

RESEARCH PAPER

Influence of β_2 -adrenoceptor gene polymorphisms on β_2 -adrenoceptor-mediated responses in human lung mast cells

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Background and purpose: Previous studies have shown that β_2 -adrenoceptor-mediated responses in human lung mast cells are highly variable. The aims of the present study were to establish whether polymorphisms of the β_2 -adrenoceptor gene (*ADRB2*) influence this variability in (a) β_2 -adrenoceptor-mediated inhibition and (b) desensitization of β_2 -adrenoceptor-mediated responses in human lung mast cells.

Experimental approach: Mast cells were isolated from human lung tissue. The inhibitory effects of the β -adrenoceptor agonist, isoprenaline (10^{-10} – 10^{-5} M), on IgE-mediated histamine release from mast cells were determined ($n = 92$). Moreover, the inhibitory effects of isoprenaline were evaluated following a desensitizing treatment involving long-term (24 h) incubation of mast cells with isoprenaline (10^{-6} M) ($n = 65$). A potential influence of polymorphisms on these functional responses was determined by genotyping 11 positions, in the promoter and coding regions, of *ADRB2* previously reported as polymorphic.

Key results: There was no influence of any of the polymorphic positions of *ADRB2* on the potency of isoprenaline to inhibit histamine release from mast cells with the exception of position 491C > T (Thr164Ile). There was no influence of any of the polymorphic positions of *ADRB2* on the extent of desensitization of the isoprenaline-mediated response following a desensitizing treatment except for position 46G > A (Gly16Arg). Analyses at the haplotype level indicated that there was no influence of haplotype on β_2 -adrenoceptor-mediated responses in mast cells.

Conclusions and implications: These data indicate that certain polymorphisms in *ADRB2* influence β_2 -adrenoceptor-mediated responses in human lung mast cells.

British Journal of Pharmacology (2007) **152**, 323–331; doi:10.1038/sj.bjp.0707400; published online 23 July 2007

Keywords: mast cells; β_2 -adrenoceptor; *ADRB2*; SNP; desensitization

Abbreviations: *ADRB2*, β_2 -adrenoceptor gene; BUP, β upstream peptide; BSA, bovine serum albumin; dNTP, deoxynucleotide triphosphate; HSA, human serum albumin; PBS, phosphate buffered saline; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism

Introduction

Recent studies have shown that the gene for the β_2 -adrenoceptor (*ADRB2*) is polymorphic (Brodde and Leineweber, 2005). At least 11 single nucleotide polymorphisms (SNPs) have been identified both within the promoter region and the coding block of the gene (Brodde and Leineweber, 2005). Polymorphisms within the promoter region may influence β_2 -adrenoceptor expression, whereas polymorphisms within the coding block have been linked to alterations in the function of the β_2 -adrenoceptor (Green *et al.*, 1995b;

Kirstein and Insel, 2004; Brodde and Leineweber, 2005). Since β_2 -adrenoceptor agonists are widely used in asthma as bronchodilators, a considerable body of work has emerged investigating whether *ADRB2* polymorphisms influence the therapeutic potential of β_2 -adrenoceptor agonists (see for example, Taylor and Kennedy, 2002).

β_2 -Adrenoceptor agonists act in asthma, primarily, by relaxing airways smooth muscle (Waldeck, 2002). However, additional beneficial effects may include the stabilization of inflammatory cell activity (Barnes, 1999). In this regard, effects on the human lung mast cell may be important and studies, both *in vivo* and *in vitro*, suggest that β_2 -adrenoceptor agonists prevent the release of spasmogenic and pro-inflammatory mediators from mast cells (Butchers *et al.*, 1980, 1991; Church and Hiroi, 1987; Lau *et al.*, 1994; Nials

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Received 24 April 2007; revised 20 June 2007; accepted 21 June 2007; published online 23 July 2007

et al., 1994; O'Connor *et al.*, 1994; Nightingale *et al.*, 1999). In the context of asthma therapy, polymorphisms in *ADRB2* might, therefore, influence how effectively β_2 -adrenoceptor agonists stabilize mast cell responses.

The potential mechanisms by which polymorphisms within *ADRB2* might influence the responses of mast cells to β -adrenoceptor agonists are manifold. For example, polymorphisms within the promoter region, most notably $-47T>C$, may influence β_2 -adrenoceptor expression (McGraw *et al.*, 1998; Scott *et al.*, 1999; Johnatty *et al.*, 2002; Westland *et al.*, 2004). This polymorphism lies within an open reading frame that, apparently, encodes for a 19-amino-acid peptide often referred to as β upstream peptide (BUP) (Parola and Kobilka, 1994). The polymorphism, $-47T>C$, leads to a change in the terminal amino acid of BUP from Cys to Arg, and this has been linked to alterations in β_2 -adrenoceptor expression (Parola and Kobilka, 1994; McGraw *et al.*, 1998). As receptor expression is known to affect the potency of agonists (MacEwan *et al.*, 1995) the polymorphism, $-47T>C$, could potentially influence agonist potency. An alternative polymorphism, $491C>T$, might also influence the ability of β -adrenoceptor agonists to stabilize mast cell responses. The polymorphism leads to a change in amino acid 164 from Thr to Ile, and β_2 -adrenoceptors that express this polymorphism have been shown to bind agonists with lower affinity and to couple to G-proteins less efficiently (Green *et al.*, 1993).

Alternative polymorphisms that could influence how effectively β -adrenoceptor agonists stabilize mast cell responses include those that have been associated with the regulation of β_2 -adrenoceptor desensitization. Polymorphisms, $46G>A$ and $79C>G$, associated with amino-acid changes at position 16 and 27 of the N-terminal region of the β_2 -adrenoceptor are thought to influence receptor downregulation (Green *et al.*, 1994, 1995a). Polymorphisms that promote enhanced levels of receptor downregulation are likely to reduce the availability of receptors and this may in turn influence the activity of β -adrenoceptor agonists.

Previous studies of our own have shown that β_2 -adrenoceptor-mediated responses in mast cells can vary extensively (Chong *et al.*, 1997, 2003; Drury *et al.*, 1998; Scola *et al.*, 2004). The aims of the present study, therefore, were to determine whether polymorphisms across the β_2 -adrenoceptor gene influence (a) β_2 -adrenoceptor-mediated inhibition of mast cells and (b) desensitization of β_2 -adrenoceptor-mediated responses. The potential influence of polymorphisms on functional responses was investigated both at the individual SNP and haplotype levels.

Methods

Lung tissue

Human lung tissue was obtained from surgical resections of patients following surgery with the approval of the Local Ethics Research Committee. Most of the patients were undergoing surgery for carcinoma. The majority of the patients were Caucasian (90%), and most were male (70%).

Genotyping

For genotypic analyses of *ADRB2*, genomic DNA was extracted from a small quantity (0.1 g) of human lung tissue using a modification of the chloroform extraction and ethanol precipitation method described elsewhere (Graham, 1978). The extracted DNA was amplified, using primers specific for the β_2 -adrenoceptor gene (Table 1), by polymerase chain reaction (PCR). The reaction constituents for PCR were the following: genomic DNA (70–100 ng), deoxynucleotide triphosphates (dNTPs) (200 μ M each), $MgSO_4$ (1 or 1.4 mM), Tris- SO_4 (pH 9.1; 60 mM), $(NH_4)_2SO_4$ (18 mM), both primers (1 μ M each) and GoTaq Flexi DNA Polymerase (1.25 U) in a final volume of 50 μ L. Conditions were essentially as described previously and involved 35 cycles of PCR (Chong *et al.*, 2000). All PCR products were visualized with ethidium bromide staining on agarose gels.

PCR products were then subjected to genotypic analysis by either automated sequencing or restriction fragment length polymorphism (RFLP). PCR products generated from primer pair 1 (Table 1) allowed determination of polymorphisms at nucleotide positions -709 , -654 , -468 , -406 and -367 , whereas products generated from primer pair 2 allowed determinations at positions -47 , -20 , 46 , 79 and 100 . PCR products subjected to automated sequencing (products of primer pairs 1 and 2) were first purified by either ethanol precipitation or using a QIAquick PCR purification kit before sequencing in-house (Applied Biosystems 48 capillary 3730 DNA Analyser, Warrington, UK). PCR products were sequenced in both forward and reverse directions. Of the 92 preparations sequenced, 56 (primer pair 1) and 28 (primer pair 2) were repeat sequenced to ensure reproducibility.

In order to determine the genotype at position 491, RFLP was performed on PCR products generated by primer pair 3 (Table 1), using the restriction enzyme *MnII*, according to methods similar to those described elsewhere (Aynacioglu *et al.*, 1999). In order to confirm the genotypes determined by RFLP for position 491, all heterozygotes ($n=4$) and a

Table 1 Primers used to amplify regions of the *ADRB2* by PCR

Primer fragment pair (bp)	Primers	Annealing conditions	Size
1	5'-CTCCAAGCCAGCGTGTGTTT-3' (sense) 5'-GTGCACAGGACTTTAGGGGA-3' (antisense)	60°C, 45 s	627
2	5'-CATAACGGGCAGAACGCACTG-3' (sense) 5'-CACAAATCCACACCATCAGAAT-3' (antisense)	56°C, 45 s	716
3	5'-GTGATCGCAGTGGATCGCTACT-3' (sense) 5'-AGACGAAGACCATGATCACCAG-3' (antisense)	58°C, 45 s	280

Abbreviation: PCR, polymerase chain reaction.

random sample of homozygotes ($n=10$) were also genotyped by automated sequencing.

Buffers

Tyrode's buffer contained the following (mM): NaCl 137, HEPES 1.2, KCl 2.7, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.04, glucose 5.6. Tyrode's-BSA was supplemented with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.5 mM, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1 mM, bovine serum albumin (BSA) 1 mg ml^{-1} , DNase $15 \mu\text{g ml}^{-1}$. Phosphate-buffered saline (PBS) contained (mM): NaCl 137, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 8, KCl 2.7, KH_2PO_4 1.5, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1, glucose 5.6, human serum albumin (HSA) $30 \mu\text{g ml}^{-1}$. The pH of Tyrode's and PBS buffers was adjusted to 7.3.

Isolation of human lung mast cells

Human lung tissue was physically and enzymatically disrupted to generate mast cell suspensions, according to a modification of the method described by Ali and Pearce (1985). The tissue was chopped vigorously for 10 min with scissors in a small volume of Tyrode's buffer. The chopped tissue was washed over a nylon mesh (100 μm pore size; Incucmesh Filtration, Warrington, UK) with 0.5–1 l Tyrode's buffer, to remove lung macrophages. The tissue was reconstituted in Tyrode's-BSA (10 ml g^{-1} of tissue) containing collagenase Ia (350 U ml^{-1} of Tyrode's-BSA) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37°C . The supernatant was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of Tyrode's-BSA (300–600 ml). The pooled filtrates were sedimented (480 g, room temperature, 10 min), the supernatant discarded and the pellets reconstituted in Tyrode's-BSA (100 ml). The pellet was washed a further two times and human lung mast cells visualized by microscopy using an Alcian blue stain (Gilbert and Ornstein, 1975).

Functional studies

In mediator release experiments, mast cells were resuspended in PBS and incubated with or without either (–)-isoprenaline bitartrate for 10 min, before challenge with a maximal releasing concentration of anti-human IgE (1:300) for a further 25 min at 37°C . The cells were then pelleted by centrifugation (400 g, room temperature, 4 min) and the supernatants saved for assessment of histamine content using an automated fluorometric method (Ennis, 1991). Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in only buffer served as a measure of spontaneous histamine release (<6%). Histamine release was, therefore, expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release. Experiments were performed in duplicate.

When long-term incubations were performed, Roswell Park Memorial Institute 1640 (RPMI 1640) buffer supplemented with penicillin/streptomycin ($10 \mu\text{g ml}^{-1}$) and gentamicin ($50 \mu\text{g ml}^{-1}$) was employed. Cells were incubated (24 h at 37°C) at a density of 0.1×10^6 mast cells ml^{-1} in six-

well plates, with or without isoprenaline (10^{-6} M). After 24 h, cells were washed three times with PBS and reconstituted in the same buffer for mediator release experiments as described above. Experiments were performed in duplicate.

Data analysis

Maximal responses (E_{max}) and potencies (pD_2) were calculated from individual fits to the data, using a classical inhibition model. P-Pharm Population PKPD software (version 1.5, InnaPhase, Ceretil, France) was used for the modelling. In a small proportion (8%) of cases, in which models could not be generated by individual fits, *post hoc* values from population estimates were used. In order to determine whether desensitizing treatments had any effect on the isoprenaline response, paired *t*-tests were performed. To determine whether genotype influenced responses, either Kruskal–Wallis or Mann–Whitney test was performed (Graph Pad InStat, version 4). Hardy–Weinberg equilibrium was determined by means of χ^2 goodness-of-fit tests.

Materials

Materials were purchased from the following sources: anti-human IgE, BSA, collagenase, DNase, HSA, (–)-isoprenaline, RPMI 1640 (Sigma, Poole, UK); agarose, gentamicin, penicillin/streptomycin (Invitrogen, Paisley, UK); dNTPs, GoTaq Flexi DNA Polymerase (Promega, Southampton, UK); *MnII* (New England Biolabs, Hitchin, UK); QIAquick (Qiagen, Sussex, UK).

Preparation of compounds

Stock solutions of (–)-isoprenaline bitartrate (10 mM) were prepared in 0.05% sodium metabisulphite (dissolved in 0.9% saline) on a weekly basis. Lyophilized polyclonal goat anti-human immunoglobulin IgE antibody was reconstituted in distilled water. All solutions were stored at 4°C .

Results

Genotyping

Ninety-two lung preparations, from which mast cells were also isolated for functional studies, were genotyped at 11 positions, seven in the promoter region (–709, –654, –468, –406, –367, –47 and –20) and four in the coding block (46, 79, 100 and 491), of *ADRB2* previously reported to be polymorphic (Brodde and Leineweber, 2005). Of these positions, three (–709, –406 and 100) were not found to be polymorphic in this population. For the remaining eight positions, the frequency of the less prominent allele ranged from 0.37 to 0.42, with the exception of nucleotide position 491, in which only four heterozygotes, and no homozygotes, expressed the less frequent allele (Table 2).

Influence of polymorphisms on function

The β -adrenoceptor agonist, isoprenaline (pD_2 , 8.6 ± 0.1 ; E_{max} , $56 \pm 2\%$), inhibited the IgE-mediated release of hista-

mine from mast cells in a concentration-dependent manner (Figure 1a). However, the degree to which isoprenaline inhibited histamine release varied substantially among the

Table 2 Allelic frequencies of polymorphic positions in *ADRB2* ($n=92$)

Position (aa)	Base	Amino acid	Frequency
-654	G	—	0.60
	A	—	0.40
-468	C	—	0.60
	G	—	0.40
-367	T	—	0.61
	C	—	0.39
-47	T	Cys	0.63
	C	Arg	0.37
-20	T	—	0.63
	C	—	0.37
46 (16)	G	Gly	0.58
	A	Arg	0.42
79 (27)	C	Gln	0.61
	G	Glu	0.39
491 (164)	C	Thr	0.98
	T	Ile	0.02

Each polymorphic position was in Hardy – Weinberg equilibrium.

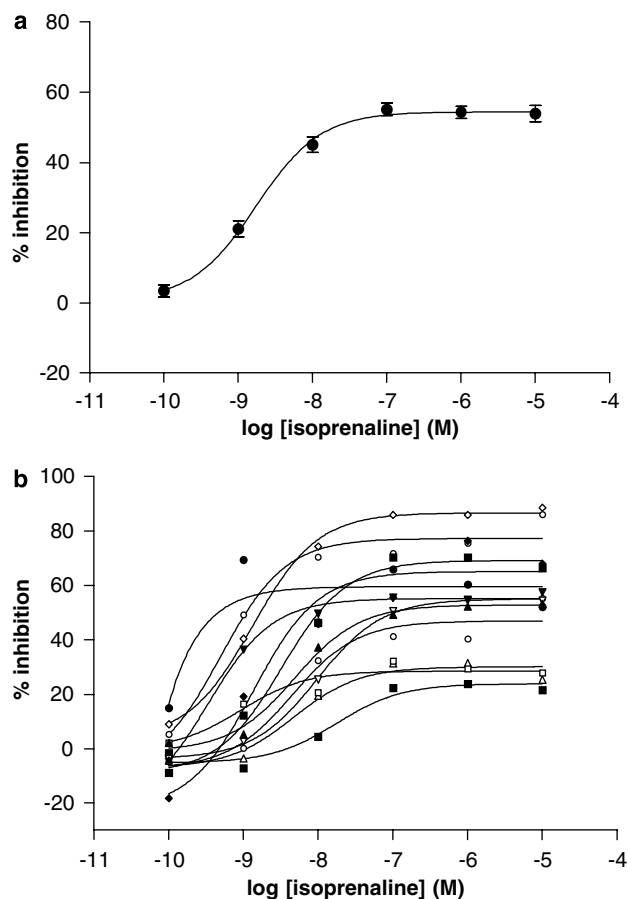


Figure 1 Effect of isoprenaline on histamine release from human lung mast cells. Cells were incubated for 10 min with or without isoprenaline, before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). (a) Results are expressed as the % inhibition of the unblocked histamine release, which was $36 \pm 1\%$ and data points are means \pm s.e. mean, $n=92$. (b) Twelve individual concentration–response curves, representative of the 92 experiments performed, are shown.

92 preparations studied (Figure 1b). A potential influence on the inhibitory activity of isoprenaline, of the eight positions (–654, –468, –367, –47, –20, 46, 79 and 491) in *ADRB2* identified in this population as polymorphic, was investigated. In all instances (see Figure 2a for example), there was no influence of individual SNPs on the potency (pD_2) and efficacy (E_{max}) of isoprenaline to inhibit histamine release, with the exception of 491C>T (Figure 2b). The polymorphism 491C>T (amino acid 164) is uncommon and, in this population, only four heterozygotes and no homozygotes were found to carry the less frequent allele. Although the maximal response of isoprenaline was not different between homozygotes (E_{max} , $56 \pm 2\%$) and heterozygotes (E_{max} , $55 \pm 6\%$), isoprenaline was significantly ($P=0.002$) less potent in mast cell preparations expressing the less frequent allele at position 491 (pD_2 , 7.5 ± 0.1) than in homozygotes (pD_2 , 8.6 ± 0.1).

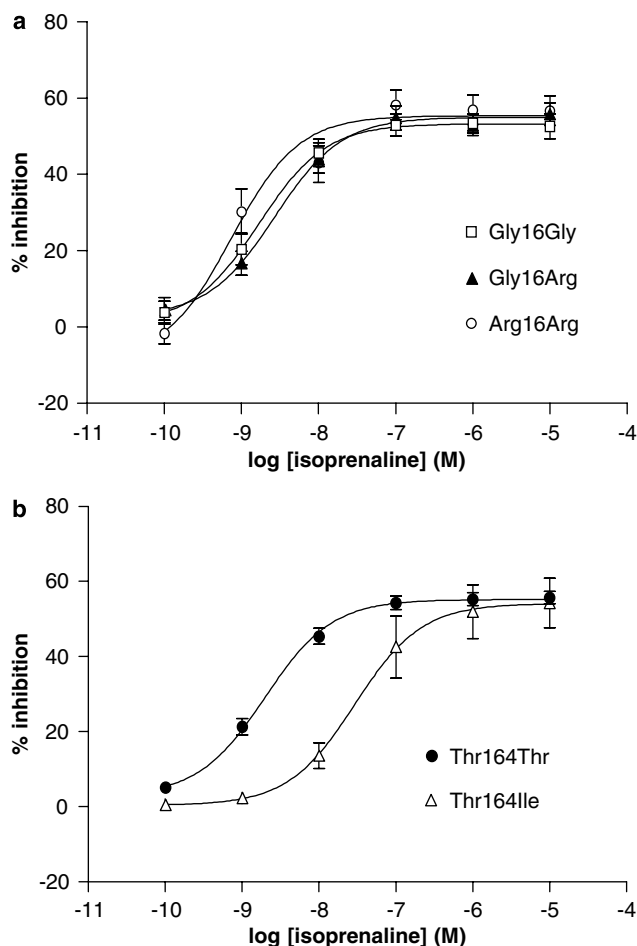


Figure 2 Influence of SNPs on inhibition. Cells were incubated for 10 min with or without isoprenaline, before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). The figure shows the influence of (a) position 46G>A (amino acid 16, Gly>Arg) and (b) position 491C>T (amino acid 164, Thr>Ile) on the isoprenaline inhibition. Results are expressed as the % inhibition of the unblocked histamine releases, which were for (a) 34 ± 2 (Gly16Gly), 38 ± 2 (Gly16Arg) and $34 \pm 4\%$ (Arg16Arg) and for (b) 37 ± 1 (Thr164Thr) and $24 \pm 7\%$ (Thr164Ile). Values are means \pm s.e. mean, for (a) 33 (Gly16Gly), 38 (Gly16Arg), and 19 (Arg16Arg) experiments and for (b) 88 (Thr164Thr) and four (Thr164Ile) experiments. SNP, single nucleotide polymorphism.

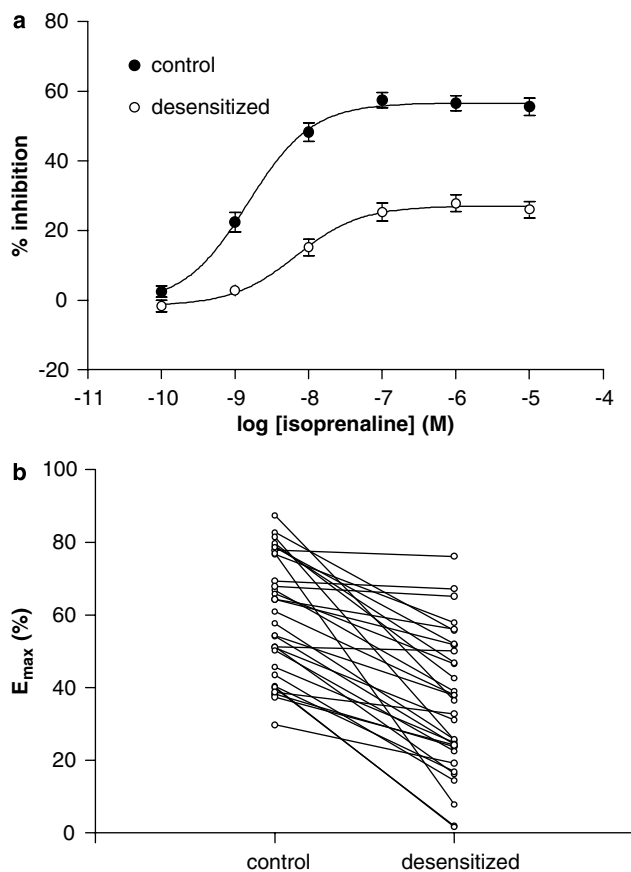


Figure 3 Effect of desensitizing treatment on the isoprenaline inhibition. (a) Mast cells were incubated for 24 h with (desensitized) or without (control) isoprenaline (10^{-6} M), after which the cells were washed extensively. Cells were then incubated for 10 min with or without isoprenaline, before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). Results are expressed as the % inhibition of the unblocked histamine releases, which were 38 ± 2 (control) and $34 \pm 2\%$ (desensitized). Values are means \pm s.e. mean, $n = 65$. (b) The figure shows the variable effect that desensitizing treatment (24 h with 10^{-6} M isoprenaline) has on E_{max} values for isoprenaline. Each line represents one of 65 experiments, although, for reasons of clarity, data from 32 experiments are shown in the figure.

Influence of polymorphisms on desensitization

Long-term (24 h) exposure of mast cells to isoprenaline (10^{-6} M) attenuated the subsequent ability of isoprenaline (10^{-10} – 10^{-5} M) to inhibit IgE-mediated histamine release from mast cells (Figure 3a). There was a 50% reduction in the efficacy (E_{max}) and a 10-fold reduction in the potency (pD_2) of isoprenaline ($P < 0.0001$) following the desensitizing treatment (Table 3). However, in the 65 preparations studied, the extent of this functional desensitization was very variable (Figure 3b). Whether individual SNPs influence the extent of desensitization was determined by comparing the change in maximal responses (% desensitization) and shifts in potency (pD_2 shift) following desensitizing treatments. This analysis indicated that there was a tendency for a number of positions to influence desensitization (Table 4). However, a statistically significant ($P < 0.05$) influence on the extent of desensitization was observed for position 46G > A only (Figure 4; Table 5).

Table 3 Effects of desensitizing treatments on the efficacy (E_{max}) and potency (pD_2) of isoprenaline

	Isoprenaline	
	E_{max} (%)	pD_2
Control	57 ± 2	8.8 ± 0.1
Desensitized	29 ± 2	7.9 ± 0.1

Desensitizing treatments (24 h) of mast cells with isoprenaline (10^{-6} M) led to statistically significant ($P < 0.0001$) reductions in E_{max} and pD_2 values for isoprenaline. Values are means \pm s.e. mean for 65 experiments, and further experimental details can be found in the legend to Figure 3.

Table 4 Influence of polymorphic positions on functional desensitization

Position	P-value	
	% Desensitization	pD_2 shift
–654	0.560	0.124
–468	0.699	0.407
–367	0.153	0.174
–47	0.079	0.175
–20	0.079	0.175
46	0.034	0.046
79	0.215	0.135

The changes in maximal response (% desensitization) and potency (pD_2 shift) of isoprenaline following a desensitizing treatment (24 h with 10^{-6} M isoprenaline) were compared statistically for the three possible genotypes at each polymorphic position. % Desensitization was calculated as follows: $(1 - [E_{max} \text{ of isoprenaline after desensitizing treatment} / E_{max} \text{ of isoprenaline}]) \times 100$. The pD_2 shift was calculated as follows: $pD_2 \text{ of isoprenaline after desensitizing treatment} - pD_2 \text{ of isoprenaline}$. No values are shown for position 491, since, in the population of 65 preparations studied, only one preparation was found to be a heterozygote.

Haplotype analysis

Classification of lung preparations ($n = 92$), by considering multiple SNPs across the β_2 -adrenoceptor gene, was also determined. This analysis demonstrated that there were a large number of different permutations of multiple SNPs across the breadth of *ADRB2*, with three prevalent homozygous haplotypes identified (Table 6). The possibility of establishment of any association between haplotypes and functional responses was investigated. There was no influence ($P = 0.38$, E_{max} comparison; $P = 0.76$; pD_2 comparison) of haplotype on the ability of isoprenaline to inhibit histamine release from mast cells (Figure 5). Furthermore, there was no influence ($P = 0.29$, % desensitization comparison; $P = 0.45$, pD_2 shift comparison) of these haplotypes on the extent of functional desensitization induced following long-term (24 h) incubation of mast cells with isoprenaline (Figure 6). When haplotype designations were simplified by considering fewer (≥ 2) polymorphic positions rather than the eight positions across the breadth of *ADRB2*, exactly the same three haplotypes emerged. Moreover, irrespective of whether stratification of haplotypes was performed inclusive of all SNPs across the gene or if fewer (≥ 2) SNPs were considered, there was little change in the complexion of groups (Figure 7z). This resulted in very little change in functional data associated with each haplotype, whether an

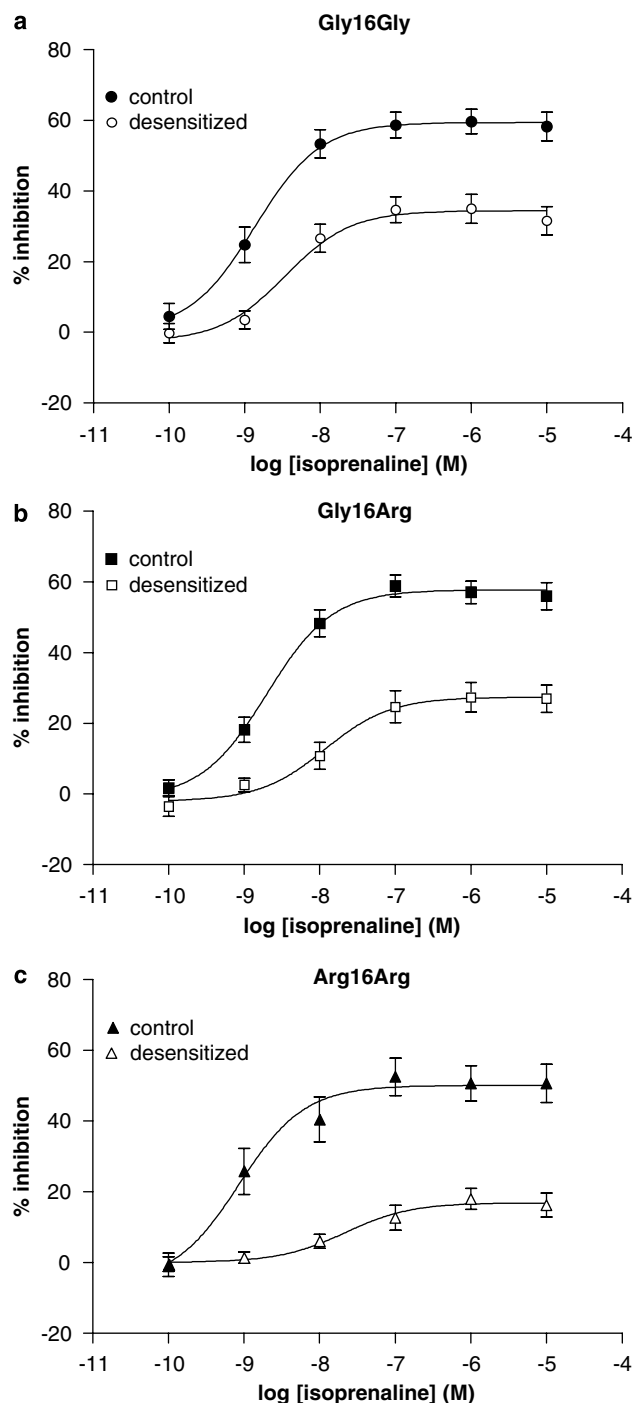


Figure 4 Influence of 46G>A (amino acid 16) on functional desensitization. Data generated from preparations expressing (a) the more common allele (Gly16Gly), (b) both alleles (Gly16Arg) and (c) the less common allele (Arg16Arg) are shown. Mast cells were incubated for 24 h with (desensitized) or without (control) isoprenaline (10^{-6} M), after which the cells were washed extensively. Cells were then incubated for 10 min with or without isoprenaline, before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). Results are expressed as the % inhibition of the unblocked histamine releases, which were for (a) 36 ± 2 (control) and $33 \pm 2\%$ (desensitized), for (b) 40 ± 2 (control) and $36 \pm 3\%$ (desensitized), and for (c) 37 ± 5 (control) and $30 \pm 4\%$ (desensitized). Values are means \pm s.e.mean, for (a) 22, (b) 29 and (c) 14 experiments.

Table 5 Influence of position 46G>A on functional desensitization

Base	Amino acid	% Desensitization	pD ₂ shift
G	Gly	42 ± 5	0.6 ± 0.2
G/A	Arg/Gly	49 ± 5	1.0 ± 0.1
A	Arg	64 ± 6	1.0 ± 0.2

Values in the table were obtained from data for Figure 4, and further details can be found in the legend to that figure. Position 46G>A leads to an amino-acid change from Gly to Arg at position 16. The % desensitization was calculated as follows: $(1 - [E_{\max} \text{ of isoprenaline after desensitizing treatment} / E_{\max} \text{ of isoprenaline}]) \times 100$. The pD₂ shift was calculated as follows: pD₂ of isoprenaline after desensitizing treatment – pD₂ of isoprenaline. There was a significant influence of genotype on the % desensitization values ($P = 0.034$) and on the pD₂ shifts ($P = 0.046$).

Table 6 Distribution of common ADRB2 haplotypes

Genotype (n)	Position							
	–654	–468	–367	–47	–20	46	79	491
0 G	C	T	T (Cys)	T	G (Gly)	C (Gln)	C (Thr)	
1 G/A	C/G	T/C	T/C	T/C	G/A	C/G	C/T	
2 A	G	C	C (Arg)	C	A (Arg)	G (Glu)	T (Ile)	
Haplotype A (13)	0	2	2	2	2	0	2	0
Haplotype B (14)	2	0	0	0	0	2	0	0
Haplotype C (5)	0	0	0	0	0	0	0	0
Genotype D (16)	1	1	1	1	1	1	1	0
Genotype E (9)	1	0	0	0	0	1	0	0
Genotype F (9)	0	1	1	1	1	0	1	0

Genotypic analysis of multiple SNPs across the length of the β_2 -adrenoceptor gene, in a population of 92 individuals, indicated that there were three main homozygous haplotypes designated A, B and C in the table. Additional common genotypic groupings, that were heterozygous at two positions or more, are also shown. There were a further 17 genotypic groupings expressed by 2 or 1 preparations that are not shown in the table.

inclusive or a simplified SNP approach was adopted to characterize the haplotype. Consequently, there was no influence ($P = 0.17$ – 0.51) of simplified haplotypes (< 8 SNPs) on (a) the inhibitory activity of isoprenaline or (b) desensitization (data not shown).

Discussion

In this study, we have attempted to investigate whether SNPs in ADRB2 influence β_2 -adrenoceptor-mediated responses in human lung mast cells. Since, in the therapeutic context, bronchodilators may target human lung mast cells (Barnes, 1999), a genetic influence on the responses of mast cells to bronchodilators could have some bearing on how effectively these drugs work *in vivo*.

Previous studies of our own have shown that the responses to β_2 -adrenoceptor agonists of mast cells isolated from different individuals can be varied (Chong *et al.*, 1997, 2003; Drury *et al.*, 1998; Scola *et al.*, 2004). We considered whether individual SNPs, across the breadth of ADRB2, might influence the ability of the β -adrenoceptor agonist, isoprenaline, to inhibit IgE-mediated histamine release. However, there was no influence of any of the polymorph-

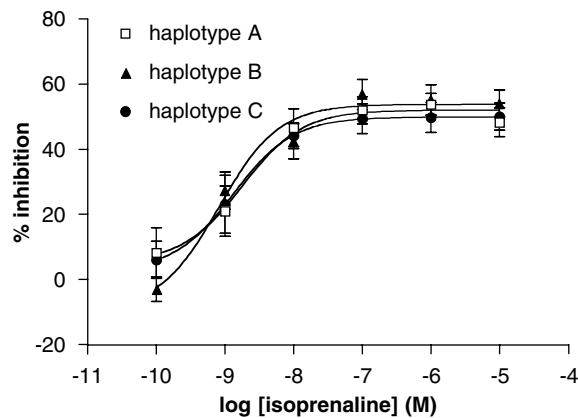


Figure 5 Influence of haplotypes on inhibition. Cells were incubated for 10 min with or without isoprenaline, before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). The figure shows the influence of (1) haplotype A, (2) haplotype B and (3) haplotype C on the isoprenaline inhibition. Results are expressed as the % inhibition of the unblocked histamine releases which were 39 ± 3 , 36 ± 5 and $40 \pm 4\%$ for (1), (2) and (3), respectively. Values are means \pm s.e.mean, for (1) 13, (2) 14 and (3) five experiments.

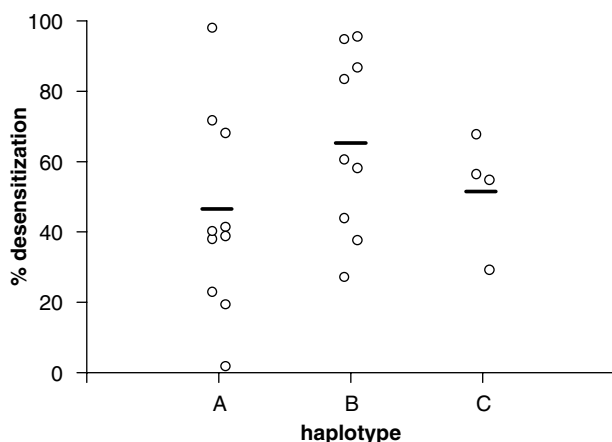


Figure 6 Influence of haplotypes on functional desensitization. Mast cells were incubated for 24 h with (desensitized) or without (control) isoprenaline (10^{-6} M) after which the cells were extensively washed. Cells were then incubated for 10 min with or without isoprenaline (10^{-10} – 10^{-5} M) before challenge for 25 min with a maximal releasing concentration of anti-IgE (1:300). E_{\max} values for the isoprenaline inhibition were determined in control and desensitized sets and % desensitization values were calculated as follows: $(1 - [E_{\max} \text{ of isoprenaline after desensitizing treatment} / E_{\max} \text{ of isoprenaline}]) \times 100$. Each point reflects data from an individual experiment and horizontal bars represent mean % desensitization values, which were 46 ± 7 ($n=10$), 65 ± 9 ($n=9$) and $52 \pm 8\%$ ($n=4$) for haplotypes A, B and C, respectively.

isms on the inhibitory responses of isoprenaline, except for position 491C>T, which leads to an amino-acid change from Thr to Ile at 164. The polymorphism is uncommon and, in this study, only four heterozygotes were found to carry the polymorphism out of a total of 92 individuals, a frequency of expression in keeping with the findings of others (Reihnsaus *et al.*, 1993; Aynacioglu *et al.*, 1999; Büscher *et al.*, 2002; Hall *et al.*, 2006). Isoprenaline was significantly

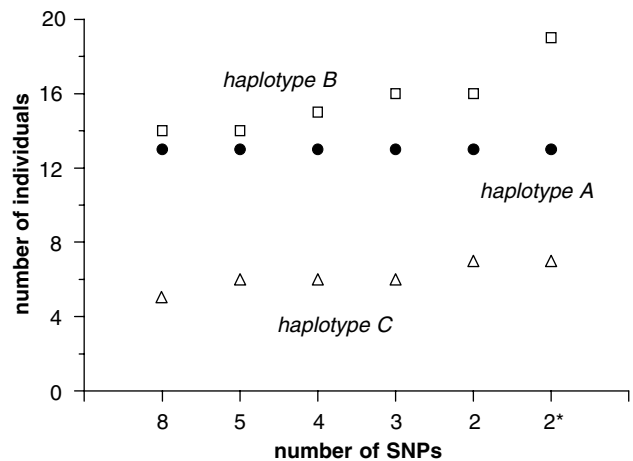


Figure 7 Haplotype designation using an inclusive or a simplified approach. Haplotypes were established by considering 8 (–654, –468, –367, –47^(BUP19), –20, 46⁽¹⁶⁾, 79⁽²⁷⁾, 491⁽¹⁶⁴⁾), 5 positions (–654, –468, –367, –47, –20), 4 (–367, –47, 46, 79), 3 (–47, 46, 79), 2 (46, 79) or 2* (–47, 46) polymorphic positions of the β_2 -adrenoceptor gene. Data show how the complexation of groups does not change (haplotype A), or changes slightly (haplotypes B and C) if all eight SNPs across the breadth of *ADRB2* or fewer SNPs are considered. This analysis was performed in a population of 92 individuals. SNP, single nucleotide polymorphism.

less potent as an inhibitor of histamine release in mast cell preparations carrying the less common allele. These findings are in general agreement with studies in transfected cells, in which it was established that agonists bind to the Ile164-expressing β_2 -adrenoceptor with lower affinity and the receptor couples less efficiently to G-protein compared to the Thr164-expressing β_2 -adrenoceptor (Green *et al.*, 1993).

Previous studies of our own have shown that overnight exposure of mast cells to β_2 -adrenoceptor agonists leads to a subsequent reduction in the ability of agonists to inhibit mediator release (Chong *et al.*, 1997, 2003; Drury *et al.*, 1998; Scola *et al.*, 2004). However, the extent of functional desensitization was highly variable. We considered whether individual SNPs might influence the extent of desensitization. In this study, an influence of 46G>A on the extent of desensitization was observed. This polymorphism leads to an amino-acid change of Gly to Arg at position 16 of the β_2 -adrenoceptor. Mast cells expressing Arg16 β_2 -adrenoceptors were significantly ($P<0.05$) more susceptible to desensitizing treatments than mast cells expressing Gly16 β_2 -adrenoceptors.

This finding is in general keeping with a previous study of ours, in a different population, in which preparations expressing Arg16 β_2 -adrenoceptors showed a greater tendency to desensitization than Gly16 β_2 -adrenoceptors (Chong *et al.*, 2000). In this same study, position 79C>G (position 27) was also found ($P=0.04$) significantly to influence desensitization and preparations expressing Gln27 β_2 -adrenoceptors were more prone to desensitization than those expressing Glu27 β_2 -adrenoceptors. While a statistically significant influence on desensitization of position 79C>G was not observed in the present study population, the same tendency was seen (Table 4). Compar-

ison of the present study with our previous report suggests that alternative sources of variability, other than SNPs within *ADRB2*, may be important and may influence desensitization. Thus, when studying different populations, although trends in desensitization may be consistent, it is possible that some differences can manifest themselves as statistically significant variations among genotypes in one study, but not in another.

Our observation that mast cells expressing Arg16 β_2 -adrenoceptors were significantly more susceptible to desensitizing treatments than mast cells expressing Gly16 β_2 -adrenoceptors is at odds with studies in transfected cells in which the reverse was reported. β_2 -Adrenoceptors expressing Gly16 were more prone to desensitization than Arg16-expressing receptors (Green *et al.*, 1994, 1995b). However, our findings are more in keeping with clinical studies showing less favourable outcomes in asthmatics expressing Arg16 β_2 -adrenoceptors taking bronchodilators regularly (Taylor *et al.*, 2000; Israel *et al.*, 2004; Palmer *et al.*, 2006).

More recently, the suggestion has been made that the analysis of haplotypes, rather than individual SNPs, may reflect a more robust analytical process (Hein, 2001; Liggett, 2006). Approaches considering an influence of haplotypes on β_2 -adrenoceptor expression and function in both transfected cells and lymphocytes have been reported. These studies have considered SNPs across the breadth of *ADRB2* as well as studies that have restricted analysis to fewer polymorphisms (Scott *et al.*, 1999; Drysdale *et al.*, 2000; Johnatty *et al.*, 2002; Lipworth *et al.*, 2002; Oostendorp *et al.*, 2005). While an influence of β_2 -adrenoceptor gene haplotypes on functional responses and desensitization has been observed (Drysdale *et al.*, 2000; Oostendorp *et al.*, 2005), these outcomes have not been found consistently (Lipworth *et al.*, 2002).

In the present study, three principal homozygous haplotypes were identified, which is in general keeping with the findings of others (Drysdale *et al.*, 2000; Lipworth *et al.*, 2002; Oostendorp *et al.*, 2005). However, there was no influence of these haplotypes on either the inhibitory activity of isoprenaline or on functional desensitization. An interesting aspect to emerge from the haplotype analysis is the similarity in the data whether 2, 3, 4, 5 or 8 SNPs are considered to define the haplotypes. This reflects the strong linkage disequilibrium that exists among alleles across the length of *ADRB2* (Dewar *et al.*, 1998; Drysdale *et al.*, 2000; Lipworth *et al.*, 2002; Silverman *et al.*, 2003). These considerations question whether extensive analysis of all SNPs across the length of *ADRB2* is necessary to investigate an influence of haplotypes when determinations of genotypes at 2, and certainly 3, SNPs may be sufficiently inclusive to delineate the three principal homozygous haplotypes.

In summary, the present study has shown that position 491C>T (Thr164Ile) influences the inhibitory response of isoprenaline in human lung mast cells. However, the infrequent nature of the polymorphism questions how much of an impact the polymorphism may have on mast cell stabilization in the clinical context. In further studies, position 46G>A (Gly16Arg) was found to influence the extent of functional desensitization in mast cells with preparations expressing Gly16 β_2 -adrenoceptors less prone

to desensitization than those expressing Arg16 β_2 -adrenoceptors. While a direct causative link cannot be made, it is of interest that asthmatics expressing Arg16 β_2 -adrenoceptors show greater asthma exacerbations with the use of bronchodilators than those expressing Gly16 β_2 -adrenoceptors (Taylor *et al.*, 2000; Israel *et al.*, 2004; Palmer *et al.*, 2006).

Although these studies may have some relevance to the clinical setting, it should be noted that the non-selective β -adrenoceptor agonist, isoprenaline, has been used throughout this study. Whether isoprenaline can reflect identically the actions of commonly used bronchodilators, such as salbutamol and salmeterol, cannot be known with certainty. Indeed, the suggestion exists that particular polymorphisms in *ADRB2* may influence certain agonists to a greater extent than others (Green *et al.*, 1993, 2001). Thus, the assumption cannot be made that the effects of isoprenaline, in this test system, are wholly representative of the actions of all bronchodilators.

To conclude, our findings indicate that certain polymorphisms in *ADRB2* may influence β_2 -adrenoceptor-mediated responses in mast cells and that these could potentially impact on how bronchodilators stabilize mast cells *in vivo*.

Acknowledgements

The authors are grateful to Mr T Locke, Mr G Cooper, Mr D Hopkinson and Mr Bhatnagar (Cardiothoracic Surgery), Dr SK Suvarna, Dr P Kitsanta and Dr C Layton (Histopathology) at the Northern General Hospital, Sheffield, for their invaluable help in providing lung tissue specimens. The in-house sequencing facility and the assistance of Aileen McDermott, Helen Langsford and Dr Franco di Giovine is gratefully acknowledged. This work was supported, in part, by Asthma UK.

Conflict of interest

The authors state no conflict of interest.

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