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

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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.

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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