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Pharmacological characterisation of the β-adrenoceptor expressed by human lung mast cells

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Abstract

The nonselective β-adrenoceptor agonist, isoprenaline (pD_2; 8.8 ± 0.2), and selective β_2-adrenoceptor agonists, clenbuterol (9.2 ± 0.4) and salbutamol (7.1 ± 0.1), inhibited the immunoglobulin E-mediated release of histamine from human lung mast cells in a concentration-dependent manner. The β_2-adrenoceptor-selective antagonist, ICI118551 (erythro-(±)-1-(7-methylindan-4-yloyl)-3-isopropylaminobutan-2-ol HCl), antagonised the isoprenaline inhibition of histamine release from human lung mast cells with high affinity (apparent pK_B; 9.5 ± 0.2), whereas high concentrations of the β_1-adrenoceptor-selective antagonist, CGP20712A (2-hydroxy-5-(2-(hydroxy-3-(4((1-methyl-4-trifluoromethyl)-1-*H*-imidazol-2-yl)-phenoxy)-propyl)-aminoethoxyl)-benzamide), were required to reverse the isoprenaline inhibition (apparent pK_B; 6.5 ± 0.3). Radioligand binding studies using [^{125}I]-iodocyanopindolol ([^{125}I]CYP) were performed on membranes derived from purified mast cells (>90% purity). Binding of [^{125}I]CYP to mast cell membranes was displaced from a single binding site with a high affinity for ICI118551 (pK_i; 8.9 ± 0.1) and low affinity for CGP20712A (pK_i; 6.0 ± 0.03), indicative of a homogeneous population of β_2-adrenoceptors. In contrast, in human lung membranes, these antagonists displaced [^{125}I]CYP from two sites indicative of a heterogeneous population of β_1-adrenoceptors (20%) and β_2-adrenoceptors (80%). These data indicate that the β-adrenoceptor expressed by human lung mast cells and mediating inhibition of mediator release from these cells is the β_2-adrenoceptor.

Keywords: Mast cell; β_2-Adrenoceptor; CGP20712A; ICI118551

1. Introduction

Bronchodilator β_2-adrenoceptor agonists continue to be used widely in the treatment of asthma (Tattersfield, 1992; Barnes, 1999). The primary action of these drugs is to relax airway smooth muscle but additional effects may include the stabilisation of inflammatory cell activity (Barnes, 1999). In this regard, the pulmonary mast cell may be an important target because a number of studies have shown that mast cells are very sensitive to the inhibitory effects of β-adrenoceptor agonists (Assem and Schild, 1969; Orange et al., 1971; Butchers et al., 1980; Church and Hiroi, 1987; Peachell et al., 1988; Chong et al., 1998), more so than alternative inflammatory cells that have been implicated in asthma (Fuller et al., 1988; Yukawa et al., 1990).

In vitro studies readily demonstrate that a variety of β-adrenoceptor agonists inhibit the release of histamine, prostaglandin D_2 and cysteinyl-leukotrienes from activated human lung mast cells (Peachell et al., 1988; Undem et al., 1988). The rank order of potency of a series of β_1- and β_2-adrenoceptor-selective agonists on the inhibition of mediator release from antigen-challenged human lung fragments and the nature of the antagonism of this inhibition by β_1- and β_2-adrenoceptor-selective antagonists, suggest that the receptor mediating the inhibition is the β_2-adrenoceptor (Butchers et al., 1980). However, despite these functional studies, no direct evidence of β_2-adrenoceptor expression by human lung mast cells, using radioligand binding techniques, has been provided.

The aim of the present study, therefore, was to determine the class of β-adrenoceptor expressed by human lung mast cells. For comparative purposes, characterisation of the β-adrenoceptors expressed by parenchymal human lung tissue was performed in parallel.

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2. Materials and methods

2.1. Buffers

Phosphate buffered saline (PBS) contained (mM): NaCl 137, $Na_2HPO_4 \cdot 12H_2O$ 8, KCl 2.7, KH_2PO_4 1.5. PBS–BSA was PBS that additionally contained: $CaCl_2 \cdot 2H_2O$ 1 mM, $MgCl_2.6H_2O$ 1 mM, glucose 5.6 mM, bovine serum albumin 1 mg/ml, DNase 15 μg/ml. PBS–HSA was PBS additionally supplemented with: $CaCl_2 \cdot 2H_2O$ 1 mM, $MgCl_2 \cdot 6H_2O$ 1 mM, glucose 5.6 mM, human serum albumin 30 μg/ml. The pH of all PBS buffers was titrated to 7.3. 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

Stock solutions (10 mM) of (−)-isoprenaline bitartrate were prepared daily in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl). The agonists, (±)-salbutamol hemisulphate, (±)-clenbuterol HCl, and (±)-dobutamine HCl, and the antagonists, (±)-propranolol HCl, ICI118551 HCl (erythro-(±)-1-(7-methylindan-4-yloyl)-3-isopropylaminobutan-2-ol HCl) and CGP20712A (2-hydroxy-5-(2-(hydroxy-3-(4((1-methyl-4-trifluoromethyl)-1-*H*-imidazol-2-yl)-phenoxy)-propyl)-aminoethoxyl)-benzamide) were prepared daily as stock solutions (10 mM) in buffer. Lyophilised polyclonal goat anti-human immunoglobulin E (IgE) antibody was reconstituted in distilled water and stored at 4 °C.

2.3. Lung tissue

Macroscopically normal tissue from lung resections of patients was obtained following surgery. Most of the patients were undergoing surgery for carcinoma. The male to female split was 70% to 30%, and 90% of the patients were white caucasians.

2.4. Isolation of human lung mast cells

Mast cells were isolated from human lung tissue by a modification of the method described by Ali and Pearce (1985). The tissue was stripped of its pleura and chopped vigorously for 15 min with scissors in a small volume of PBS buffer. The chopped tissue was washed over a nylon mesh (100-μm pore size; Cadisch and Sons, London, UK) with 0.5–1 l of PBS buffer to remove lung macrophages. The tissue was reconstituted in PBS–BSA (10 ml/g tissue) containing collagenase Ia (350 Units/ml PBS–BSA) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37 °C. The supernatant (containing some mast cells) was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of PBS–BSA buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with PBS–BSA (300–600 ml). The pooled filtrates were sedimented ($120 \times g$, room temperature, 8 min), the supernatant discarded and the pellets reconstituted in PBS–BSA (100 ml). The pellet was washed a further two times. The dispersion procedure generated 0.2 to 1×10^6 mast cells per gram of lung tissue at 3% to 13% purity. These preparations were employed for the histamine release experiments. For the receptor binding studies, mast cells were purified further by flotation over Percoll density gradients and immunomagnetic bead separations according to methods that have been described in detail elsewhere (Weston et al., 1997). Mast cells were visualised by microscopy using an alcian blue stain (Gilbert and Ornstein, 1975).

2.5. Histamine release

Histamine release experiments were performed in PBS–HSA. Histamine release from mast cells was initiated immunologically with an optimal releasing concentration (1:300) of antibody to human IgE (anti-IgE). Secretion was allowed to proceed for 25 min at 37 °C after which time the cells were pelleted by centrifugation ($400 \times g$, room temperature, 3 min). Histamine released into the supernatant was determined by a modification (Ennis, 1991) of the automated fluorometric method of Siraganian (1974). When the effects of agonists (isoprenaline, salbutamol, clenbuterol or dobutamine) were studied, cells were incubated with an agonist for 10 min at 37 °C before the addition of stimulus and then samples were processed as indicated above. When antagonists (ICI118551 or CGP20712A) were studied, cells were co-incubated with isoprenaline and without or with an antagonist for 10 min at 37 °C before the addition of stimulus. Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in buffer alone served as a measure of spontaneous histamine release (<6%). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release.

2.6. Radioligand binding

Membrane fractions were prepared from purified (≥90% purity) human lung mast cell preparations ($\geq 3 \times 10^6$ cells) by homogenising in ice-cold Tris buffer using an Ultra Turrax homogeniser for 20 s followed by 4 strokes ($\times 4$) of a Teflon homogeniser. The homogenate was centrifuged ($500 \times g$, 40 min), the supernatant was harvested and subjected to further centrifugation ($40{,}000 \times g$, 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. When membrane fractions of human lung tissue (0.2 g) were prepared, the procedure was as for mast cells with the exception that the centrifugation step at $500 \times g$ was for 10 min. In saturation binding assays, the membrane preparations were assayed for β-adrenoceptor

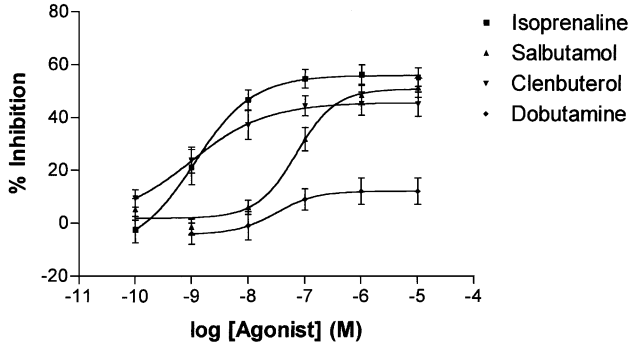

Fig. 1. Effect of β-adrenoceptor agonists on histamine release from human lung mast cells. Cells were incubated without or with either isoprenaline, salbutamol, clenbuterol or dobutamine before challenge with anti-human IgE (1/300). Values are expressed as the percent inhibition of the control histamine release which was 33 ± 4%. Values are means ± S.E.M. from nine experiments for all agonists except dobutamine, which was studied in only five of these nine experiments.

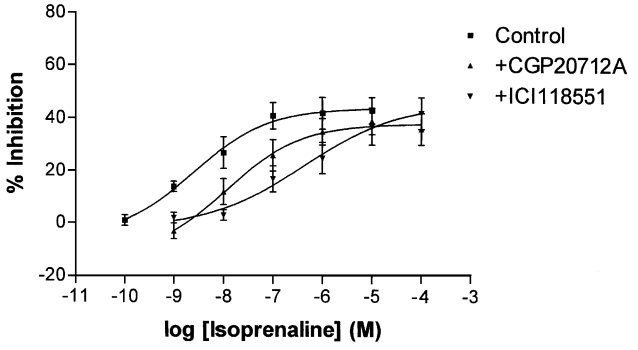

Fig. 2. Effect of antagonists on the isoprenaline inhibition of histamine release. Mast cells were incubated without (control) or with either ICI118551 (30 nM) or CGP20712A (3 μM) and without or with isoprenaline before challenge with anti-human IgE (1/300). Values are expressed as the percent inhibition of the control histamine releases, which were 39 ± 4% (control), 33 ± 6% (ICI118551) and 35 ± 5% (CGP20712A). Values are means ± S.E.M. from six experiments.

binding sites using [^{125}I]-iodocyanopindolol ([^{125}I]CYP). Membrane suspensions (100 μl) were incubated (1 h, 37 °C) using a range of radioligand concentrations (0.03125–2 nM) in a total volume of 250 μl. Nonspecific binding was determined by displacement with propranolol (1 μM). Specific binding, expressed as a percentage of the total binding, was 74 ± 4% (mast cell membranes) and 84 ± 2% (lung tissue membranes) at a [^{125}I]CYP concentration of 0.0625 nM. Preliminary studies indicated that the approximate K_D for [^{125}I]CYP was 0.05 nM and this concentration was used in competition binding assays. The subtype(s) of β-adrenoceptor present in membranes was determined in competition studies with the use of the antagonists propranolol (β_1/β_2), ICI118551 (β_2-adrenoceptor-selective) and CGP20712A (β_1-adrenoceptor-selective). Membrane preparations (100 μl) were incubated (1 h, 37 °C) with a range of antagonist concentrations in the presence of [^{125}I]CYP (0.05 nM) in a total volume of 250 μl. 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. Protein content of the membranes was determined by the method of Lowry et al. (1951).

Table 1
Maximal inhibition (E_{max}) and potencies (pD_2) for the inhibition of histamine release from human lung mast cells by β-adrenoceptor agonists

Agonist	E_{max} (%)	pD_2
Isoprenaline	56 ± 3	8.8 ± 0.2
Salbutamol	52 ± 4	7.1 ± 0.1
Clenbuterol	45 ± 4	9.2 ± 0.4
Dobutamine	12 ± 8	7.3 ± 0.2

Values were calculated from the data in Fig. 1 and further details may be found in the legend to that figure. E_{max} values are expressed as the percent inhibition of histamine release. Values are means ± S.E.M.

2.7. Materials

The following were purchased from the sources indicated: anti-human IgE, bovine serum albumin, collagenase, DNase,

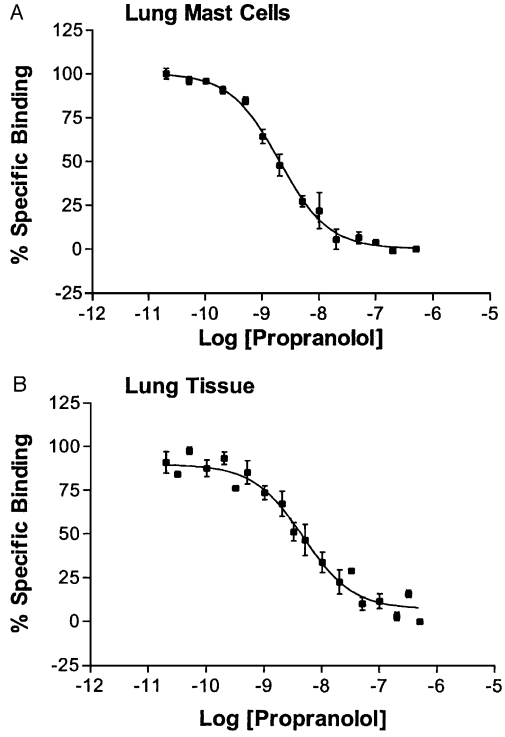

Fig. 3. Competition binding with propranolol in (A) human lung mast cell and (B) human lung tissue membranes. Membranes were incubated with [^{125}I]CYP (0.05 nM) and increasing concentrations of propranolol. For mast cell membranes, the Hill slope was 0.98 ± 0.10 and the pK_i value for propranolol was 9.0 ± 0.1. In lung tissue membranes, the Hill slope was 0.92 ± 0.07 and the pK_i value for propranolol was 8.7 ± 0.1. Values are means ± S.E.M. from five lung preparations and four mast cell preparations at 96 ± 1% purity.

human serum albumin, Percoll, isoprenaline, salbutamol, clenbuterol, dobutamine (all Sigma, Poole, UK); [^{125}I]CYP (New England Nuclear, Stevenage, UK). CGP20712A and ICI118551 were kindly supplied as gifts from Ciba-Geigy (Basel, Switzerland) and AstraZeneca (Macclesfield, UK), respectively.

2.8. Data analysis

In functional studies, antagonist affinities were estimated using the following formula: $pK_B = \log(\text{dose ratio} - 1) - \log(\text{antagonist concentration})$, where pK_B is the $-$ logarithm of the apparent dissociation constant and the dose ratio is the ratio of EC_{50} values in the presence and absence of antagonist. Maximal inhibition (E_{max} values) and potencies (pD_2 values, defined as the $-$ logarithm of the EC_{50}) were determined using GraphPad Prism software. Saturation and competition binding curves were analysed using the same software. Statistical significance was assessed utilising Student's *t* test.

3. Results

3.1. Functional studies

The nonselective β-adrenoceptor agonist, isoprenaline, inhibited the release of histamine from human lung mast cells activated with anti-human IgE (Fig. 1). The selective β_2-adrenoceptor agonists, clenbuterol and salbutamol, also inhibited histamine release although these agonists showed variable intrinsic activity among mast cell preparations acting as full agonists, relative to isoprenaline, in some preparations and as partial agonists in others. The non-selective agonist, dobutamine, inhibited histamine release weakly. Maximal inhibitory effects (E_{max}) and potencies (pD_2) for these agonists are provided in Table 1.

The β_2-adrenoceptor-selective antagonist, ICI118551 (30 nM), caused over a hundredfold rightward shift in the isoprenaline concentration–response curve for the inhibition of histamine release from human lung mast cells (Fig. 2). The β_1-adrenoceptor-selective antagonist, CGP20712A (3 μM), also shifted the isoprenaline concentration–response curve for the inhibition of histamine release (Fig. 2). However, CGP20712A was a thousandfold less potent than ICI118551 and affinity estimates (pK_B values) for the antagonists were 6.5 ± 0.3 and 9.5 ± 0.2, respectively.

3.2. Radioligand binding studies

In order to establish the β-adrenoceptor subtype(s) present in human lung mast cells, competition binding assays with several antagonists were performed on membranes generated from purified mast cells using the radioligand [^{125}I]CYP (0.05 nM). In these studies, membranes

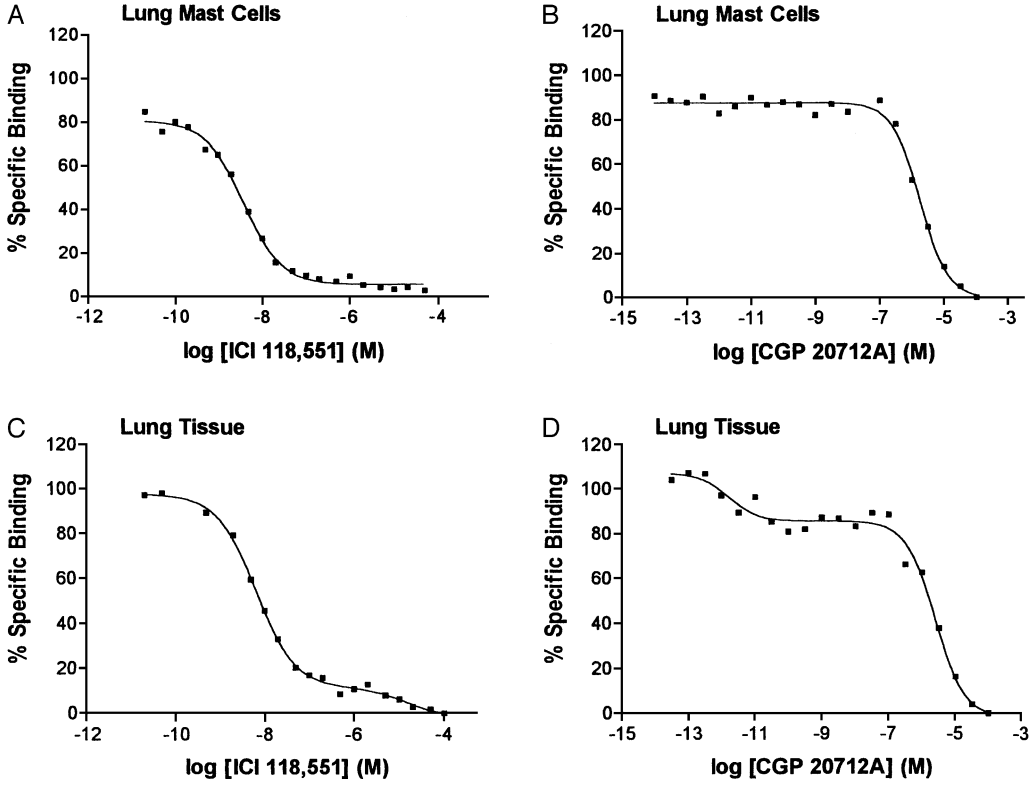

Fig. 4. Competition binding with ICI118551 or CGP20712A in human lung mast cell and human lung tissue membranes. Membranes were incubated with [^{125}I]CYP (0.05 nM) and increasing concentrations of either ICI118551 (A, C) or CGP20712A (B, D). The figure depicts representative curves from a total of four experiments each performed in duplicate. Mean data can be found in Table 2.

Table 2
pK_i values for ICI118551 and CGP20712A as antagonists of [^{125}I]CYP binding to human lung mast cell and human lung tissue membranes

	Mast cells		Lung tissue	
	ICI118551	CGP20712A	ICI118551	CGP20712A
pK_i high	8.9 ± 0.1	6.0 ± 0.03	8.9 ± 0.2	10.9 ± 0.8
pK_i low	–	–	6.6 ± 0.6	5.8 ± 0.2
Hill slope	1.05 ± 0.06	0.93 ± 0.06	0.64 ± 0.07	0.33 ± 0.11
%High affinity site	–	–	80 ± 2	21 ± 2

Data for mast cells best fitted displacement from a single binding site and Hill slopes were close to unity. Data for lung tissue best fitted displacement from two binding sites, the larger population of sites having a high affinity for ICI118551 and a low affinity for CGP20712A. Hill slopes for lung tissue were low and significantly different ($P<0.001$) from unity. The mast cell purity was 99 ± 1%. Values are means ± S.E.M. from four experiments.

from whole human lung tissue were prepared and studied in a comparative context. Propranolol displaced [^{125}I]CYP binding with a high affinity from a single binding site in both mast cells (pK_i, 9.0 ± 0.1; Fig. 3A) and human lung tissue (pK_i, 8.7 ± 0.1; Fig. 3B). Hill slopes for propranolol competition were close to unity for both types of membrane (mast cells, 0.98 ± 0.10; human lung, 0.92 ± 0.07).

In competition studies with ICI118551 or CGP20712A, assays were performed on membranes generated from purified mast cells and lung tissue derived from the same donor. Representative displacement curves are shown in Fig. 4 and mean data (pK_i values, $n=4$) are provided in Table 2. In membranes from mast cells, both ICI118551 and CGP20712A displaced [^{125}I]CYP with steep and monophasic competition curves (Fig. 4A and B), the displacement data best fitting a one-site model of binding and with Hill slopes close to unity (Table 2). The β_2-adrenoceptor-selective antagonist, ICI118551, had a high affinity at this binding site while the β_1-adrenoceptor-selective antagonist, CGP20712A, was a thousandfold less potent (Table 2). These data indicate a homogeneous population of β_2-adrenoceptors in mast cells. In contrast, for membranes prepared from human lung, competition curves for ICI118551 were

Table 3
Dissociation constants (K_D values) and density (B_{max}) of [^{125}I]CYP binding sites in membranes prepared from mast cells and lung tissue

	n	K_D (nM)	B_{max} (fmol/mg protein)
Mast cells	8	0.04 ± 0.01	9.4 ± 3.0
Lung tissue	12	0.05 ± 0.01	33.2 ± 6.4

Data are means ± S.E.M. of the indicated number of experiments, each performed in duplicate. Human lung tissue membranes possess significantly ($P<0.001$) higher densities of β-adrenoceptors than mast cell membranes.

shallow (Fig. 4C) and the biphasic competition curves yielded significantly better fits to a two-site model. Nonlinear regression analysis of these curves revealed the presence of 80% high affinity sites (β_2-adrenoceptors) with the remaining sites having a low affinity for ICI118551 (β_1-adrenoceptors). Competition curves for the highly selective β_1-adrenoceptor antagonist, CGP20712A, confirmed the presence of a minor population of β_1-adrenoceptors in human lung tissue. Displacement of [^{125}I]CYP binding by this antagonist also fitted to a two-site model and the competition curves were shallow (Fig. 4D, Table 2). The majority of sites had a low affinity for CGP20712A (β_2-adrenoceptors) and the data suggest the presence of a population (21%) of β_1-adrenoceptors (high affinity sites) similar to that estimated using ICI118551.

In order to determine the density of β-adrenoceptors in membranes from mast cells and lung tissue, saturation binding assays were performed (Fig. 5). Total β-adrenoceptor density (B_{max}) was over threefold higher in lung tissue than in mast cell membranes (Table 3). Dissociation constants (K_D values) for [^{125}I]CYP were similar in membranes from mast cells and human lung tissue. In five cases, B_{max} values were determined in membranes generated from mast cells and lung tissue derived from the same donor. In these five cases, although the density of receptors in mast cell membranes was always lower than that found in lung tissue membranes, there was a good positive correlation ($r=0.83$) between the density of receptors in mast cell membranes and in lung tissue membranes (Fig. 6).

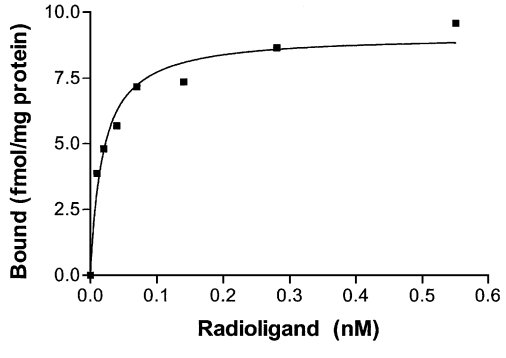

Fig. 5. Representative saturation binding curve of the radioligand, [^{125}I]CYP, to human lung mast cell membranes. The purity of the mast cell preparation was 97%, receptor density (B_{max}) was 9.2 fmol/mg protein and the K_D for [^{125}I]CYP was 0.03 nM. Specific binding of [^{125}I]CYP was 87% at a concentration of 0.0625 nM.

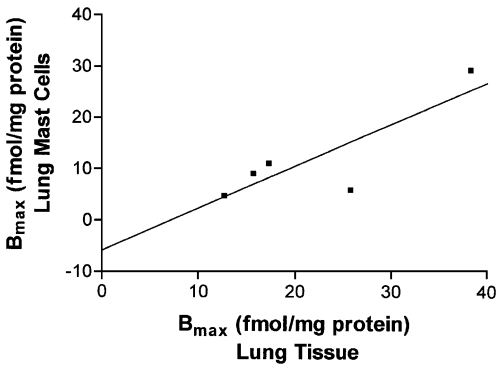

Fig. 6. Correlation of receptor densities (B_{max}) in human lung mast cells and human lung. Mast cell and lung tissue membranes were prepared from the same five donors. Mast cell purities ranged from 92% to 100%. The correlation coefficient is $r=0.83$.

4. Discussion

A large number of studies have shown that β-adrenoceptor agonists inhibit the release of mediators from activated human lung mast cells (Assem and Schild, 1969; Orange et al., 1971; Butchers et al., 1980; Church and Hiroi, 1987; Peachell et al., 1988; Chong et al., 1998). Based on the rank order of potency of a variety of β_1- and β_2-adrenoceptor-selective agonists, it has been suggested that the receptor mediating this inhibition is the β_2-adrenoceptor (Butchers et al., 1980). Data from the present study are in agreement with these suggestions as the rank order of potency and the intrinsic activities of several agonists, as inhibitors of histamine release from activated mast cells, are similar to those observed in alternative systems including cell lines transfected with the β_2-adrenoceptor (MacEwan et al., 1995; McDonnell et al., 1998; Hopkinson et al., 2000).

Further confirmation that the β-adrenoceptor mediating inhibition of mediator release is the β_2-adrenoceptor has been provided in the present study through the demonstration that the β_2-adrenoceptor-selective antagonist, ICI1 18551, was considerably more potent than the β_1-adrenoceptor-selective antagonist, CGP20712A, at reversing the inhibition of histamine release by isoprenaline. However, although estimates of the affinities (pK_B) for ICI118551 (9.5 ± 0.2) and CGP20712A (6.5 ± 0.3) are within the range expected for the β_2-adrenoceptor, these values are at the higher end of the range reported (Dooley et al., 1986; Zerkowski et al., 1986; Yamazaki et al., 1998). Interestingly, higher affinities have been shown for alternative β-AR antagonists (e.g. propranolol, atenolol) employed to antagonise the isoprenaline inhibition of histamine release from antigen-challenged human lung fragments (Butchers et al., 1980).

While these functional studies strongly suggest the presence of β_2-adrenoceptors in human lung mast cells, we sought to provide confirmation by performing radioligand binding assays. In these studies, we also investigated the β-adrenoceptor population in human lung tissue. This served as a valuable control as a number of studies have shown that, although the predominant β-adrenoceptor in lung tissue is the β_2-adrenoceptor, some β_1-adrenoceptors are also present (Sano et al., 1993; Nishikawa et al., 1996).

Binding of [^{125}I]CYP to human lung mast cell membranes was antagonised by the β_1/β_2-adrenoceptor antagonist, propranolol, in a concentration-dependent manner. The high affinity ($pK_i = 9.0 \pm 0.1$) obtained for propranolol indicates the presence of β_1-adrenoceptors and/or β_2-adrenoceptors in these membranes and that neither β_3-adrenoceptors nor atypical-adrenoceptors are present (Emorie et al., 1989; Arch, 1997). Very similar results were obtained for [^{125}I]CYP and propranolol ($pK_i = 8.7 \pm 0.1$) when used with membranes generated from human lung tissue, leading to the same conclusions concerning the sub-types of β-adrenoceptor present in this system.

Data from studies employing the β_2-adrenoceptor-selective antagonist, ICI118551, in mast cell membranes show that nanomolar concentrations of ICI118551 ($pK_i = 8.9 \pm 0.1$) displaced radioligand from a single binding site, indicating that β_2-adrenoceptors alone are present in human lung mast cells. The data with the very selective β_1-adrenoceptor antagonist, CGP20712A, confirmed the finding that β_2-adrenoceptors alone are present in mast cell membranes as low concentrations ($< 10^{-8}$ M) of the antagonist did not displace the radioligand, indicating an absence of β_1-adrenoceptors, whereas high concentrations did displace the radioligand from a single binding site ($pK_i = 6.0 \pm 0.03$), showing the presence of β_2-adrenoceptors. In contrast, studies of radioligand binding to membranes derived from lung tissue and competition of this binding by ICI118551 and CGP20712A show that lung tissue membranes contain a heterogeneous population of β_2-adrenoceptors and β_1-adrenoceptors. Indeed, determination of the relative proportions of β_2- and β_1-adrenoceptors (4:1) was essentially identical irrespective of which of the two antagonists was used and similar to values reported in the literature (Sano et al., 1993; Nishikawa et al., 1996). These data provide assurances that the methodologies employed in the present study are capable of accurately discriminating between β-adrenoceptor sub-types and making it unlikely, therefore, that β-adrenoceptors, other than the β_2-adrenoceptor, are present in mast cell membranes.

Measurements of β-adrenoceptor density indicate that receptor density varies markedly among mast cell preparations (range 3 to 30 fmol/mg protein). It is likely that β_2-adrenoceptor density may influence mast cell function and studies in transfected cell systems show that the potency and intrinsic activity of agonists is increased in cells expressing higher levels of β_2-AR (MacEwan et al., 1995; McDonnell et al., 1998). Moreover, our own previous studies have shown that variable receptor reserves exist for the isoprenaline inhibition of histamine release among human lung mast cell preparations (Drury et al., 1998). It is probable that the variability in receptor density observed in the present study may explain, at least in part, the differences in receptor reserve observed previously among mast cell preparations. It is also noteworthy that mast cell membranes contain about threefold fewer β_2-adrenoceptor binding sites than lung tissue as a whole, when taking into account the fact that about a fifth of the sites in lung tissue are probably β_1-adrenoceptors. This could indicate that mast cells contain fewer β_2-adrenoceptors than alternative pulmonary cells and this could have some bearing on the relative responses of different pulmonary cells to β_2-adrenoceptor agonists.

In summary, the present study has shown that the β-adrenoceptor mediating inhibition of histamine release from human lung mast cells is the β_2-adrenoceptor. Radioligand binding studies confirm that mast cell membranes contain a population of β_2-adrenoceptors and, furthermore, that this is the only β-adrenoceptor sub-type expressed by these cells.

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