Polymorphisms of the 5′ Leader Cistron of the Human β_2-Adrenergic Receptor Regulate Receptor Expression

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Abstract

Cellular expression of the β_2-adrenergic receptor (β_2AR) is controlled in part by a 19–amino acid peptide that regulates mRNA translation. This peptide is encoded by a short open reading frame, termed the 5′ leader cistron (5′LC), which is 102 bp upstream of the β_2AR coding block. In 176 normal subjects we found a single nucleotide polymorphism resulting in either Arg (previously denoted wild-type) or Cys at position 19 of this peptide. Allele frequencies were 0.37 for Arg and 0.63 for Cys. To determine if these variants altered β_2AR expression, COS-7 cells were transfected with polymorphic constructs consisting of 1,989 bp encompassing the 5′LC and the β_2AR coding block exactly as found in the human gene. β_2AR density, as determined by [^{125}I]CYP radioligand binding, was 72% higher in cells transfected with the 5′LC-Cys19 construct as compared with those transfected with the 5′LC-Arg19 construct and 110% higher when a cotransfection technique with a luciferase construct was used to control for transfection efficiency. Levels of the two mRNA transcripts were not different, confirming in vitro studies that the upstream peptide regulates receptor expression at the translational level. In human airway smooth muscle cells that natively express β_2AR, receptor expression was approximately twofold higher in those bearing the Cys versus the Arg polymorphism, confirming the phenotype in a relevant cell type. Linkage disequilibrium was observed between the 5′LC-Cys polymorphism and the β_2AR coding block polymorphisms Arg16 and Gln27 ($P < 0.0001$), although several different haplotypes were identified. Thus, β_2AR expression in the human population is controlled by a common polymorphism of this 5′LC, and may be responsible for interindividual variation in βAR responsiveness. (*J. Clin. Invest.* 1998. 102:1927–1932.) Key words: mutations • G protein–coupled receptors • catecholamines • translation

Introduction

β_2-adrenergic receptors (β_2AR)[1] are G protein–coupled receptors that are activated by endogenous catecholamines. These receptors are widely distributed and play important roles in regulating cardiac, vascular, pulmonary, and metabolic functions. Studies of such physiologic functions of β_2AR in humans have revealed several observations. First, there appears to be substantial interindividual variation in responsiveness, and secondly receptor function appears to be dynamically regulated as indicated by intraindividual variation. Recently, we have delineated significant genetic variability in the structure of the β_2AR in the human population due to polymorphisms (1, 2). These occur in the amino terminus of the receptor at amino acids 16 (Arg or Gly) and 27 (Gln or Glu) and in the fourth transmembrane spanning domain at amino acid 164 (Thr or Ile). In recombinant cell studies (3, 4), and in primary cultures of cells endogenously expressing these variants (5), clear phenotypic differences have been shown between the polymorphic receptors. The Gly16 receptor was found to undergo enhanced agonist-promoted downregulation of receptor number as compared with the Arg16 receptor (3). In contrast, the Glu27 receptor was found to undergo very little agonist-promoted downregulation compared with the Gln27 receptor (3). These variants are common in the population (1). The Ile164 receptor, which occurs in the heterozygous state in $\sim$5% of the population, displays depressed coupling to the stimulatory G protein, G_s (4).

Subsequent studies have assessed the role of the aforementioned polymorphic β_2AR in diseases such as asthma (for review see reference 6), based on the role of β_2AR in modulating bronchial smooth muscle tone. In these studies, no differences in the frequencies of any of these polymorphisms between nonasthmatics and asthmatics have been reported. However, polymorphisms at positions 16 and 27 were found to act as significant disease modifiers (7–10). In the majority of the above cited studies, the presumption has been that the clinical phenotypes of those with the Gly16 polymorphism were due to enhanced downregulation of this receptor (as compared with those with the Arg16 receptor) by endogenous catecholamines. Thus, responsiveness in individuals with this polymorphism has been considered depressed due to this tonic downregulation. A similar scenario is considered in those with the Glu27 variant, where responsiveness is greater than those with the Gln27 receptor, presumably due to its minimal downregulation by catecholamines. An amplification of these differences may occur during chronic agonist administration, as has been shown recently in asthma (11). Although this paradigm may in fact be correct, we have been prompted to consider whether polymorphisms in a region directly responsible for receptor expression (i.e., not dependent on agonist) might also be present in the human population. The β_2AR is encoded by an intronless gene on chromosome 5q31 (12). Receptor transcripts have a 5′ leader region harboring an open reading frame (ORF) that encodes a 19–amino acid peptide (13). Recent in vivo and in vitro studies have shown that this peptide impedes translation of β_2AR mRNA, and thus regulates cellular expression of the receptor (14). Given the importance of this 5′ leader cistron (LC) in controlling β_2AR expression, we examined this region in the human population for genetic vari-

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1. *Abbreviations used in this paper:* β_2AR, β_2-adrenergic receptor; LC, leader cistron; ORF, open reading frame.

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ability. We indeed identified a frequent polymorphism in a key position and found that genetic variation at this position significantly regulates β_2AR cellular expression.

Methods

Identification of polymorphisms. The study was approved by the University of Cincinnati College of Medicine Institutional Review Board. Genomic DNA was derived by the cetylmethyl ammonium bromide method (15) from peripheral blood obtained from 176 healthy Caucasian subjects without histories of chronic disease.

In this paper, the first nucleotide of the initiator methionine codon of the β_2AR coding block is denoted as nucleotide 1 and the adjacent 5′ residue as nucleotide −1. The 5′LC is thus localized to nucleotides −102 to −42. PCR was carried out using primers that provided for a product spanning this region (sense: 5′-AAGGACACCAC-CTCCAGCTTLAG-3′, antisense: 5′-GCGCATGGCTTACTATT-GGGLA-3′). Each reaction contained 1 U of *AmpliTaq* polymerase (Perkin Elmer, Norwalk, CT), 500 ng genomic DNA, 250 μM of each dNTP, and 37.5 pmol of each primer in a final volume of 50 μl. After an initial denaturation step of 98°C for 2 min, 35 cycles of 98°C for 30 s, 56°C for 30 s, and 72°C for 30 s were carried out, followed by a 7-min final extension at 72°C. 10 μl of the PCR reaction was electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining. The remaining PCR product was purified with a commercial kit (Promega, Madison, WI) and was sequenced by an automated sequencer (ABI 377 Prism) using dye-terminator chemistry (Fig. 1 *A*).

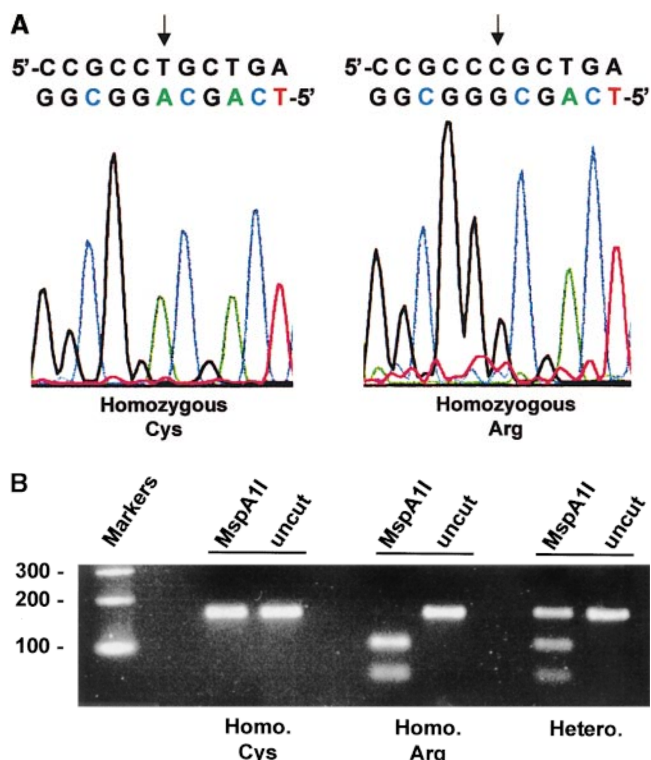

Figure 1. Identification of polymorphisms in the 5′LC of the human β_2AR. PCR products were sequenced as described in Methods. A variance (either C or T) at nucleotide −47 was found, encoding either Arg or Cys, respectively, at amino acid 19 of the peptide. (*A*) Representative antisense sequencing results from two homozygous individuals. (*B*) Representative restriction digests of a 163-bp PCR product digested with MspA1I. A C at position −47 results in two bands of 103 and 60 bp, while a T ablates the site, resulting in no digestion.

After sequencing 46 individuals, a single polymorphism (C or T at position –47) was identified. A rapid detection method was subsequently developed based on the loss of an MspA1I restriction endonuclease site when T is present (Fig. 1 *B*). The polymorphisms at nucleotides 46 and 79 (amino acids 16 and 27 of the receptor) were detected by the genetic bit analysis technique (16).

Transfection studies. The identified polymorphism results in either Arg or Cys in the translated peptide at amino acid 19 of the 5′LC. To test whether this variation altered receptor expression, cells were transiently transfected with two receptor constructs. These consisted of a contiguous stretch of 1,989 bp exactly as found in the human gene, comprising a 93-bp region upstream of the LC, the LC with its stop codon, an additional intervening 41 bp of 5′ sequence, the β_2AR coding sequence, and 557 bp of 3′ untranslated region. This cDNA was subcloned into the expression vector pBC12BI at the BamHI site. The two constructs, denoted 5′LC-Arg19 and 5′LC-Cys19, differed only by the nucleotide at position −47. Of note, both constructs encoded for Gly at position 16 and Glu at position 27 in the β_2AR coding block, which is the most common β_2AR genotype. COS-7 cells were transfected by a DEA-Dextran method with 15 μg of either construct as described previously (17). Cells were maintained in DME in 10% FCS at 37°C in a 95% air, 5% CO_2 atmosphere. 2 d after transfection, the confluent cells were harvested for determination of receptor expression by radioligand binding as described below. In another set of experiments, cells were cotransfected with the above constructs and a luciferase expression vector driven by the human β-actin promoter to provide for transient expression of firefly luciferase, which acted as a control for transfection efficiency. Expression of luciferase was determined by measurement of luminescence in cell lysates.

Human airway smooth muscle cell lines. To determine if the 5′LC polymorphisms affected β_2AR expression in cells endogenously expressing this receptor, human airway smooth muscle cells were obtained by rapid autopsy from individuals without lung disease. These cells were maintained in primary culture, genotyped, and radioligand binding carried out as described below. The methods used to establish these lines have been described previously (5).

Ribonuclease protection assays. Total cellular RNA was prepared from transfected cells by an acid guanidinium thiocyanate-phenol-chloroform extraction reagent (Molecular Research, Cincinnati, OH) and β_2AR transcripts delineated by ribonuclease protection assays in a manner similar to that described previously (18). A template for the synthesis of riboprobes was prepared by subcloning a cDNA encoding the β_2AR ORF into the HindIII/XbaI sites of plasmid pSP72 (Promega). Plasmid linearized with EcoNI was then used for in vitro transcription reactions with T7 polymerase and [^{32}P]UTP to generate a 563-bp antisense riboprobe corresponding to the distal 500 bp of the β_2AR ORF. In addition, a radiolabeled antisense riboprobe for β-actin was generated from a commercial template (Ambion Inc., Austin, TX) using T7 polymerase. Ribonuclease protection assays were performed as reported previously (18) by hybridizing 20 μg of total cellular RNA with both the actin and β_2AR riboprobes. The hybridized products were digested with RNase A and T1, after which protected fragments were separated by electrophoresis on 6% polyacrylamide gels containing 8 M urea. Radiographic bands corresponding to the protected fragments were visualized with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and conventional autoradiography. Band density was quantitated on the PhosphorImager with the ImageQuant software package (Molecular Dynamics). To account for minor differences in sample loading and recovery, the measured value for the β_2AR-protected fragment was normalized to that of β-actin in the same sample.

Receptor studies. β_2AR expression was quantitated by radioligand binding with [^{125}I]cyanopindolol ([^{125}I]CYP) using 1 μM propranolol to define nonspecific binding. Cells were disrupted by scraping with a rubber policeman in cold 5 mM Tris, 2 mM EDTA, pH 7.4 buffer and the particulates were centrifuged at 40,000 *g* for 10 min and resuspended in 75 mM Tris, 12 mM $MgCl_2$, 2 mM EDTA, pH 7.4

buffer. Membranes (~ 10 μg) were incubated in triplicate with a saturating concentration of [^{125}I]CYP (400 pM) without or with propranolol in the aforementioned buffer for 2 h at 25°C. The reaction was stopped by dilution and rapid vacuum filtration over Watman GF/C filters. Radioactivity was measured in a gamma counter at 80% efficiency. Protein was quantitated by the copper bicinchoninic method (19). In an additional set of experiments, COS cells were cotransfected with a β-actin–driven luciferase expression vector so that receptor expression could be normalized for transfection efficiency. Cells from half of a 150-mm culture dish were scraped and used for membrane preparations as described above. Lysates prepared from the remaining cells were then assayed for luciferase expression using a commercial luciferase assay system (Promega). Luminescence was measured using a Monolight 2010 luminometer (Analytical Luminescence Laboratory) and expressed per milligram of protein in the lysate. To account for differences in transfection efficiency, the value for [^{125}I]CYP binding (fmol/mg protein) was divided by the value for luciferase expression (light units/mg protein). The corrected value is thus reported as fmol/light unit (fmol/LU). Adenylyl cyclase activities were determined in membrane preparations as described previously (17).

Statistical analysis. The distributions of genotypes at nucleotides −47, 46, and 79 were analyzed by Fisher's exact test. Agreement between genotypes observed and those predicted by the Hardy-Weinberg equilibrium was assessed by a χ^2 test. Results from other studies were compared by paired or unpaired t tests as appropriate. $P < 0.05$ was considered significant. Data are presented as mean±SE.

Results

These studies were undertaken to delineate polymorphisms in the β_2AR 5′LC (14) which encodes the peptide MRLPGVRSRPAEPPRGSAR. One polymorphism was found within this region. This was at nucleotide −47, within the most 3′ codon of the sequence, where either a cytosine or thymidine was found (Fig. 1). This results in the encoded residue at amino acid 19 being either Arg or Cys. In 176 normal, unrelated subjects, homozygous Arg was found in 13% and homozygous Cys was found in 39%, with the remainder being heterozygotes. Thus, the allele frequencies were 0.37 for Arg and 0.63 for Cys in this population (Table I). The number of homozygous and heterozygous alleles that were found was no different from that predicted by the Hardy-Weinberg relationship ($P = 0.98$).

The relevance of this variation was assessed in COS-7 cells, where constructs that were identical except for the polymorphisms were used for transfection. These constructs consisted of a contiguous stretch of residues exactly as found in the human gene from nucleotides −194 to +1795. COS-7 cells were transfected with 15 μg of either the 5′LC-Arg19 or 5′LC-Cys19 constructs and 2 d later radioligand binding was carried out to determine the expression of β_2AR protein. As shown in Fig. 2 *A*, the 5′LC-Cys19 construct resulted in 72% higher levels of expression over that obtained with the 5′LC-Arg19 construct (4,666±302 vs. 2,711±294 fmol/mg, $n = 4$, $P < 0.01$). This is consistent with the aforementioned studies (14), which showed that the Arg leader polypeptide inhibited β_2AR translation. Additional studies were carried out with cotransfection of the above constructs and one encoding for luciferase, which allowed for control of any possible differences in transfection efficiency in the two plates. Radioligand binding and luciferase assays were then carried out and receptor levels were expressed as fmol/LU. Again, as shown in Fig. 2 *B*, the 5′LC-Cys19 construct provided for higher β_2AR expression (130% increase) as compared with the Arg construct (656±90 vs. 288±45 fmol/LU, $n = 4$, $P < 0.01$). Finally, adenylyl cyclase activities were determined in membranes from cells transfected with the two constructs. Consistent with the enhanced expression, isoproterenol-stimulated activities were higher in membranes obtained from transfection with the 5′LC-Cys19 construct as compared with the 5′LC-Arg19 construct (66.2±4.5 vs. 49.7±4.9 pmol/min/mg, $P < 0.02$, $n = 5$ experiments).

We reasoned that if the degree of DNA transfection was indeed the same between the two constructs, and the difference in β_2AR cellular expression was due to differences in peptide-mediated inhibition of translation (rather than transcription or mRNA stability), then there would be no differences in β_2AR mRNA levels between cells transfected with one construct versus the other. To assess this, mRNA levels were determined by a quantitative ribonuclease protection assay which included a probe for β-actin as a control. These results are shown in Fig. 3. In four independent experiments, β_2AR mRNA levels were found to be identical between cells transfected with the two constructs (as were transcripts for β-actin).

Table I. Distribution of Polymorphisms at Position 19 of the β_2AR 5′ Cistron

	No.	%
Homozygous Arg	23	13
Heterozygous	85	48
Homozygous Cys	68	39

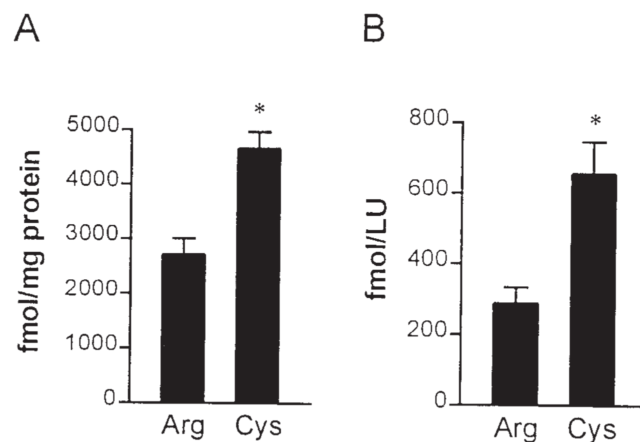

Figure 2. β_2AR expression in cells transfected with constructs containing polymorphic 5′LCs. COS-7 cells were transfected with 15 μg of either the 5′LC-Arg19 or 5′LC-Cys19 constructs as described in Methods. (*A*) β_2AR expression was determined by radioligand binding with [^{125}I]CYP performed in triplicate and is presented as fmol receptor/mg membrane protein. (*B*) Cells were cotransfected with either of the aforementioned 5′LC constructs and a construct encoding firefly luciferase, to control for transfection efficiency. Receptor density was measured by [^{125}I]CYP binding and luciferase activity was measured by luminescence. β_2AR expression is presented here as fmol receptor/LU. Using either approach, β_2AR expression was greater with the 5′LC-Cys19 as compared with the 5′LC-Arg19 construct. Shown are the results of four independent transfection experiments performed using each protocol (*$P < 0.01$).

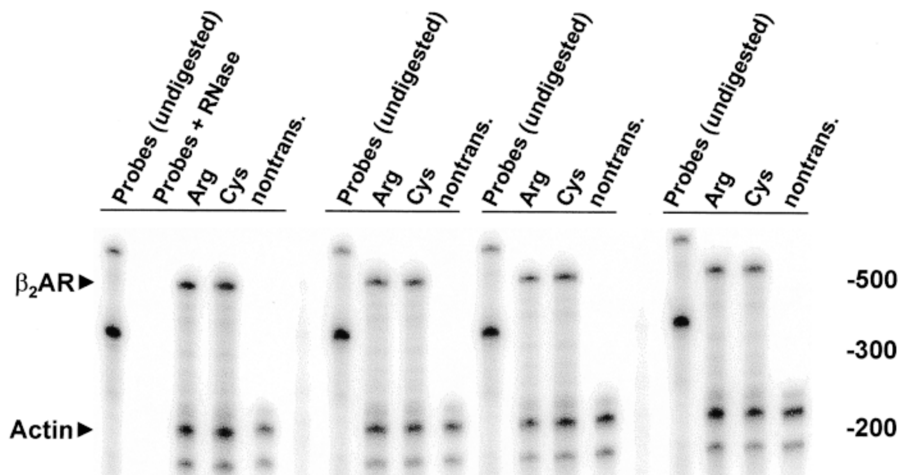

Figure 3. β_2AR mRNA expression in cells transfected with constructs containing polymorphic 5′LCs. COS-7 cells were transfected as in Fig. 2 and ribonuclease protection assays were carried out to identify β_2AR and β-actin transcripts. No differences in β_2AR mRNA expression were noted between cells transfected with constructs encoding for the Arg or Cys 5′LC variants (mean ratio Cys/Arg mRNA = 1.11±0.04, P = NS). Shown are data from four independent transfection experiments.

Having shown that the 5′ cistron polymorphisms had differential effects on β_2AR expression in a recombinant system, we wondered whether receptor expression correlated with the 5′LC polymorphism in cells that endogenously express the β_2AR. We used human airway smooth muscle because it expresses exclusively β_2AR, and the receptors on this cell have known physiologic relevance. Of note, linkage disequilibrium exists between the 5′LC and the coding block polymorphisms (see below), and we had a limited number of human airway smooth muscle lines available that were homozygous for the 5′LC polymorphisms. Thus, we did not have matches at positions 16 and 27 between the two cell lines used. (The 5′LC-Arg cells were homozygous for Gly and Glu at coding positions 16 and 27, whereas the 5′LC-Cys cells were Arg and Gln.) Nevertheless, a clear difference in β_2AR expression was found. As shown in Fig. 4, for cells homozygous for the 5′LC-Arg19 polymorphism, [^{125}I]CYP binding revealed β_2AR expression of 44±10 fmol/mg. As was seen with the recombinant studies, those with the 5′LC-Cys polymorphism had higher expression levels, which amounted to 84±12 fmol/mg (n = 5, $P < 0.02$).

To assess linkage disequilibrium between the previously described polymorphisms at nucleotides 46 and 79 (amino acids 16 and 27 of the receptor protein) and the 5′LC polymorphisms, some of the subjects underwent genotyping at all three sites. For this analysis, only those that were homozygous were used so as to unequivocally assign haplotypes. These results (Table II) show linkage disequilibrium between the Cys 5′ cistron polymorphism and the Gln27 coding block polymorphism ($P < 0.0001$) and to a lesser extent the Arg16 coding block polymorphism. The frequencies of other genotypic combinations at these three positions are shown in Table III.

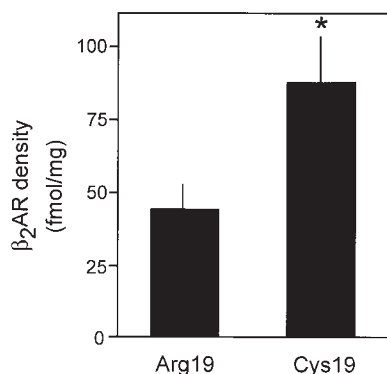

Figure 4. The 5′LC polymorphisms alter β_2AR expression in human airway smooth muscle cells. DNA was extracted and genotyped at nucleotide −47 as described in Methods from primary cultures of human airway smooth muscle. Receptor expression was determined by [^{125}I]CYP binding. The genotypes at the other β_2AR polymorphic loci are provided in Results. Shown are mean± SE for five independent experiments with each cell line. *$P < 0.02$.

Discussion

These studies were undertaken to assess the genetic variability, and consequences thereof, of the short ORF in a 5′ upstream region of the human β_2AR. This 5′LC encodes a 19-amino acid polypeptide (MRLPGVRSRPAEPRRGSAR) that has been shown by Parola and Kobilka to modify translation of β_2AR mRNA and thus expression of the receptor (14). Studies by these investigators were carried out in both COS-7 cells and in a cell-free expression system, with receptors being quantitated by [^{125}I]CYP binding and ^{35}S labeling. In the cell system, mutational inactivation of the 5′ short ORF (by mutating its start codon) increased β_2AR expression twofold. In vitro translation of β_2AR mRNA was increased 2.4-fold by inactivation of this short ORF. In addition, a synthetic peptide corresponding to this 5′ short ORF inhibited in vitro translation. The polymorphism that we identified was at the most 3′ codon, resulting in either an Arg (considered wild-type, see reference 13) or Cys in this position. Interestingly, in the earlier cited work, mutations of the four positively charged arginines in the carboxy-terminal portion of the peptide to alanines increased β_2AR translation by 50%. This is consistent with reports that arginine-rich peptides inhibit translation (20).

Table II. Haplotypes of β_2AR Polymorphisms in the 5′ Cistron and the Coding Block

	5′ cistron		
Coding block	Arg19	Cys19	P value
Arg16	0	19	< 0.0001
Gly16	19	6	
Gln27	0	42	< 0.0001
Glu27	18	0	

Shown are the numbers of subjects in each group.

Table III. Genotypes at Three Polymorphic Loci of the β_2AR (n = 130 Subjects)

Genotype				
5′LC19	cb16	cb27	No.	% total
Het	Het	Het	41	31.5
Het	Gly	Het	25	19.2
Cys	Arg	Gln	19	14.6
Arg	Gly	Glu	18	13.8
Cys	Het	Gln	17	13.1
Cys	Gly	Gln	6	4.6
Het	Het	Gln	1	0.8
Arg	Gly	Het	1	0.8
Cys	Het	Het	1	0.8
Het	Gly	Gln	1	0.8

5′LC, β_2AR 5′ leader cistron; *cb*, β_2AR coding block; *Het*, heterozygous. Arg, Cys, Gln, and Glu represent homozygous alleles at these positions.

The polymorphism identified in the current report results in a change in the positively charged carboxy-terminal arginine to a neutral cysteine. This resulted in an increase in receptor expression which is remarkably similar in magnitude to that observed in the aforementioned study when the 5′LC was mutated such that it was not encoded or lacked the arginines. In agreement with the evidence presented in that study showing that the peptide inhibits translation, we found no differences in the levels of mRNA from cells transfected with the two constructs. Our results cannot be explained by different efficiencies of transfection, since mRNA levels were not different, and expression of luciferase, whose expression was not expected to be regulated by the peptide, was not different between the two sets of cells. Thus, we can infer that the differences in expression of the receptor are due to the effects of the encoded wild-type or mutated 5′LC peptide on receptor translation.

To confirm the importance of this variation in the 5′LC of the β_2AR, we studied human airway smooth muscle cells which endogenously express β_2AR, comparing receptor expression between lines bearing the two different 5′LC alleles. In these studies (Fig. 4) the 5′LC-Cys19 polymorphism had ~ 100% higher expression level compared with cells expressing 5′LC-Arg19. This is similar in magnitude to the 72% and 130% increases observed in the two COS-7 transfection systems (Fig. 2). Thus, it appears that genetic variation at this position of the β_2AR 5′LC regulates receptor expression in physiologically relevant cells, endogenously expressing the receptor under control of the native promoter. While it is recognized that other factors also influence expression in the intact lung, taken together these studies strongly implicate these 5′LC polymorphisms as critical components to lung (and likely other organ systems) β_2AR expression in vivo.

As introduced earlier, several studies have been carried out examining potential relationships between polymorphisms in the β_2AR coding regions and phenotypic characteristics in diseases such as asthma, hypertension, and obesity. We wondered whether one could retrospectively gain additional insight into these results by attempting to assign a 5′LC genotype based on the genotypes at the nearby nucleotides encoding amino acids 16 and/or 27 of the β_2AR coding region. Therefore, we determined genotypes at all three loci in 130 normal individuals. Analysis of homozygous genotypes revealed that the 5′LC-Cys19 polymorphism most commonly occurs with Gln27 and Arg16. It is interesting to note that some combinations of the 5′LC and the coding block polymorphisms are likely to display extreme phenotypes. For example, the 5′LC-Arg19 in combination with the Gly16 β_2AR polymorphism would be expected to have low expression at baseline, and then after the enhanced downregulation afforded by Gly16 would have even lower expression after agonist exposure. Thus, the overall physiologic responsiveness in individuals with this haplotype would be expected to be substantially less under these conditions than, for example, those with the 5′LC-Cys19 and coding block Arg16 genotype, where baseline expression is higher and the receptor undergoes less downregulation. However, the consequences of this or other haplotypes have not been specifically tested. Nor is it known whether any interactions between the 5′LC and coding block polymorphisms occur.

As indicated in Tables II and III, several different possible genotypic combinations were in fact detected in our population. Given that some of the findings in clinical studies with the coding block polymorphisms are of marginal statistical significance (8, 11), it would be interesting to consider whether these results would be different if stratification of the patients with the 5′ cistron polymorphisms was considered. Having now demonstrated that these polymorphisms are relevant to receptor expression, new studies or retrospective genotyping is indicated. Our results may explain the recent findings of Martinez et al. (9) who found that individuals with the coding block Arg16 polymorphism were much more likely to have a bronchodilatory response to the β-agonist albuterol than those with the Gly16 genotype. These subjects had been withdrawn from β-agonists before the study, so the presumption has been that the Gly16 receptor was downregulated due to endogenous catecholamines, and thus the depressed response to albuterol. However, we now know that those individuals with the Gly16 coding block polymorphism likely have the Arg19 5′LC polymorphism which results in lower expression of β_2AR. Thus, it would be expected that the initial response to agonist would be depressed in these subjects. Similarly, a recent study has shown linkage and association between serum total IgE levels and the Gln27 coding block polymorphism. Given that activation of β_2AR increases IgE production (21), the basis for this correlation with Gln27, which downregulates more than the Glu27 receptor, was not clear. However, those with the Gln27 coding block polymorphism likely have the Cys19 polymorphism in the 5′LC (which results in increased β_2AR expression), so these observations with IgE levels now appear to make sense from a mechanistic standpoint.

Studies over the last several years of mutations/polymorphisms of G protein–coupled receptors in the human population have revealed several classes of genetic variations. One class is composed of mutations that alter receptor function or expression and are the direct cause of a disease. These diseases are typically rare and the mutation is not found in healthy (nonaffected) individuals. Examples of this class are mutations of the luteinizing hormone receptor that cause constitutive activation and results in familial male precocious puberty (22), the calcium sensing receptor which causes constitutive activation and results in familial hypoparathyroidism (23), and the V2 vasopressin receptor that causes depressed receptor function and results in nephrogenic diabetes insipidus (24). A second class of genetic variation of G protein–coupled receptors

comprises those that alter receptor function or expression but do not appear to be the direct cause of a disease. Here, the variation can be common in apparently healthy individuals. Studies to date indicate that these polymorphisms may act as disease modifiers (6) with the physiologic consequences becoming apparent when receptor function is critical for compensation in the diseased state or for the response to therapy. As such, they may be responsible for certain clinical subsets of a given disease (such as different phenotypes of asthma or hypertension) or represent the basis of differential responsiveness to therapeutic agents. The coding block β_2AR polymorphisms previously described represent this class of genetic variation. In asthma, the disease in which the role of β_2AR polymorphisms has been studied most extensively, the frequency of β_2AR coding block polymorphisms is no different between asthmatics and normal individuals. Whether the 5′LC polymorphisms are associated with asthma has not been tested to date. However, given the linkage disequilibrium between the common coding block polymorphisms and the 5′LC polymorphisms, we suspect that it is unlikely that one of the latter will be uniquely confined to a disease population. On the other hand, within asthmatic cohorts, the coding block polymorphisms are associated with the response to β-agonists (9), tachyphylaxis to β-agonists (11), airway responsiveness to bronchoconstrictive agents (8), and certain asthmatic phenotypes (10, 25). In complex diseases that are likely multigenetic, polymorphisms may also directly contribute to the disease, but only in the context of multiple other mutations. This may be the case for β_2AR polymorphisms in hypertension, where it has been reported in African-Caribbeans that the Gly16 variant is more common in hypertensives than normotensives (26). Similarly, the Glu27 receptor is associated with obesity (27) but it is unlikely that this polymorphism is a major cause of obesity given its high prevalence in the normal population. These examples may thus represent a third class of G protein–coupled receptor genetic variations, or a subset of the second class as delineated above.

In summary, we have detected a common polymorphism in normal individuals in the β_2AR 5′LC. This variation results in either Arg or Cys being encoded at the terminal amino acid (position 19) of the peptide. The Cys polymorphism results in increased expression of β_2AR in both recombinant and endogenously expressing cells as compared with the Arg polymorphism. Thus, this 5′LC variation may represent the genetic basis of variable physiologic sympathetic responses, variation in disease phenotypes, or differences in therapeutic efficacy of β-agonists or antagonists.

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