

Molecular and genetic basis of β_2 -adrenergic receptor function

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The β_2 -adrenergic receptor has been cloned, mutated, and recombinantly expressed such that many structural features involved in receptor function have been defined. Agonists bind in a pocket formed by transmembrane spanning domains 3, 5, and 6, where key contact points initiate receptor activation. An interaction with the β -hydroxyl group of β -agonists and Asn293 of the latter transmembrane domain is the basis of the stereoselectivity of R- vs S-isomers of catecholamine-like agonists. Sites within the receptor that serve to dampen the signal with continuous agonist exposure have also been identified and include sites for phosphorylation by protein kinase A and G-protein-coupled receptor kinases and structural features that direct the receptor toward degradation (downregulation). Several regions of the β_2 -adrenergic receptor show genetic diversity within the human population, such that expression, coupling, and agonist regulation may be different in individuals with these polymorphisms. (*J Allergy Clin Immunol* 1999;103:S42-6.)

Key words: Polymorphism, allele, adenylyl cyclase, desensitization, isomers, agonist, G-protein

The β_2 -adrenergic receptor (β_2 AR) is a member of a large superfamily of cell surface receptors that carry out their signal transduction to the interior of the cell by coupling to heterotrimeric G proteins.¹ The β_2 AR couples to the stimulatory G protein termed G_s , which acts to stimulate the effector adenylyl cyclase catalyzing the conversion of adenosine triphosphate to cyclic AMP (cAMP). cAMP acts as a classic second messenger in the cell through its activation of protein kinase A, which phosphorylates multiple targets. In the heart, activation of β_2 AR results in an increased rate and force of contraction of cardiac muscle. In the lungs, β_2 AR stimulation acts to relax airway smooth muscle.

STRUCTURE OF THE β_2 AR

The primary amino acid sequence of the human β_2 AR, and its proposed membrane topology, is shown in Fig 1. The amino-terminus is localized extracellularly; the carboxy terminus is intracellular. The protein traverses the membrane 7 times, with these transmembrane-spanning domains (TMDs) thought to form α helices. Three extracellular and 3 intracellular loops are situated as shown. Because of the palmitoylation of the indicated cysteine in

Abbreviations used

β_2 AR:	β_2 -adrenergic receptor
β ARK:	β adrenergic receptor kinase
cAMP:	Cyclic 3' 5' adenosine monophosphate
5'-LC:	5'-leader cistron
GRK:	G protein coupled receptor kinase
TMD:	Transmembrane domain

the cytoplasmic tail, which anchors this portion to the membrane, some have considered that this stretch of intracellular residue represents a fourth intracellular loop.

For the β_2 AR, agonists such as isoproterenol appear to bind to the receptor at specific contact points in the TMDs, in a ligand binding "pocket."²⁻⁶ The critical interactions are at Asp113 (TMD3), which interacts with the amine head group, Ser204, 207 (TMD5), which interacts with the hydroxyl groups on the catechol ring, and Asn293 (TMD6), which interacts with the β -hydroxyl group of catecholamines. The Asn293 interaction⁶ is also the basis of the stereoselectivity of R- vs S-isomers of catecholamines (Fig 2). The regions of receptor interaction with G_s are the second intracellular loop, the amino and carboxy terminal portions of the third intracellular loop, and the proximal portion of the cytoplasmic tail.

REGULATION OF β_2 AR FUNCTION

With continuous agonist exposure, the aforementioned signaling undergoes a diminution over time, which is termed *desensitization*.⁷⁻⁹ Desensitization is a common biologic phenomenon found with many different signaling transduction systems. Clinically, desensitization is termed *tachyphylaxis* when receptor function is lost over time as the result of continuous or repetitive therapeutic administration of a drug. Regarding prolonged use of β -agonists in patients with asthma or chronic obstructive pulmonary disease, there is intense debate as to whether clinically significant tachyphylaxis to the bronchodilating effect actually occurs. The mechanism of agonist-promoted desensitization of β_2 AR can be divided into 3 general processes: phosphorylation, sequestration, and downregulation of receptor number. Phosphorylation of β_2 AR occurs rapidly after agonist exposure and is mediated in part by the cAMP-dependent protein kinase A. This phosphorylation occurs at sites in the third intracellular loop and the cytoplasmic tail of the receptor. Such phosphorylation appears to directly interfere with receptor coupling to G_s . Note that any mechanism that increases cAMP in the cell will activate protein kinase A and

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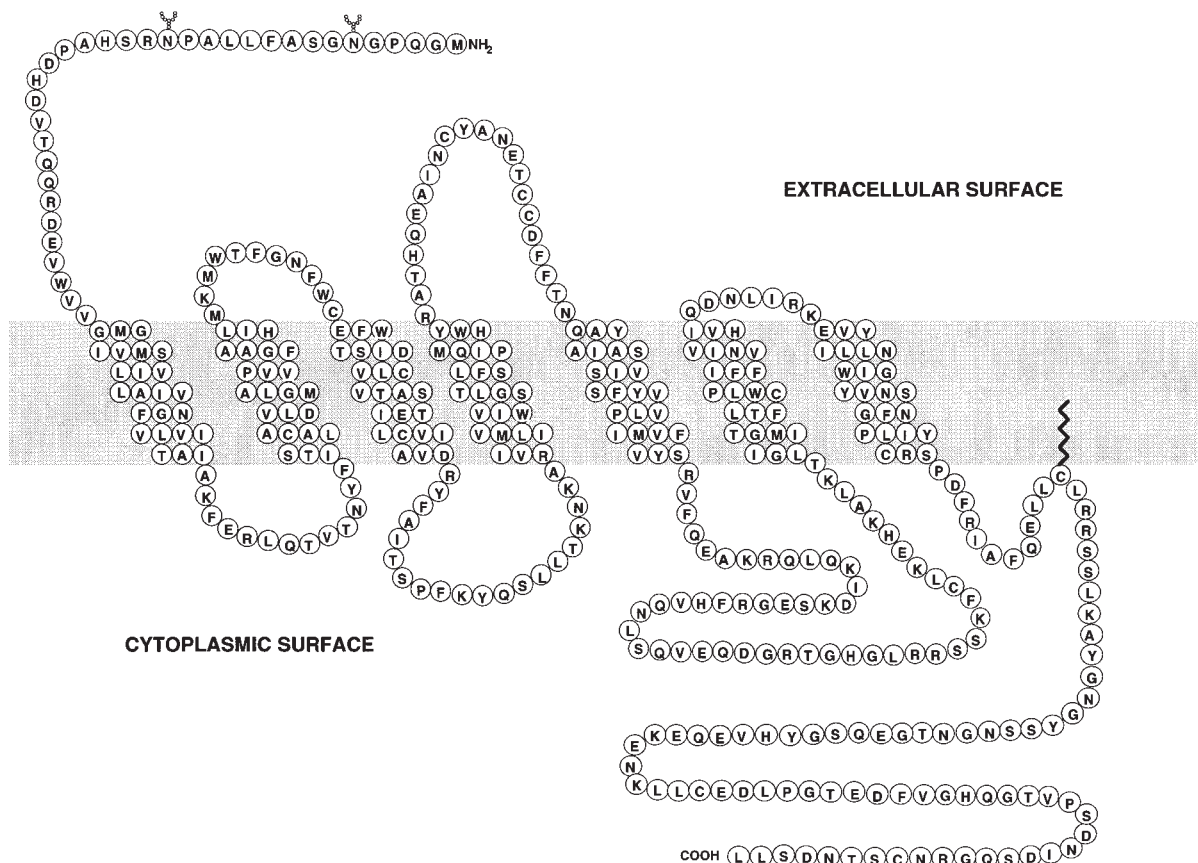


FIG 1. Primary amino-acid sequence and proposed membrane topography of the human β_2 AR. The single letter amino acid nomenclature is used. Shown is the receptor that is termed *wild-type*.

thus phosphorylate and desensitize β_2 AR. So, such desensitization can occur even with no β -agonist pre-exposure. Protein kinase A is thus one mechanism by which heterologous desensitization of the β_2 AR can occur. Another kinase, actually a family of kinases, can phosphorylate the β_2 AR in a non-cAMP-dependent manner.¹⁰ The first of these kinases to be identified has been termed the β AR kinase (β ARK; also now termed *GRK2*). Phosphorylation of β_2 AR by β ARK is triggered by the conformational change induced (or stabilized) by agonist occupancy of the receptor. Such phosphorylation results in the binding of another protein, β arrestin, which serves to decrease coupling of the receptor to G_s . The sites for phosphorylation of the β_2 AR by β ARK include serines and threonines in the cytoplasmic tail (Fig 1). Because this form of desensitization is strictly agonist dependent, it is referred to as homologous desensitization. Other members of this family of kinases, termed G protein coupled receptor kinases (GRKs), are rhodopsin kinase (GRK1), β ARK2 (also called GRK3), and GRKs 4-6. Each GRK has unique properties, and most serve to phosphorylate many members of the G protein coupled receptor superfamily.

After more prolonged exposure to agonist (seconds to minutes), a proportion of β_2 AR internalize into subcellu-

lar compartments. This process is termed *sequestration*. These sequestered receptors are not available for signal transduction and thus, except in cases of significant receptor reserve, can act as a mechanism of desensitization. There is also evidence that sequestration is involved in dephosphorylation of GRK-mediated phosphorylation of the receptor and trafficking of the receptor to a degradation process. With prolonged exposure to agonist (hours to days) the total complement of β_2 AR is decreased, regardless of compartment. This is termed *receptor downregulation*. Decreased transcription, enhanced messenger RNA degradation, and enhanced receptor protein degradation have been implicated in agonist-promoted β_2 AR downregulation.

It should be noted that although the times of onset of these processes are different, long-term agonist-promoted functional desensitization likely represents the combined actions of all 3 mechanisms.¹¹

AGONIST PLEIOTROPY

Traditional views of agonist-receptor activation centered on the receptor being in the inactivated state (ie, not in a conformation favorable for G_s coupling) when not bound by agonist. Results of experiments such as those

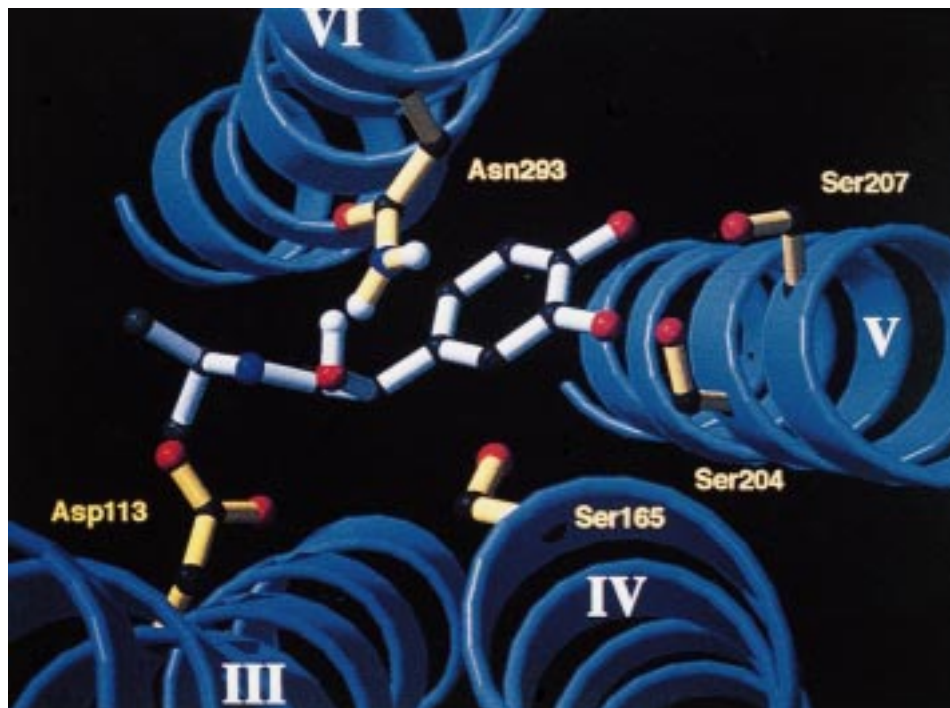


FIG 2. Proposed positioning of the R-isomer of the agonist isoproterenol in the binding pocket of the β_2 AR. The interaction at Asn293 determines the stereoselectivity for R- versus S-isomer. (From Wieland K, Zuurmond HM, Krasel C, Izerman AP, Lohse MJ. Involvement of Asn-293 in stereospecific agonist recognition and in activation of the β_2 -adrenergic receptor. *Proc Natl Acad Sci USA* 1996;93:9276-81. Copyright 1996 National Academy of Sciences, U.S.A.)

shown in Fig 3 have led to a modification of this concept. In this experiment, we permanently expressed the human β_2 AR in Chinese hamster fibroblasts (which do not express endogenous β AR) to various levels.¹² As can be seen, basal adenylyl cyclase activities were linearly related to the expression level. This indicated that some fraction of β_2 AR, even in the absence of agonist, can assume the active conformation. Agonist binding, though, stabilizes this conformation, and ultimately a significant proportion of receptors are in the active conformation, and agonist stimulation of adenylyl cyclase is observed. In its simplest case, then, the receptor toggles between inactive and active conformations, with the latter being significantly more likely in the presence of agonist. Neutral antagonists do not affect the equilibrium between inactive and active conformations, but block the binding of agonists. On the other hand, inverse agonists (also called negative antagonists) shift the equilibrium more toward the inactive conformation, and thus can serve to lower basal activation of an effector, such as adenylyl cyclase. Studies with the α_2 AR, the β_2 AR, and other G protein coupled receptors have indicated that some receptors can couple to more than one effector pathway.¹ Thus a receptor could spontaneously achieve 2 active conformations. One conformation would be favorable for coupling to one G protein while another couples to a second G protein. Conceivably then, an agonist might stabilize both states of the receptor, or could preferentially stabilize one

state over another, thus promoting activation of a single pathway. This concept, termed agonist *pleiotropy*, has been shown in studies with structurally diverse agonists and mutated receptors. Given that many of the aforementioned events such as phosphorylation by GRKs and sequestration are dependent on the receptor adopting the "agonist-occupied" conformation, it is also conceivable that spontaneous conformations favoring one event over another occur. Thus it is possible that certain agonists could stabilize one conformation but not another. In essence, this concept furthers the notion that synthetic agonists with diverse structures could interact with receptors in unique ways, preferentially activating clinically advantageous or disadvantageous pathways.

PHARMACOGENETICS OF β_2 AR

We have recently shown that the β_2 AR displays significant structural variance in the human population because of genetic polymorphisms in the β_2 AR gene.¹³ In the coding block, there are 4 loci where variation has been detected (Table I; Fig 4, A). In addition, we have recently found¹⁴ a polymorphism in the 5' upstream portion of the human β_2 AR, in a small open reading frame (termed the β_2 AR 5'-leader *cistron*) that encodes for a peptide that regulates receptor expression (Table I; Fig 4, B).

Using site-directed mutagenesis followed by recombinant expression in Chinese hamster fibroblasts (CHW-

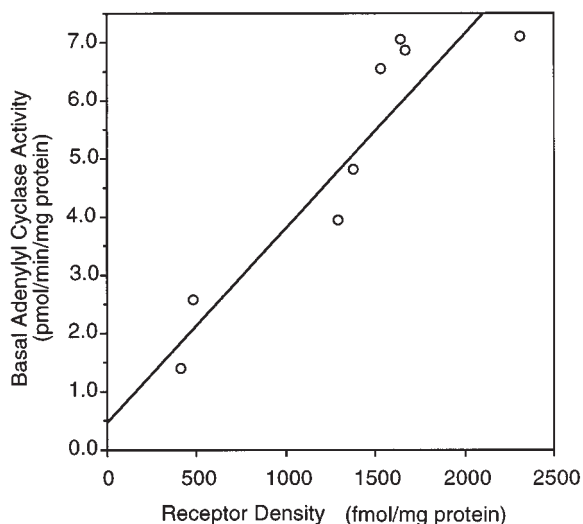


FIG 3. Signaling of β_2 AR in the absence of agonist. Chinese hamsters fibroblasts (CHW) expressing different levels of β_2 AR (expressed as fmol per mg on the x-axis) were homogenized, and basal adenylyl cyclase activities were determined in membranes. As is shown, a direct relationship between expression and basal activity is present.

1102 cells), we have delineated the functional significance of the coding block polymorphisms.^{12,15} For purposes of this discussion, wild-type human β_2 AR is considered to be Arg16, Gln27, Val34, and Thr164, based on the initial cloning results.¹⁶ The Gly16 and Glu27 receptors bound agonists and antagonists with wild-type affinities and coupled normally to adenylyl cyclase. However, the Gly16 variant displays increased isoproterenol-promoted downregulation of the receptor.¹⁵ In contrast, the Glu27 receptor shows no downregulation whatsoever.¹⁵ These experiments have also been performed with human airway smooth muscle cells, which endogenously express the various polymorphic β_2 AR. The results with these cells confirmed the downregulation phenotype observed with the transfected cell system: enhanced downregulation with Gly16 and minimal downregulation with Glu27.¹⁷ The Met34 receptor shows no difference in any parameter compared with the wild-type Val34 receptor. The Ile164 receptor showed decreased affinity for the agonists isoproterenol, epinephrine, and norepinephrine and some but not all antagonists studied.¹² This polymorphic receptor was also significantly uncoupled from the stimulation of adenylyl cyclase.¹²

Recently we have also found a polymorphism in the 5' leader cistron (5'-LC) at the carboxy terminus (position 19), where Cys or Arg can be found (Fig 4, B). These are denoted 5'-LC Arg19 and 5'-LC Cys19. To assess the relevance of this variation, constructs consisting of each variant of the 5'-LC, with the intervening downstream sequence, the β_2 AR coding block, and 3' untranslated sequence were made in a mammalian expression vector. When transfected into COS-7 cells, the 5'-LC Cys19 construct consistently provided for higher β_2 AR expression levels as determined by radioligand binding.¹⁴ Lev-

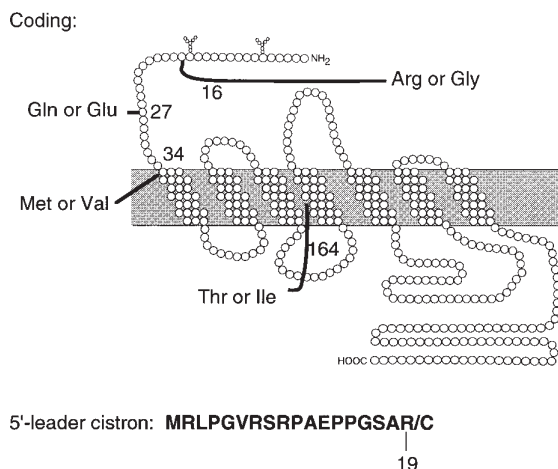


FIG 4. Localization of polymorphisms at amino acid positions 16, 27, 34, and 164 of the receptor and 19 of the 5'-LC.

TABLE I. Polymorphisms of the β_2 AR

Nucleic acid position	Amino acid position	Wild-type	Polymorphism (allele frequency)	Polymorphism phenotype
-47	19 (5'-LC)	Arg	Cys (0.63)	↑Expression
46	16	Arg	Gly (0.61)	↑Downregulation
79	27	Gln	Glu (0.43)	↓Downregulation
100	34	Val	Met (<0.001)	Wild-type
491	164	Thr	Ile (0.05)*	↓Coupling

*Heterozygote frequency; allele frequency not clear, because no individuals with homozygous Ile164 have been found to date.

els of mRNA, as determined by quantitative ribonuclease protection assays, were the same in cells transfected by the 2 constructs. Thus the differences were considered due to the 2 peptides having different abilities to alter translation of the β_2 AR message. We also assessed β_2 AR expression in human airway smooth muscle cells bearing 5'-LC Arg19 or 5'-LC Cys19. Consistent with the transfection studies, these endogenously expressing cells with the Cys variant displayed approximately 2-fold higher expression of β_2 AR compared with those with the Arg variant.

So, as shown in Table I, the various β_2 AR genotypes result in a number of phenotypes in cell systems, including differences in expression, coupling, and downregulation. There are several important points about these polymorphic variants that are not yet clear. First, little is known about the interactions between polymorphic loci. That is, a number of combinations of different polymorphisms have been detected in the human population, with some having not been studied at the cellular level to date. Second, for the common polymorphisms at positions 16 and 27, agonist-promoted downregulation (which is enhanced with Gly16 and minimal with Glu27) has only been studied with the agonist isoproterenol. Although we have presumed that agonists commonly used in the treatment of asthma are likely to result in similar findings, this has not yet been established.

In asthma, the polymorphisms at positions 16 and 27 have been evaluated as potential risk factors, disease modifiers, and genetic factors influencing the response to β -agonist. Except for a single report¹⁸ in children with asthma, where Glu27 was found to be more prevalent in patients with asthma than in normal controls, all other studies have shown that β_2 AR polymorphisms are not significant risk factors for the development of the syndrome. However, studies have shown that polymorphisms at positions 16 and 27 are associated with the initial responsiveness to albuterol,¹⁹ tachyphylaxis to formoterol,²⁰ severity,²¹ bronchial hyperresponsiveness,²² elevated IgE levels,²³ and nocturnal asthma.²⁴ To date, studies with sufficient numbers of patients with Ile164 have not been published, nor have there been any reports on the clinical consequences of the variation in the 5'-LC.

FUTURE DIRECTIONS

Significant progress has been made at the molecular, genetic, and cellular levels in understanding the basis of human β_2 AR structure and function. The challenge for the future is the development of agonists (or other agents that can favorably modify the receptor) that are highly efficacious with fewer adverse effects than the currently used agonists and that take into account the genetic variability of the receptor in the population.

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