ELSEVIER

European Journal of Pharmacology 421 (2001) 141–147

ejp

www.elsevier.nl/locate/ejphar

The Ile164 β_2-adrenoceptor polymorphism alters salmeterol exosite binding and conventional agonist coupling to G_s

Stuart A. Green [a], Deborah A. Rathz [b], Amy J. Schuster [a], Stephen B. Liggett [a,b,*]

[a] *Department of Medicine, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Room G062, Cincinnati, OH 45267-0564, USA*
[b] *Department of Pharmacology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA*

Received 22 March 2001; received in revised form 2 May 2001; accepted 11 May 2001

Abstract

β_2-adrenoceptors (β_2AR) are polymorphic at amino acid 164 (Thr or Ile) of the fourth transmembrane domain. In transfected fibroblasts, six agonists commonly used in the treatment of bronchospasm were studied. Isoproterenol, albuterol, metaproterenol, terbutaline, formoterol, and salmeterol displayed decreased binding affinities (K_is were 1.2–3.0-fold higher) and a significant degree of impaired maximal stimulation of adenylyl cyclase (~40%), was observed with all agonists for the Ile164 receptor. The ratios of signal transduction efficiencies (Tau function, Ile164/Thr164) varied from a low of 0.17 for terbutaline to 0.49 for salmeterol. In addition, Ile164 bound salmeterol at the exosite, as delineated in perfusion washout studies, at a decreased level ($31 \pm 4.8\%$ vs. $49 \pm 4.4\%$ retained salmeterol, respectively, $P = 0.02$). In cAMP production studies under perfusion conditions, this decreased exosite binding caused a ~50% decrease in the duration of action of salmeterol at Ile164 ($t_{1/2} = 21.0 \pm 3.6$ vs. 46.8 ± 4.1 min for Thr164, $P = 0.001$). The durations of action for isoproterenol and formoterol under similar perfusion conditions were not different between the two receptors. These in vitro results indicate the Ile164 polymorphic receptor represents a pharmacogenetic locus for the most commonly utilized agonists in the treatment of asthma with a unique phenotype for salmeterol. © 2001 Published by Elsevier Science B.V.

Keywords: Mutation; G-protein; Adenylyl cyclase

1. Introduction

The β_2-adrenoceptor (β_2AR) is expressed in many cell-types in the body subserving a wide range of physiologic functions in the sympathetic nervous system (Green and Liggett, 1996a; Liggett, 1997). β_2AR expressed on bronchial smooth muscle act to relax the muscle (Liggett, 1997), affording bronchodilation and increased airflow. β_2AR agonists, directed at these airway receptors, are the most commonly utilized therapeutic agents for the treatment of bronchospasm in asthma and chronic obstructive lung disease (COPD) (Tattersfield, 1997). Of particular concern within these populations are those who overuse β-adrenoceptor agonists or have significantly depressed responsiveness, since these individuals may be at risk of increased morbidity and mortality (Spitzer et al., 1992; Suissa et al., 1994; Sears et al., 1990; Beasley et al., 1999).

In the human population, the β_2AR displays genetic heterogeneity (Reihsaus et al., 1993), with nonsynonymous single nucleotide polymorphisms occurring at four loci within the coding region. The common polymorphisms occur at amino acid positions 16 (Arg or Gly) and 27 (Gln or Glu). At amino acid position 164, either Thr or Ile can be found, with the latter occurring with a heterozygous frequency of ~5%. This polymorphism is localized within the fourth transmembrane spanning domain (TMD) of the receptor, and influences the binding "pocket" for conventional agonists which is formed by the TMDs (Green et al., 1993). Using site-directed mutagenesis and recombinant expression in Chinese hamster fibroblasts, we have shown that the affinity of the Ile164 receptor for epinephrine, norepinephrine and isoproterenol is decreased. Furthermore, the coupling of Ile164 to G_s as assessed in adenylyl cyclase assays, in response to the agonist epinephrine, is depressed compared to that of the wild-type Thr164 receptor (Green et al., 1993). This functional phenotype appears to be due to altered physical coupling of the receptor to $G\alpha_s$ and thus decreased formation of the high-affinity agonist-receptor-$G\alpha_s$ complex (Green et al., 1993). The consequences of the Ile164 receptor have also been ex-

* Corresponding author. University of Cincinnati College of Medicine, 231 Albert Sabin Way, Room G062, Cincinnati, OH 45267-0564, USA. Tel.: +1-513-558-4831; fax: +1-513-558-0835.
E-mail address: stephen.liggett@uc.edu (S.B. Liggett).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01049-4

plored in transgenic mice (Turki et al., 1996). In these studies, the Thr164 and the Ile164 receptors were overexpressed in the heart (where β_2AR exert positive inotropic and chronotropic effects) and in vitro adenylyl cyclase signaling and in vivo cardiac function assessed. In response to the β-adrenoceptor agonist isoproterenol, adenylyl cyclase activities were reduced as were the inotropic and chronotropic responses.

All the above functional studies have been carried out only with the aforementioned prototypic β-adrenoceptor agonists. However, the β-adrenoceptor agonists currently available for the treatment of bronchospasm have a wide range of structures and pharmacologic properties. Structurally, these vary from minimal substitutions at the catechol ring and the amine head groups (albuterol) to complex substitutions such as the aralkyloxalkyl substitution at the amine group found with salmeterol (Derom and Pauwels, 1997). These drugs differ in their subtype specificities, potencies and efficacies for activation of β_2AR, which has led to the notion that these synthetic agents can interact with the receptor in agonist-specific ways (Derom and Pauwels, 1997). Indeed, the long duration of action of salmeterol ($\sim$12 h) has been shown to be due to binding of the aforementioned side chain to TMDs (including TMD4), which anchors the agonist to the receptor providing for repetitive binding events (Green et al., 1996b; Rong et al., 1999). Such binding has been termed exosite binding (Liggett and Green, 1996), since it lies outside the critical contact points for conventional agonists (Strader et al., 1989, 1995; Wieland et al., 1996), which are in TMDs 3, 5 and 6. In contrast, a similar long duration of action found with formoterol is thought to be due to a lipid depot effect in the cell membrane (Nials et al., 1993). Given this agonist pleiotropy, we have considered that the phenotype of the Ile164 receptor may be dependent on agonist structure. We have thus pursued the pharmacogenomic properties of this receptor using the common β-adrenoceptor agonists used for the treatment of asthma and COPD.

2. Materials and methods

2.1. Mutagenesis and cell transfections

Site-directed mutagenesis was carried out as described previously (Green et al., 1993) such that a cytosine or a thymine is present at nucleotide 491 of the human β_2AR open reading frame encoding Thr or Ile, respectively, at amino acid 164. The constructs consisted of these coding blocks cloned into the mammalian expression vector pcDNA/Neo1. Both receptors had Arg at position 16 and Gln at position 27. Chinese hamster fibroblasts (CHW 1102 cells) were permanently transfected using a calcium phosphate technique as described (Green and Liggett, 1994a). Cells were grown in Dulbecco's modified Eagles medium supplemented with 10% fetal calf serum, and 100 units/ml penicillin, and 100 μg/ml streptomycin in a 95% air, 5% CO_2 atmosphere at 37°.

2.2. Radioligand binding and adenylyl cyclase activity studies

Cells were washed twice with phosphate buffered saline (PBS) and then detached by scraping in 5 mM Tris (pH 7.40) 2 mM EDTA buffer at 4°C. Particulates were centrifuged at $40{,}000 \times g$ for 15 min and resuspended in various buffers as indicated. Expression levels of β_2AR were determined by [^{125}I]cyanopindolol (^{125}I-CYP) radioligand binding on membranes as described (Green and Liggett, 1994a) for 2 h at 25°C. Competition assays were performed using 12 concentrations of drug, and 40 pM ^{125}I-CYP in 75 mM Tris (pH 7.40), 2 mM EDTA, 12 mM $MgCl_2$ buffer containing 100 μM GTP. Agonists studied were: (−) isoproterenol, (±) albuterol, (±) metaproterenol, (±) terbutaline, (±) formoterol and (±) salmeterol. Radioligand binding assays were stopped by dilution with cold 20 mM Tris pH 7.5 buffer and rapid vacuum filtration over glass fiber filters. For adenylyl cyclase assays, membranes were incubated with 30 mM Tris, pH 7.4, 2.0 mM $MgCl_2$, 0.8 mM EDTA, 120 μM ATP, 60 μM GTP, 2.8 mM phosphoenolpyruvate, 2.2 μg/ml myokinase, 100 μM cAMP, and 1 μCi [α-^{32}P]ATP for 30 min at 37°C as described (Green et al., 1993). [^{32}P]cAMP was separated from [α-^{32}P]ATP by chromatography over alumina columns. A [^{3}H]cAMP standard included in the stop buffer accounted for individual column recovery. Activities were determined in the presence of vehicle (basal), the indicated concentrations of agonists, or 100 μM forskolin. Protein determinations were by the copper bicinchoninic method (Smith et al., 1985).

2.3. Salmeterol studies

As previously described in detail (Green et al., 1996b), we utilized an assay which assesses the maintenance of binding of salmeterol to the β_2AR during extensive washing. Briefly, attached cells expressing wild-type or Ile164 receptors were exposed in 30-mm culture dishes to 10 μM isoproterenol, salmeterol, formoterol or vehicle for 10 min at 37°C. Cells were then washed by aspiration of the media and two exchanges with PBS, and then continuously perfused at 25° with PBS at a rate of 20 ml/min for salmeterol and formoterol. Because the effects of isoproterenol decayed very rapidly during such washout, the perfusion rate was decreased to 5 ml/min for studies with this agonist. At the indicated times, perfusions were stopped, media added, and the dishes incubated for 10 min at 37°. The media were then aspirated and stored for cAMP determination by radioimmunoassay. The dishes were then returned to the perfusion apparatus for additional washing. The levels of cAMP at each time point were normalized to the response observed following the initial exposure to

Table 1
Results of [^{125}I]CYP competition radioligand binding studies with wild-type and Ile164 β_2AR
Results are from four independent experiments.

Agonist	K_i (nM)		K_i mutant/ K_i wild type
	Thr164	Ile164	
Isoproterenol	166.9 ± 9.4	494.6 ± 43.0[a]	3.0 ± 0.2
Terbutaline	7216 ± 571.5	16636 ± 1951.6[a]	2.3 ± 0.3
Albuterol	1239.3 ± 39.9	1978.0 ± 236.5	1.6 ± 0.2
Formoterol	28.0 ± 1.2	67.1 ± 1.9[a]	2.4 ± 0.2
Metaproterenol	14123.3 ± 502.8	23576.7 ± 2792.7	1.7 ± 0.2
Salmeterol	3.1 ± 0.7	3.3 ± 0.2	1.2 ± 0.3

[a] $P < 0.05$ vs. Thr164.

salmeterol. Time course data for these functional experiments were fit to an exponential decay function, such that the maximal change and the time to half-maximal change ($t_{1/2}$) could be calculated. In addition, radioligand binding with ^{125}I-CYP was performed after 20 min of perfusion in order to determine the number of receptors not occupied by salmeterol. Results from the binding experiments are presented as exosite binding, defined as the proportion of receptors occupied by salmeterol (and thus unavailable for binding to ^{125}I-CYP), compared to receptor density determined in cells pretreated with vehicle.

2.4. Statistical analysis

Results are presented as means ± S.E. Comparisons were by paired or unpaired two-way *t*-tests, as appropriate, with significance imparted when $P < 0.05$. Signal transduction efficiencies were calculated from the adenylyl cyclase dose–response curves using the Tau function as described (Black et al., 1985; Lohse et al., 1990) and the Tau ratios (Ile164/WT) are presented. Curve fitting was carried out using Prizm software (GraphPad, San Diego, CA).

3. Results

Initial studies focused on conventional ligand binding affinities and stimulation of adenylyl cyclase for the six agonists. The results of competition binding studies are shown in Table 1. As previously described (Green et al., 1993), isoproterenol had a lower affinity for the Ile164 receptor, as indicated by a ~3-fold higher K_i as compared to that for the Thr164 receptor. The other agonists had K_is that varied between 1.2- and 2.4-fold greater with the Ile164 receptor compared to the wild-type β_2AR. The differences were statistically significant for isoproterenol, terbutaline and formoterol. Adenylyl cyclase studies were carried out with membranes from permanently transfected cells expressing the Thr164 or Ile164 receptors at 1051 ± 70 and 931 ± 47 fmol/mg, respectively. As previously delineated, membranes from the Ile164 receptor expressing cells had slightly lower basal adenylyl cyclase activities compared to wild-type, consistent with a decreased spontaneous activation (Green et al., 1993). As shown in Fig. 1 and Table 2, all agonists studied exhibited less maximal stimulation of adenylyl cyclase with the Ile164 receptor compared to the wild-type (Thr164) β_2AR. The defect is receptor specific, in that the forskolin-stimulated adenylyl cyclase responses were identical between membranes from the two cell lines (362 ± 50 vs. 345 ± 48 pmol/min/mg, respectively). The phenotype was present with highly efficacious agonists (isoproterenol, formoterol), partial ago-

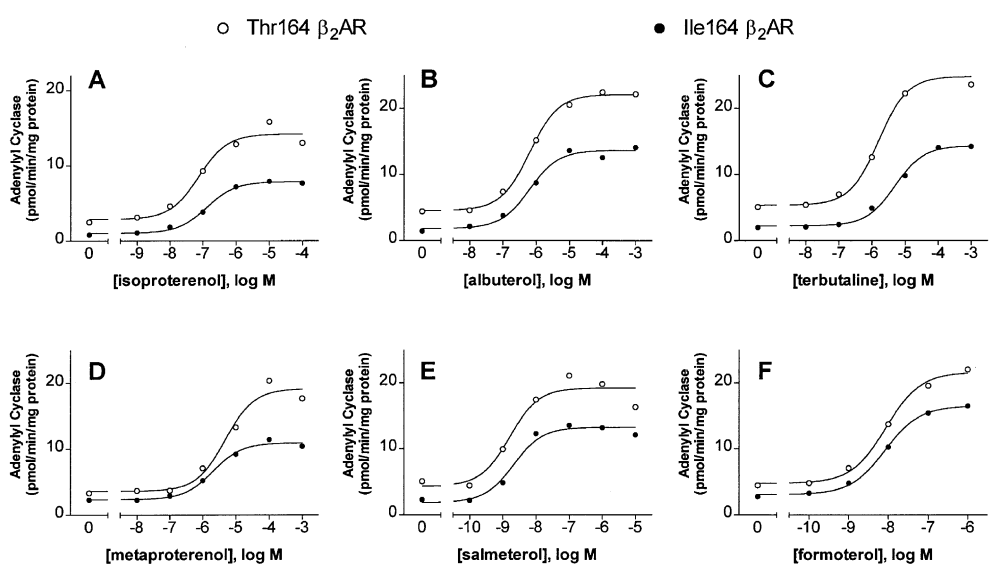

Fig. 1. Agonist activation of adenylyl cyclase of wild type and Ile164 β_2AR. All agonist-stimulated activities were statistically less ($P < 0.01$) with the Ile164 receptor compared to wild type (Thr164) β_2AR. Results shown are representative of three to five independent experiments performed in each group with the indicated agonist. See Table 2 for mean data.

Table 2
Agonist-promoted adenylyl cyclase activities for wild-type and Ile164 β_2AR expressed in CHW cells
Results are mean ± S.E. from three to five independent experiments. Basal activities are the means derived from all the experiments.

	Receptor	Basal	ISO	ALB	TERB	MET	SALM	FORM
WT	R_{MAX} (pmol/min/mg)	4.72 ± 0.34	17.9 ± 1.6	23.8 ± 1.1	22.9 ± 0.9	17.3 ± 1.0	18.7 ± 1.0	19.5 ± 0.5
	EC_{50} (nM)		44 ± 7	600 ± 16	1731 ± 107	3764 ± 489	3.01 ± 0.51	9.07 ± 0.35
Ile164	R_{MAX} (pmol/min/mg)	2.68 ± 0.13[a]	11.9 ± 2.3[a]	15.2 ± 0.9[a]	15.3 ± 0.5[a]	12.1 ± 0.7[a]	12.5 ± 0.5[a]	14.5 ± 0.3[a]
	EC_{50} (nM)		104 ± 31[a]	627 ± 10	3628 ± 899[a]	3028 ± 459	3.72 ± 0.47	6.82 ± 0.61
	Tau Ratio (Tau WT/Tau Ile164)		0.23	0.20	0.17	0.27	0.49	0.39

ISO, isoproterenol; ALB, albuterol; TERB, terbutaline; MET, metaproterenol; SALM, salmeterol; FORM, formoterol.
[a] $P < 0.05$ compared to wild type.

nists (albuterol, terbutaline, metaproterenol, salmeterol) and agonists with long durations of action (formoterol, salmeterol). From these agonist dose–response studies, the Tau values were calculated (Black et al., 1985; Lohse et al., 1990) as measures of signal transduction efficiencies. This function takes into account both potency and efficacy. The ratios of Tau for the Ile164 receptor to that of the wild-type β_2AR for each agonist are shown in Table 2. The Ile164 defect was approximately the same (Tau ratio 0.17–0.27) for isoproterenol, albuterol, terbutaline and metaproterenol. Salmeterol and formoterol activation were also impaired, but to a somewhat lesser extent (0.49 and 0.39). So, in terms of activating adenylyl cyclase, no commonly utilized β-adrenoceptor agonist used for treating bronchospasm "escapes" the defect imposed by the Thr to Ile substitution.

We next turned our attention to the interaction of salmeterol with the two receptors. As introduced earlier, we have shown that the prolonged duration of action of this agonist is due in part to an interaction between the long side chain of the amine head group of salmeterol and the fourth TMD of the β_2AR (Green et al., 1996b). Given that the non-conservative Ile164 substitution occurs within the mid-portion of TMD4, we considered that this interaction may be perturbed in the polymorphic receptor. To address this, cells expressing the Thr164 or Ile164 receptors were exposed to a saturating concentration of salmeterol to allow for initial binding, washed to remove the drug from the media, and then perfused with buffer to determine retention of the agent via radioligand binding and cAMP measurements. This method has been previously utilized in transfected cells (Green et al., 1996b; Clark et al., 1996), and variations of the approach have been used with endogenously expressing tissues (Nials et al., 1993) and intact trachea (Ball et al., 1991). Such retention does not occur with the β_1AR subtype, but can be conferred to the β_1AR via mutagenesis by substituting the β_2AR TMD4 (Green et al., 1996b). Moreover, the binding and functional studies carried out under these

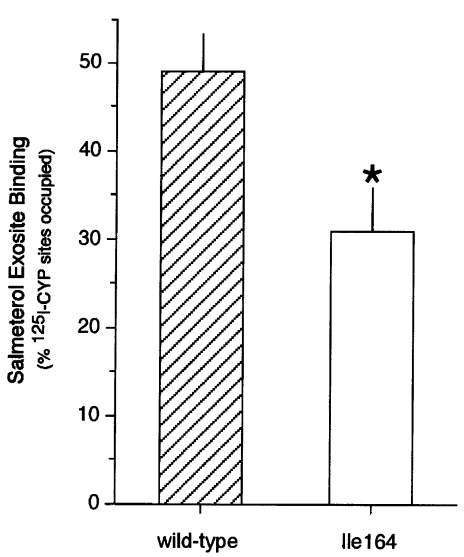

Fig. 2. Retention of salmeterol during washout is reduced with Ile164 compared to wild type β_2AR. Cells in culture expressing the two receptors were exposed to vehicle or 10 μM salmeterol for 10 min, the media aspirated, washed twice with PBS, and then the cells were perfused with PBS at a rate of 20 ml/min. At 20 min, cells were detached, membranes prepared, and [^{125}I]CYP saturation binding carried out (see Materials and methods) to determine the number of unoccupied sites remaining. The Ile164 receptor retained less salmeterol than wild type β_2AR. Results are from five independent experiments. $^{*}P < 0.02$.

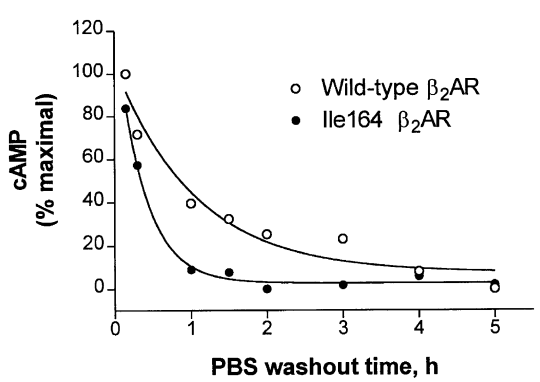

Fig. 3. Functional consequences of decreased salmeterol exosite binding to the Ile164 receptor. Cells expressing each receptor underwent the same exposure to salmeterol with washout as described in Fig. 2, except that at the indicated times the perfusion was stopped, and cAMP accumulation over 10 min determined (see Materials and methods). Results are expressed as a percentage of the initial cAMP response to salmeterol. The decay of cAMP stimulation from retained salmeterol was accelerated with Ile164 compared to wild type β_2AR. Shown are the results from a single experiment performed in triplicate which are representative of five performed. For five such experiments, the $t_{1/2}$ was 21.0 ± 3.6 vs. 46.8 ± 4.1 min, $P = 0.001$. Washout studies with isoproterenol and formoterol showed no differences (see Table 3).

Table 3
Kinetics of cAMP production from cells expressing WT and Ile164 β_2AR under perfusion conditions
For studies with salmeterol and formoterol, the perfusion rate was 20 ml/min; for isoproterenol the rate was 5 ml/min. Results are the $t_{1/2}$ values from five independent experiments.

	Isoproterenol	Salmeterol	Formoterol
Wild-type	17.3 ± 4.9	46.8 ± 4.1	36.4 ± 12.7
Ile164	15.3 ± 1.9	21.0 ± 3.6[a]	44.5 ± 10.9

[a] $P < 0.05$.

conditions show this interaction exclusively with salmeterol, as opposed to agents such as albuterol, and the other long-acting β-adrenoceptor agonist formoterol which appears to have prolonged activation by another mechanism (Green et al., 1996b; Ball et al., 1991). The results of radioligand binding studies under these conditions are depicted in Fig. 2. As shown, after extensive washing by perfusion, 49 ± 4.4% of Thr164 receptors remain occupied by salmeterol. In contrast, 31 ± 4.8% of the Ile164 receptors remained occupied under the same conditions ($n = 5$, $P = 0.02$). This difference was not due to an altered salmeterol binding affinity at the active site as determined in ^{125}I-CYP competition studies (Table 1), where the K_is for the wild-type and the Ile164 receptors were virtually identical (3.1 ± 0.7 vs. 3.3 ± 0.2 nM). The functional consequences of this decreased exosite binding were assessed by measuring cAMP accumulation due to the initial exposure and then at various times during perfusion (Fig. 3 and Table 3). As shown, the cAMP response decayed during high volume washout with the wild-type receptor, consistent with the loss of salmeterol occupied receptors over time under these conditions. However, with the Ile164 receptor, the decay was substantially accelerated with a mean $t_{1/2} = 21.0 \pm 3.6$ min compared to 46.8 ± 4.1 min for wild-type receptor ($n = 6$, $P = 0.001$). In contrast, the $t_{1/2}$ for isoproterenol (5 ml/min perfusion) did not differ between the β_2AR wild-type and the Ile164 receptor. Formoterol also displayed a long duration of action under these washout conditions, but the $t_{1/2}$ did not differ between the two receptors (Table 3). These studies with isoproterenol and formoterol indicate that the altered duration of action for salmeterol with the Ile164 receptor is specific to salmeterol rather than a generalized effect.

4. Discussion

Variation in the β_2AR gene in the human population has led to the hypothesis that the known interindividual variability in the clinical response to therapeutic β-adrenoceptor agonists may be, in part, due to such genetic variation. Our approach to studying polymorphisms of the β_2AR (Green et al., 1993, 1994b; Drysdale et al., 2000) and other adrenoceptors (Mason et al., 1999; Small et al., 2000) has been to carry out in vitro studies in recombinant cells or transgenic mice before embarking on clinical studies. Determining the consequences of the polymorphism on cellular signaling thus provides a basis for hypothesis-driven clinical investigations. To date, such studies with β_2AR polymorphisms at positions 16 and 27, as well as haplotypes composed of these and 5′ UTR polymorphisms, have shown that certain variants are associated with altered responsiveness (Martinez et al., 1997; Drysdale et al., 2000), and tachyphylaxis (Israel et al., 2000), to β-adrenoceptor agonists.

The Ile164 polymorphism, which is the least common of the functional β_2AR coding polymorphisms, has not been studied in a clinical setting. The current work focuses on the interaction between the Ile164 and the most commonly utilized therapeutic β-adrenoceptor agonists. We were particularly intrigued with the fact that this polymorphism occurs in TMD4. In a previous study (Green et al., 1996b), we showed that TMD4 is necessary for the prolonged activation of receptor by the unique agonist salmeterol. This agonist has a prolonged duration of action in humans (~ 12 h), which is thought to be due to a "tethering" of the molecule, via its aryloxyalkyl side chain, to the β_2AR such that multiple binding/activation events occur. This site has been considered to lie outside the active sites for traditional agonist binding, and has been termed the salmeterol exosite. Given that salmeterol is also highly hydrophobic, an interaction with the lipid bilayer of the cell membrane has also been considered. In a previous study (Green et al., 1996b), we showed that a chimeric receptor, consisting of the β_2AR with a substituted β_1AR TMD4 had unaltered binding affinity for salmeterol in ^{125}I-CYP competition studies. However, while the wild-type β_2AR retained salmeterol under aggressive perfusion washout conditions, this property was substantially lost with the chimeric receptor. Since both receptors were recombinantly expressed in the same cell-type, we reasoned that any cell membrane interaction with salmeterol would be equivalent between the two receptors. In further support of the notion that the exosite lies within the β_2AR, substitution of a portion of the β_2AR TMD4 into the β_1AR (which normally has no exosite binding) partially confers exosite binding properties. Additional studies (Rong et al., 1999; Isogaya et al., 1998) have shown that salmeterol likely binds to TMDs 6 and 7 as well. While the above studies do not exclude binding of salmeterol to the lipid bilayer, it is interesting to note that with salmeterol analogues which have the oxygen of the side chain at positions 2 or 8 carbons from the nitrogen, the long duration of action is lost (Johnson, 1995). Yet, the hydrophobicity of these analogues is the same as the parent compound. Taken together, it seems reasonable to conclude that at least one component of the molecular mechanism of salmeterol's long duration of action is an interaction with TMD4.

Our results show two important aspects of Ile164 pharmacogenomics. First, all the β-adrenoceptor agonists commonly utilized for the treatment of asthma and COPD display depressed stimulation of adenylyl cyclase. Thus, individuals with this polymorphism would be expected to have a decreased bronchodilatory response to any of these agents. It is possible that adequate efficacy could be obtained by an increased dosage, but this will require explicit clinical testing. Secondly, patients with Ile164 would be expected to have a shorter duration of action for salmeterol compared to those with the wild type β_2AR. Based on the $t_{1/2}$ of the functional washout studies, the predicted duration of action of salmeterol in these individuals may be $\sim$50% ($\sim$6 h) of the duration observed in those with wild-type receptor. Those with Ile164 would also have a decreased bronchodilatory response based on the adenylyl cyclase studies. So, the overall clinical effectiveness of salmeterol may well be substantially less in those with the polymorphic receptor. Again, this needs to be specifically studied in a clinical trial. It should be noted that we have recently found that when Ile164 is present, the polymorphic allele at amino acid position 16 is likely to be Gly (Drysdale et al., 2000). This may be a particularly unfavorable combination, as tachyphylaxis to another β-adrenoceptor agonist (formoterol) has been reported to be associated with the Gly16 genotype (Tan et al., 1997).

In summary, we have delineated the pharmacologic phenotype of the β_2AR Ile164 polymorphic receptor in regard to its interaction with the commonly utilized β-adrenoceptor agonists. All agonists tested displayed decreased stimulation of adenylyl cyclase with the Ile164 receptor compared to wild-type β_2AR. In addition, the unique interaction of salmeterol with the receptor is altered with the Ile164 variant, such that duration of action is decreased. Patients with this genotype may be candidates for atypical dosing regiments, different β-adrenoceptor agonists engineered to bypass the defect, or alternative agents working via other receptors or mechanisms. These data suggest that in the future a panel of polymorphic sites of various genes may be useful in developing individualized therapies based on pharmacogenomic loci.

Acknowledgements

The authors thank Nicole Tepe for the technical assistance and Esther Getz for the manuscript preparation. Supported by NIH grants GM61376, HL45967, and HD07463.

References

Ball, D.I., Brittain, R.T., Coleman, R.A., Denyer, L.H., Jack, D., Johnson, M., Lunts, L.H.C., Nials, A.T., Sheldrick, K.E., Skidmore, I.F., 1991. Salmeterol, a novel, long-acting β_2-adrenoceptor agonist: characterization of pharmacological activity in vitro and in vivo. Br. J. Pharmacol. 104, 665–671.

Beasley, R., Pearce, N., Crane, J., Burgess, C., 1999. Beta-agonists: what is the evidence that their use increases the risk of asthma morbidity and mortality? J. Allergy Clin. Immunol. 104, S18–S30.

Black, J.W., Leff, P., Shankley, N.P., 1985. An operational model of pharmacological agonism: the effect of E/[A]curve shape on agonist dissociation constant estimation. Br. J. Pharmacol. 84, 561–571.

Clark, R.B., Allal, C., Friedman, J., Johnson, M., Barber, R., 1996. Stable activation and desensitization of β_2-adrenergic receptor stimulation of adenylyl cyclase by salmeterol: evidence for quasi-irreversible binding to an exosite. Mol. Pharmacol. 49, 182–189.

Derom, E., Pauwels, R., 1997. Bronchodilatation. In: Pauwels, R., O'Byrne, P.M. (Eds.), Beta$_2$-Agonists in Asthma Treatment. Marcel Dekker, New York, pp. 161–178.

Drysdale, C.M., McGraw, D.W., Stack, C.B., Stephens, J.C., Judson, R.S., Nandabalan, K., Arnold, K., Ruano, G., Liggett, S.B., 2000. Complex promoter and coding region β_2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc. Natl. Acad. Sci. U. S. A. 97, 10483–10488.

Green, S., Liggett, S.B., 1994a. A proline-rich region of the third intracellular loop imparts phenotypic β_1-versus β_2-adrenergic receptor coupling and sequestration. J. Biol. Chem. 269, 26215–26219.

Green, S.A., Liggett, S.B., 1996a. Molecular basis of G-protein coupled receptor signalling. In: Liggett, S.B., Meyers, D. (Eds.), The Genetics of Asthma. Marcel Dekker, New York, pp. 67–90.

Green, S.A., Cole, G., Jacinto, M., Innis, M., Liggett, S.B., 1993. A polymorphism of the human β_2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. J. Biol. Chem. 268, 23116–23121.

Green, S., Turki, J., Innis, M., Liggett, S.B., 1994b. Amino-terminal polymorphisms of the human β_2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry 33, 9414–9419.

Green, S.A., Spasoff, A.P., Coleman, R.A., Johnson, M., Liggett, S.B., 1996b. Sustained activation of a G protein coupled receptor via "anchored" agonist binding: molecular localization of the salmeterol exosite within the β_2-adrenergic receptor. J. Biol. Chem. 271, 24029–24035.

Isogaya, M., Yamagiwa, Y., Fujita, S., Sugimoto, Y., Nagao, T., Kurose, H., 1998. Identification of a key amino acid of the beta2-adrenergic receptor for high affinity binding of salmeterol. Mol. Pharmacol. 54, 616–622.

Israel, E., Drazen, J.M., Liggett, S.B., Boushey, H.A., Cherniack, R.M., Chinchilli, V.M., Cooper, D.M., Fahy, J.V., Fish, J.E., Ford, J.G., Kraft, M., Kunselman, S., Lazarus, S.C., Lemanske, R.F., Martin, R.J., McLean, D.E., Peters, S.P., Silverman, E.K., Sorkness, C.A., Szefler, S.J., Weiss, S.T., Yandava, C.N., 2000. The effect of polymorphisms of the β_2-adrenergic receptor on the response to regular use of albuterol in asthma. Am. J. Respir. Crit. Care. Med. 162, 75–80.

Johnson, M., 1995. Salmeterol. Med. Res. Rev. 15, 225–257.

Liggett, S.B., 1997. Molecular basis of G-protein coupled receptor signalling. In: Crystal, R., West, J.B., Weibel, E.R., Barnes, P.J. (Eds.), The Lung: Scientific Foundations. Raven Press, New York, pp. 19–36.

Liggett, S.B., Green, S.A., 1996. Molecular Biology of the β_2-adrenergic receptor: focus on interactions of agonist with receptor. In: Pauwels, R., Lofdahl, C.G., O'Byrne, P. (Eds.), Beta$_2$-Agonists in Asthma Treatment. Marcel Dekker, New York, pp. 19–34.

Lohse, M.J., Benovic, J.L., Caron, M.G., Lefkowitz, R.J., 1990. Multiple pathways of rapid β_2-adrenergic receptor desensitization. J. Biol. Chem. 265, 3202–3209.

Martinez, F.D., Graves, P.E., Baldini, M., Solomon, S., Erickson, R., 1997. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J. Clin. Invest. 100, 3184–3188.

Mason, D.A., Moore, J.D., Green, S.A., Liggett, S.B., 1999. A gain-of-function polymorphism in a G-protein coupling domain of the human β_1-adrenergic receptor. J. Biol. Chem. 274, 12670–12674.

Nials, A.T., Summer, M.J., Johnson, M., Coleman, R.A., 1993. Investigations into factors determining the duration of action of the β_2-adrenoceptor agonist, salmeterol. Br. J. Pharmacol. 108, 507–515.

Reihsaus, E., Innis, M., MacIntyre, N., Liggett, S.B., 1993. Mutations in the gene encoding for the β_2-adrenergic receptor in normal and asthmatic subjects. Am. J. Respir. Cell Mol. Biol. 8, 334–339.

Rong, Y., Arbabian, M., Thiriot, D.S., Seibold, A., Clark, R.B., Ruoho, A.E., 1999. Probing the salmeterol binding site on the β_2-adrenergic receptor using a novel photoaffinity ligand, [^{125}I]Iodoazidosalmeterol. Biochemistry 38, 11278–11286.

Sears, M.R., Taylor, D.R., Print, C.G., Lake, D.C., Qingoing, L., Flannery, E.M., Yates, D., Lucas, M.K., Herbison, G.P., 1990. Regular inhaled beta-agonist treatment in bronchial asthma. Lancet 336, 1391–1396.

Small, K.M., Forbes, S.L., Rahman, F.F., Bridges, K.M., Liggett, S.B., 2000. A four amino acid deletion polymorphism in the third intracellular loop of the human α_{2C}-adrenergic receptor confers impaired coupling to multiple effectors. J. Biol. Chem. 275, 23059–23064.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.

Spitzer, W.O., Suissa, S., Ernst, P., Horwitz, R.I., Habbick, B., Cockcroft, D., Boivin, J.-F., McNutt, M., Buist, A.S., Rebuck, A.S., 1992. The use of β-agonists and the risk of death and near death from asthma. N. Engl. J. Med. 326, 501–506.

Strader, C.D., Candelore, M.R., Hill, W.S., Sigal, I.S., Dixon, R.A.F., 1989. Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. J. Biol. Chem. 264, 13572–13578.

Strader, C.D., Gaffney, T., Sugg, E.E., Candelore, M.R., Keys, R., Patchett, A.A., Dixon, R.A.F., 1995. Allele-specific activation of genetically engineered receptors. J. Biol. Chem. 266, 5–8.

Suissa, S., Ernst, P., Boivin, J.F., Horwitz, R.I., Habbick, B., Cockroft, D., Blais, L., McNutt, M., Buist, A.S., Spitzer, W.O., 1994. A cohort analysis of excess mortality in asthma and the use of inhaled beta-agonists. Am. J. Respir. Crit. Care Med. 149, 604–610.

Tan, S., Hall, I.P., Dewar, J., Dow, E., Lipworth, B., 1997. Association between beta 2-adrenoceptor polymorphism and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics. Lancet 350, 995–999.

Tattersfield, A.E., 1997. Clinical studies of beta-agonists in adults. In: Pauwels, R., O'Byrne, P.M. (Eds.), Beta$_2$-Agonists in Asthma Treatment. Marcel Dekker, New York, pp. 283–317.

Turki, J., Lorenz, J.N., Green, S.A., Donnelly, E.T., Jacinto, M., Liggett, S.B., 1996. Myocardial signalling defects and impaired cardiac function of a human β_2-adrenergic receptor polymorphism expressed in transgenic mice. Proc. Natl. Acad. Sci. U. S. A. 93, 10483–10488.

Wieland, K., Zuurmond, H.M., Krasel, C., Ijzerman, A.P., Lohse, M.J., 1996. Involvement of Asn-293 in stereospecific agonist recognition and in activation of the β_2-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. 93, 9276–9281.