Pulmonary Pharmacology & Therapeutics 23 (2010) 71–77

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Influence of β_2-adrenoceptor gene polymorphisms on β_2-adrenoceptor expression in human lung

Linda J. Kay [a], S. Kim Suvarna [b], Anne-Marie Scola [a], Amin Rostami-Hodjegan [a], Russell Chess-Williams [c], Peter T. Peachell [a,*]

[a] Academic Unit of Respiratory Medicine, University of Sheffield, The Medical School (Floor M), Beech Hill Road, Sheffield S10 2RX, UK
[b] Department of Histopathology, Northern General Hospital, Herries Road, Sheffield S5 7AU, UK
[c] Faculty of Health Sciences and Medicine, Bond University, Queensland 4229, Australia

ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form
13 October 2009
Accepted 26 October 2009

Keywords:
Asthma
Human lung
ADRB2
β_2-adrenoceptor
SNP

ABSTRACT

Background: The aim of the present study was to establish whether polymorphisms, especially those within the promoter region, of the β_2-adrenoceptor gene (*ADRB2*) influence β_2-adrenoceptor expression in human lung.
Methods: The density of β-adrenoceptors in human lung tissue ($n = 88$) was determined by saturation binding using the radioligand, iodinated cyanopindolol. Discrimination of β_1- and β_2-adrenoceptors was determined using the highly selective β_1-adrenoceptor antagonist, CGP20712A. Genotype was determined at 5 positions of *ADRB2* previously reported as polymorphic. Potential influences of single nucleotide polymorphisms (SNPs) within the promoter region (−367, −47) and coding block (46, 79, 491) of *ADRB2* on β_2-adrenoceptor expression were investigated.
Results: The density of β_2-adrenoceptors was variable among the 88 lung preparations studied ranging from 17 to 177 fmol/mg protein (mean ± S.E.M., 72 ± 4 fmol/mg protein). There was no influence of genotype on β_2-adrenoceptor expression for any of the polymorphisms studied except at position 491. The polymorphism at position 491C > T, leading to a change from thr to ile at amino acid 164, is uncommon. Preparations genotyped as heterozygous (126 ± 15 fmol/mg protein; $n = 5$) expressed significantly ($P = 0.0005$) higher levels of β_2-adrenoceptor than those that were homozygous (69 ± 4 fmol/mg protein; $n = 83$).
Conclusion: With the exception of position 491, these data indicate that polymorphisms of *ADRB2* do not influence β_2-adrenoceptor expression in human lung.

1. Introduction

A large number of studies have shown that the gene for the β_2-adrenoceptor (*ADRB2*) is polymorphic [1]. Single nucleotide polymorphisms (SNPs) have now been found not only within the promoter region and the coding block [1] but also within the 3′ untranslated region [2] of *ADRB2*. Additional studies have demonstrated that some of these polymorphisms may influence both receptor function and expression [1,3,4]. This could, potentially, impact quite heavily on how effectively agonists activate β_2-adrenoceptors. This is of particular interest in the context of asthma therapy since β_2-adrenoceptor agonists are the most commonly prescribed drugs used to treat asthma [5]. Consequently, a considerable body of work has emerged geared toward establishing whether polymorphisms in *ADRB2* influence the therapeutic benefits of β_2-adrenoceptor agonists in asthma [6].

β_2-adrenoceptor agonists act in asthma primarily as bronchodilators relaxing airway smooth muscle but additional effects could include the stabilization of pulmonary cell activity [7,8]. One factor that could influence the effectiveness of β_2-adrenoceptor agonists in the treatment of asthma is the extent of receptor expression in the lung. Receptor density is known to influence both the potency and efficacy of agonists [9]. This may be of especial importance in asthma since many of the β-adrenoceptor agonists used clinically, such as salbutamol and salmeterol, are partial agonists [10]. Partial agonists are unable to attain maximal responses despite full receptor occupancy and responses are particularly dependent on the size of the functional receptor population [9]. Should receptor expression be compromised, the response to a partial agonist will

Abbreviations: *ADRB2*, β_2-adrenoceptor gene; BUP, beta upstream peptide; dNTP, deoxynucleotide triphosphate; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

* Corresponding author. Tel.: +44 114 271 2063; fax: +44 114 271 1863.
E-mail address: p.t.peachell@shef.ac.uk (P.T. Peachell).

1094-5539/$ – see front matter
doi:10.1016/j.pupt.2009.10.013

be further attenuated whereas a full agonist may continue to evoke maximal responses.

Polymorphisms within the promoter region of *ADRB2* have been associated with the regulation of receptor expression [11–15]. In particular, a polymorphism at position −47 has been strongly linked to β_2-adrenoceptor expression [11]. Position −47 is found within an open-reading frame, just upstream of the start site for the β_2-adrenoceptor, that encodes a putative 19 amino acid peptide that is sometimes referred to as beta upstream peptide (BUP) [16]. The polymorphism (−47T > C) leads to a change in the terminal residue of BUP from cys to arg. BUP-arg has been reported to attenuate β_2-adrenoceptor expression [11]. In addition to position −47, position −367 within the promoter region has also been linked to influencing receptor expression as the polymorphism (−367T > C) interrupts a potential binding site for transcription factors [12,14].

An influence of polymorphisms on β_2-adrenoceptor expression has been assessed in transfected cells and lymphocytes with equivocal outcomes [11–15,17]. The aim of the present study was to perform a comprehensive analysis of the influence of polymorphisms across *ADRB2* on β_2-adrenoceptor expression in human lung tissue.

2. Materials and methods

2.1. Buffers

Tris buffer contained (mM): Tris 50, NaCl 154, $MgCl_2 \cdot 6H_2O$ 10, EDTA 2. The pH of Tris buffer was titrated to 7.4.

2.2. Preparation of compounds

CGP20712A (2-hydroxy-5-(2-(hydroxy-3-(4((1-methyl-4-trifluoromethyl)-1-*H*-imidazol-2-yl)-phenoxy)-propyl)-amino-ethoxyl)-benzamide) and (±)-propranolol HCl, were prepared daily as stock solutions (10 mM) in Tris buffer.

2.3. Lung tissue

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

2.4. Radioligand binding

Membrane fractions were prepared from lung tissue by methods that have been described previously [18,19]. The tissue (100–150 mg) was homogenised in ice-cold Tris buffer using an Ultra Turrax homogeniser for 20 s followed by four strokes (×4) of a Teflon homogeniser. The homogenate was centrifuged (500*g*, 10 min) and the supernatant was harvested and subjected to further centrifugation (40,000*g*, 15 min) in an ultra-centrifuge (L80, Beckman). The pellet was washed and the high-speed centrifugation step repeated. The pellet was resuspended in Tris buffer and used in receptor binding assays. All procedures were carried out at 4 °C.

In saturation binding assays, the membrane preparations were assayed for β-adrenoceptor binding sites using [^{125}I]-cyanopindolol. Membrane suspensions (100 μl) were incubated (1 h, 37 °C) using a range of radioligand concentrations (0.01563–2 nM) in a total volume of 250 μl. Non-specific binding was determined by displacement with propranolol (1 μM). Specific binding, expressed as a percentage of the total binding, was 87 ± 1% at a [^{125}I]-cyanopindolol concentration of 0.0625 nM. The subtypes of β-adrenoceptor present in membranes were determined in competition studies with the use of the highly selective β_1-adrenoceptor antagonist, CGP20712A [20]. Membrane preparations (100 μl) were incubated (1 h, 37 °C) with CGP20712A (10^{-14}–10^{-4} M) in the presence of [^{125}I]-cyanopindolol (0.0625 nM) in a total volume of 250 μl. Although full displacement curves using an extensive range of CGP20712A concentrations were performed for some preparations ($n = 23$), further studies indicated that the use of a single concentration (10 nM) of the antagonist was sufficiently discriminatory to determine the split of β-adrenoceptors present in lung membranes and this approach was used in the majority of instances.

Additions of ice-cold Tris buffer were used to terminate the reactions followed by rapid filtration through Whatman GF/B glass fibre filters. The filters were rapidly washed four times with 3 ml ice-cold buffer and the radioactivity remaining on filters measured in a Packard Cobra auto-gamma counter. All saturation binding experiments were performed in duplicate. All competition binding experiments were performed either in duplicate or quadruplicate. Twenty-five of the 88 lung preparations that were studied in these binding experiments were repeat assayed to ensure data reproducibility. These were, primarily, preparations that were at either end of the population's spectrum such as preparations that expressed very high or very low levels of β-adrenoceptors. Protein content of the membranes was determined by the method of Lowry et al. [21].

2.5. Genotyping

For genotypic analyses of the β_2-adrenoceptor, genomic DNA was extracted from a small quantity of human lung tissue using a modification of the chloroform extraction and ethanol precipitation method described elsewhere [22]. The extracted DNA was amplified, using primers specific for *ADRB2* (see Table 1), by polymerase chain reaction (PCR). The reaction constituents for PCR were, genomic DNA (70–100 ng), dNTPs (200 μM of 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 μM of each) and ELONGASE enzyme mix (1 μl) in a final volume of 50 μl. Conditions were essentially as described previously and involved 35 cycles of PCR [23]. All PCR products were visualised 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 (see Table 1) permitted determination of polymorphisms at nucleotide position −367 whereas products generated from primer pair 2 allowed determinations at positions −47, 46 and 79. 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 (Applied

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

Primer pair	Primers	Annealing conditions	Fragment size (bp)
1	5′-CTCCAAGCCAGCGTGTGTTT-3′ (sense) 5′-GTGCACAGGACTTTAGGGGA-3′ (antisense)	60 °C, 45 s	627
2	5′-CATAACGGGCAGAACGCACTG-3′ (sense) 5′-CACAATCCACACCATCAGAATG-3′ (antisense)	56 °C, 45 s	716
3	5′-GTGATCGCAGTGGATCGCTACT-3′ (sense) 5′-AGACGAAGACCATGATCACCAG-3′ (antisense)	58 °C, 45 s	280

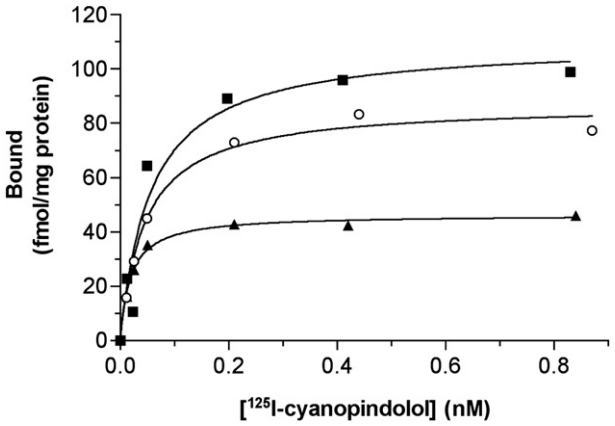

Fig. 1. Saturation binding curves of the radioligand [^{125}I]-cyanopindolol to human lung membranes. Specific binding was determined by evaluating binding of the radioligand to membranes in the absence and presence of the β-adrenoceptor antagonist, propranolol (1 μM). Saturation curves were determined by non-linear regression analysis. Representative data for three lung preparations are shown for which receptor densities (B_{max}) were 110, 87 and 46 fmol/mg protein and K_D values for [^{125}I]-cyanopindolol were 0.06, 0.05 and 0.02 nM (squares, circles and triangles, respectively).

Biosystems, 373 Stretch). Alternatively, 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 MnlI according to methods similar to those described elsewhere [24]. In order to confirm the genotypes determined by RFLP for position 491, all heterozygotes ($n = 5$) and a random sample of homozygotes ($n = 9$) were also genotyped by automated sequencing.

2.6. Materials

Materials were purchased from the following sources: QIAquick (Qiagen, Sussex, UK), Tris (Calbiochem, Nottingham, UK); propranolol (Sigma, Poole, UK); [^{125}I]-cyanopindolol (New England Nuclear, Stevenage, UK); agarose, ELONGASE enzyme mix (Invitrogen, Paisley, UK); dNTPs (Promega, Southampton, UK); MnlI (New England Biolabs, Hitchin, UK). CGP20712A was kindly supplied as a gift from Ciba-Geigy (Basel, Switzerland).

2.7. Data analysis

Receptor densities (B_{max}) and radioligand affinity (K_D) were determined by non-linear regression analysis of saturation curves (GraphPad Prism, version 4). To determine whether β-adrenoceptor density varies among lung regions, repeated measures ANOVA was performed. To determine whether genotype influences β-adrenoceptor density either Kruskal–Wallis or Mann–Whitney tests were performed (Graph Pad Instat, version 4). Hardy–Weinberg equilibrium was determined by means of χ^2 goodness-of-fit tests.

3. Results

3.1. β-adrenoceptor expression in human lung

The density of β-adrenoceptors in membranes generated from lung tissue was determined by saturation binding using the radioligand [^{125}I]-cyanopindolol (0.01563–2 nM). The total β-adrenoceptor density was variable among the 88 lung preparations studied ranging from 19 to 180 fmol/mg protein (mean ± S.E.M.,

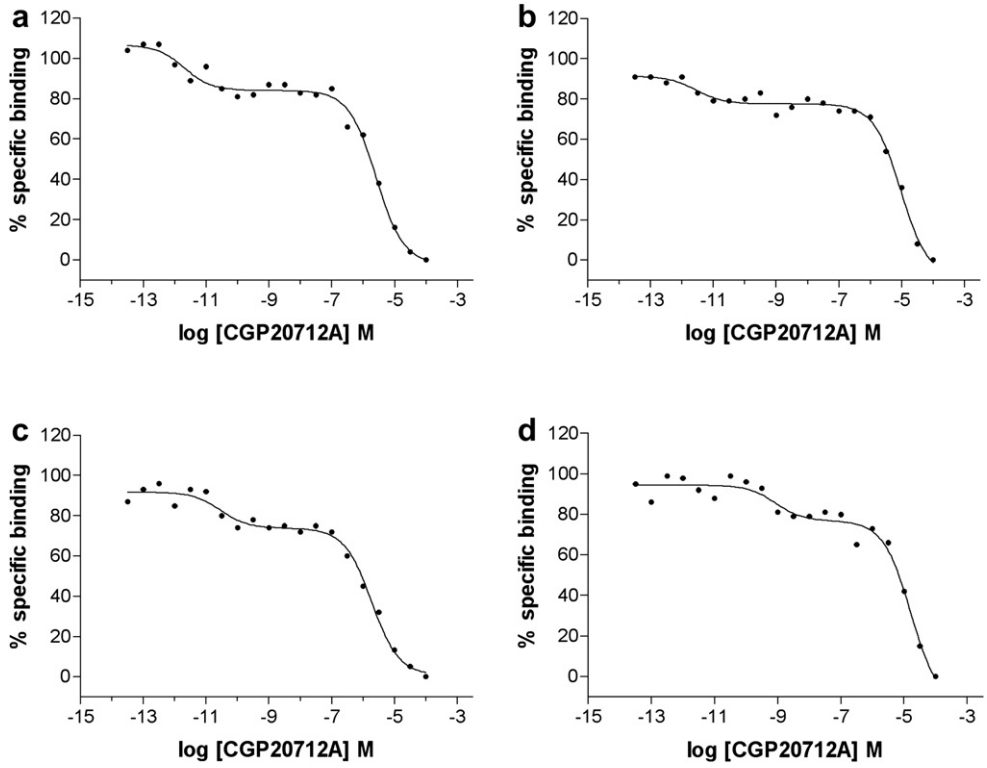

Fig. 2. Distribution of β-adrenoceptor subtypes in human lung. Membranes were prepared from lung samples ($n = 88$) and discrimination of β_2- and β_1-adrenoceptors was determined by competition of the radioligand [^{125}I]-cyanopindolol (0.0625 nM) with increasing concentrations of the highly β_1-adrenoceptor-selective antagonist, CGP20712A. The figure shows representative data in which each panel depicts competition binding for an individual preparation. CGP20712A displayed both a high (11.0 ± 0.5) and lower (5.6 ± 0.1) pK_i associated with interactions at β_1- and β_2-adrenoceptors, respectively. The percentage of β_1-adrenoceptors in these preparations ranged from 14% to 21%.

Table 2
β-Adrenoceptor density in different zones of human lung.

	Zones			
	Upper	Middle	Lower	Central
β-AR density (fmol/mg protein)	80 ± 13	90 ± 16	75 ± 10	114 ± 18
β_2-AR (%)	92 ± 1	95 ± 4	95 ± 2	94 ± 2
K_D (nM)	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01

Tissue was sampled from peripheral regions of upper, middle and lower zones and from deep, central zones of lung following pneumonectomies. Membranes were prepared from these samples and β-adrenoceptor (β-AR) density was determined by saturation binding assays using the radioligand [^{125}I]-cyanopindolol (0.01563–2 nM). The K_D values for [^{125}I]-cyanopindolol, determined in different zones of lung, are provided in the table and were not significantly different, statistically ($P > 0.05$), from each other. Discrimination of β_2- and β_1-adrenoceptors was determined by competition of the radioligand with CGP20712A. There was no difference, statistically ($P > 0.05$), among zones of lung in the density (fmol/mg protein) of (a) total β-adrenoceptors, (b) β_2-adrenoceptors, or (c) β_1-adrenoceptors. Values are means ± S.E.M. for seven experiments.

80 ± 4 fmol/mg protein). Representative saturation binding data are provided (Fig. 1).

The density of β_2- and β_1-adrenoceptors was assessed by competition binding utilizing the highly selective β_1-adrenoceptor antagonist CGP20712A (Fig. 2). These experiments demonstrated that of the total β-adrenoceptors, 91 ± 10% were β_2-adrenoceptors ($n = 88$).

3.2. β-Adrenoceptor expression in different zones of human lung

The samples used in these studies were sub-pleural and taken from the periphery of either upper, middle or lower zones of lung following lobectomies. In order to determine whether the variability in β-adrenoceptor density and subtype distribution was due to the area of lung being sampled, binding assays were performed on tissue sampled from the periphery of upper, middle and lower zones, as well as deep, central regions of lung, following pneumonectomies (Table 2). There was no difference, statistically ($P > 0.05$), among regions of lung in the density (fmol/mg protein) of (a) total β-adrenoceptors, (b) β_2-adrenoceptors, or (c) β_1-adrenoceptors.

3.3. Influence of SNPs on β_2-adrenoceptor expression

In order to establish whether polymorphisms within *ADRB2* influence receptor expression in human lung, the 88 lung preparations whose β_2-adrenoceptor densities had been determined (see Section 3.1), were genotyped at 5 positions of *ADRB2* (−367, −47, 46, 79 and 491) known to be polymorphic. For these positions, the frequency of the less prominent allele was either 0.40 or 0.41 with the exception of nucleotide position 491 in which only 5 heterozygotes and no homozygotes of the less frequent allele were detected (Table 3).

Table 3
Allelic frequencies of polymorphic positions in *ADRB2* ($n = 88$).

Position (aa)	SNP	Amino acid	Frequency
−367	T	–	0.59
	C	–	0.41
−47	T	cys	0.60
	C	arg	0.40
46 (16)	G	gly	0.60
	A	arg	0.40
79 (27)	C	gln	0.59
	G	glu	0.41
491 (164)	C	thr	0.97
	T	ile	0.03

Each polymorphic position was in Hardy–Weinberg equilibrium.

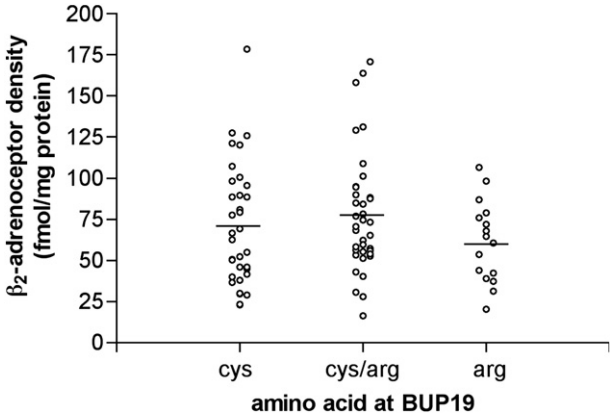

Fig. 3. Influence of position −47T > C on β_2-adrenoceptor expression in human lung. Polymorphisms at −47 lead to changes in the terminal amino acid (cys to arg) of a putative 19 amino acid peptide, BUP, that has been linked to β_2-adrenoceptor expression. There was no difference ($P = 0.35$) in the expression of β_2-adrenoceptors in human lung genotyped as BUP-cys (71 ± 6 fmol/mg protein; $n = 34$), BUP-cys/arg (77 ± 6 fmol/mg protein; $n = 38$) or BUP-arg (61 ± 6 fmol/mg protein; $n = 16$). Horizontal bars show mean values.

Position −47, within the promoter region, has been reported to influence β_2-adrenoceptor expression [11]. However, our data demonstrate that there was no influence ($P > 0.05$) of position −47 on β_2-adrenoceptor expression in the 88 lung preparations that were studied (Fig. 3). Position −367 within the promoter region of *ADRB2* has also been linked to influencing receptor expression [12,15]. However, in human lung this position had no influence on β_2-adrenoceptor expression (Table 4).

The potential influence of coding block SNPs on β_2-adrenoceptor expression in lung was evaluated (Table 4). None of the positions had an influence with the exception of position 491 (amino acid position 164) in which preparations genotyped as heterozygous expressed significantly ($P = 0.0005$) higher levels of β_2-adrenoceptor than homozygotes (Fig. 4).

4. Discussion

β_2-adrenoceptor agonists continue to be significantly important drugs used to manage asthma [5]. Therapeutically, β_2-adrenoceptor agonists act as bronchodilators but additional benefits may include the stabilization of inflammatory cell activity [7,8]. As agonists that mediate effects through pulmonary β_2-adrenoceptors, the actions of these drugs are likely to be influenced by the extent of β_2-adrenoceptor expression in the lung. In the present study, we found vast differences, as much as tenfold, in the extent of β-adrenoceptor expression among lung tissue specimens. Our intention was to

Table 4
Influence of SNPs on β_2-adrenoceptor expression in human lung.

	β_2-Adrenoceptor density (fmol/mg protein)				
Genotype	−367	−47	46	79	491
0	65 ± 6	71 ± 6	71 ± 6	67 ± 6	69 ± 4
1	84 ± 6	77 ± 6	74 ± 6	79 ± 6	126 ± 15*
2	63 ± 6	61 ± 6	71 ± 8	63 ± 6	–

For each polymorphic position, homozygotes for the more common allele are represented by set 0 and for the less common allele by set 2 and for heterozygotes by set 1. Further information concerning alleles and allele frequencies can be found in Table 3. None of the polymorphisms influenced β_2-adrenoceptor expression except position 491 (amino acid 164) in which preparations that were heterozygous ($n = 5$) at this position expressed significantly (*$P = 0.0005$) higher levels of receptor than homozygotes ($n = 83$).

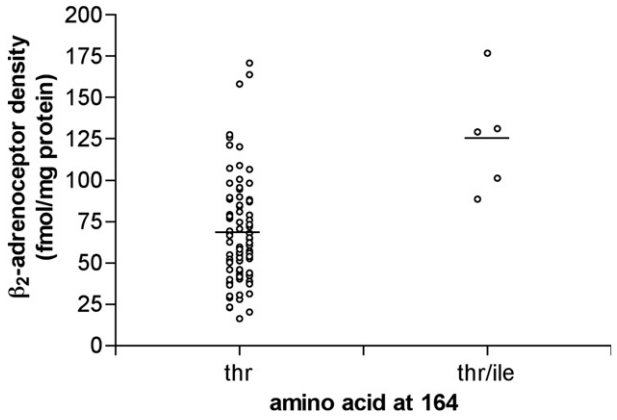

Fig. 4. Influence of position 491C > T on β_2-adrenoceptor expression in human lung. Polymorphisms at 491 lead to changes (thr to ile) in the amino acid at position 164. The expression of β_2-adrenoceptors in human lung was significantly ($P = 0.0005$) higher in preparations genotyped as heterozygous (thr/ile; 126 ± 15 fmol/mg protein; $n = 5$) compared to homozygous preparations (thr; 69 ± 4 fmol/mg protein; $n = 83$). Horizontal bars show mean values. In order to confirm these data, the 5 preparations, heterozygous at position 491, were reassessed for β_2-adrenoceptor density. Following reassessment, mean β_2-adrenoceptor density determinations were similar (125 ± 26 fmol/mg protein) and were significantly ($P = 0.006$) different from densities seen in homozygous preparations.

determine whether this wide difference in the degree of receptor expression might be genetically influenced.

Polymorphisms within the promoter region of *ADRB2*, especially those at −47T > C (BUP-cys to BUP-arg) and −367T > C, have been associated with differences in β_2-adrenoceptor expression in transfected cells [11–15]. By contrast, a study using lymphocytes isolated from asthmatics demonstrated that there was no association between the polymorphism at −47 and β_2-adrenoceptor expression [17]. While lymphocytes were used in this study as a representation of lung β_2-adrenoceptors, it is known that the behaviour and expression of β_2-adrenoceptors can be influenced by the tissue or cell in which the receptor is expressed [25–27]. Nevertheless, findings from the present study are in accord with those reported for lymphocytes since there was no influence of position −47 on β_2-adrenoceptor expression in human lung. Indeed, neither of the SNPs (−47 and −367) found upstream from the coding block and that, conceivably, could have an influence on β_2-adrenoceptor expression were associated with differences in receptor density in this study.

A potential influence of SNPs within the coding block of *ADRB2* was also considered. Thus, polymorphisms at positions 46 and 79, corresponding with amino acid positions 16 and 27 of the β_2-adrenoceptor, were examined to establish whether any link existed with receptor expression. Positions 16 and 27 have been associated with differences in the degree of β_2-adrenoceptor down-regulation [28,29] such that expression of a particular genotype at either of these two positions could, potentially, impact on the degree of receptor expression in lung. However, in the present study, there was no influence of either of these two positions on β_2-adrenoceptor expression.

In addition, we also considered an alternative polymorphism, 491C > T, within the coding block corresponding with a change in amino acid at position 164 of the β_2-adrenoceptor from thr to ile [30]. In keeping with a number of studies [24,30–32], the appearance of the less common allele was very infrequent with only 5 preparations out of 88 genotyped as heterozygous at this position with none homozygous for the less common allele. Lung preparations genotyped as thr164ile expressed significantly greater levels, almost twice as many, β_2-adrenoceptors as those genotyped as thr164thr. While interesting, these data were unexpected since no precedents exist in the literature for such an outcome. One possible explanation for the markedly greater β_2-adrenoceptor density in lungs genotyped as thr164ile could be due to a compensatory mechanism. In transfected cells, 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 [33]. This could mean that diminished constitutive or promoted down-regulation occurs of the ile164-expressing β_2-adrenoceptor due to impaired activation of the protein kinases (protein kinase A and G-protein receptor kinase) that are thought to be involved in receptor desensitization [34–36]. Consequently, this could result in a relative increase in β_2-adrenoceptors in preparations expressing the less frequent allele at

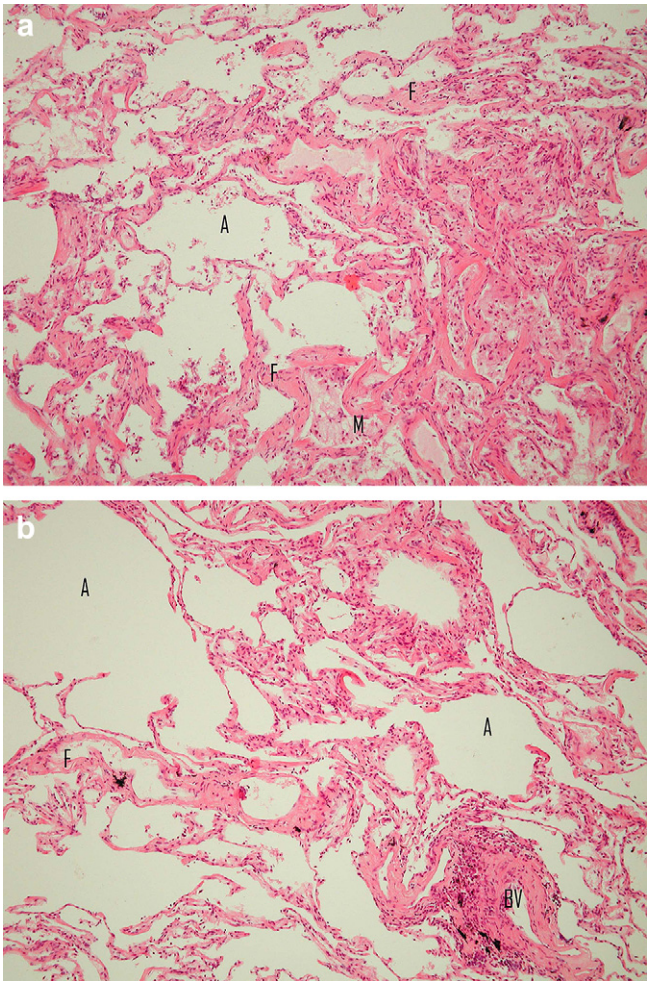

Fig. 5. Human lung histology. Twelve human lung preparations known to express high, low and intermediary levels of β_2-adrenoceptor were examined histologically. Sections of lung tissue were fixed in buffered formalin and prepared through paraffin embedding for analysis. The samples were scored for oedema, fibrosis, emphysema, acute and chronic inflammation, blood vessel and airway integrity. This analysis was performed blind by a single observer (SKS). Although there were slight morphological differences among the 12 lungs studied, these were judged non-significant regarding any inflammation component. The two representative sections shown are lung preparations expressing β_2-adrenoceptor densities of (a) 29 fmol/mg protein and (b) 171 fmol/mg protein. (a) Photomicrograph of lung 551, showing slight interstitial fibrosis (F) and mucus inspissation (M) in the lung. A very minor interstitial accumulation of chronic inflammatory cells was noted without accumulation or frank suppuration. Minimal airspace dilatation was seen, (A). (b) Photomicrograph of lung 516, showing alveolated lung with some emphysematous changes (variable airspace dilatation, (A)) and only very minor interstitial expansion (F). A prominent blood vessel (BV) is noted and there is some minor soot black pigment noted. Again, a very mild chronic inflammatory cell population was seen in the interstitium (×200, Haematoxylin and Eosin).

position 164. While such a scheme is, admittedly, speculative, depressed desensitization of the ile164-expressing β_2-adrenoceptor in transfected cells has been reported [33].

Overall, these findings indicate that common polymorphisms in *ADRB2* do not influence β_2-adrenoceptor expression in human lung. This suggests that a direct genetic influence is not responsible for the wide variability in β_2-adrenoceptor density observed and that alternative influences contribute to the variability. We explored the possibility that the underlying lung pathophysiology might influence β_2-adrenoceptor expression by blinded histological review of the samples taken. Microscopic analysis of different lung tissue samples known to express a range of β_2-adrenoceptor densities, was performed by a single observer (SKS). The analysis indicated minimal differences among samples in oedema, inflammatory cell infiltration, fibrosis and emphysema (see Fig. 5). These minimal differences are often seen in background resected lung tissues and are judged unlikely to have any material impact on β-adrenoceptor density.

One alternative factor that could potentially influence β_2-adrenoceptor expression in lung is prior exposure to medications, in particular, to β_2-agonists and corticosteroids. Exposure to β_2-agonists could lead to β_2-adrenoceptor down-regulation [18,34–37] and corticosteroids have been shown to increase β_2-adrenoceptor expression in a variety of test systems [38–41]. In the present study, tissue was donated anonymously and drug histories were, therefore, unavailable. However, in an unrelated study in which 250 individuals had donated lung tissue for study and for whom details exist, 2% were on corticosteroids, 5% were on β_2-agonists and 4% were on a combination of corticosteroids and β_2-agonists. If an assumption is made that a similar proportion of the present study population was exposed to these drugs, it seems unlikely that exposure to corticosteroids and β_2-agonists is a substantially confounding influence. Indeed, whether prior exposure to β_2-agonists has a substantial effect on receptor density in the lung is a moot point since reports have shown that the pulmonary β_2-adrenoceptor is unusually resistant to down-regulation [25,26,42].

In summary, the present study has shown that common polymorphisms both within the promoter region and the coding block of *ADRB2* do not influence the expression of β_2-adrenoceptors in human lung. By contrast, there was an association between the rare polymorphism at position 491 (amino acid 164) and β_2-adrenoceptor expression. However, the possibility that other recently identified SNPs in *ADRB2*, 5′ and 3′ of the region studied [2], might influence receptor expression cannot be excluded.

In conclusion, these findings indicate that common polymorphisms in *ADRB2* do not influence β_2-adrenoceptor expression in human lung. This suggests that a direct genetic influence is not responsible for the wide variability in β_2-adrenoceptor density observed and that alternative influences contribute to the variability.

Acknowledgements

The authors are grateful to Mr. J Edwards, Mr. J Rao, Mr. T Locke, Mr. G Cooper and Mr. D Hopkinson (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.

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