesteros JA, Konvicka K, Davidson JS, Weinstein H, Millar RP, Sealfon SC. A reciprocal mutation supports helix 2 and helix 7 proximity in the gonadotropin-releasing hormone receptor. Mol Pharmacol. 1994; 2:165–170. [PubMed: 8114667]

Zhu G, Wu LH, Mauzy C, Egloff AM, Mirzadegan T, Chung FZ. Replacement of lysine-181 by aspartic acid in the third transmembrane region of endothelin type B receptor reduces its affinity to endothelin peptides and sarafotoxin 6c without affecting G protein coupling. J Cell Biochem. 1992; 50:159–164. [PubMed: 1429881]

Zhukovsky EA, Oprian DD. Effect of carboxylic acid side chains on the absorption maximum of visual pigments. Science. 1989; 246:928–930. [PubMed: 2573154]

Zoffmann S, Gether U, Schwartz TW. Conserved HisVI-17 of the NK-1 receptor is involved in binding of non-peptide antagonists but not substance P. FEBS Lett. 1993; 336:506–510. [PubMed: 7506676]

Zvyaga TA, Min KC, Beck M, Sakmar TP. Movement of the retinylidene Schiff base counterion in rhodopsin by one helix turn reverses the pH dependence of the metarhodopsin I to metarhodopsin II transition. J Biol Chem. 1993; 268:4661–4667. [PubMed: 8444840]

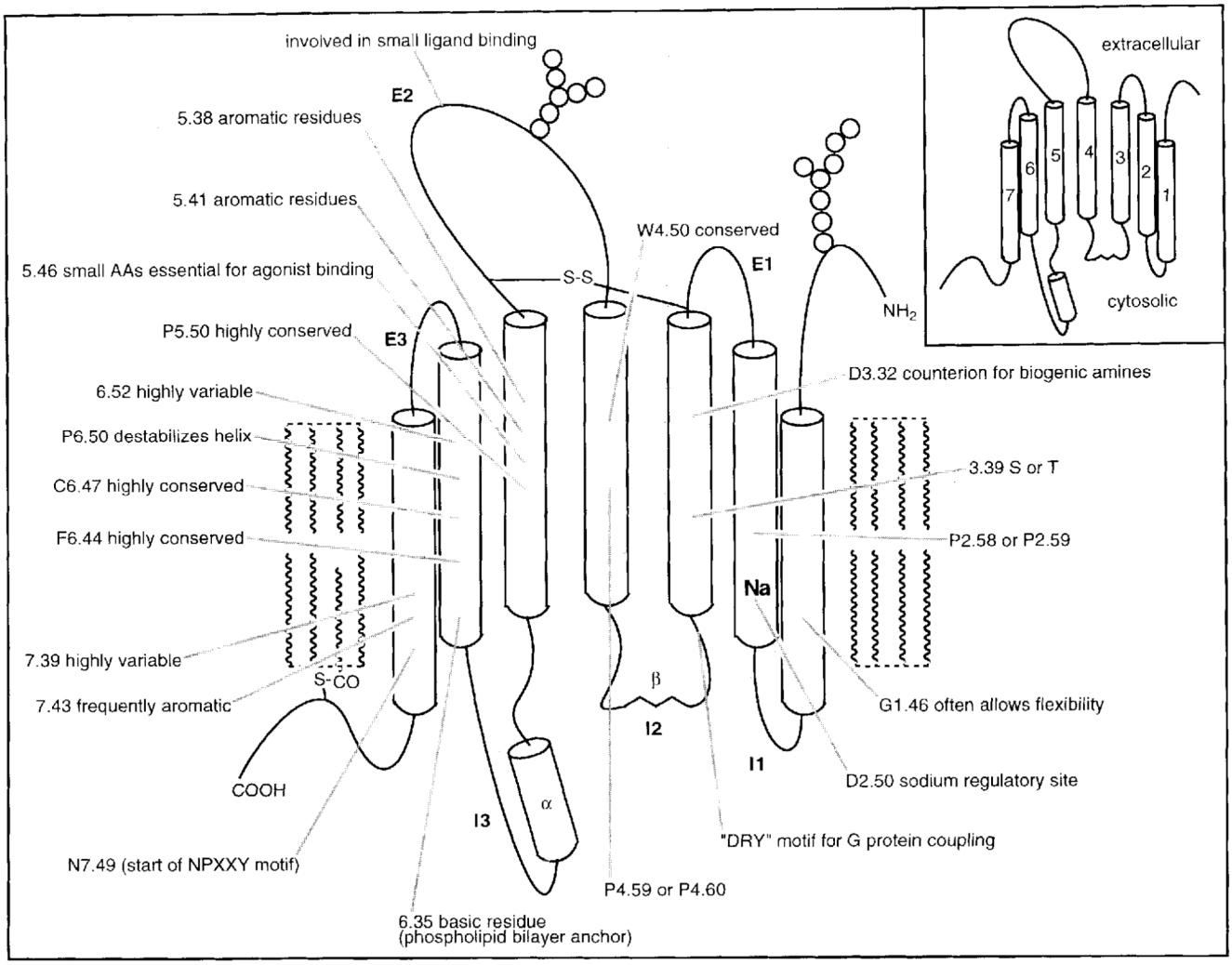


Figure 1.

Schematic presentation of the general topology of GPCRs. NT = N-terminus; CT = C-terminus; Ix = intracellular loop x; Ex = extracellular loop x; α indicates a supposed α-helical fragment of I3; β indicates a supposed β-pleated sheet substructure in I2; S-S indicates a possible cystine bond between TM3 and TM5; stacked circles indicate tentative ribosylation sites. Glycosylation has been shown to occur at either or both NT and E2; S-CO denotes a possible acylation site in CT. The numbering of the TMs is shown in the inset in the upper right corner.

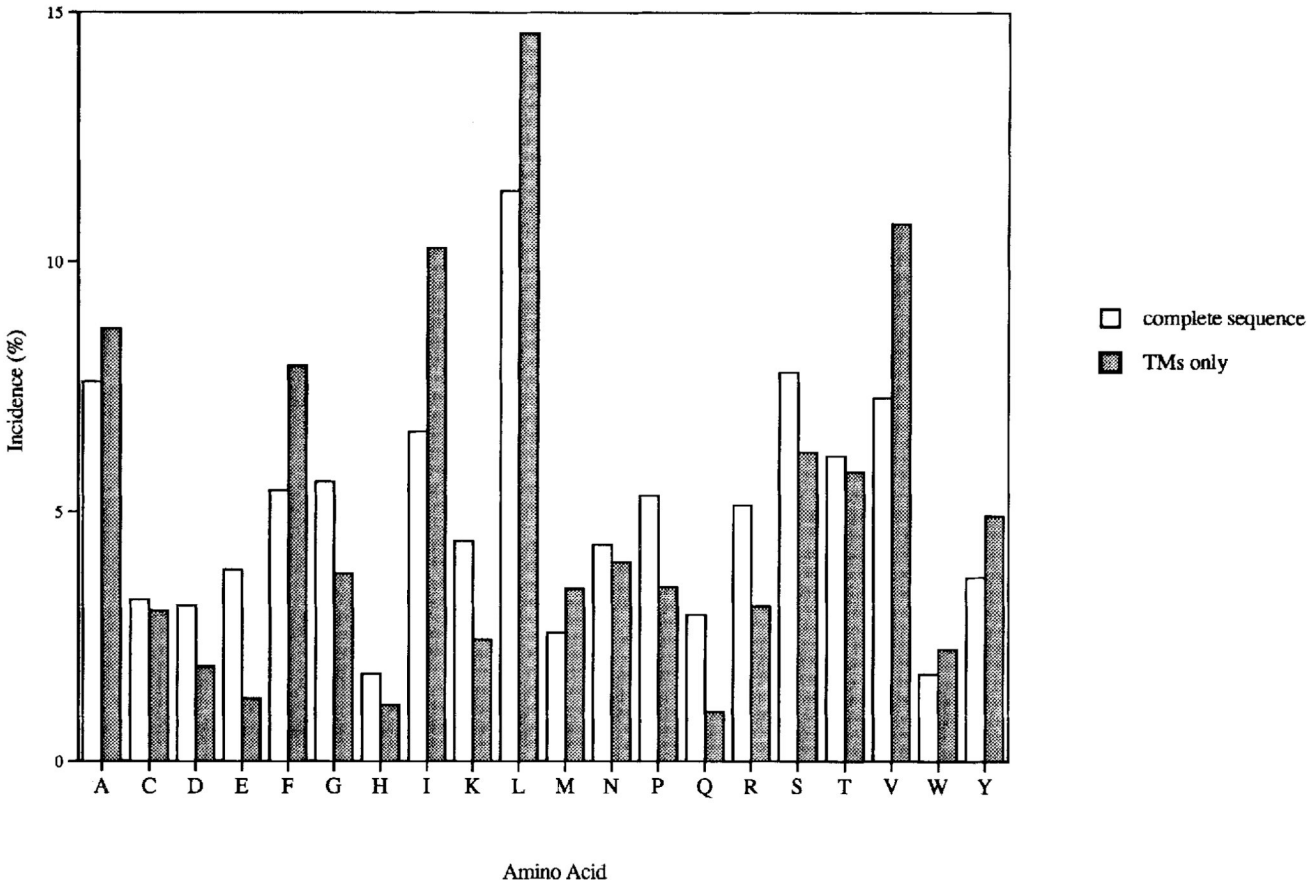


Figure 2.
Amino acid distribution throughout the GPCRs used in Table 3.

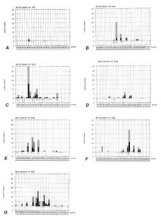


Figure 3.
Mutation distribution in the transmembrane regions of GPCRs from Table 3. Open bars (□) represent the number of ineffective mutants reported; shaded bars (▧) represent the number of reports where agonist affinity (AG) is affected; closed bars (■) represent the number of reports where antagonist affinity (AN) is affected.

TABLE 1

Cloned G Protein-Coupled Receptors

Class	Refcode	Name (if not human: species)
Peptide/cytokine	P30968	Gonadotropin-releasing hormone: GRH
	P34981	Thyrotropin-releasing hormone: TRH
	P25116	Thrombin: THR
	P23945	Follicle stimulating hormone: FSH
	P16473	Thyrotropin: TSH
	P22888	Luteinizing hormone: LH/CG
	Q01718	Adrenocorticotropic hormone: ACTH/MC2
	Q01726	Melanocyte Stimulating hormone: MSH/MC1
	B46647	Melanocortin MC3
	P32245	Melanocortin MC4
	P33032	melanocortin MC5
	U14108	Melatonin
	P30559	Oxytocin
	P37288	Vasopressin V_{1A}
	A55089	Vasopressin V_{1B}
	P30518	Vasopressin V_2
	P32238	Cholecystokinin CCK-A
	P32239	Cholecystokinin CCK-B
	P25929	Neuropeptide Y: NPY1
	P25103	Neurokinin NK1
	P21452	Neurokinin NK2
	P29371	Neurokinin NK3
	P25101	Endothelin ET-A
	P24530	Endothelin ET-B
	P28336	Neuromedin B: NMB
	P30872	Somatostatin SSTR1
	P30874	Somatostatin SSTR2
	P32745	Somatostatin SSTR3
	P31391	Somatostatin SSTR4
	P35346	Somatostatin SSTR5
	P25024	Interleukin IL-8A
	P25025	Interleukin IL-8B
	P30556	Angiotensin AT_{1a}
	P29089	Angiotensin AT_{1b} (*Rattus norvegicus*)
	JC2435	Angiotensin AT_2
	U12512	Bradykinin BK1
	P30411	Bradykinin BK2
	P21462	Formyl-Met-Leu-Phe peptide: fMLP
	JC2194	Vaso-intestinal peptide VIP-IVR8

Class	Refcode	Name (if not human: species)
	S38397	Vaso-intestinal peptide VIP-PACAP
	P32241	Vaso-intestinal peptide VIP1
	JC2195	Vaso-intestinal peptide VIP-IVR5
	P35000	Vaso-intestinal peptide VIP2 (*Rattus norvegicus*)
	JC2463	Vaso-intestinal peptide VIP-T1
	L04308	Parathyroid hormone
	L23332	Corticotropin releasing hormone
	U10037	Glucagon
	U20178	Secretin
lipid mediator	P34995	Prostaglandin E_2 PGE-EP1
	P35408	Prostaglandin E_2 PGE-EP2
	U19487	Prostaglandin E_2 PGE-EP3
	L24470	Prostaglandin $F_{2\alpha}$:PGF
	P21731	Thromboxane A_2: TXA_2
	P25105	Platelet-activating factor: PAF
biogenic amine	P08172	Muscarinic acetylcholine M2
	P08173	Muscarinic acetylcholine M4
	P11229	Muscarinic acetylcholine M1
	P20309	Muscarinic acetylcholine M3
	P08912	Muscarinic acetylcholine M5
	P35367	Histamine H_1
	P25021	Histamine H_2
	P08908	Serotonin $5HT_{1A}$
	P28222	Serotonin $5HT_{1B}$
	P28221	Serotonin $5HT_{1D}$
	P28566	Serotonin $5HT_{1E}$
	P30939	Serotonin $5HT_{1F}$
	P28223	Serotonin $5HT_{2A}$
	X77307	Serotonin $5HT_{2B}$
	P28335	Serotonin $5HT_{2C}$
	X81411	Serotonin $5HT_{5A}$
	P35365	Serotonin $5HT_{5B}$ (*Rattus norvegicus*)
	P31388	Serotonin $5HT_6$ (*Rattus norvegicus*)
	P34969	Serotonin $5HT_7$
	P25100	Adrenergic α_{1A}
	P35368	Adrenergic α_{1B}
	P35348	Adrenergic α_{1C}
	P08913	Adrenergic α_{2A}
	P18089	Adrenergic α_{2B}
	P18825	Adrenergic α_{2C1}

Class	Refcode	Name (if not human: species)
	P35369	Adrenergic α_{2C2}
	P08588	Adrenergic β_1
	P07550	Adrenergic β_2
	P13945	Adrenergic β_3
	P21728	Dopamine D_{1A}
	P14416	Dopamine D_2
	P35462	Dopamine D_3
	P21917	Dopamine D_4
	P21918	Dopamine D_5
nucleoside	P30542	Adenosine A_1
	P29274	Adenosine A_{2a}
	P29275	Adenosine A_{2b}
	P33765	Adenosine A_3
nucleotide	Z49205	P_2 purinergic P_{2Y}/$P2Y_1$
	P41231	P_2 purinergic P_{2U}/$P2Y_2$
	U41070	P_2 purinergic P_{2Y}/$P2Y_5$
	D63665	P_2 purinergic P_{2Y}/$P2Y_6$
cyclic AMP	P13773	Cyclic AMP-1 (*Dictyostelium discoideum)*
	P34907	Cyclic AMP-2 (*Dictyostelium discoideum*)
	P35352	Cyclic AMP-3 (*Dictyostelium discoideum)*
octopamine	P22270	Octopamine (*Drosophila melanogaster*)
opsin	P03999	Blue opsin
	P04001	Green opsin
	P04000	Red opsin
	P08100	Rhodopsin
	M92039	Violet opsin (*Gallus gallus*)
	L03781	Opsin (*Limulus polyphemus*)
opioid	P41143	δ opioid
	P41145	κ opioid
	P35372	μ opioid
sensory (olfactory)	145774	OLF1 (*Ictalurus punctatis*)
	H45774	OLF3 (*Ictalurus punctatis*)
	E45774	OLF8 (*Ictalurus punctatis*)
	D45774	OLF32A (*Ictalurus punctatis*)
	C45774	OLF32B (*Ictalurus punctatis*)
	B45774	OLF32C (*Ictalurus punctatis*)
	A45774	OLF32D (*Ictalurus punctatis*)
	F45774	OLF47 (*Ictalurus punctatis*)
	G45774	OLF202 (*Ictalurus punctatis*)
	P37067	OLFCOR1 (*Gallus gallus*)

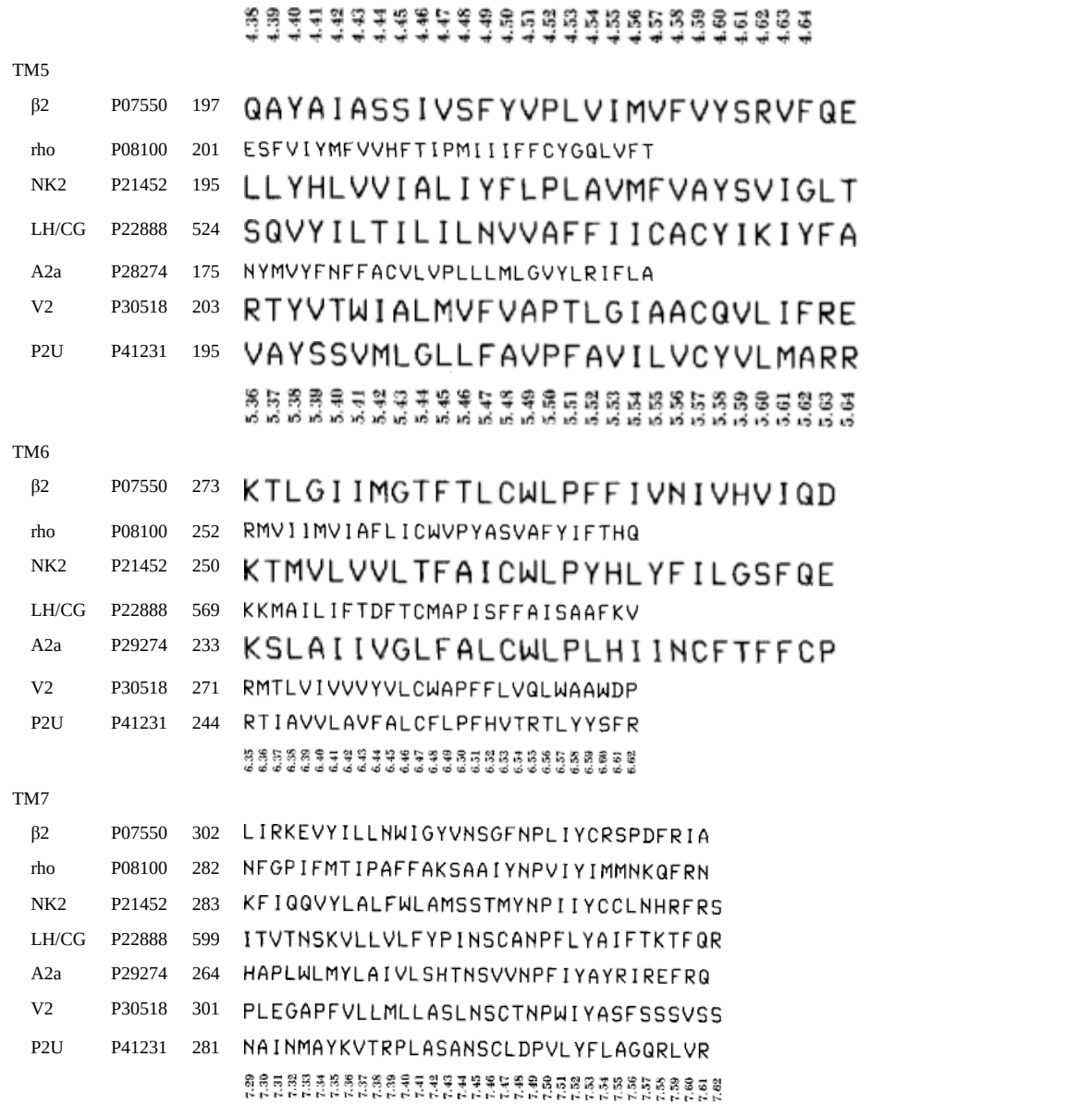
Class	Refcode	Name (if not human: species)
	P37068	OLFCOR2 (*Gallus gallus*)
	P37069	OLFCOR3 (*Gallus gallus*)
	P37070	OLFCOR4 (*Gallus gallus*)
	P37071	OLFCOR5 (*Gallus gallus*)
	P37072	OLFCOR6 (*Gallus gallus*)
	P30955	OLF (*Canis familiaris*)
	P34982	OLF07E
	P30953	OLF07I
	P30954	OLF07J
	P23275	OLFOR3 (*Mus musculus*)
	S47014	OLF (*Rattus norvegicus*)
	P23265	OLFF3 (*Rattus norvegicus*)
	P23266	OLFF5 (*Rattus norvegicus*)
	P23267	OLFF6 (*Rattus norvegicus*)
	P23268	OLFF12 (*Rattus norvegicus*)
	P23269	OLF13 (*Rattus norvegicus*)
	P23270	OLF17 (*Rattus norvegicus*)
	P23271	OLF18 (*Rattus norvegicus*)
	P23272	OLF19 (*Rattus norvegicus*)
	P23273	OLF114 (*Rattus norvegicus*)
	P23274	OLF115 (*Rattus norvegicus*)
	X80391	OLFOR17–40
(gustatory)	D12820	GUST27 (*Rattus norvegicus*)
metabotropic	P23385	MG1 (*Rattus norvegicus*)
glutamate	JC2132	MG5A
	JC2131	MG5B
	P31424	MG5 (*Rattus norvegicus*)
	P31423	MG4 (*Rattus norvegicus*)
	P35400	MG7 (*Rattus norvegicus*)
	P35349	MG6 (*Rattus norvegicus*)
	P31421	MG2 (*Rattus norvegicus*)
	P31422	MG3 (*Rattus norvegicus*)
calcium	U20769	Ca^{2+} -sensitive GPCR
calcitonin	P30988	calcitonin
pheromone	Q00619	MAM2 (*Schizosaccharomyces pombe*)
	P06842	STE2 (*Saccharomyces cerevisiae*)
	P31302	PRA1 (*Ustilago maydis*)
	P31303	PRA2 (*Ustilago maydis*)
	P31397	MAP3 (*Schizosaccharomyces pombe*)
	P06783	STE3 (*Saccharomyces cerevisiae*)
orphan	P41146	OPRX
receptors	Z28332	AERG (*Anthopleura elegantissima*)

Class	Refcode	Name (if not human: species)
	P35412	GP01 (*Mus musculus)*
	P30951	R334 (*Rattus norvegicus*)
	P35413	GP21 (*Mus musculus*)
	P30731	GCRC (*Mus musculus*)
	P30098	TXKR
	P31392	G10D (*Rattus norvegicus*)
	M64749	RDC1
	P32302	BLR1
	L06797	CL5
	P30991	LCR1
	P32248	EBI1
	P35411	RBS1 (*Rattus norvegicus*)
	P32249	EBI2
	P32250	GCRT (*Gallus gallus*)
	P35414	APJ
	P23749	RTA (*Rattus norvegicus*)
	S77867	UHR (*Rattus norvegicus*)
	P25089	FMRL1
	P25090	FMRL2
	U14910	RPE
	QQBED3	HHRF1
	QQBED2	HHRF2
	QQBET9	HHRF3
	U03882	MCP-1A
	U03905	MCP-1B
	P35350	PPR1 (*Bos taurus*)
	QQBEQ4	ALB (*saimiriine herpes virus 1*)
	U17473	Calcitonin-like

TABLE 2

Alignment of Transmembrane Domains of Selected GPCRs

TM1			
β2	P07550	33	VVGMGIVMSLIVLAIVFGNVLVITAIAKFER
rho	P08100	37	FSMLAAYMFLLIVLGFPINFLTLYVTVQHKK
NK2	P21452	33	LALWAPAYLALVLVAVTGNAIVIWIILAHRR
LH/CG	P22888	359	YDFLRVLIWLINILAIMGNMTVLFVLLTSRY
A2a	P29274	6	SSVYITVELAIAVLAILGNVLVCWAVWLNSN
V2	P30518	37	ARAELALLSIVFVAVALSNGLVLAALARRGR
P2U	P41231	33	YVLLPVSYGVVCVLGLCLNAVGLYIFLCRLK
			1.32 1.33 1.34 1.35 1.36 1.37 1.38 1.39 1.40 1.41 1.42 1.43 1.44 1.45 1.46 1.47 1.48 1.49 1.50 1.51 1.52 1.53 1.54 1.55 1.56 1.57 1.58 1.59 1.60 1.61 1.62
TM2			
β2	P07550	69	NYFITSLACADLVMGLAVVPFGAAHILM
rho	P08100	73	NYILLNLAVADLFMVLGGFTSTLYTSLH
NK2	P21452	69	NYFIVNLALADLCMAAFNAAFNFVYASH
LH/CG	P22888	395	RFLMCNLSFADFCMGLYLLLIASVDSQT
A2a	P29274	52	NYFVVSLAAADIAVGVLAIPFAITISTG
V2	P30518	75	HVFIGHLCLADLAVALFQVLPQLAWKAT
P2U	P41231	69	TTYMFHLAVSDALYAASLPLLVYYYARG
			2.40 2.41 2.42 2.43 2.44 2.45 2.46 2.47 2.48 2.49 2.50 2.51 2.52 2.53 2.54 2.55 2.56 2.57 2.58 2.59 2.60 2.61 2.62 2.63 2.64 2.65 2.66 2.67
TM3			
β2	P07550	117	EFWTSIDVLCVTASIETLCVIAVDRY
rho	P08100	111	NLEGFFATLGGEIALWSLVVLAIERY
NK2	P21452	117	YFQNLFPITAMFVSIYSMTAIAADRY
LH/CG	P22888	440	STAGFFTVFASELSVYTLTVITLERW
A2a	P29274	78	LFIACFVLVLTQSSIFSLLAIAIDRY
V2	P30518	113	RAVKYLQMVGMYASSYMILAMTLDRH
P2U	P41231	107	KLVRFLFYTNLYCSILFLTCISVHRC
			3.26 3.27 3.28 3.29 3.30 3.31 3.32 3.33 3.34 3.35 3.36 3.37 3.38 3.39 3.40 3.41 3.42 3.43 3.44 3.45 3.46 3.47 3.48 3.49 3.50 3.51
TM4			
β2	P07550	146	TKNKARVIILMVWIVSGLTSFLPIQMH
rho	P08100	149	GENHAIMGVAFTWVMALACAAPPLAGW
NK2	P21452	144	SAPSTKAVIAGIWLVALALASPQCFYS
LH/CG	P22888	479	RLRHAILIMLGGWLFSSLIAMLPLVGV
A2a	P29274	117	TGTRAKGIIAICWVLSFAIGLTPMLGW
V2	P30518	152	SGAHWNRPVLVAWAFSLLLSLPQLFIF
P2U	P41231	146	RARYARRVAGAVWVLVLACQAPVLYFV



*A short receptor name, the accession number (SwissProtein), and the first residue number for the sequence are indicated at each line. The sequence identifiers (extended notation) are indicated underneath each column. Interconversion of notation schemes can be achieved by applying the following: (1.50) = 117H = 130O = I:18B; (2.50) 209H = 224O = II:14B; (3.50) = 326H = 340O = III:25; (4.50) = 406H = 420O = IV:11B; (5.50) = 511H = 520O = V:14B; (6.50) = 615H = 620O = VI:18B; (7.50) = 722H = 730O = VII:18B; where H = notation as proposed by Hibert et al [1991]; O = notation as proposed by Oliveira et al [1993]; and B = notation as proposed by Baldwin [1993].

TABLE 3

Genetically Engineered and Naturally Occurring Point Mutations in GPCRs

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
m5/m2	Human	T37A(1.39)	AG & AN & act. restored	Liu et al., 1995
V2	Human	L44F(1.39)	decr. act.	Knoers et al., 1994
NK3	Rat	L81V(1.41)	n.e.	Wu et al., 1994
NK3	Rat	F88L(1.48)	n.e.	Wu et al., 1994
rho	Bovine	T58R(1.53)	decr. act.	Min et al., 1993
V2	Human	L62P(1.57)	decr. act.	Knoers et al., 1994
TXA_2	Human	R60L(2.36)	decr. act. (PI)	Hirata et al., 1994
β_2	Human	C77S(2.48)	n.e	Fraser et al., 1989b
β_2	Hamster	C77V(2.48)	AG & AN n.a.	Dixon et al., 1987
β_2	Hamster	C77V(2.48)	AG & AN n.a.	Dohlman et al., 1990
m1	Rat	C69S(2.48)	decr. AG; AN & act. n.a.	Savarese et al., 1992
$5HT_{1A}$	Rat	D82N(2.50)	decr. AG; AN & act. n.a.	Ho et al., 1992
$5HT_{1A}$	Human	D82N(2.50)	decr. AG; AN & act. n.a.	Ho et al., 1992
$5HT_2$	Rat	D120N(2.50)	decr. AG & AN; decr. act.	Wang et al., 1993a
α_2	Porcine	D79N(2.50)	decr. act.; AG & AN n.a.; Na	Horstman et al., 1990
β_2	Human	D79N(2.50)	decr. act.; AG & AN n.a.	Wang et al., 1991
α_{2a}	Murine	D79N(2.50)	incr. AG; decr. act. (K); act. n.a. (AC)	Suprenant et al., 1992
AT_{1a}	Rat	D74E(2.50)	AG & AN n.a.; decr. act.	Bihoreau et al., 1993
AT_{1a}	Rat	D74N(2.50)	AG n.a.; AN aff.; decr. act.	Bihoreau et al., 1993
β_2	Hamster	D79A(2.50)	decr. AG; AN n.a.	Strader et al., 1987a
β_2	Hamster	D79A(2.50)	decr. AG; AN & act. n.a.	Strader et al., 1988
β_2	Hamster	D79E(2.50)	decr. AG; AN & act. n.a.	Strader et al., 1989
β_2	Human	D79N(2.50)	decr. AG; AN n.a.; decr. act.	Wang et al., 1989
β_2	Human	D79N(2.50)	decr. AG; AN & act. n.a.	Chung et al., 1988
D_2	Rat	D80A,E(2.50)	decr. AG & act.; Na; AN n.a.	Neve et al., 1991
ET-A	Human	D126A(2.50)	selec. AG; decr. act. (PLC)	Rose et al., 1995
ET-B	Human	D147A(2.50)	AG & AN n.a.; decr. act. (PI)	Rose et al., 1995
GRH	Human	N87D(2.50)	decr. AG; decr. act. (PI)	Davidson et al., 1994b
LH/CG	Rat	D383N(2.50)	decr. AG & act.	Ji and Ji, 1991
LH/CG	Rat	D383N(2.50)	decr. AG & act.; Na	Quintana et al., 1993
m1	Rat	D71N(2.50)	inc. AG; AN n.a.; decr. eff.	Fraser et al., 1989b
NK1	Human	E78A(2.50)	n.e.	Rosenkilde et al., 1994
rho	Bovine	D83A(2.50)	n.e.	Zhukovsky and Oprian, 1989
SST2	Murine	D89N(2.50)	AG & act. n.a.	Kong et al., 1993
TRH	Murine	D71A(2.50)	decr. AG & AN & act.	Perlman et al., 1992
V2	Human	D85N(2.50)	decr. act.	Knoers et al., 1994
ET-A	Human	Y129A(2.53)	sub. selec. AG	Krystek et al., 1994
ET-B	Human	H150A,Y(2.53)	selec. AG & AN	Rose et al., 1995

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
NK3	Rat	V121M(2.53)	incr. AN; spec. selec.	Wu et al., 1994
V2	Human	V88H(2.53)	decr. act.	Knoers et al., 1994
V2	Human	V88M(2.53)	n.e.	Bichet et al., 1993
NK1	Human	M81A(2.54)	n.e.	Fong et al., 1994b
$5HT_2$	Human	F125L(2.55)	n.e.	Choudhary et al., 1993
ET-A	Human	D133A(2.57)	decr. AN; AG & act. n.a.	Rose et al., 1995
ET-B	Human	D154A(2.57)	n.e.	Rose et al., 1995
m3	Rat	S120A(2.57)	decr. AN; AG & act. n.a.	Wess et al., 1991
NK1	Human	N85A(2.57)	decr. AN; AG n.a.	Rosenkilde et al., 1994
MSH	Murine	E92K(2.60)	const. act.	Robbins et al., 1993
m1	Human	Y82F(2.61)	AG n.a.	Drubbisch et al., 1992
NK1	Human	N89A(2.61)	n.e.	Rosenkilde et al., 1994
NK1	Human	Y92A(2.64)	decr. AN; AG n.a.	Rosenkilde et al., 1994
NK3	Rat	G133A(2.65)	incr. AN; spec. selec.	Wu et al., 1994
NK1	Human	N96A(2.68)	n.e.	Rosenkilde et al., 1994
V2	Human	P108M,V(2.73)	n.e.	Pan et al., 1992
AT_{1a}	Rat	C101G(3.25)	decr. AG	Yamano et al., 1992
β_2	Hamster	C106V(3.25)	n.e.	Dixon et al., 1987
rho	Bovine	C110A(3.25)	decr. stab.	Davidson et al., 1994a
β_2	Hamster	E107A(3.26)	n.e.	Strader et al., 1987a
D_2	Human	D108C(3.26)	AN n.a.; SH +	Javitch et al., 1995
m1	Rat	D99N(3.26)	decr. AG & AN; act. n.a.	Fraser et al., 1989b
P_{2U}	Human	K107I(3.26)	n.e.	Erb et al., 1995
V_2	Human	R113W(3.26)	decr. AG; act. n.a.; imp. proc.?	Birnbaumer et al., 1994
D_2	Human	I109C(3.27)	AN n.a.; SH+	Javitch et al., 1995
D_2	Human	F110C(3.28)	decr. AN; SH +	Javitch et al., 1995
NK2	Human	Q109H(3.28)	decr. AG; AN n.a.	Bhogal et al., 1994
rho	Bovine	E113A,Q(3.28)	destab. Schiff base	Lin et al., 1992
rho	Bovine	E113Q(3.28)	const. act.	Cohen et al., 1992
rho	Bovine	E113Q(3.28)	const. act.	Robinson et al., 1992
D_2	Human	V111C(3.29)	AN n.a.; SH ++	Javitch et al., 1995
NK1	Human	N109A(3.29)	n.e.	Fong et al., 1994b
P_{2U}	Human	K110L(3.29)	n.e.	Erb et al., 1995
TRH	Murine	T102V(3.29)	n.e.	Perlman et al., 1994b
D_2	Human	T112C(3.30)	AN n.a ; SH−	Javitch et al., 1995
TRH	Murine	Y103F(3.30)	n.e.	Perlman et al., 1994b
D_2	Human	C113C(3.31)	decr. AN; SH−	Javitch et al., 1995
$5HT_{1A}$	Human	D116N(3.32)	decr. AG; AN & act. n.a.	Ho et al., 1992
$5HT_2$	Rat	D155N(3.32)	decr. AG & AN	Wang et al., 1993a
α_2	Human	D113N(3.32)	decr. act. & AN	Wang et al., 1991
β_2	Hamster	D113E(3.32)	decr. AG & AN; act. n.a.	Strader et al., 1988

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
β_2	Hamster	D113E(3.32)	decr. AG & AN; AN as part. AG	Strader et al., 1989
β_2	Hamster	D113N(3.32)	decr. AG & AN	Strader et al., 1987a
β_2	Hamster	D113Q(3.32)	decr. AG & AN; act. n.a.	Strader et al., 1988
β_2	Hamster	D113S(3.32)	decr. AN aff.; AG mod.	Strader et al., 1991
D_2	Human	D114C(3.32)	decr. AN; SH ++	Javitch et al., 1995
D_2	Rat	D114G,N(3.32)	decr. AG & AN	Mansour et al., 1992
H_1	Human	D107A(3.32)	decr. AN & act.	Ohta et al., 1994
H_1	Human	D107N(3.32)	decr. AG & AN & act.	Ohta et al., 1994
H_1	Human	D107E(3.32)	decr. AN & act.	Ohta et al., 1994
H_2	Canine	D98N(3.32)	decr. AG & AN & act.	Gantz et al., 1992
m1	Rat	D105(3.32)	binds aff. reag.	Kurtenbach et al., 1990
m1	Rat	D105E(3.32)	decr. AG; AN & act. n.a.; uncoupling?	Page et al., 1995
m1	Rat	D105N(3.32)	decr. AG & AN	Fraser et al., 1989b
m1	Rat	D105N(3.32)	decr. AG & AN & act.	Page et al., 1995
m2	Rat	D103E(3.32)	decr. AG; AN & act. n.a.; uncoupling?	Page et al., 1995
NK1	Human	P112A(3.32)	n.e.	Fong et al., 1994b
rho	Bovine	A117F(3.32)	Blue shift	Ridge et al., 1992
TRH	Murine	Q105A,E,L,V(3.32)	decr. AG; act. n.a.; perturb. BD	Perlman et al., 1994b
D_2	Human	V115C(3.33)	decr. AN; SH ++	Javitch et al., 1995
ET-B	Rat	K181D(3.33)	decr. AG; act. n.a.	Zhu et al., 1992
m3	Rat	Y148F(3.33)	decr. AG; AN & act. n.a.	Wess et al., 1991
m3	Rat	Y148F(3.33)	decr. AG	Wess et al., 1992b
TRH	Murine	Y106F(3.33)	decr. AG; act. n.a.	Perlman et al., 1994a
D_2	Human	M116C(3.34)	AN n.a.; SH−	Javitch et al., 1995
D_2	Human	M116L(3.34)	AG & AN n.a.	Mansour et al., 1992
AT_1	Human	N111A(3.35)	incr. AG; AG selec.; decr. AN	Perlman et al., 1995b
β_2	Hamster	C116V(3.35)	AG & AN n.a.	Dixon et al., 1987
D_2	Human	M117C(3.35)	AN n.a.; SH−	Javitch et al., 1995
D_2	Human	M117C,G(3.35)	AG & AN n.a.	Mansour et al., 1992
A_{2a}	Human	T88A,R,S(3.36)	decr. AG; AN n.a.	Jiang et al., 1995
D_2	Human	C118(3.36)	AN n.a.; SH + +	Javitch et al., 1995
NK1	Rat	L116V(3.36)	spec. selec. AN	Jensen et al., 1994
NK1	Human	V116L(3.36)	reversed selec. AN	Fong et al., 1992b
NK2	Human	M117L(3.36)	decr. AN; AG n.a.	Bhogal et al., 1994
A_{2a}	Human	Q89A(3.37)	inc. AG & AN; selec. AN	Jiang et al., 1995
A_{2a}	Human	Q89H,R(3.37)	decr. AN; AG n.a.; perturb. BD	Jiang et al., 1995
A_{2a}	Human	Q89L,N,S(3.37)	incr. AG; AN n.a.; perturb. BD	Jiang et al., 1995
D_2	Human	T119C(3.37)	decr. AN; SH−	Javitch et al., 1995
rho	Bovine	E122A,Q(3.37)	blue shift	Ridge et al., 1992
rho	Bovine	E122D(3.37)	n.e.	Ridge et al., 1992

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
rho	Bovine	E122Q(3.37)	n.e.	Lin et al., 1992
rho	Bovine	E122Q(3.37)	blue shift	Zvyaga et al., 1993
TRH	Murine	N110A,S(3.37)	decr. AG	Perlman et al., 1994b
A_{2a}	Human	S90A(3.38)	incr. AG; AN n.a.	Jiang et al., 1995
D_2	Human	A120C(3.38)	AN n.a.; SH−	Javitch et al., 1995
A_{2a}	Human	S91A(3.39)	n.e.	Jiang et al., 1995
β_2	Hamster	S120A(3.39)	decr. expr.	Dixon et al., 1987
β_2	Hamster	S120A(3.39)	imp. proc.	Strader et al., 1989a
D_2	Human	S121C(3.39)	decr. AN; SH +	Javitch et al., 1995
D_2	Human	I122C(3.40)	AN n.a.; SH +	Javitch et al., 1995
D_2	Human	L123C(3.41)	AN n.a.; SH−	Javitch et al., 1995
rho	Bovine	W126A,L(3.41)	blue shift	Ridge et al., 1992
rho	Bovine	W126F(3.41)	n.e.	Ridge et al., 1992
V2	Human	Y128S(3.41)	decr. act.	Pan et al., 1992
V2	Human	Y128S(3.41)	decr. act.	Bichet et al., 1993
V2	Human	Y128S(3.41)	decr. act.	Faa et al., 1994
D_2	Human	N124C(3.42)	AN n.a.; SH−	Javitch et al., 1995
TRH	Murine	Y106F(3.42)	decr. AG	Perlman et al., 1994a
D_2	Human	L125C(3.43)	decr. AN; SH +	Javitch et al., 1995
β_2	Hamster	C125V(3.44)	AG & AN n.a.	Dohlman et al., 1990
D_2	Human	C126(3.44)	AN n.a.; SH−	Javitch et al., 1995
D_2	Human	A127C(3.45)	AN n.a.; SH−	Javitch et al., 1995
V2	Human	A132D(3.45)	decr. act.	Bichet et al., 1993
D_2	Human	I128C(3.46)	AN n.a.; SH−	Javitch et al., 1995
D_2	Human	S129C(3.47)	dec. AN; SH +	Javitch et al., 1995
D_2	Human	I130C(3.48)	AN n.a.; SH−	Javitch et al., 1995
$5HT_2$	Rat	D172N(3.49)	decr. AG & AN	Wang et al., 1993a
α_2	Porcine	D130N(3.49)	AG & AN n.a.	Horstman et al., 1990
α_{2a}	Human	D130N(3.49)	AN n.a.; decr. AG & act.	Wang et al., 1991
β_2	Human	D130N(3.49)	AN n.a.; incr. AG; decr. act.	Fraser et al., 1988
LH/CG	Rat	E441D,Q(3.49)	AG & AN & act. n.a.; imp.proc.	Wang et al., 1993b
m1	Rat	D122N(3.49)	act. & AN n.a.; incr. AC	Fraser et al., 1989b
rho	Bovine	E134Q(3.49)	decr. act.	Franke et al., 1992
rho	Bovine	E134Q(3.49)	n.e.	Lin et al., 1992
rho	Bovine	R135L,W(3.50)	decr. act.	Zvyaga et al., 1993
V2	Human	R137H(3.50)	decr. act.	Bichet et al., 1993
V2	Human	R137H(3.50)	AG n.a.; decr. act.	Rosenthal et al., 1993
V2	Human	R143P(3.56)	decr. act.	Tsukaguchi et al., 1993
α_{2a}	Human	I150V(4.42)	n.e.	Wang et al., 1991
H_1	Human	S155A(4.47)	n.e.	Moguilevsky et al., 1995

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
m3	Rat	W192F(4.50)	decr. AG & AN; act. n.a.	Wess et al., 1993
β_2	Hamster	S161A(4.53)	n.e.	Strader et al., 1989a
iodop	Chicken	S177A(4.53)	red shift (int. w. ionone ring)	Lin et al., 1994
rho	Bovine	A164S(4.53)	n.e.	Chan et al., 1992
V2	Human	S167L(4.53)	decr. act.	Knoers et al., 1994
NK2	Human	L160F(4.54)	n.e.	Bhogal et al., 1994
β_2	Human	T164I(4.56)	decr. AG & eff.	Turki et al., 1995
β_2	Hamster	S165A(4.57)	imp. proc.	Strader et al., 1989a
m3	Rat	P201A(4.59)	decr. AG & AN; act. n.a.	Wess et al., 1993
NK1	Human	P164A(4.59)	n.e.	Fong et al., 1994b
NK1	Human	Q165A(4.60)	decr. AG & AN	Fong et al., 1994b
NK1	Human	Q165N,S(4.60)	AN selec.; decr. AG	Fong et al., 1994b
NK2	Human	C167G(4.61)	n.e.	Bhogal et al., 1994
AT_{1a}	Rat	R167A(4.63)	decr. AG & AN	Yamano et al., 1995
NK1	Human	Y168A(4.63)	n.e.	Fong et al., 1994b
AT_{1a}	Rat	H166A(4.64)	decr. AG & AN	Yamano et al., 1995
NK1	Human	S169A(4.64)	AN selec.	Fong et al., 1994b
V2	Human	R181C(4.67)	decr. act.	Bichet et al., 1993
AT_{1a}	Rat	E173A(4.71)	n.e.	Yamano et al., 1995
V_2	Human	C185C(4.71)	decr. act.	Bichet et al., 1993
rho	Bovine	C187A(4.76)	decr. stab.; imp. proc.	Davidson et al., 1994a
rho	Human	C187S(4.76)	decr. act.; imp. proc.?	Karnik et al., 1988
rho	Human	C187Y(4.76)	decr. act.; imp. proc.?	Richards et al., 1995
AT_{1a}	Rat	H183A(5.26)	n.e.	Yamano et al., 1995
AT_{1a}	Rat	E185A(5.28)	n.e.	Yamano et al., 1995
NK2	Human	G190N,K(5.31)	n.e.	Bhogal et al., 1994
NK2	Human	G191K(5.32)	n.e.	Bhogal et al., 1994
NK1	Human	E193L(5.35)	decr. AN	Gether et al., 1994
NK2	Human	L194E,T(5.35)	n.e.	Bhogal et al., 1994
V2	Human	R202C(5.35)	decr. act.	Bichet et al., 1993
NK1	Human	K194L(5.36)	decr. AN	Gether et al., 1994
V2	Human	Y205C(5.38)	decr. act.	Bichet et al., 1993
m3	Rat	T231A(5.39)	decr. AG; AN & act. n.a.	Wess et al., 1991
m3	Rat	T231A(5.39)	decr. AG	Wess et al., 1992b
NK1	Human	H197A,Y,S,K(5.39)	decr. AN; AG n.a.	Fong et al., 1993a
NK1	Human	H197F,Q(5.39)	n.e.	Fong et al., 1993a
NK2	Human	F198A,L(5.39)	decr. AG & AN	Bhogal et al., 1994
A_{2a}	Human	F180A(5.41)	n.e.	Kim et al., 1995
$5HT_{1A}$	Human	S198A(5.42)	decr. AG; AN & act. n.a.	Ho et al., 1992
$5HT_{1A}$	Rat	S198A(5.42)	decr. AG; AN & act. n.a.	Ho et al., 1992
A_{2a}	Human	N181S(5.42)	AG selec.; AN n.a.	Kim et al., 1995

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
α_{2a}	Human	S200A(5.42)	decr. AG; AN & act. n.a.	Wang et al., 1991
AT_{1a}	Rat	K199A(5.42)	decr. AG; AN n.a.	Yamano et al., 1995
AT_{1a}	Rat	K199Q(5.42)	decr. AG; AG selec.	Yamano et al., 1992
β_2	Hamster	S203A(5.42)	imp. proc.	Strader et al., 1989a
D_1	Human	S198A(5.42)	decr. AG & AN; act. n.a.; imp. proc.?	Pollock et al., 1992
D_2	Rat	S194A(5.42)	AG & AN n.a.	Mansour et al., 1992
H_1	guinea pig	T203A(5.42)	n.e.	Leurs et al., 1994
H_1	Human	T194A(5.42)	decr. AG; incr. AN; act. n.a.	Ohta et al., 1994
H_1	Human	T194A(5.42)	decr. AG; incr. AN; act. n.a.	Moguilevsky et al., 1995
H_2	Canine	D186A(5.42)	decr. AN & act.; AG n.a.	Gantz et al., 1992
H_2	Canine	D186N(5.42)	decr. AN & act.; AG n.a.	Gantz et al., 1992
m3	Rat	T234A(5.42)	decr. AG & act.; AN n.a.	Wess et al., 1991
NMB	Rat	I216S(5.42)	decr. AG & act.	Fathi et al., 1993
$5HT_{1A}$	Human	T199A(5.43)	decr. AG; act. n.a.	Ho et al., 1992
A_{2a}	Human	F182A(5.43)	decr. AG & AN & act.	Kim et al., 1995
A_{2a}	Human	F182W(5.43)	decr. AG; AN n.a.	Kim et al., 1995
A_{2a}	Human	F182Y(5.43)	decr. AG; AN n.a. (selec.?)	Kim et al., 1995
α_{2b}	Murine	S182C(5.43)	incr. AN	Link et al., 1992
α_2-C10	Human	C201S(5.43)	spec. selec	Link et al., 1992
β_2	Hamster	S204A(5.43)	decr. AG; AN n.a.	Strader et al., 1989b
D_1	Human	S199A(5.43)	decr. AG & AN; act. n.a.	Pollock et al., 1992
m3	Rat	T234A(5.43)	decr. AG; act. n.a.	Wess et al., 1992b
NK2	Human	I202V(5.43)	decr. AG; AN n.a.	Bhogal et al., 1994
$5HT_{2A}$	Human	S242A(5.46)	spec. selec. AG; incr. AN	Kao et al., 1992
α_{2a}	Human	S204A(5.46)	decr. AG; AN & act. n.a.	Wang et al., 1991
β_2	Human	S207A(5.46)	decr. AG; AN n.a.	Strader et al., 1989b
D_1	Human	S202A(5.46)	AG selec.; AN & act. n.a.	Pollock et al., 1992
D_2	Rat	S197A(5.46)	AG & AN selec.	Mansour et al., 1992
H_1	Human	N198A(5.46)	decr. AG; AN & act. n.a.	Ohta et al., 1994
H_1	guinea pig	N207A(5.46)	decr. AG & AN; AG selec.; act.n.a.	Leurs et al., 1994
H_1	Human	N198A(5.46)	decr. AG & act.; AN n.a.	Moguilevsky et al., 1995
H_2	Canine	T190A(5.46)	decr. AN; act. n.a.	Gantz et al., 1992
H_2	Canine	T190C(5.46)	decr. AN; act. n.a.	Gantz et al., 1992
rho	Bovine	H211C,F(5.46)	n.e.; conf. change?	Weitz and Nathans, 1992
rho	Bovine	H211F(5.46)	n.e.	Cohen et al., 1992
m3	Rat	P242A(5.50)	decr. AG; AN & act. n.a.	Wess et al., 1993
LH/CG	Human	I542L(5.56)	decr. AG; Const.act. (AC)	Laue et al., 1995
AT_{1a}	Rat	Y215F(5.58)	AN n.a.; decr. AG & act. (PI)	Hunyady et al., 1995
β_2	Hamster	Y219L(5.58)	AG & AN n.a.	Strader et al., 1989a

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
m3	Rat	R252H(5.60)	n.e.	Blüml et al., 1994a
m3	Rat	I253A(5.61)	n.e.	Blüml et al., 1994a
m3	Rat	Y254A,F,W(5.62)	decr. act. (PI)	Blüml et al., 1994d
m3	Rat	Y254S(5.62)	AG & AN n.a.; decr. act.	Blüml et al., 1994a
rho	Bovine	C240(5.75)	crosslinking to transducin (α)	Resek et al., 1994
LH/CG	Human	D564G(6.30)	const.act. (AC)	Laue et al., 1995
α_{1b}	Hamster	A293X(6.34)	incr. AG; const. act.	Kjelsberg et al., 1992
α_{2a}	Human	T348C,E,K(6.34)	incr. AG.; const. act.; AN n.a.	Ren et al., 1993
TSH	Rat	A623E,K(6.34)	incr. AG; decr. act.	Kosugi et al., 1992
TSH	Human	A623I(6.34)	const. act.	Parma et al., 1993
TSH	Human	A623V(6.34)	const.act (AC); act. n.a. (PI)	Paschke et al., 1994
LH/CG	Human	M571I(6.37)	const.act. (AC)	Laue et al., 1995
AT_{1a}	Human	A572V(6.38)	const. act. (AC); incr. AG	Yano et al., 1995
TSH	Human	F631C(6.42)	AG & act n.a. (PI); const. act. (AC)	Kosugi et al., 1994
LH/CG	Human	T577I(6.43)	const.act. (AC)	Laue et al., 1995
TSH	Human	T632I(6.43)	AG & act n.a. (PI); const. act. (AC)	Kosugi et al., 1994
TSH	Human	T632I(6.43)	const. act. (AC); act.n.a. (PI)	Paschke et al., 1994
V2	Human	ΔV279(6.43)	decr. act.	Tsukaguchi et al., 1993
V2	Human	ΔV279(6.43)	decr. act.	Faa et al., 1994
LH/CG	Human	D578G(6.44)	AG n.a.; const. act. (AC)	Shenker et al., 1993
LH/CG	Human	D578G(6.44)	const.act. (AC)	Laue et al., 1995
LH/CG	Rat	D556N(6.44)	AG & act. n.a.	Ji and Ji, 1991
LH/CG	Human	D578Y(6.44)	high const.act. (AC) [phenotypic]	Laue et al., 1995
rho	Bovine	F261Y(6.44)	red shift	Chan et al., 1992
rho	Fish	Y261F(6.44)	blue shift	Yokoyama et al., 1995
TSH	Human	D633E(6.44)	AG & act n.a. (PI); const. act. (AC)	Kosugi et al., 1994
TSH	Human	D633Y(6.44)	AG & act. n.a. (PI); const. act. (AC)	Kosugi et al., 1994
SST5	Rat	G258F(6.46)	n.e.	Ozenberger and Hadcock, 1995
β_2	Hamster	C285S(6.47)	decr. act.; AG & AN n.a.	Fraser et al., 1989a
β_2	Hamster	C285V(6.47)	AG & AN n.a.	Dohlman et al., 1990
LH/CG	Human	C581R(6.47)	decr. AG; const.act. (AC)	Laue et al., 1995
m3	Rat	T502A(6.47)	n.e.	Wess et al., 1991
AT_{1a}	Rat	W253A(6.48)	decr. AG; AN n.a.	Yamano et al., 1995
m3	Rat	W503F(6.48)	decr. AG & AN: act. n.a.	Wess et al., 1993
rho	Bovine	W265A,F,Y(6.48)	blue shift	Ridge et al., 1992
TRH	Murine	W279A(6.48)	n.e.	Perlman et al., 1995a
m3	Rat	P505A(6.50)	n.e.	Wess et al., 1993
V2	Human	P286L(6.50)	decr. act.	Faa et al., 1994
V2	Human	P286R(6.50)	decr. act.	Pan et al., 1992
v2	Human	P286R(6.50)	decr. act.	Bichet et al., 1993
$5HT_2$	Human	F339L(6.51)	selec. AN; AG n.a.	Choudhary et al., 1993

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
AT_{1a}	Rat	H256A(6.51)	n.e.	Yamano et al., 1995
CCK-B	Canine	L355V(6.51)	selec. AN; act. n.a.	Beinborn et al., 1993
CCK-B	Human	V319I(6.51)	selec. AN; AG n.a.	Beinborn et al., 1993
CCK-B	Human	V319L(6.51)	rev. selec. AN; AG n.a.	Beinborn et al., 1993
m3	Rat	Y506F(6.51)	decr. AG; selec. AN; act. n.a.	Wess et al., 1991
m3	Rat	Y506F(6.51)	decr. AG; act. n.a.	Wess et al., 1992b
NK2	Human	Y266F(6.51)	n.e.	Bhogal et al., 1994
rho	Bovine	Y268F(6.51)	n.e.	Ridge et al., 1992
$5HT_2$	Human	F340L(6.52)	selec. AN; decr. AG & act.	Choudhary et al., 1993
A_1	Bovine	H251L(6.52)	decr. AN; AG n.a.	Olah et al., 1992
A_{2a}	Human	H250A(6.52)	decr. AG & AN & act.	Kim et al., 1995
A_{2a}	Human	H250F(6.52)	selec. AG; AN n.a.	Kim et al., 1995
A_{2a}	Human	H250Y(6.52)	selec. AG; AN n.a.	Kim et al., 1995
iodop	Chicken	T282A(6.52)	red shift (int. w. ionone ring)	Lin et al., 1994
m3	Rat	N507A(6.52)	decr. AN & AG; act. n.a.	Blüml et al., 1994b
m3	Rat	N507D(6.52)	decr. AN; AG selec.	Blüml et al., 1994b
m3	Rat	N507S(6.52)	const. act.	Blüml et al., 1994b
NK1	Human	H265A(6.52)	decr. AN; AG & act. n.a.	Zoffmann et al., 1993
NK1	Human	H265A,F(6.52)	AN selec.; spec. selec.	Fong et al., 1994a
NK1	Human	H265F,Q(6.52)	n.e.	Zoffmann et al., 1993
NK1	Human	H265Q,S,Y(6.52)	AN selec.; spec. selec.	Fong et al., 1994a
rho	Bovine	A269T(6.52)	red shift	Chan et al., 1992
SST5	Rat	F265Y(6.52)	AG& sub. select.; act. n.a.	Ozenberger and Hadcock, 1995
TRH	Murine	R283H,K(6.52)	decr. AG; act. n.a.	Perlman et al., 1995a
TRH	Murine	R283A,E,L,S(6.52)	decr. AG & act.	Perlman et al., 1995a
AT_{1a}	Rat	F259A(6.54)	decr. AG; AN n.a.	Yamano et al., 1995
A_{2a}	Human	N253A(6.55)	decr. AG & AN & act.	Kim et al., 1995
A_{2a}	Human	N253Q(6.55)	decr. AG & AN	Kim et al., 1995
A_{2a}	Human	N253S(6.55)	decr. AG & AN	Kim et al., 1995
AT_{1a}	Rat	T260A(6.55)	n.e.	Yamano et al., 1995
P_{2U}	Human	R265L(6.55)	decr. AG	Erb et al., 1995
iodop	Chicken	Y274(6.57)	Stabiliz. Schiff base	Lin et al., 1994
AT_{1a}	Rat	D263A(6.58)	decr. AG & AN	Yamano et al., 1995
NK1	Human	P271G(6.58)	n.e.	Gether et al., 1994
NK2	Human	G273P(6.58)	decr. AG & AN	Bhogal et al., 1994
NK2	Human	G273T(6.58)	decr. AG; AN n.a.	Bhogal et al., 1994
A_{2a}	Human	F257A(6.59)	decr. AG & act.	Kim et al., 1995
NK1	Human	Y272T,A(6.59)	decr. AN; selec.	Gether et al., 1994
NK2	Human	S274T,Y(6.59)	n.e.	Bhogal et al., 1994
AT_{1a}	Rat	C274G(7.25)	decr. AG	Yamano et al., 1992

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
AT_{1a}	Rat	D278A(7.29)	decr. AG; AG selec.; AN n.a.	Hjorth et al., 1994
AT_1	Human	D278A(7.29)	decr. AG; AG selec.; AN n.a.	Perlman et al., 1995b
AT_{1a}	Rat	D281A(7.32)	decr. AG; AG selec; incr. AN	Hjorth et al., 1994
AT_1	Human	D281A(7.32)	decr. AG; AG selec.; incr. AN	Perlman et al., 1995b
A_{2a}	Human	Y271A(7.36)	decr. AG & act.	Kim et al., 1995
m2/m5	Human	T423A(7.36)	imp. proc.	Liu et al., 1995
m2/m5	Human	T423,E,H,N(7.36)	AG & AN & act. restored	Liu et al., 1995
P_{2U}	Human	K289I(7.36)	reversed selec. AG	Erb et al., 1995
TXA_2	Human	L291F(7.36)	decr. AG & AN; imp. proc.	Funk et al., 1993
NK1	Human	I290S(7.38)	reversed selec. AN	Fong et al., 1992a
NK1	Human	I290S(7.38)	decr. AG; spec. selec.	Sachais et al., 1993
NK1	Rat	S290I(7.38)	spec. selec. AN	Jensen et al., 1994
NK2	Human	L292I(7.38)	AG selec.; decr. AN	Bhogal et al., 1994
NK2	Human	L292S(7.38)	AG selec.; AN n.a.	Bhogal et al., 1994
$5HT_{1A}$	Human	N385V(7.39)	decr. AN; AG n.a.	Guan et al., 1992
$5HT_{1B}$	Human	T355N(7.39)	incr. aff.; spec. selec.	Oksenberg et al., 1992
$5HT_{1B}$	Human	T355N(7.39)	decr. AG & AN; spec. selec.	Parker et al., 1993
A_{2a}	Human	I274A(7.39)	decr. AG & AN; act. n.a.	Kim et al., 1995
α_2-C10	Human	F412N(7.39)	AN & AG sub. selec.	Suryanarayana et al., 1991
β_2	Human	N312A(7.39)	dec. AN; act n.a.; AN sel.	Suryanarayana et al., 1993
β_2	Human	N312F(7.39)	imp. proc.	Suryanarayana et al., 1991
β_2	Human	N312F(7.39)	imp. proc.	Suryanarayana et al., 1993
β_2	Human	N312Q,T(7.39)	decr. AG & AN; act. n.a.	Suryanarayana et al., 1993
m3	Rat	Y529F(7.39)	decr. AG; selec. AN; act. n.a.	Wess et al., 1991
m3	Rat	Y529F(7.39)	decr. AG	Wess et al., 1992b
P_{2U}	Human	R292L(7.39)	decr. AG	Erb et al., 1995
rho	Bovine	A292D(7.39)	n.e.	Ridge et al., 1992
rho	Human	A292E(7.39)	const. act.	Dryja et al., 1993
TRH	Murine	R306A,E,L(7.39)	decr. AG; act. n.a.	Perlman et al., 1995a
TRH	Murine	R306K(7.39)	n.e.	Perlman et al., 1995a
β_2	Turkey	W330(7.40)	photochem. lab.	Wong et al., 1988
m3	Rat	W530F(7.40)	n.e.	Wess et al., 1993
EP_3	Murine	R309E,V(7.40)	decr. AG	Huang and Tai, 1995
EP_3	Human	R309K(7.40)	incr. AG	Huang and Tai, 1995
TXA_2	Human	R295Q(7.40)	decr. AG & AN	Funk et al., 1993
A_1	Human	T277S,A(7.42)	decr. AG; AN n.a.; spec. sel.	Townsend-Nicholsen and Schofield, 1994
A_{2a}	Human	S277A(7.42)	decr. AG; AN & act. n.a.	Kim et al., 1995
A_{2a}	Human	S277C(7.42)	decr. AG; AN n.a.	Jiang et al., 1995
A_{2a}	Human	S277N,T(7.42)	n.e.	Kim et al., 1995

Receptor	Species	Mutation (identifier)	Effect on function[a]	Reference
m1	Rat	C407S(7.42)	decr. AG; act. n.a.	Savarese et al., 1992
A_1	Bovine	H278L(7.43)	decr. AG & AN	Olah et al., 1992
A_{2a}	Human	H278A(7.43)	decr. AG & AN; act. n.a.	Kim et al., 1995
A_{2a}	Human	H278F,Q,Y(7.43)	decr. AG & AN	Kim et al., 1995
β_2	Hamster	Y316S(7.43)	imp. proc.?	Strader et al., 1989a
m3	Rat	Y533F(7.43)	decr. AG & AN; act. n.a.	Wess et al., 1991
m3	Rat	Y533F(7.43)	decr. AG	Wess et al., 1992b
rho	Bovine	K296A,E,G(7.43)	const. act.	Robinson et al., 1992
rho	Bovine	K296A,E,G,H(7.43)	const. act.	Cohen et al., 1992
rho	Human	K296E(7.43)	const. act.	Li et al., 1995
TXA_2	Human	w299L(7.44)	decr. AN; AG n.a.	Funk et al., 1993
TXA_2	Human	W299R(7.44)	decr. AG & AN	Funk et al., 1993
AT_1	Human	N294A(7.45)	decr. AN; AG n.a.	Perlman et al., 1995b
β_2	Hamster	N318K(7.45)	decr. AG; AN n.a.	Strader et al., 1987a
A_{2a}	Human	S281A(7.46)	decr. AG & AN & act.	Kim et al., 1995
A_{2a}	Human	5281N(7.46)	inc. AG; decr. AN; selec. AN	Jiang et al., 1995
A_{2a}	Human	S281T(7.46)	selec. AG & AN	Kim et al., 1995
β_2	Hamster	S319A(7.46)	decr. AG; AN n.a.	Strader et al., 1989a
m3	Rat	T537A(7.47)	n.e.	Wess et al., 1991
GRH	Human	D318N(7.49)	n.e.	Davidson et al., 1994b
β_2	Hamster	P323S(7.50)	imp. proc.	Strader et al., 1987a
m3	Rat	P540A(7.50)	decr. act.; AG & AN n.a.	Maggio et al., 1993
m3	Rat	P540A(7.50)	incr. AG; AN n.a.; decr. act.	Wess et al., 1993
GRH	Human	S320A(7.51)	n.e.	Davidson et al., 1994b
m1	Rat	C417S(7.52)	n.e.	Savarese et al., 1992
β_2	Human	C327R(7.54)	decr. AG & act.	O'Dowd et al., 1988
β_2	Human	C327S(7.54)	AG & AN n.a.	Fraser et al., 1989a
α_2	Porcine	D432N(7.58)	AG & AN n.a.	Horstman et al., 1990
rho	Bovine	C322S(7.69)	n.e.	Karnik et al., 1993
rho	Bovine	C323S(7.70)	n.e.	Karnik et al., 1993

[a] decr., decreased; incr., increased; n.a., not affected, ≤3-fold difference with wild type receptor; n.e., no effect or ≤3-fold difference with wild type receptor; imp. proc., impaired processing, either translational/posttranslational/transport; AG;, agonist (affinity); AN, antagonist (affinity); act., activity per se; aff., any measure of affinity (K_d/K_i/EC_{50}/IC_{50}); eff., efficacy; perturb. BD, size-dependent perturbation of binding domain; (rev.) selec., (reversed) ligand selectivity; spec. selec., species selectivity; sub. selec., subtype selectivity; const. act., constitutive activity; (K), potassium channel activity; (AC), adenylyl cyclase activity; (PI), phosphatidyl inositol turnover activity; (PLC), phospholipase C activity; SH, probed with sulfhydryl reagent: − = does not react, + = reacts, + + = reacts strongly; Na, affects sodium modulatory capacity; part. AG, partial agonist(s)/agonism; conf. change, involved in conformational change(s); photochem. lab., photochemically labeled; stab, (photochemical) stability. Notes 1 "decr./incr." denotes a >3-fold difference with the wild type receptor, regardless whether a smaller difference is deemed significant by statistical analysis. A 3-fold difference in affinity corresponds to 0.5 log unit on the concentration scale. A 3-fold decrease in affinity corresponds to a loss of approximately 1 kcal/mol in binding energy, which is less than the loss of 1 hydrogen bond (typically between 3 and 6 kcal/mol). 2 Affinity is defined by the equilibrium constants derived from any ligand-receptor interaction. In the absence of such values as K_d, (equilibrium dissociation constant), K_i (equilibrium inhibitory displacement constant) or IC_{50} (concentration of inhibitory ligand that displaces 50% of the marker ligand) from binding studies, EC_{50} or IC_{50} (concentration of ligand that displays a semi-maximal excitatory or inhibitory effect, respectively) values derived from functional assays may be used. Although such functional parameters are frequently obtained under non-equilibrium conditions, for

simplicity they will be treated equivalent to equilibrium constants for the purpose of this paper. 3 The term ‘activity’ in this paper is used solely for the pupose of identifying effects on the activity of the receptor-effector system, and does not reflect leftward or rightward shifts of dose-response curves. Neither does it reflect ‘intrinsic activity’, or other parameters involved in the actions of partial agonists. Consequently, the failure of a system to respond to stimulation by virtue of a loss of affinity, should not be considered a loss of activity. However, it is virtually impossible to distinguish between lack of response due to severe loss of affinity (the system will not respond at supramaximal stimulation), and that due to an impaired response system (the system will not respond to any stimulation whatsoever).