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PHARMACOLOGY AND PHYSIOLOGY OF HUMAN ADRENERGIC RECEPTOR POLYMORPHISMS

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■ **Abstract** Adrenergic receptors are expressed on virtually every cell type in the body and are the receptors for epinephrine and norepinephrine within the sympathetic nervous system. They serve critical roles in maintaining homeostasis in normal physiologic settings as well as pathologic states. These receptors are also targets for therapeutically administered agonists and antagonists. Recent studies have shown that at least seven adrenergic receptor subtypes display variation in amino acid sequence in the human population due to common genetic polymorphisms. Variations in potential regulatory domains in noncoding sequence are also present. Here, we review the consequences of these polymorphisms in terms of signaling, human physiology and disease, and response to therapy.

INTRODUCTION

Adrenergic receptors are the targets for epinephrine and norepinephrine and thus represent a critical component in the sympathetic nervous system for maintenance of homeostasis and response to disease. Adrenergic receptors are members of the superfamily of cell surface receptors that carry out signaling via coupling to guanine nucleotide binding proteins (G-proteins). G-protein coupled receptors (GPCRs), including adrenergic receptors, are targets for many therapeutic agonists and antagonists in current use. The family of human adrenergic receptors consists of nine subtypes: α_{1A}-, α_{1B}-, α_{1D}AR; α_{2A}-, α_{2B}-, α_{2C}AR; and β_1-, β_2-, and β_3AR. Although several of these receptors can couple to more than one G-protein, the classic coupling pathways for α_1AR, α_2AR, and βAR are via G_q (stimulation of phopholipase C), G_i (inhibition of adenylyl cyclase), and G_s (stimulation of adenylyl cyclase), respectively. As such, assays measuring inositol phosphate stimulation, inhibition or stimulation of adenylyl cyclase activities, or cAMP levels are often utilized to ascertain receptor function. However, other signals may be physiologically relevant in various cell types. For example, the α_2AR also couples to stimulation of adenylyl cyclase, inhibition of inwardly rectifying potassium channels, stimulation of voltage gated calcium channels, increases in intracellular

calcium release, and stimulation of mitogen activated protein (MAP) kinase. The signaling pathways of adrenergic receptors and their organ and cell-type distribution have been extensively described (1, 2).

For many years it has been recognized that physiologic responses, expression, and function of adrenergic receptors, as well as the response to adrenergic receptor agonists and antagonists, display marked interindividual variation within the human population. Although environmental factors and the heterogeneity of diseases, such as asthma and hypertension, for which these drugs are utilized are undoubtedly important, recent efforts have been under way to discern the impact of genetic variations of the receptor genes as a basis for some of this interindividual variation.

A variability in DNA sequence that occurs with an allele frequency of $>1\%$ in the population is termed a polymorphism. This is in contrast to mutations, which are rare variants and may be the single basis of an inherited disease (i.e., cystic fibrosis, nephrogenic diabetes insipidus). In such cases, these mutations are necessary and sufficient for the disease. On the other hand, polymorphisms may have no effect, have effects that are clinically silent but can be delineated with physiologic testing, have an increased prevalence in certain diseases and thus act as low-level risk factors, can act to modify diseases, or can alter the response to therapy. Whereas nucleotide deletions and insertions are observed in the human genome, by far the most common polymorphisms are single nucleotide substitutions (single nucleotide polymorphisms, frequently abbreviated as SNPs). Within coding regions of genes, polymorphisms may encode different amino acids (denoted as nonsynonymous polymorphisms) or because of redundancy in the genetic code, may have no effect on the encoded amino acid (synonymous polymorphisms). Polymorphisms occur in 5′ UTR, promoter, 3′ UTR, and introns as well, and in general, these are more common than coding polymorphisms.

Seven of the nine adrenergic genes have been found to have nonsynonymous or noncoding polymorphisms (Figure 1). In this paper, the genetics, signaling consequences, and clinical relevance (when known) of these polymorphisms are reviewed. Particular attention is devoted to the effects of receptor polymorphisms on cellular signaling, which provides a molecular mechanism for the physiologic and pharmacologic phenotypes observed in the limited number of human studies and provides a basis for additional clinical studies. The issues regarding initial discovery and subsequent rapid detection of adrenergic receptor polymorphisms (3), "in silico" methods (4), sample size considerations (4), and transfected cell-based characterization methods (5) are described elsewhere.

β_1-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

There are two nonsynonymous coding polymorphisms of the β_1AR found in the human population (Figure 1, Table 1). At nucleotide 145, variation is present that results in either Ser or Gly at amino acid position 49. This position is within

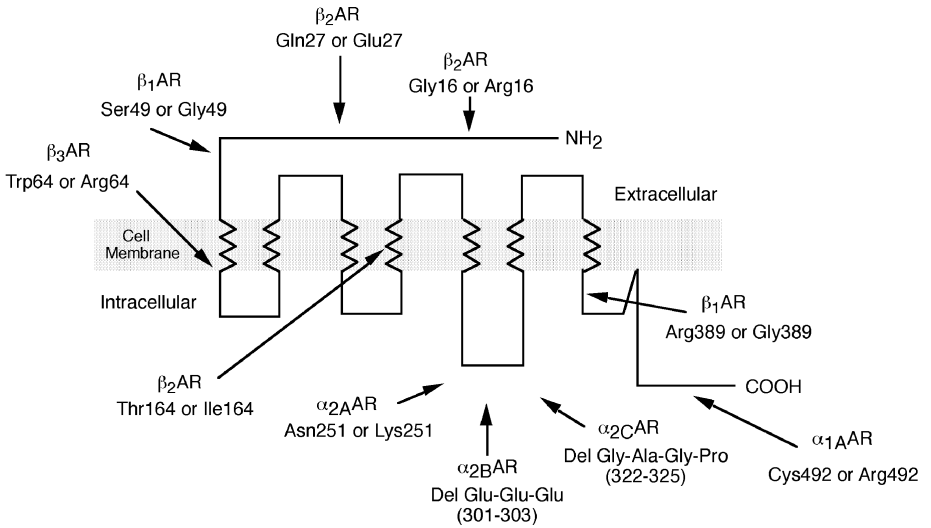

Figure 1 Localization of nonsynonymous polymorphisms of human adrenergic receptors. Shown is a prototypic adrenergic receptor and the approximate location of amino acid substitutions or deletions.

the extracellular amino terminus, ∼9 amino acids from the cell membrane. The minor allele is Gly, which has a frequency of ∼15% (Table 1). We have found no differences in the frequency of this β_1AR polymorphism between Caucasians and African Americans (6). Polymorphic variation of the β_1AR also occurs at nucleotide 1165, resulting in either Arg or Gly being encoded at amino acid 389. This locus of variability is in the proximal portion of the carboxy terminus. This region, between the seventh transmembrane spanning domain and the palmitoylated cysteine(s), is predicted to be an α-helix. The first human β_1AR to be cloned actually represents the minor allele (Gly), and has been referred to as the wild-type receptor. Indeed, virtually all recombinant structure/function studies have been performed with the Gly389 β_1AR. The allele frequency of Gly389 differs between Caucasians and African Americans, with the latter ethnic group having a higher frequency (∼42% versus ∼27%, Table 1). In a preliminary study, several very uncommon variations have been reported by one group (7) at amino acids 399, 402, 404, and 418. Apparently, these have been detected as heterozygotes in one or two subjects in their cohorts. Verification and pharmacologic studies have not been reported to date.

The localization of the common amino acid substitutions at positions 49 and 389 (Figure 1) suggested potential phenotypes of the polymorphic receptors. For the position 49 variation, it seemed unlikely that agonist binding or G-protein coupling would be affected. However, expression or trafficking of GPCRs can be affected by mutations imposed in the amino terminus. In this regard, we were hampered by the

TABLE 1 Adrenergic receptor polymorphisms

Receptor	Position		Alleles		Minor allele frequency (%)	
	Nucleotide	Amino acid	Major	Minor	Caucasians	African Americans
α_{1A}AR	1441	492	Cys[a]	Arg	46	70
α_{2A}AR	753	251	Asn	Lys	0.4	5
α_{2B}AR	901–909	301–303	No deletion	Delete Glu-Glu-Glu	31	12
α_{2C}AR	964–975	322–325	No deletion	Delete Gly-Ala-Gly-Pro	4	38
β_1AR						
	145	49	Ser	Gly	15	13
	1165	389	Arg	Gly	27	42
β_2AR						
	46	16	Gly	Arg	39	50
	79	27	Gln	Glu	43	27
	491	164	Thr	Ile	2–5	2–5
β_3AR	190	64	Trp	Arg	10	?

[a]In African-Americans, Arg is the major allele.

well-recognized observation in recombinantly expressed cells, that β_1ARs display very little agonist-promoted internalization or agonist-promoted down-regulation via protein degradation (8). On the other hand, in many endogenous expressing cells, such as the cardiomyocyte, agonist-promoted down-regulation of the β_1AR clearly occurs, which is thought to be due primarily to decreases in mRNA. Taken together, it is clear that the recombinant approach, with cells transiently or stably expressing the β_1AR, represents only a model system. Nevertheless, because the cDNAs used for transfections are in identical expression vectors and differ only at nucleotide 145, the system can be useful to differentiate potential phenotypes between the Ser49 and Gly49 receptors.

HEK293 cells stably expressing the two β_1ARs have been utilized for the majority of studies with the position 49 polymorphism (9). Agonist and antagonist-binding affinities, as well as coupling to adenylyl cyclase, were not different between Ser49 and Gly49 β_1AR. Agonist-promoted internalization was minimal ($\sim$28% after 30 min of exposure to 10 μM isoproterenol) and not different between the two receptors. However, with 24 h of exposure to isoproterenol, a clear difference in receptor down-regulation was observed (9). The Ser49 receptor showed no down-regulation; indeed, receptor expression actually increased with this receptor (Figure 2*A*). In contrast, the Gly49 receptor underwent a 24 $\pm$ 3% decrease in expression in paired studies. These findings were confirmed in multiple cell lines and were independent of the initial levels of receptor expression. The basal

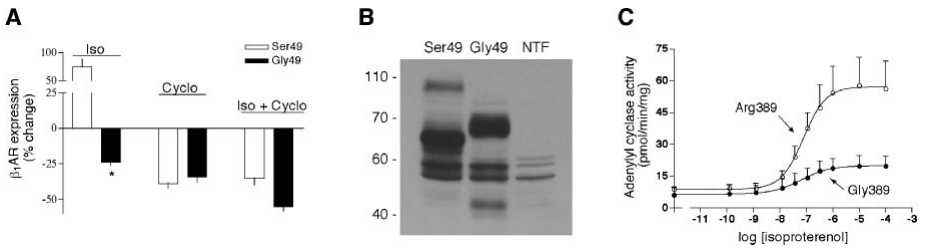

Figure 2 Characteristics of β_1AR polymorphisms in transfected cells. (*A*) Agonist-promoted down-regulation phenotypes of the Ser49 and Gly49 β_1ARs. (*B*) Western analysis of the Ser49 and Gly49 β_1ARs. (*C*) Adenylyl cyclase activities of the Arg389 and Gly389 β_1ARs. ISO = isoproterenol, Cyclo = cycloheximide.

(non-agonist promoted) turnover rates were assessed by treating cells with the protein synthesis inhibitor cycloheximide. Under these conditions, both receptors displayed a similar decrease in expression after 18 h of exposure, consistent with basal degradation rates being equivalent. To approximate the scenario in myocytes, where receptor synthesis is markedly reduced due to a decrease in transcripts, cells were treated with both cycloheximide and isoproterenol. Here again, the Gly49 receptor displayed greater down-regulation than Ser49 (55 $\pm$ 3% versus 36 $\pm$ 5%, Figure 2*A*).

Western blots have shown differences in the immunoreactive bands between the two receptors (Figure 2*B*) (9). Additional studies have indicated that a high molecular weight band ($\sim$105 kDa) was observed only with the Ser49 receptor and represents a high degree of glycosylation (rather than nonglycosylated receptor dimers). The major glycosylated forms also differed between the Ser49 ($\sim$63 kDa) and Gly49 ($\sim$69 kDa) receptors. Both were sensitive to tunicamycin and N-glycosidases. Although the loss of Ser in the amino terminus might represent a loss of an O-glycosylation site, this was not the case, as the immunoreactive bands were not affected by O-glycosidase. Of note, the site for N-linked glycosylation of the β_1AR is amino acid position 15. Thus, it is not clear how a polymorphism at position 49 can sufficiently alter the conformation of the amino terminus so as to alter glycosylation at Asn15. Although there are limitations to this model system, the data indicate that the Gly49 receptor displays enhanced agonist-promoted down-regulation compared to the Ser49 receptor. This effect appears to occur after the internalization process and may be related to altered intracellular trafficking to degradation pathways, perhaps due to differences in receptor glycosylation status.

The Arg389 and Gly389 receptors were stably expressed in Chinese hamster fibroblasts (CHW cells) (10). In the presence of guanine nucleotide, agonist competition studies in cell membranes revealed no differences in affinity binding constants. However, functional studies revealed a marked difference in coupling of the two receptors to adenylyl cyclase (Figure 2*C*). As is shown, maximal agonist stimulation of adenylyl cyclase of Gly389 is only $\sim$1/3 that of the Arg389

receptor. In agonist competition studies carried out with membranes in the absence of GTP, high- and low-affinity binding sites could only be detected with the Arg389 receptor. This indicates impaired formation of the agonist-receptor-G_s complex with the Gly389 receptor. In addition, agonist stimulation of [^{35}S]GTPγS was less with Gly389 compared to the Arg389 receptor (10). Thus, the decreased adenylyl cyclase stimulation is due to a less favorable receptor conformation for agonist stabilization and coupling to $G_{\alpha s}$. Residue 389 is within a G_s coupling domain of the β_1AR, and Gly is considered likely to disrupt the predicted α-helix in this region. Thus, depressed agonist-promoted stimulation of adenylyl cyclase is consistent with the location of the polymorphism and the nature of the substitution. Because the Gly389 receptor was originally denoted as the wild-type, the Arg389 receptor was characterized as a gain-of-function variant. Because Gly is the minor allele, we have now denoted it as the polymorphism, which has a phenotype of depressed functional coupling.

Three SNPs in the 5′ promoter region of the human β_1AR have been reported at positions −93, −210, and −2146 (11). However, they are rare (allele frequencies 1%–10%) and have not been studied as to functional significance. The −2146 allele is in strong linkage disequilibrium with the position 145 locus in the coding block (11).

Human Studies

There have been several clinical association studies addressing whether β_1AR polymorphisms are risk factors for disease, modifiers of disease, or modifiers of the response to βAR agonists or antagonists. Most of these have centered on cardiovascular phenotypes. In regard to heart failure, the basis of these inquiries include studies with various animal models, showing that prolonged stimulation of cardiac β_1AR via drug infusions, enhanced presynaptic norepinephrine release, or transgenic expression of β_1AR results in severe cardiomyopathy (12–15). Patients with heart failure show improvements (albeit to variable extents) in contractility, morbidity, and mortality when treated with β-blockers (16). In case-control studies, no difference in allele frequencies of the position 49 or 389 genotypes was noted in one study (17), but another study by Podlowski et al. (7) showed an increase in the frequency of the Gly49 allele in idiopathic dilated cardiomyopathy. In this latter study, no individuals with Gly49 were found (i.e., allele frequency of 0%) in the control (normal) population. This is in marked contrast to data from a number of other studies that show an allele frequency of ∼10%–15% for the Gly49 polymorphism in normals (6, 11, 17–19) and in patients with various forms of heart failure (11, 17, 20). Given that the Podlowski study had only 37 patients and 40 controls, their finding of no controls with Gly49, combined with the results of these other studies, make this proposed association seem erroneous.

In considering β_1AR polymorphisms as risk factors for heart failure, it would seem that the combination of increased norepinephrine release from cardiac presynaptic nerves and a hyperactive β_1AR would be a condition whereby the probability

of catecholamine-evoked cardiomyopathy might be the greatest. Indeed, African Americans with the combination of a dysfunctional α_{2C}AR polymorphism (α_{2C}AR Del322-325, see below), which is localized to cardiac presynaptic nerves and is responsible for basal norepinephrine release, and the Arg389 polymorphism, which has enhanced coupling compared to the Gly389 polymorphism, have a marked risk for heart failure (odds ratio = 10.11, 95% CI = 2.11−48.53, p = 0.004) (21). The effect is synergistic, rather than additive. In fact, the odds ratio for heart failure was 0.55 (p = 0.23) for the β_1AR Arg389 polymorphism alone. It thus appears that Arg389 is a risk factor for heart failure, but only in conjunction with α_{2C} Del322-325. The Gly49 allele has been shown to be associated with a small (5 beats/min) but statistically significant decrease in resting heart rate (19). Interestingly, in this study the position 389 polymorphism was not associated with heart rate or hypertension. In another study, however, Arg389 was found to be associated with increased diastolic blood pressure in discordant sibling-pairs and hypertension (defined as elevated systolic or diastolic pressure) in a case-control analysis (22). In addition, Arg389 has been shown to be associated with increased systolic blood pressure (∼12 mmHg) in patients with heart failure (20).

Concerning disease modification and drug response, the β_1AR Gly49 allele has been associated with improved five-year survival (risk = 2.34, 95% CI = 1.3−4.20, p = 0.003) (17). This finding is consistent with the phenotype delineated in transfected cells (9) because down-regulation of the β_1AR may serve as a chronic protective effect in the failing heart (23). Another study explored potential relationships between β_1AR polymorphisms and patients with heart failure and exercise performance (20). The primary outcome variable was oxygen consumption (VO_2), which is considered a relevant measure of the capacity of the heart to increase cardiac output as well as overall cardiovascular status. The results revealed a highly clinically significant decreased VO_2 in patients homozygous for Gly389 compared to those homozygous for Arg389 ($VO_2 = 14.5 \pm 0.6$ versus 17.7 ± 0.4 ml/kg/min, respectively; p = 0.006). Heterozygous individuals had intermediate levels of VO_2. The position 49 polymorphisms were also associated with a difference in exercise capacity but only when heterozygous and homozygous Gly49 individuals were considered as one group. However, a robust relationship between haplotypes consisting of the combination of polymorphisms at positions 49 and 389 and VO_2 was found. It was concluded that the major determinant of exercise capacity, in regards to β_1AR polymorphisms, is at the 389 locus based on allele frequency and a greater absolute effect, and that haplotypes may provide an even greater predictive value. In another study, exercise-induced increases in heart rates, and systolic time intervals have been reported to be the same between groups of normal individuals with Arg389 or Gly389 (24), but VO_2 was not measured. This may suggest that these β_1AR variants play a more prominent role when β_1ARs are desensitized, as occurs in heart failure. No studies to date have assessed the potential for β_1AR polymorphisms to effect responsiveness to β-blockers in heart failure. However, one study showed no association between blood pressure or heart rate responses to β-blockers in the treatment of hypertension (25).

β_2-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

In the coding region of the human β_2AR, nine polymorphisms have been identified, three of which are nonsynonymous (Table 1) (26). A nonsynonymous variation at codon 34 has been reported (26), but the allele frequency is <1%. As shown in Figure 1, the common nonsynonymous polymorphisms occur at nucleotides 47 (amino acid 16) and 79 (amino acid 27). Both display differences in allele frequencies between Caucasians and African-Americans (Table 1) (27). Of note, the Arg16 receptor was first cloned and has been referred to as wild-type, but is in fact the minor allelic variant.

Prior to a full appreciation of the linkage disequilibrium between the position 16 and 27 polymorphisms, all combinations of the two (Arg16/Gln27, Gly16/Gln27, Arg16/Glu27, and Gly16/Glu27) were studied in recombinant CHW cells (28). Given their location in the amino terminus of the receptor, it was not unexpected to find no differences in agonist binding or agonist-stimulated adenylyl cyclase activities. In addition, receptor synthesis rates and agonist-promoted internalization were not different between the receptors. However, the extent of agonist-promoted down-regulation was affected by these substitutions (Figure 3*A*) (28). Cells were exposed to 10 μM isoproterenol or carrier for up to 24 h in culture, washed, membranes prepared, and [^{125}I]CYP radioligand binding utilized to quantitate receptor density. The Arg16/Gln27 receptor underwent 26 $\pm$ 3% down-regulation. In contrast, the Gly16/Gln27 receptor displayed 41 $\pm$ 3% down-regulation. Furthermore, the rare Arg16/Glu27 receptor failed to downregulate. The Gly16/Glu27 receptor displayed a similar level of down-regulation (39 $\pm$ 4%) compared to the Gly16/Gln27 receptor. Taken together, the data suggest that the major polymorphic

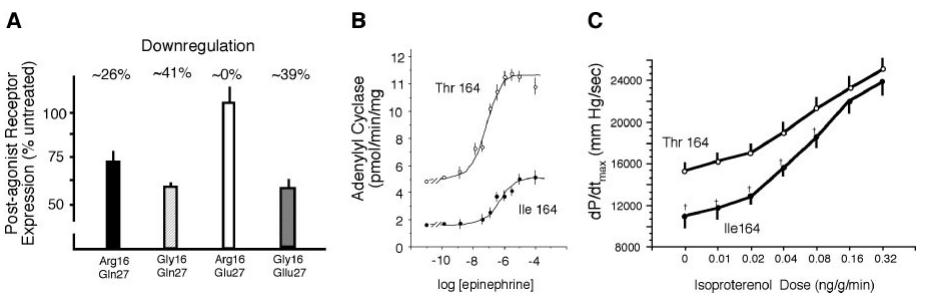

Figure 3 Characteristics of the β_2AR polymorphisms in transfected cells or transgenic mice. (*A*) Agonist-promoted down-regulation phenotypes of the position 16 and 27 polymorphisms in various combinations. (*B*) Adenylyl cyclase activities of the Thr164 and Ile164 β_2ARs. (*C*) Contractility of hearts from transgenic mice with targeted expression of the Thr164 and Ile164 β_2ARs.

locus that affects agonist-promoted down-regulation is at position 16. That is, whenever Gly16 is present, down-regulation is enhanced compared to Arg16. The molecular basis of these phenotypes is not clear, but it appears to occur after the internalization process, prior to or during passage through the degradation pathway. Efforts to further explore this mechanism are somewhat hampered in that little is known about how β_2ARs are targeted to degradative pathways.

In a study of cultured human airway smooth muscle cells natively expressing several of the β_2AR genotypic combinations (29), down-regulation (decrease in receptor density) promoted by 24 h of agonist exposure followed the same pattern as that observed in the transfected cell studies (28). However, another study using human airway smooth muscle cells showed a somewhat different phenotype (30). In this study, changes in receptor expression were not determined, but rather changes in function (cell stiffness and cAMP accumulation) after agonist exposure were delineated. In these studies, the presence of any Glu27 allele was associated with enhanced desensitization of these functions. This finding was observed with both 24-h exposures as well as 1-h exposures to agonist in culture. Because agonist-promoted down-regulation of β_2AR density requires many hours (minimal of $\sim$6 h for earliest detection regardless of cell-type), one must conclude that these protocols serve to study event(s) other than down-regulation alone. Based on the location of residue 27 and the known time courses of the short- and long-term desensitization processes, it is difficult to assign a common mechanistic basis for these observations. The effect of the position 16 genotype could not be fully assessed due to the distribution of genotypes. In another study with natively expressing polymorphic β_2AR, human lung mast cell function (agonist-promoted inhibition of histamine release) was examined (31). Desensitization of this response after 24 h of agonist exposure showed results that were the opposite of that predicted by the study where receptor expression was quantitated (29). Even though the above three studies utilized different protocols and outcome measures, one must also consider that polymorphisms in other genes whose products are involved in the various pathways being investigated may account for some of these apparent discrepancies.

The Ile164 polymorphism is localized to the fourth transmembrane spanning domain of the receptor (32). The Ile164 allele is uncommon in all populations studied to date. The heterozygous frequency is $\sim$2%–5% (Table 1). We have never found an individual homozygous for Ile164. When expressed in CHW cells, a two- to threefold decrease in affinities for agonists, and some antagonists, was observed in membrane competition studies in the presence of guanine nucleotide. Further agonist competition studies in the absence of GTP revealed very little accumulation of high-affinity binding with Ile164, such that the curves were essentially monophasic. In contrast, the Thr164 receptor displayed biphasic curves with readily resolved high- and low-affinity binding constants. Thus, the Ile164 receptor has depressed formation of the agonist-receptor-G_s complex as a result of this substitution in the ligand-binding pocket of the receptor. This was manifested as a decrease in basal and agonist-stimulated adenylyl cyclase activities

(Figure 3*B*) (32). The lower basal activity is consistent with the current concept concerning spontaneous activation of GPCRs in the absence of agonist. With high expression levels, the basal phenotype of an uncoupled receptor would thus be more readily observed. Indeed, expression levels for the Thr164 and Ile164 receptors were ~1000 fmol/mg. In contrast, the studies described above with the β_1AR Arg389 and Gly389 receptor were at ~250 fmol/mg, where an increase in agonist-stimulated, but not basal, levels was observed, due to the lower expression levels.

The Thr164 and Ile164 β_2AR have also been studied in targeted transgenic mice (33). For these studies, the α–myosin heavy chain promoter was utilized to direct expression to the heart. Expression levels were ~1000 fmol/mg for both receptors. Adenylyl cyclase studies in cardiac membranes revealed the same signaling phenotype as was observed using the recombinant CHW cells. The endogenous βAR in the heart were still present, but the levels of adenylyl cyclase were below those of the transgenic mice so that the phenotypic differences between the two receptors could be delineated. Both sets of transgenic mice displayed normal development, and at one year showed no evidence of cardiac pathology. Invasive studies (cardiac catheterization) revealed lower heart rates and contractility ($^{+}$dP/dt) in the Ile164 mice compared to the Thr164 mice (Figure 3*C*). It is interesting to note that the physiologic responses of the Ile164 mice were found to be essentially the same as those of nontransgenic mice despite ~40-fold overexpression (33). This suggests that physiologically, Ile164 may be even more impaired than that indicated by the adenylyl cyclase studies. We conclude that the phenotype of the Ile164 β_2AR receptor is that of depressed basal and agonist-promoted function, which likely has physiologic relevance in target tissues.

The degree of variability of the β_2AR gene has been further explored by interrogating ~1100 bp of the 5′ upstream region of this intronless gene (34). This region includes a short open reading frame, denoted the 5′-leader cistron. The encoded peptide from the cistron (β_2AR upstream protein or BUP) alters translation of the β_2AR and a SNP that alters the most 3′ amino acid (Cys to Arg) was found. In addition, the promoter and 5′ UTR regions contain multiple potential *cis*-acting elements. For this study, sequencing did not extend into the 3′ UTR and in fact terminated after the synonymous SNP at nucleotide 523. The results are shown in Table 2. Altogether, 13 SNPs were identified. Of the 2^{13} (8192) possible haplotypic combinations, only 12 were found. As can be seen, a number of these haplotypes are uncommon, with four major haplotypes identified. For some haplotypes, there were marked differences (>20-fold) in frequency between various ethnic groups. To ascertain the relevance of these haplotypes to receptor function, the two most common Caucasian homozygous haplotypes (2/2 and 4/4) were studied in a transient expression system. The constructs used for transfection lacked the typical eukaryotic promoters, but instead used the two β_2AR 5′ upstream sequences as the promoter in the exact context found in the native gene. As shown in Figure 4, the levels of β_2AR mRNA and protein expression were indeed different between haplotypes 2 and 4. As is discussed below, these haplotypes were associated with a

TABLE 2 Haplotypes of the human beta-2 adrenergic receptor

														Frequency (%)			
Nucleotide:	-1023	-709	-654	-468	-406	-367	-47	-20	46	79	252	491	523	Ca	A-A	As	H-L
Alleles:	G/A	C/A	G/A	C/G	C/T	T/C	T/C	T/C	G/A	C/G	G/A	C/T	C/A				
Haplotype																	
1	A	C	G	C	C	T	T	T	A	C	G	C	C	0.7	25.0	12.5	10.0
2	A	C	G	G	C	C	C	C	G	G	G	C	C	48.3	6.3	10.0	26.7
3	G	A	A	C	C	T	T	T	A	C	G	C	C	0.7	0.0	0.0	0.0
4	G	C	A	C	C	T	T	T	A	C	G	C	C	33.0	29.7	45.0	40.0
5	G	C	A	C	C	T	T	T	G	C	G	C	C	1.4	0.0	0.0	0.0
6	G	C	G	C	C	T	T	T	G	C	A	C	A	13.2	31.3	30.0	13.3
7	G	C	G	C	C	T	T	T	G	C	A	T	A	1.0	1.6	0.0	3.3
8	G	C	A	C	C	T	T	T	A	C	A	C	A	0.7	0.0	0.0	0.0
9	A	C	G	C	T	T	T	T	A	C	G	C	C	0.0	4.7	0.0	0.0
10	G	C	G	C	C	T	T	T	G	C	A	C	C	0.7	0.0	0.0	3.3
11	G	C	G	C	C	T	T	T	G	C	G	C	C	0.3	0.0	2.5	0.0
12	A	C	G	G	C	T	T	T	A	C	G	C	C	0.0	1.6	0.0	3.3
Location:	5'	5'	5'	5'	5'	5'	AA19 BUP Cys/Arg	5'	AA16 Gly/Arg	AA27 Gln/Glu	syn	AA164 Thr/Ile	syn				

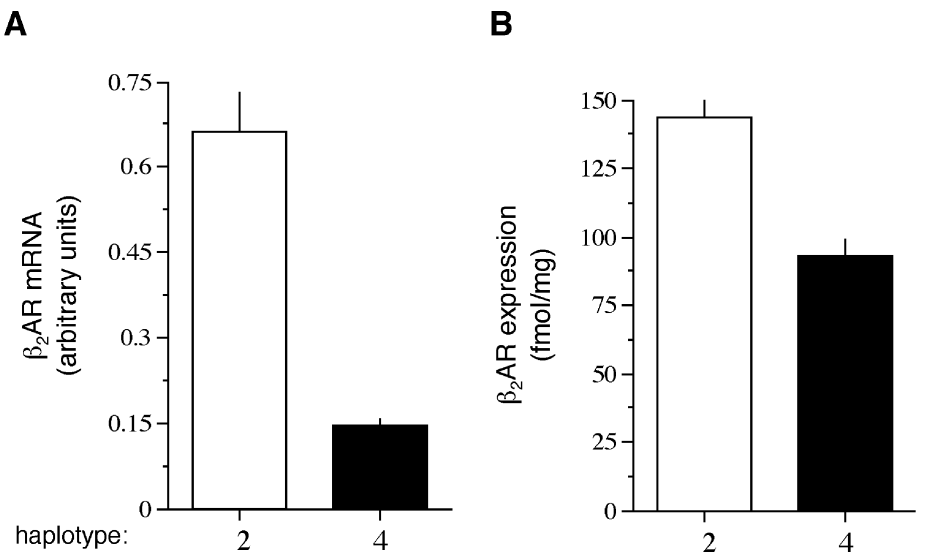

Figure 4 β_2-adrenergic receptor haplotypes affect receptor expression. See Table 2 and text for localization of the polymorphisms that encompass haplotypes 2 and 4.

differential response to albuterol in asthmatics of the same direction and magnitude as found in these in vitro studies (34). Thus the β_2AR gene is highly polymorphic, with all the nonsynonymous SNPs resulting in receptors that have distinct characteristics that include ligand binding, functional coupling, and agonist-promoted regulation. In addition, 5′ upstream SNPs, likely in combination with each other and the coding SNPs, may act to direct expression of the receptor. Additional studies, however, are necessary to delineate the nature of these effects and interactions and to discover which SNPs are primarily responsible for the expression phenotype.

Human Studies

Many clinical association studies have been carried out with β_2AR SNPs or haplotypes, examining if they are risk factors or modifiers of disease or determinants of the response to β-agonists. The majority have been within the context of asthma (26, 27, 34–56) because β_2ARs expressed on airway smooth muscle regulate bronchomotor tone and β-agonists are utilized for treating asthma since they serve to bronchodilate. In assessing the body of studies collectively, it is clear that most, but not all, have shown some association between β_2AR genotypes and various asthma phenotypes. These have included severity and other clinical subsets, hyperresponsiveness, bronchodilator response to β-agonist, tachyphylaxis to β-agonist, "asthma control," and others. A few of these asthma studies are discussed here in some detail in order to highlight important points relevant to genotype-phenotype

association studies in asthma and similar complex syndromes. In a study by Israel et al., relationships between position 16 and 27 polymorphisms and tachyphylaxis to the β-agonist albuterol were sought in a cohort of mild-moderate asthmatics (49). Patients were treated as needed with albuterol (i.e., only when symptoms occurred) or on a regular schedule (four times/day) with a standard dose of inhaled albuterol. The outcome measure was the change in morning and evening peak expiratory flow rates, with worsening rates over the 16-week treatment plus the 4-week washout period being considered evidence for tachyphylaxis. In the analysis, only homozygous patients were considered. Morning and evening peak expiratory flow rates declined only in patients receiving regular albuterol who had the homozygous Arg16 genotype. Gly16 patients showed no decline, nor did Arg16 patients who received intermittent β-agonist.

The above results from this clinical study appeared to be in contrast to what would be predicted from the recombinant cell culture studies (28). In the latter studies, the Gly16 β_2AR underwent the greatest degree of down-regulation after prolonged exposure to agonist in the culture medium. However, we have proposed (57) that the results of the Israel et al. study are consistent with the recombinant studies if one considers a dynamic, rather than static, model of receptor regulation. The concept is relevant to a variety of regulatory processes and is depicted in a generic manner in Figure 5. In the static model, the expression/function of β_2AR is not modulated by endogenous agonist (epinephrine). As such, when challenged by chronic β-agonist administration, the Gly16 receptor ("SNP B" in the model) would display the greatest degree of desensitization, clinically manifested

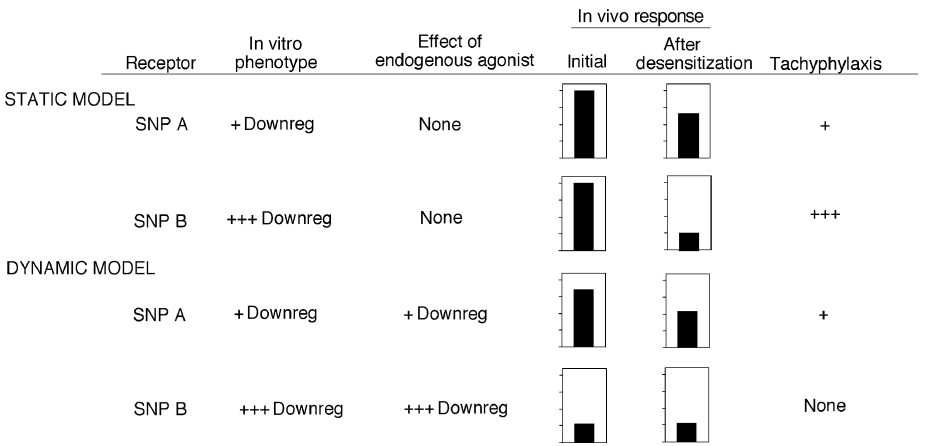

Figure 5 Comparison of tachyphylaxis phenotypes in the static versus dynamic models of receptor regulation. The models differ as to whether endogenous agonist "pre-regulates" receptor function prior to exogenous agonist exposure. The predicted clinical effect (tachyphylaxis) differs depending on the model. For β_2AR, SNP A is the Arg16 receptor and SNP B is Gly16. The bars represent arbitrary physiologic responses, such as FEV_1.

as tachyphylaxis. However, ample evidence points to the fact that GPCRs, including the β_2AR, are under constant regulation by their cognate endogenous agonists, thereby providing a means to integrate the multitude of incoming signals and to ultimately modulate complex physiological functions. Under such circumstances, one can consider that the Gly16 β_2AR, which is the most sensitive to agonist-promoted down-regulation, may be substantially, or maximally, downregulated by endogenous epinephrine before exposure to exogenous agonist such as albuterol. As such, patients with this genotype would show no tachyphylaxis because further down-regulation by albuterol does not occur. In contrast, the Arg16 receptor ("SNP A" in the model) undergoes relatively less down-regulation by endogenous epinephrine, but with exposure to chronic exogenous albuterol, undergoes down-regulation. The difference between the pre- and post-albuterol state is thus measurable, and so patients with Arg16 in fact display tachyphylaxis (Figure 5). A recent study of desensitization of vascular blood flow to isoproterenol supports the dynamic model in the context of β_2AR polymorphisms as well (58). The above concepts emphasize the need to carry out both in vitro and human studies to fully appreciate the effects and mechanisms of polymorphisms on differential responses to therapeutics (59, 60).

In addition to considering the impact of polymorphisms within the context of the intact individual, recent studies have also shown the importance of taking into account the context of polymorphisms within a functional unit (i.e., the gene) rather than in isolation. In the most straightforward illustration, one could have a scenario whereby a SNP at one position could negate, or accentuate, the effect of a SNP at another position. Thus, knowing the SNP at only one position may not provide ample predictive power. To address this, we examined the haplotypes of the β_2AR gene (34) as described above (see also Table 2). The common Caucasian haplotype pairs were 2/2, 4/4, 2/4, 2/6, and 4/6. In a cohort of 131 Caucasian asthmatics, the forced expiratory volume in one second (FEV_1) was determined before and 30 min after inhalation of a standard dose of albuterol. Initially, analysis was conducted to assess whether any one SNP was associated with the bronchodilator response (considered as a continuous variable). No associations were found. However, haplotype pair was found to be significantly associated with the FEV_1 response ($p = 0.007$ by ANCOVA). The greatest differences were between those with haplotype pair 4/6 and 4/4 (19.1 versus 8.5 change in % predicted FEV_1). We had no patients who were homozygous 6/6. Those with haplotypes 2/2 and 2/6 had intermediate responses between those with 4/4 and those with 4/6. The data with the 4/4 and 2/2 individuals were consistent with the transfection studies (see above and Figure 4) that showed greater expression of β_2AR mRNA and protein expression with the haplotype 2 construct. This study emphasizes, particularly in somewhat heterogenous groups, that β_2AR haplotypes provide greater predictive power of the bronchodilator response to β-agonists than individual SNPs. A similar finding was made by Weir et al. (27) when asthma severity was examined. Here, only haplotypes composed of chromosomally phased SNPs at positions 16 and 27 revealed an association. In another study, a three-SNP haplotype, but not individual

β_2AR SNPs, was strongly predictive of protection against bronchial hyperreactivity (47). Taken together, these studies indicate, at least with some phenotypic traits, the need for more extensive information about β_2AR genetic variation than individual SNPs or unphased genotypic combinations of a few SNPs to further enhance predictive power.

Some (61), but not all (62–67), studies have shown relationships between β_2AR polymorphisms and hypertension, presumably because of the expression of these receptors on vascular smooth muscle that serve to vasodilate. Indeed, several studies have revealed a relationship between genotype and some measure of vascular relaxation in response to agonist infusion (62, 68, 69). Perhaps the most definitive study, by Boerwinkle and colleagues (65), which utilized sib-pairs from 55 pedigrees and ~2500 individuals from 589 families, revealed that the β_2AR polymorphisms are susceptibility loci for essential hypertension. The risk was greater for Gly16 and Glu27 alleles, the latter having an odds ratio for occurrence of hypertension of 1.80 (95% CI = 1.08−3.0, p = 0.023). Given the heterogeneity of hypertension, it is not surprising to find that β_2AR polymorphisms are responsible for a relatively small effect. However, in combination with other genetic variants and substantial environmental interactions, β_2AR polymorphisms appear to represent one component in this complex syndrome.

Several studies have been carried out examining β_2AR polymorphisms and heart failure. The basis of these studies is the growing body of evidence that myocardial β_2AR signals somewhat differently than β_1AR, with the former having anti-apoptotic effects (70). In addition, β_2ARs have positive inotropic and chronotropic effects in the human heart, and on vascular smooth muscle they contribute to vascular tone, particularly during exercise. An initial analysis of cases and controls showed no association between β_2AR genotypes at positions 16, 27, and 164 and heart failure. However, longitudinal follow-up for three years revealed that individuals with the Ile164 allele had a rapid progression to either death or transplantation (adjusted relative risk = 4.81, 95% CI = 2.0−11.5, $p < 0.001$) (71). Indeed, one year survival was 42% for patients with Ile164 compared to 70% for Thr164 patients. There was no significant confounding effect of age, sex, etiology of failure, medication use, or initial left ventricular ejection fraction. A subsequent study (72) examined patients early in the course of the disease, matched for clinical and demographic parameters, but either homozygous for Thr164 or heterozygous for Ile164. Exercise capacity was measured using a graded treadmill protocol, with VO_2 as the primary outcome measure. Those with Ile164 had substantially depressed VO_2 (15.0 $\pm$ 0.9 versus 17.9 $\pm$ 0.9 ml/kg/min). The odds ratio of having $VO_2 \leq 14$ ml/kg/min was 8.0 ($p = 0.009$). Importantly, these patients could not be differentiated by standard clinical tests. Of note, a $VO_2 \leq 14$ is one of the criteria for placement on the cardiac transplantation list. So, even at early stages, with more intense investigation a pathophysiologic effect of Ile164 is observed. A study by Brodde et al. (73) with normal volunteers showed a decreased responsiveness (heart rate and systolic time interval) to infusions of the β_2AR agonist terbutaline in those with the Ile164 allele compared to Thr164 homozygotes. This

indicates that this polymorphism has physiologic effects even in the absence of a disease such as heart failure.

There have been several studies assessing potential associations between β_2AR polymorphisms and obesity (74). These were based on the fact that β_2ARs are expressed on white adipose tissue where activation results in lipolysis. In obese women, homozygosity for Glu27 was associated with ~20 kg higher fat mass and ~50% larger fat cells in the obese compared to nonobese women (75). However, in isolated adipocytes from such individuals, Glu27 was not associated with increased sensitivity or maximal glycerol release from terbutaline exposure ex vivo. Instead, the sensitivity was related to the position 16 polymorphisms. In another study, obesity in males was shown to be positively associated with the Gln27 polymorphisms (or negatively associated with Glu27) (76). It was concluded that gender may play a role in the influence of β_2AR genotype on obesity. Interestingly, the effect of position 27 polymorphisms may be modified by exercise (77) or, stated another way, may identify patients likely to achieve weight loss with exercise (78). Other studies, however, have failed to observe any relationship, or only a small risk, for obesity (79–82). Studies have also suggested associations with dyslipoproteinemia (79, 83) and type II diabetes (79). At this juncture, then, it is difficult to ascertain the role of β_2AR polymorphisms as predisposing factors for obesity. This is most likely due to the extreme clinical heterogeneity of the syndrome, gender effects, interaction with other genes, influences of other related disease such as diabetes, and environmental influences.

β_3-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

One nonsynonymous polymorphism of the β_3AR gene has been reported (84) that results in a substitution of Arg for Trp (the major human allele) at amino acid position 64. This residue is localized either to the most distal residue within the first transmembrane spanning domain or the most proximal residue of the first intracellular loop (Figure 1) of the receptor. It is interesting to note that in virtually all β_3AR genes cloned from various species, Arg is found at position 64. In most cases that we are aware of, the human major allele is the one found in the other species, rather than the apparent situation with the β_3AR. This suggests very strong, human-specific, evolutionary pressure for dominance of the Trp residue. Indeed, the pharmacologic properties between rodent and human β_3AR are quite different (85), suggesting that the receptor subserves somewhat different functions in the two species. The Arg64 β_3AR polymorphism occurs with an allele frequency of ~8%–10% in Caucasian populations; to our knowledge the frequency in African or African-American populations has not been reported. However, Japanese and Alaskan Eskimos have higher allele frequencies (74).

Two studies with discrepant results have been published on the pharmacologic effect of the Arg64 substitution in the β_3AR using recombinant expression. In CHO(dhfr-) cells, Strader and colleagues (86) found no differences in agonist

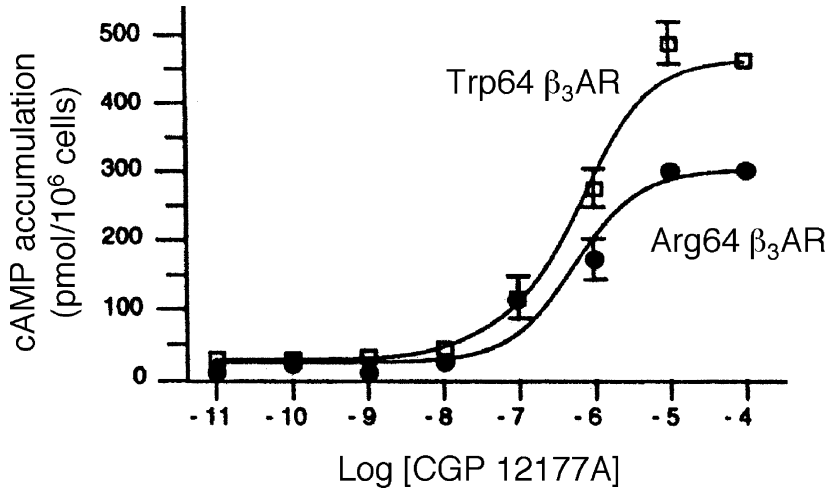

Figure 6 Adenylyl cyclase activities of the β_3AR polymorphism in transfected cells.

binding parameters or agonist stimulation of cellular cAMP accumulation between the Trp64 and Arg64 receptors. In contrast, another group has reported a decrease in the maximal stimulation of cAMP accumulation in CHO-K1 and HEK293 cells with the Arg64 β_3AR compared to its allelic counterpart (Figure 6). The CHO-K1 cells expressing the Arg64 β_3AR displayed lower agonist-promoted cAMP accumulation compared to the Trp64 cells. However, forskolin-stimulated accumulation was also depressed. In HEK-293 cells, the forskolin-stimulated cAMP levels were similar between the cell lines, with agonist-stimulated cAMP levels being modestly depressed with the Arg64 β_3AR (Figure 6). The reduction was observed with all agonists tested, albeit to different extents. Agonist affinity was not tested. The reasons for the discrepancy between these two studies are not clear. In human fat cells, an ~10-fold increase in EC_{50} (i.e., a decrease in sensitivity) to a β_3AR agonist in stimulation of lypolysis ex vivo has been reported (87). No differences in β_1AR- or β_2AR-mediated lipolysis were noted. β_3AR expression levels were not measured, so it is not clear whether the polymorphism may alter expression of the receptor in adipocytes. Taken together, it appears that there may be a difference in coupling between Arg64 and Trp64 β_3ARs, but additional studies may help to clarify the pharmacologic characteristics and mechanistic basis for the phenotype.

Human Studies

Quite a few studies have been published examining potential relationships between indices of obesity or type II diabetes and the Arg64 β_3AR polymorphism (74). The basis for such studies is the expression of the β_3AR in brown adipose tissue, where receptor activation increases thermogenesis, and in white adipose tissue,

where activation results in lipolysis. The β_3AR is also expressed in several other tissues, such as heart and gall bladder, but its relevance at these sites is not well established. Well over 30 studies have been published in regard to indices of obesity or type II diabetes, with ∼one half reporting positive associations and the remainder failing to note associations. Many of these studies had large numbers of subjects and had similar study designs yet reached very different conclusions. The reasons for such extensive discrepancies in this field are not entirely clear. Again, the heterogeneity of obesity, gender, and racial factors, and the presence of comorbid conditions have made interpretation of these studies as a group difficult. An interaction with other obesity-related genes is highly likely. To our knowledge, effects of a β_3AR agonist administered for weight loss in humans, stratified by the Arg64 and Trp64 genotypes, have not been published.

α_1-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

There was some initial confusion as to the pharmacologic classification of the cloned α_1AR subtypes. The current classification is as follows: α_1A (originally designated as the $\alpha_{1c}$when cloned), α_{1B} (same as cloned α_{1b}), and α_{1d} (cloned α_{1d} but also designated by some as the $\alpha_{1A/D}$ or $\alpha_{1a/d}$). In this review, the current classification as indicated above is utilized.To date, only one polymorphism in an α_1AR subtype gene that alters the amino acid sequence has been described (Table 1). This polymorphism, initially identified as a Pst I restriction fragment length polymorphism (RFLP), is located within the α_{1A}AR gene and corresponds to either a C or T at nucleotide 1441 encoding Arg or Cys at amino acid 492 (88). This residue is located in the carboxy terminus of the receptor (Figure 1). The frequency of the Cys492 allele was found to be more common in Caucasians than in African Americans, with frequencies of 54% and 30%, respectively (89). For the α_{1B}AR gene, sequence analysis of exonic regions from 51 individuals revealed only two synonymous polymorphisms (90). Studies designed to identify polymorphisms of the α_{1D}AR have not been described.

Comparison of the Arg492 and Cys492 α_{1A}AR function has been investigated using transfected CHO cells stably expressing each receptor (91). Radioligand binding studies using [^{125}I]-HEAT showed no differences in agonist or antagonist binding. In addition, receptor-mediated calcium signaling, as well as the extent of receptor desensitization following agonist exposure, were also found to be similar for both receptors. Given the location of this SNP and the nature of the substitution, these findings were not altogether unexpected.

Human Studies

Although functional differences between the Arg492 and Cys492 receptors have yet to be identified, the potential relevance of α_{1A}AR genetic variation to multiple pathophysiological conditions has been examined. In these studies, the prevalence

of each polymorphic allele was ascertained in control individuals and patients with hypertension, benign prostatic hypertrophy, schizophrenia, or clozapine-induced urinary incontinence. Despite compelling evidence to support a role for α_{1A}AR in these conditions, no association of either polymorphic allele was found (89, 91–93). A trend showing an increase in the frequency of the Arg492 allele in patients with Alzheimer's disease has been reported, but the significance of this observation remains to be explored (94).

In summary, the Arg492 and Cys492 α_{1A}ARs have been shown to have similar pharmacological and functional characteristics, results consistent with a lack of association of either polymorphic allele with multiple pathophysiological conditions involving α_{1A}AR function. At this time, a complete interrogation of sequence spanning all coding and noncoding regions of each α_1AR subtype, performed using an appropriately powered repository of ethnically diverse DNA samples, has not been described. Thus, relevant α_1AR polymorphisms may remain unidentified.

α_{2A}-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

Direct sequence analysis of overlapping PCR products identified a SNP within the coding region of the α_{2A}AR gene (95). This polymorphism consists of a C to G transversion at nucleotide 753 that results in an Asn to Lys change at amino acid 251, a highly conserved residue within the third intracellular loop of the receptor (Figure 1). The Lys251 α_{2A}AR allele was found to be relatively rare, with frequencies of 0.4% and 5% in Caucasians and African-Americans, respectively (Table 1).

The consequences of this polymorphism on ligand binding and receptor coupling were assessed in CHO cells stably expressing either the Asn251 or Lys251 α_{2A}AR (95). Ligand binding was not altered by the presence of the polymorphism as shown by virtually identical dissociation binding constants for the α_2AR antagonist [^{3}H]yohimbine and no differences in binding to the agonist epinephrine. Activation of the polymorphic Lys251 receptor with the agonist epinephrine, however, resulted in ~40% increase in [^{35}S]GTPγS binding. This enhanced agonist-promoted G-protein coupling was also evident in multiple signaling pathways, with the Lys251 receptor showing increased agonist-promoted inhibition of forskolin-stimulated adenylyl cyclase activity and activation of MAP kinase (Figure 7). In each case, basal receptor function was equivalent for each receptor, whereas enhanced function was observed with several full and partial agonists in selected assays. It is interesting to note that the gain of function was much more for MAP kinase stimulation (~280%) compared to inhibition of adenylyl cyclase (~30%), which highlights the need to assess multiple pathways when determining phenotype. In addition to the Lys251 polymorphism within the coding region of the α_{2A}AR, several polymorphisms have also been identified that are located within noncoding regions of the gene (96–99).

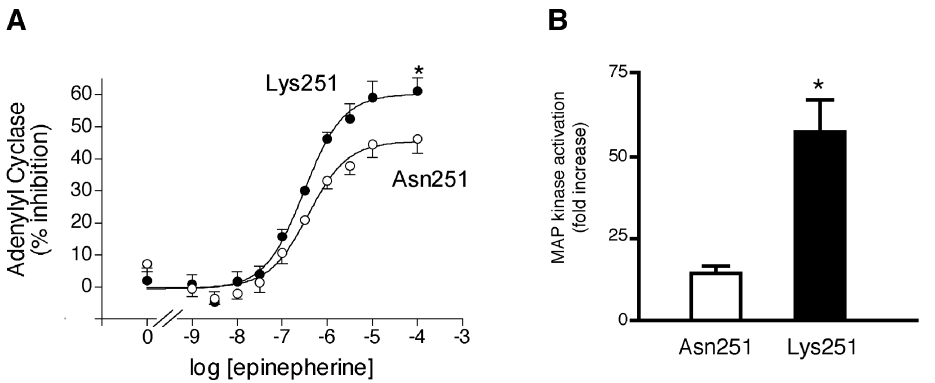

Figure 7 Enhanced coupling of the Lys251 α_{2A}AR polymorphism in transfected cells.

Human Studies

To date, no associations have been established for the Lys251 α_{2A}AR polymorphism and clinical phenotypes in which α_{2A}ARs are thought to play a role. One case-control study has been performed to ascertain the frequency of this polymorphism in patients with essential hypertension (95); however, considering the gain-of-function phenotype of the polymorphic receptor and the known centrally mediated hypotensive functions of the α_{2A}AR (100), results showing a lack of association with this polymorphism and essential hypertension are not surprising.

Although the functional consequences of the noncoding α_{2A}AR polymorphisms remain unknown, several clinical studies have been performed to examine potential associations of these polymorphisms with various disease states. For most of the noncoding polymorphisms, no associations have been found with multiple pathological conditions linked to α_{2A}AR function, including hypertension, panic and mood disorders, schizophrenia, and obesity (92, 99, 101–105). In contrast, several clinical phenotypes, including hypertension, body fat distribution, and glucose metabolism, have been shown to be associated with a Dra I restriction fragment length polymorphism within the 3′UTR (106–109). However, because the functional relevance of this polymorphism has not been determined, the significance of these results remains unclear.

α_{2B}-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

One polymorphic form of the α_{2B}AR, consisting of a three–amino acid deletion (denoted Del301-303) located within the third intracellular loop of the receptor, has been described (110). This polymorphism consists of an in-frame nine-bp deletion beginning at nucleotide 901 that results in the loss of three Glu residues at positions

301–303 (Figure 1). PCR amplification of the region spanning this polymorphism followed by agarose gel electrophoresis to distinguish the insertion/deletion alleles based on size was performed to determine the frequency of each allele in various populations. In doing so, the deletion allele was found to occur at a frequency of 31% in Caucasians and 12% in African-Americans (Table 1).

As with the polymorphisms of the other α_2AR subtypes, the effects of the α_{2B}AR deletion polymorphism on ligand binding and receptor function were assessed in CHO cells stably expressing the wild-type and the polymorphic Del301-303 receptors (110). Saturation binding with the antagonists [^{3}H]yohimbine and [^{125}I]aminoclonidine, as well as competition binding with the agonists epinephrine, revealed that deletion of amino acids 301–303 had little effect on ligand binding. In addition, receptor coupling to G_i, as determined by agonist-promoted inhibition of forskolin-stimulated adenylyl cyclase activity, showed only a modest decrease in Del301-303 receptor function as compared to the wild-type α_{2B}AR, manifested as an ~18 % decrease in the maximal inhibition of adenylyl cyclase activity and a ~twofold increase in the EC_{50} for this response. Because these deleted Glu amino acids are localized to an acidic region of the third loop that had been found to be essential for GRK-mediated phosphorylation and agonist-promoted desensitization (111), studies were carried out to investigate these functions. COS-7 cells were transiently transfected to express GRK2 and the two α_{2B}AR receptors and whole-cell agonist stimulated phosphorylation studies carried out (110). In such experiments, the Del301-303 receptor displayed ~56% of wild-type agonist-promoted phosphorylation (Figure 8*A*). For the wild-type α_{2B}AR receptor, agonist-promoted phosphorylation results in receptor desensitization, which is manifested as an increase in the EC_{50} for agonist-mediated inhibition of adenylyl cyclase activity. At a concentration of agonist equal to the EC_{50}, the differences in inhibition of adenylyl cyclase between control and agonist pre-exposed cells represents 54% desensitization with the wild-type α_{2B}AR (Figure 8*B*). In contrast to the

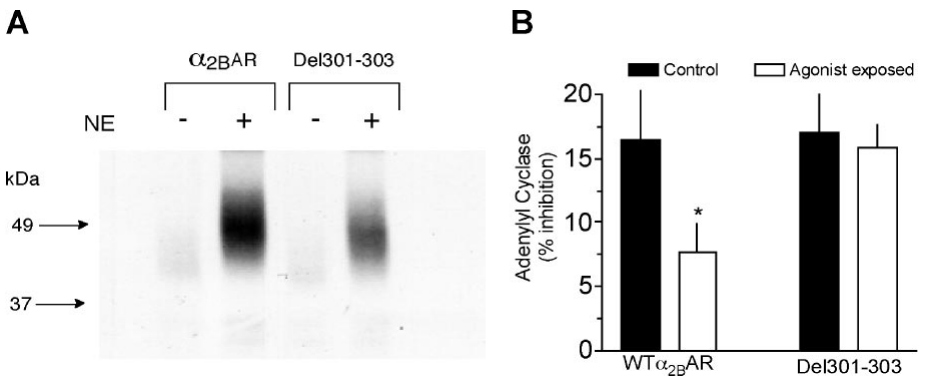

Figure 8 Altered agonist-promoted phosphorylation and desensitization of the Del301-303 α_{2B}-adrenergic receptor polymorphism in transfected cells.

desensitization observed with the wild-type receptor, the α_{2B}Del301-303 displayed a complete absence of desensitization (Figure 8*B*). This total loss of desensitization, despite only a partial loss of phosphorylation, is consistent with other studies of GRK-mediated phosphorylation of the α_{2A}AR and α_{2B}AR, which indicate that "full" phosphorylation (i.e., all sites are phosphorylated) is necessary to evoke desensitization (111, 112).

Human Studies

Several clinical studies have been performed to assess the role of the α_{2B}AR deletion polymorphism as a risk factor for disease. In terms of cardiovascular disease, it has been hypothesized that the presence of the deletion polymorphism may lead to increased vasoconstriction in humans (113, 114). This is based on the fact that α_{2B}AR have been shown to mediate the hypertensive effects of α_2AR stimulation (115) and that the polymorphic loss of three glutamic acid residues from the third intracellular loop of the receptor results in a complete loss of receptor desensitization (110). In a recent prospective study of Finnish men, individuals homozygous for the deletion polymorphism had 2.2 times the risk (95% CI = 1.1−4.4, p = 0.02) for experiencing acute coronary events, defined as prolonged chest pain or acute myocardial infarction. Thus, the presence of the deletion polymorphism may be a risk factor for the occurrence of acute coronary events (114). In contrast, no significant associations for this polymorphism have been observed for patients with essential hypertension (113, 114). In addition, because α_2ARs are also known to influence energy metabolism through inhibition of insulin secretion and lipolysis, several studies have been performed to investigate the potential roles of the α_{2B}AR deletion polymorphism in clinical parameters related to obesity. Heinonen et al. have shown that the deletion polymorphism is associated with reduced basal metabolic rates in obese patients (116), whereas Sivenius et al. found an increase in body weight in nondiabetic individuals homozygous for this polymorphism (117). In another study, a lack of association for either the α_{2B}AR deletion polymorphism or the β_3AR Arg64 polymorphism alone with various clinical parameters of obesity was noted, but a significant interaction of these two variants on fat mass and percentage of fat in Caucasian women was found (118).

α_{2C}-ADRENERGIC RECEPTOR POLYMORPHISMS

Localization and Characterization

One polymorphic form of the α_{2C}AR (Figure 1), consisting of a four–amino acid deletion (denoted Del322-325), has been identified within the third intracellular loop of this receptor (119). This polymorphism consists of a 12-bp in-frame deletion beginning at nucleotide 964 that results in the deletion of amino acids 322–325 (Gly-Ala-Gly-Pro). The Del322-325 allele was found to be common in African-Americans, with an allele frequency of ∼40%. In contrast, this polymorphism

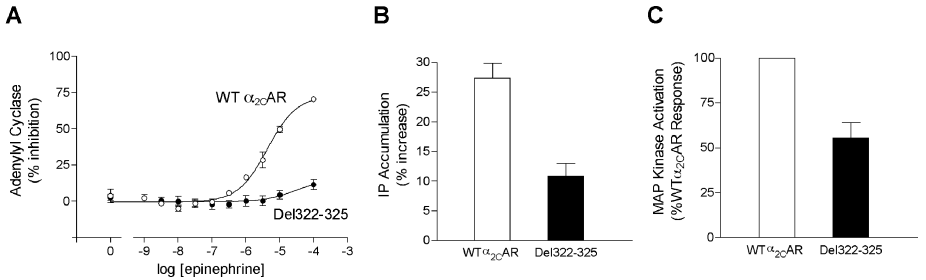

Figure 9 Decreased function of the Del322-325 α_{2C}AR polymorphism in transfected cells. IP = inositol phosphates.

was found to be relatively rare in Caucasians, with an allele frequency of ∼4% (Table 1).

The consequences of the Del322-325 α_{2C}AR polymorphism have been studied in CHO cells permanently expressing equivalent levels of wild-type (i.e., no deletion) and the α_{2C}Del322-325 receptor (119). In competition binding studies with the agonist epinephrine, carried out in the absence of GTP, the deletion receptor showed reduced high-affinity agonist binding, indicating an impaired ability of this receptor to form the high-affinity agonist-receptor-Gi/Go complex. Indeed, additional analyses showed impaired coupling of the polymorphic receptor to multiple signaling pathways. In cell lines expressing high levels of receptor (∼1200 fmol/mg), a ∼50% reduction in agonist-promoted inhibition of adenylyl cyclase was observed for the Del322-325 compared to the wild-type receptor. The impaired function of the deletion receptor was even more striking (∼86% reduction) in cell lines with low levels of receptor expression (∼500 fmol/mg) (Figure 9*A*). In addition, agonist-promoted coupling to activation of MAP kinase as well as accumulation of inositol phosphates via activation of phospholipase C were impaired 71% and ∼60%, respectively (Figure 9*B*,*C*).

Human Studies

In a recent study, the prevalence of the Del322-325 α_{2C}AR polymorphism in control and heart failure patients was ascertained. The study was based on the location and function of this subtype in cardiac presynaptic nerve terminals. Presynaptic autoinhibition of norepinephrine release is regulated by both the α_{2A}- and α_{2C}AR subtypes. The α_{2A}AR regulates release due to high-frequency stimulation, whereas the α_{2C}AR regulates release from low-frequency (basal) stimulation (13). In various animal models, prolonged stimulation of cardiac β_1AR via drug infusions, ablation of α_{2A}- and α_{2C}AR, or transgenic expression of β_1AR results in severe cardiomyopathy (12–15). We then considered that a defective α_{2C}AR, which would lead to chronic increased norepinephrine release, may predispose individuals to heart failure. The major findings in this case-control study were in

African-Americans, where the prevalence of the α_{2C}Del322-325 polymorphism is common. The odds ratio for heart failure in individuals with Del322-325 was found to be 5.65 (95% CI = 2.67−11.95, $p<0.0001$). Indeed, 53% of African-Americans with heart failure were homozygous for α_{2C}Del322-325, compared to 18% of controls. The association held for both idiopathic dilated and ischemic cardiomyopathies. As introduced earlier, a significant gene-gene interaction was observed with the α_{2C}Del322-325 and the β_1AR Arg389 alleles. The latter receptor has ∼threefold enhanced stimulation of adenylyl cyclase compared to the β_1AR Gly389. Thus, from a biological standpoint, the enhanced risk (odds ratio ∼10, see above) resulting from the synergistic actions of these receptor variants makes sense. Additional studies with β_1AR, α_2AR, and polymorphisms of other biologically linked genes need to be carried out in order to fully appreciate the genetic component in these complex heart failure and other cardiovascular syndromes.

CONCLUSIONS

Adrenergic receptors display a substantial degree of polymorphic variation in multiple different structural domains of the encoded proteins. Although not as extensively investigated, 5′ and 3′ noncoding regions also are variable. Both recombinant expression systems and human studies have indicated that many of the polymorphisms alter some aspect of receptor signaling. Clinical studies have further revealed that these polymorphisms may be risk factors for disease, modify disease, or alter the response to therapy. In most cases, the diseases studied are complex, with multiple phenotypes and environmental influences. Investigations utilizing haplotypes of these receptors and polymorphisms of other genes within common signal transduction pathways may lead to a further refinement in our understanding of their physiologic, pathologic, and pharmacologic importance.

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