Evidence for an atypical, or β_3-adrenoceptor in ferret tracheal epithelium

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1 A preparation of the ferret trachea *in vitro* was used to examine the effects of three selective β-adrenoceptor agonists on lysozyme secretion from submucosal gland serous cells and epithelial albumin transport into tracheal mucus following sustained, submaximal stimulation of mucus production with methacholine (20 μM)

2 Prenalterol, salbutamol and BRL 37344 all enhanced methacholine-induced albumin output. BRL 37344 was 10,000 times more potent than salbutamol, and salbutamol was slightly more potent than prenalterol. The concentrations required to increase albumin output by 100% ($EC_{100\%}$) were 1.4 nM, 0.7 mM and approximately 1.0 mM for BRL 37344, salbutamol and prenalterol, respectively. All three agonists inhibited methacholine-induced lysozyme output, with salbutamol being 60 times more potent than BRL 37344, and BRL 37344 being approximately 100 times more potent than prenalterol.

3 The selective β_2-adrenoceptor antagonist, ICI 118551, inhibited the increase in albumin output produced by BRL 37344, but was much more potent at inhibiting the response to salbutamol; the pA_2 for ICI 118551 was 5.55 and 7.18 ($P < 0.001$) when the agonist was BRL 37344 and salbutamol, respectively. ICI 118551 also attenuated the inhibition of lysozyme output produced by the two agonists, but was 10–30 times more potent at inhibiting this response than the albumin response to BRL 37344 and salbutamol.

4 The greater potency (4–5 orders of magnitude) of BRL 37344, compared to the β_1- (prenalterol) and β_2 - (salbutamol) adrenoceptor selective agonists, in stimulating methacholine-induced albumin transport suggests that tracheal epithelium possess an atypical, or β_3-adrenoceptor similar to that previously reported for adipocytes and gastrointestinal smooth muscle. The weak antagonism of the response to BRL 37344 by ICI 118551 would also be consistent with an atypical adrenoceptor mediating the albumin transport response. Inhibition of methacholine-induced serous cell lysozyme output would appear to be mediated predominantly by β_2-adrenoceptors.

5 In view of the possible beneficial protective effects of albumin in airway surface liquid, selective β_3-agonists like BRL 37344 might have potential value in the prevention and/or treatment of inflammatory airway disease.

Keywords: Trachea; β-adrenoceptors; salbutamol; BRL 37344; albumin; lysozyme; epithelium; submucosal glands

Introduction

Ahlquist (1948) first proposed that there was more than one adrenoceptor. His hypothesis was based on a study of the relative abilities of several adrenoceptor agonists to cause either contraction or relaxation of smooth muscle. Receptors on the smooth muscle were designated either α or β depending on whether catecholamines produced either excitatory or inhibitory responses, respectively. This initial classification was corroborated by the finding that certain antagonists can selectively block the effects of sympathomimetic agents at α- or β- adrenoceptors. β-Adrenoceptors were later subdivided into β_1 and β_2 on the basis of the differences in potency (10 to 50 fold) between adrenaline and noradrenaline in different tissues (Lands *et al.*, 1967), and many antagonists that discriminate between β_1 and β_2 -receptors have since been developed.

Although most functional studies can be reconciled with the involvement of β_1 or β_2-adrenoceptors, or a mixture of the two, there have been an increasing number of reports incompatible with such a simple division (see Zaagsma & Nahorski, 1990). Some of the strongest evidence for a third, or atypical β-adrenoceptor came from studies with a series of novel agonists (e.g. BRL 26830, BRL 35135) that had potent and selective thermogenic anti-obesity activities in animal models. The active metabolites of these compounds (e.g. BRL 37344) were found to be 20 to 400 times more potent on brown adipose tissue (BAT) thermogenesis than on clas-

sical β_1- (atrial rate) and β_2- (tracheal relaxation) functions (Arch *et al.*, 1984). These potency ratios, and the low pA_2 values of conventional selective and non-selective β-adrenoceptor antagonists (Arch *et al.*, 1984; Stock & Sudera, 1989; Zaagsma & Hollenga, 1991), suggest that BAT possesses an atypical adrenoceptor. A human gene has now been identified that encodes for a third (i.e. β_3) adrenoceptor (Emorine *et al.*, 1989), and cells transfected with this receptor are 10 times more sensitive to noradrenaline than adrenaline, resistant to blockade by conventional β-antagonists and highly sensitive to the novel, thermogenic β-agonists. Thus, the atypical adrenoceptor on BAT is now thought to be the same, or similar to the human β_3-adrenoceptor, and thermogenic BAT-selective agonists, such as BRL 37344, are commonly refered to as β_3-agonists. Apart from brown (and white) adipocytes, there is evidence also for atypical, or β_3-adrenoceptors in the gastrointestinal tract that mediate relaxation of guinea-pig gastric fundus (Coleman *et al.*, 1987), inhibition of longitudinal muscle tension in guinea-pig ileum (Bond & Clarke, 1988) and relaxation of rat distal colon (McLaughlin & McDonald, 1989).

In the airways, activation of β_2-adrenoceptors leads to relaxation of tracheal and bronchial smooth muscle and β-agonists are still the standard treatment used for obstructive airways diseases such as asthma. However, asthma is now characterized as a disease of chronic inflammation, involving

mucosal tissues such as submucosal glands and epithelium, as well as airway smooth muscle. β-Adrenoceptors have been shown to be present on the epithelium and glands (Barnes *et al.*, 1982), and β₂-agonists such as salbutamol are weak stimulants of gland secretion (Webber & Widdicombe, 1989) and ion transport across the epithelium (Feldman *et al.*, 1990). However, relatively little is known of the effects of β-agonists on airway mucosal tissues and the receptors, or receptor subtypes mediating these effects.

In the present study, the ferret whole trachea *in vitro* preparation (Webber & Widdicombe, 1987) was used to examine the effects of agonists selective for each of the β-receptor subtypes on submucosal gland secretion (lysozyme secretion from submucosal gland serous cells) and the active transport of albumin across the tracheal epithelium (Webber & Widdicombe, 1989). Since these agents are only weak secretagogues in their own right, baseline mucus secretion was promoted with the muscarinic agonist, methacholine. The effects of a selective β₂-antagonist on the responses to the agonists was also studied.

Methods

The ferret trachea in vitro

Ferrets of either sex, weighing 0.5–1.5 kg, were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (50 mg kg⁻¹, Sagatal, May & Baker). The trachea was exposed and cannulated about 5 mm below the larynx with a perspex cannula containing a conical collecting well (Webber & Widdicombe, 1987). The ferret was then killed with an overdose of sodium pentobarbitone injected into the heart. The chest was opened along the midline and the trachea exposed to the carina, cleared of adjacent tissue, removed and cannulated just above the carina. The trachea was mounted, laryngeal end down, in a jacketed organ bath with Krebs-Henseleit buffer restricted to the submucosal side. The composition of the Krebs-Henseleit solution was (mM): NaCl 120.8, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 7H₂O 1.2, NaHCO₃ 24.9, CaCl₂ 2.4, glucose 5.6. The buffer was maintained at 37°C and gassed with 95% O₂/5% CO₂. The lumen of the trachea remained air-filled. Before the start of an experiment each trachea was allowed to equilibrate for 20 min, and during this time changes of bathing medium were made every 5 min. Secretions were carried by gravity and mucociliary transport to the lower cannula, where they pooled and could be withdrawn periodically into a polyethylene catheter which was inserted into the lower cannula to form an airtight seal. The catheters containing the secretions were sealed at both ends with bone wax, numbered and stored frozen until required.

After defrosting, the secretions were washed out of the catheters into labelled plastic vials with 0.5 ml distilled H₂O. The vials were frozen and stored for use in the albumin and lysozyme assays. Preliminary experiments had shown that frozen storage for up to 6 months does not affect the enzymatic activity of lysozyme or the albumin content of the samples. Secretion volumes were estimated by the differences in weights of the catheters with secretions and dried without secretions, and the secretion rates were expressed as μl min⁻¹ (assuming 1 g of secretion was equivalent to 1 ml).

Assay for lysozyme

The lysozyme concentrations of the mucus samples were measured by a turbidimetric assay which relies on the ability of lysozyme to break down the cell wall of the bacterium *Micrococcus lysodeikticus*. Addition of lysozyme to a solution of the bacteria reduces the turbidity of the solution, thereby leading to a fall in optical density (OD) measured at 450 nm.

A stock suspension of *M. lysodeikticus* of 3 mg ml⁻¹ was prepared. When diluted 10 fold (the dilution in the assay)

this suspension gives an OD of approximately 0.6 at 450 nm. To produce a standard curve, various concentrations of hen egg white lysozyme (0.5 to 100 ng ml⁻¹) were incubated in duplicate in 1.5 ml potassium phosphate buffer (50 mM, pH 7.4) containing *M. lysodeikticus* (0.3 mg ml⁻¹), sodium azide (1 mg ml⁻¹) and bovine serum albumin (BSA, 1 mg ml⁻¹). The BSA was included in the assay for its protein stabilizing effects and the sodium azide was added to prevent the growth of bacteria in the incubating solutions. The reaction mixtures were incubated for 18 h at 37°C. After incubation, the OD of each solution was measured at a wavelength of 450 nm with potassium phosphate buffer (pH 7.4) containing BSA (1 mg ml⁻¹) as a blank. The standard curve was constructed by plotting the fall in OD (reduction in turbidity) against the concentration of lysozyme in the solution.

To estimate the concentration of lysozyme in a mucus sample, 20 μl of sample were incubated in 1.5 ml potassium phosphate buffer (50 mM, pH 7.4), exactly as described above for the known concentrations of lysozyme used in the preparation of the standard curve. The lysozyme concentrations (equivalent to hen egg white lysozyme) of the 20 μl samples and hence of the original mucus samples were estimated from the standard curve. The rate of output of lysozyme was then calculated by dividing the total amount of lysozyme in a mucus sample by the time over which the sample accumulated.

Albumin transport

To examine the effect of β-receptor stimulation on the transport of albumin across the ferret trachea, BSA was added to the buffer bathing the submucosal surface of the trachea in a concentration of 4 mg ml⁻¹. Fluorescent BSA (0.02–0.03 mg ml⁻¹) was also added to the buffer as a marker and enabled an estimate to be made of the total amount of albumin which appeared in the mucus samples.

The fluorescence of the mucus samples was measured with a fluorimeter, using an excitation wavelength at 550 nm and an emission wavelength of 490 nm. The fluorescent albumin concentration of the mucus samples was estimated from a standard curve relating fluorescence (arbitrary units) to the concentration of fluorescent BSA (range 25 ng ml⁻¹ to 3 μg ml⁻¹). The total concentration of albumin in the mucus samples was obtained by multiplying the fluorescent albumin concentration (estimated from the standard curve) by the ratio of non-fluorescent to fluorescent albumin used in the experiment. The rate of output of albumin was determined by dividing the total amount of albumin in a mucus sample by the time over which that sample accumulated.

Experimental protocol

Effect of β-agonists on responses to methacholine Previous studies have shown that methacholine produces concentration-dependent increases in lysozyme and albumin outputs from the ferret trachea (Webber & Widdicombe, 1987; 1989). After a 30 min control period, methacholine (20 μM) was added to the buffer bathing the trachea. This concentration of methacholine produces 70–80% of the respective maximum responses for lysozyme and albumin outputs. Mucus samples were taken every 30 min until a steady 'maintained' mucus volume output was obtained (typically 2.5–3 h). After each 30 min period the buffer surrounding the trachea was replaced with fresh buffer containing methacholine.

In the first series of experiments, and after a maintained mucus volume output to methacholine had been established, five concentrations of a β-agonist were added in ascending order to the methacholine-containing buffer surrounding the trachea. The concentrations of agonist used in these experiments covered a wide concentration range (at least four log units). Each concentration of β-agonist was left in contact with the trachea for 30 min. After 30 min, the secretion produced was withdrawn and processed. The buffer surrounding the trachea was then replaced with fresh buffer

containing methacholine and the next concentration of β-agonist.

In order to compare the relative potencies of salbutamol and BRL 37344 more accurately, a second series of experiments was carried out. In these experiments, the same procedure was used as described above, but with a much narrower concentration range of the β-agonists (one to two log units). The range used for each agonist was chosen to cover the linear segment of the concentration-response that would produce a 100% increase in albumin transport ($EC_{100\%}$), or a 50% inhibition of lysozyme secretion ($IC_{50\%}$); the $EC_{100\%}$ and $IC_{50\%}$ values obtained were used in the next series of experiments.

Effect of ICI 118551 on responses to salbutamol and BRL 37344 As in the previous experiments, a steady mucus volume output was obtained with methacholine, and then the concentration of salbutamol or BRL 37344 producing a 100% increase in methacholine-induced albumin output ($ED_{100\%}$, determined in the previous experiment) was added with methacholine in the buffer. This concentration of salbutamol or BRL 37344 was present in the buffer with the methacholine for the remainder of the experiment. Four concentrations of the β_2-antagonist, ICI 118551 (0.1–100 μM), were then added in ascending order to the buffer surrounding the trachea. Each concentration of ICI 118551 was left in contact with the trachea for 30 min, after which time the secretion was withdrawn and processed. In another series of experiments, exactly the same procedure was followed using concentrations of salbutamol and BRL 37344 that produced a 50% inhibition of methacholine-induced lysozyme output ($IC_{50\%}$). Finally, an attempt was made to estimate an approximate pA_2 value for ICI 118551 by determining the concentration-response for albumin secretion using BRL 37344 and salbutamol, with and without ICI 118551 (10 μM) in the incubation buffer.

Analysis of results

The change in methacholine-induced lysozyme or albumin output produced by a β-agonist was calculated as the difference in output obtained between the period immediately before the β-agonist was added and the period when the agonist was in the organ bath, expressed as a percentage. The $EC_{100\%}$ and $IC_{50\%}$ values for salbutamol and BRL 37344, and the $IC_{50\%}$ values for ICI 118551 (concentration producing a 50% inhibition of response to salbutamol or BRL 37344) were obtained by linear regression analysis of the data. MacKay's method (1978) was used to calculate the pA_2 for ICI 118551 from the $ED_{100\%}$ concentration-ratio of both salbutamol and BRL 37344.

Drugs

The following drugs were used sources are given in parenthesis: Salbutamol (Glaxo); prenalterol methacholine (Hässle); ICI 118551 (ICI); BRL 37344 (SKB).

Results

Baseline values

The mean baseline outputs of lysozyme and albumin in all experiments ($n = 48$) before the addition of any drugs were 29 ± 15 ng min^{-1} and 0.15 ± 0.09 μg min^{-1} respectively.

Effects of methacholine

In the 30 min period immediately after the addition of methacholine, the lysozyme and albumin outputs increased from baseline levels to 358 ± 33 ng ml^{-1} ($n = 48$) and 2.12 ± 0.16 μg min^{-1} ($n = 48$), respectively. On continued applica-

tion of methacholine the lysozyme and albumin outputs fell, but reached a steady maintained level after 2.5–3 h. The mean level of the maintained methacholine-induced lysozyme and albumin output immediately before the addition of any further drugs was 168 ± 17 ng min^{-1} and 0.64 ± 0.07 μg min^{-1}.

Concentration-response effects of β-adrenoceptor agonists

Prenalterol (10 μM–1 mM), salbutamol (0.1 μM–1 mM) and BRL 37344 (0.1 nM–10 μM) produced concentration-dependent increases in methacholine-induced albumin output (Figure 1a). Judging from the concentration required to produce a 100% increase in albumin output, BRL 37344 was approximately five orders of magnitude more potent than salbutamol, which was only slightly more potent than prenalterol. All three agonists also produced concentration-dependent reductions in methacholine-induced lysozyme output. However, the potency order was changed, with salbutamol being approximately 60 fold more potent than BRL 37344, which was 100 fold more potent than prenalterol.

Based on these initial results, more precise $EC_{100\%}$ values for salbutamol and BRL 37344-induced enhancement of methacholine-stimulated albumin output were determined with a narrower range of concentrations (salbutamol 0.1–1 mM; BRL 37344 0.3–10 nM). These produced linear responses (Figure 2), and estimated $EC_{100\%}$ values of 1.4 nM and 0.74 mM for BRL 37344 and salbutamol, respectively. These $EC_{100\%}$ concentrations of BRL 37344 and salbutamol increased the concentration of albumin in the mucus from ($n = 6$) 1.8 ± 0.4 to 9.2 ± 1.3 μg μl^{-1}, and from 2.1 ± 0.3 to 8.4 ± 1.1 μg μl^{-1}, respectively (cf. 4 μg μl^{-1} in submucosal buffer).
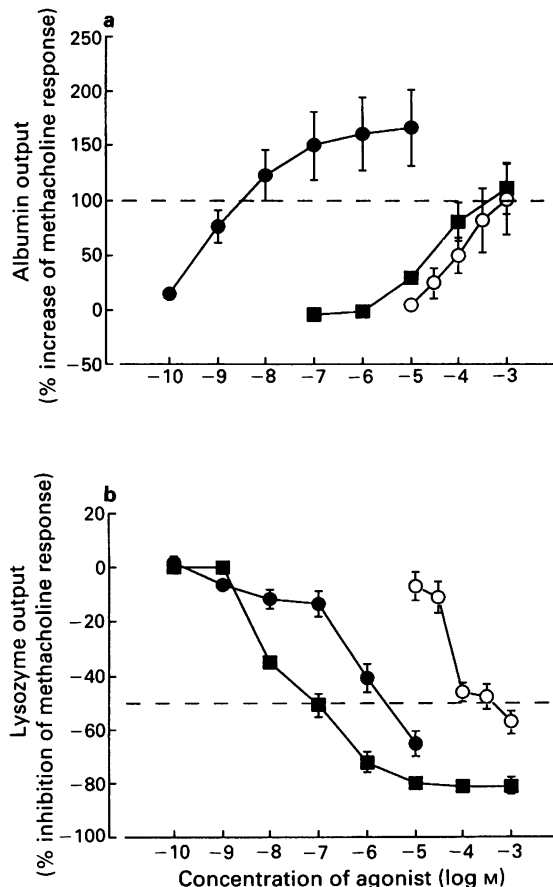

Figure 1 Concentration-response curves showing the effect of BRL 37344 (●), salbutamol (■) and prenalterol (○) on maintained methacholine-induced albumin (a) and lysozyme (b) outputs. Points are the means of 6–8 determinations, vertical bars = s.e.mean.
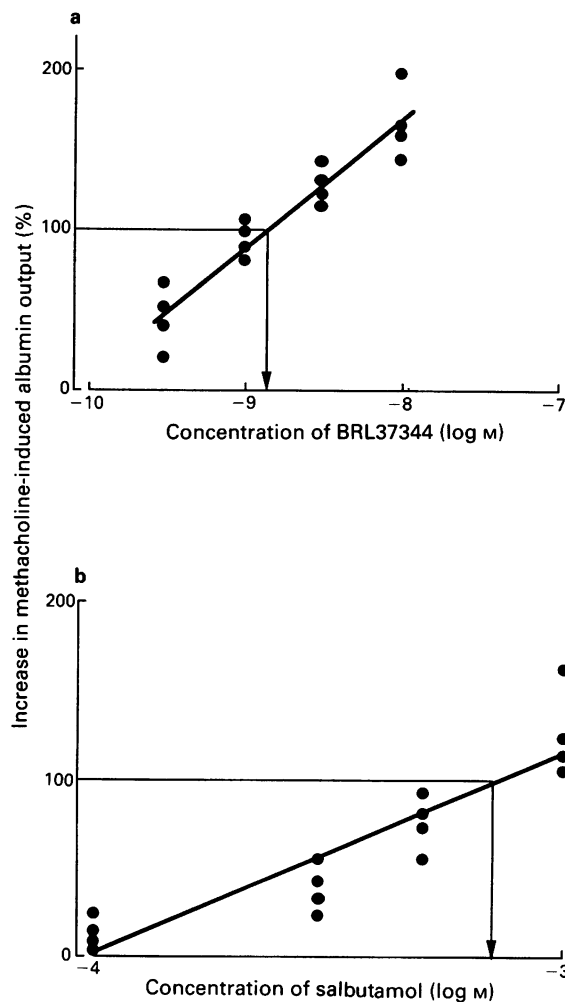
Figure 2   Linear regression analysis of the increase in methacholine-induced albumin output by (a) BRL 37344 and (b) salbutamol. Individual data points are shown. The arrows indicate the concentrations of BRL 37344 and salbutamol which increase albumin output by 100% ($EC_{100\%}$).
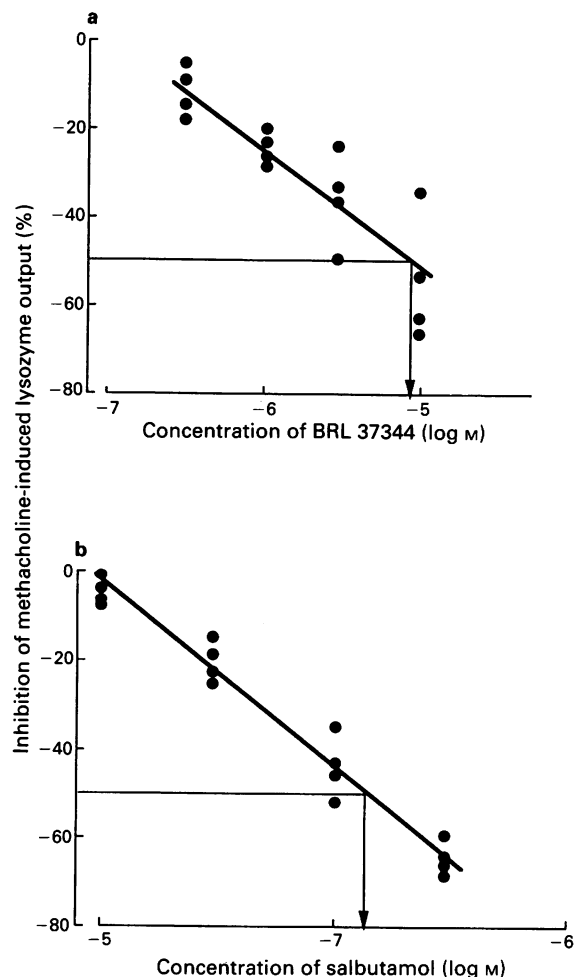

Figure 3   Linear regression analysis of the inhibition of methacholine-induced lysozyme output produced by (a) BRL 37344 and (b) salbutamol. Individual data points are shown. The arrows indicate the concentrations of BRL 37344 and salbutamol which inhibit lysozyme output by 50% ($IC_{50\%}$).

A different range of concentrations (salbutamol 10 nM–1 μM; BRL 37344 0.3–10 μM) was used to produce the results for inhibition of lysozyme secretion shown in Figure 3. The estimated $IC_{50\%}$ values were 0.14 μM and 8.14 μM for salbutamol and BRL 37344, respectively.

Effects of ICI 118551 on responses to salbutamol and BRL 37344

ICI 118551 produced a concentration-dependent inhibition of albumin output in response to $EC_{100\%}$ concentrations of BRL 37344 and salbutamol (Figure 4). However, the concentration of ICI 118551 required to inhibit the response to BRL 37344 by 50% ($IC_{50\%}$) was 10 times greater (4.3 μM) than that (0.43 μM) required to inhibit the salbutamol response. ICI 118551 also produced a concentration-dependent attenuation of the inhibitory effects of $IC_{50\%}$ concentrations of BRL 37344 and salbutamol on lysozyme secretion (Figure 5). In this case, ICI 118551 was 30 times more potent at inhibiting the lysozyme response to salbutamol than to BRL 37344; $IC_{50\%}$ for the antagonist was 25 nM and 0.8 μM for salbutamol and BRL 37344, respectively.

Having obtained these results with different concentrations of ICI 118551, a single concentration (10 μM) of the antagonist was selected and the $EC_{100\%}$ concentration-ratio for stimulation of albumin secretion by salbutamol and BRL 37344 determined. These data were used to calculate a pA_2

for ICI 118551 and produced a mean value of 5.55 ± 0.07 ($n = 4$) when BRL 37344 was the agonist, but a significantly ($P < 0.001$) higher value (7.18 ± 0.06; $n = 4$) when salbutamol was the agonist.

Discussion

In the present study, agonists presumed from previous work to be relatively selective for each of the three β-adrenoceptor subtypes all produced a concentration-dependent inhibition of methacholine-induced lysozyme output. Lysozyme is a specific marker for submucosal gland serous cell secretion (Bowes & Corrin, 1977), and these results therefore suggest that β-receptor activation (of whatever subtype) results in an inhibition of serous cell secretion produced by muscarinic receptor stimulation. These results are in direct contact to the effects of β-agonists on baseline secretion. Thus, the β_2-agonist, salbutamol, is a weak stimulant of baseline mucus volume output and lysozyme output from the ferret trachea (Webber & Widdicombe, 1989), dobutamine (β_1-agonist) and salbutamol both increase the output of radiolabelled sulphated glycoprotein from cat trachea (Peatfield & Richardson, 1982), and the β_2-agonist, terbutaline, increases the output of radiolabelled macromolecules from ferret trachea (Borson et al., 1984). It is not clear how activation of β-adrenoceptors can increase baseline submucosal gland secre-
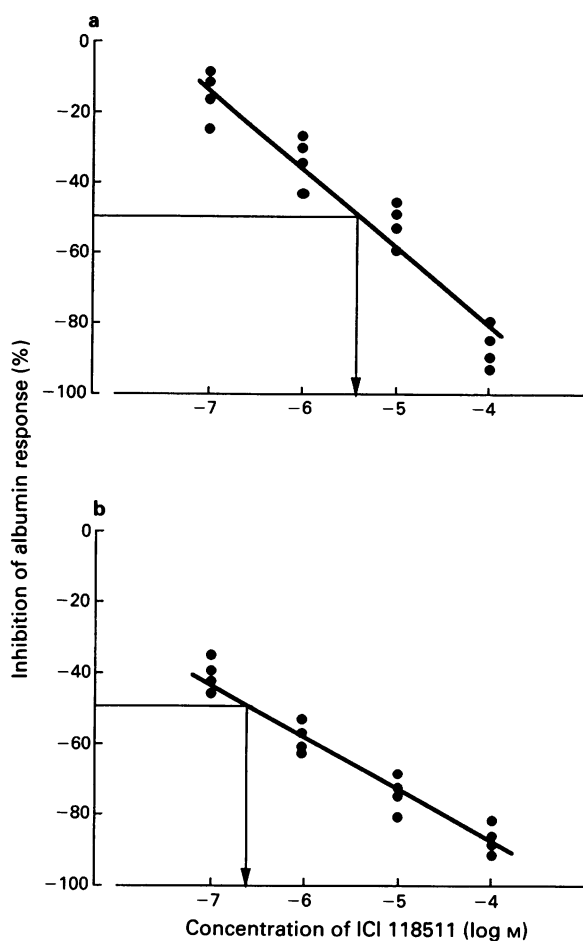
Figure 4 The effect of ICI 118511 on the enhancement of methacholine-induced albumin output produced by $EC_{100\%}$ concentrations of (a) BRL 37344 (1.4 nM) and (b) salbutamol (0.7 mM). Individual data points are shown. The arrows indicate the concentration ($IC_{50\%}$) of ICI 118551 inhibiting the response to BRL 37344 or salbutamol by 50%.
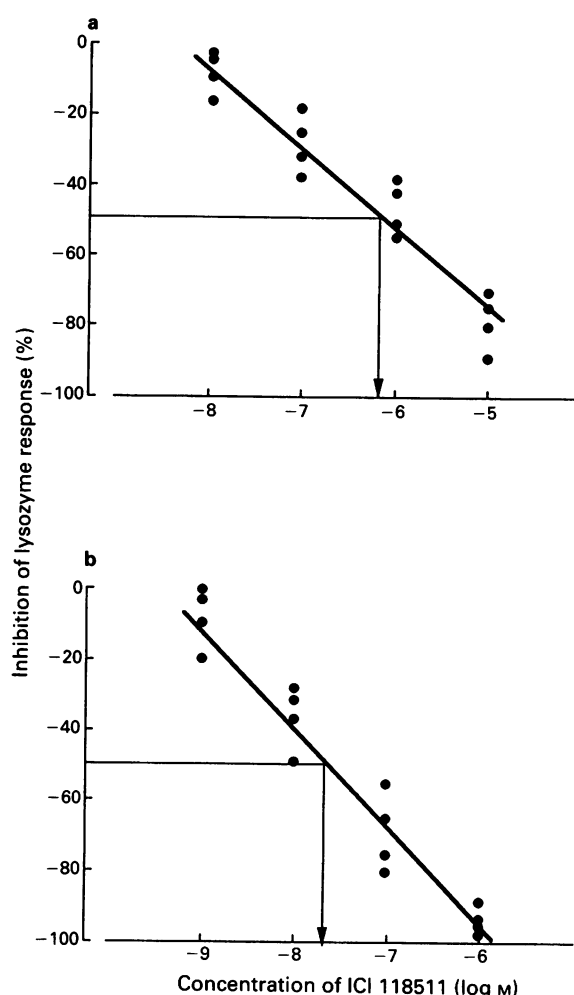

Figure 5 The effect of ICI 118551 on the inhibition of methacholine-induced lysozyme output produced by (a) BRL 37344 and (b) salbutamol. Individual data points are shown. The arrows indicate the concentration ($IC_{50\%}$) of ICI 118551 inhibiting the response to BRL 37344 or salbutamol by 50%.

tion, while also being capable of inhibiting the secretion resulting from muscarinic stimulation. The most likely explanation is that an intracellular messenger of the β-adrenoceptor response (possibly adenosine 3':5'-cyclic monophosphate, cyclic AMP) antagonizes the intracellular regulation of muscarinic-induced secretion (possibly involving IP_3 and release of Ca^{2+} from intracellular stores). In other tissues, such as airway smooth muscle, it is known that β-adrenoceptor-induced release of cyclic AMP can inhibit phosphoinositide hydrolysis, and thus the release of intracellular Ca^{2+} by IP_3 (Offer et al., 1990).

Salbutamol was 50 times more potent than BRL 37344 and 10,000 times more potent than prenalterol at inhibiting methacholine-induced lysozyme output. The high potency of salbutamol in the present study strongly suggests that this agonist is inhibiting submucosal gland serous cell secretion by activation of β2-receptors. The selective β2-adrenoceptor antagonist, ICI 118551, produced concentration-dependent inhibitions of the lysozyme response to BRL 37344 and salbutamol. However, ICI 118551 was 30 times more potent at inhibiting the lysozyme response to salbutamol. A 50% inhibition of the salbutamol response was achieved at a concentration of 25 nM providing further evidence that salbutamol inhibits muscarinic-induced lysozyme output by activation of β2-receptors. The low potency of BRL 37344 and the extremely low potency of prenalterol on lysozyme secretion was probably due to residual non-selective activity on β2-receptors, although effects on β3- and β1-adrenoceptors, respectively, cannot be completely ruled out.

All three selective β-agonists produced concentration-dependent increases in albumin output above that sustained by methacholine, suggesting enhancement of muscarinic-receptor-stimulated active transport of albumin across the tracheal epithelium into the lumen. Salbutamol alone (i.e. without methacholine) is also known to stimulate the baseline transport of albumin across the ferret and rabbit tracheal epithelium (Webber & Widdicombe, 1989; Price et al., 1990), but this may be because it also increases mucus production, whereas BRL 37344 and prenalterol alone have no effect on mucus volume. Thus, it is not possible to determine whether the latter two agonists stimulate albumin production by themselves, since it is not possible to collect sufficient mucus for albumin analysis. However, BRL 37344 was approximately 10,000 times more potent than salbutamol, and 100,000 times more potent than prenalterol at enhancing methacholine-induced albumin output.

It is now generally accepted that activation of BAT by BRL 37344 and other thermogenic β-agonists occurs via the atypical, or β3-adrenoceptor (Zaagsma & Hollenga, 1991), where BRL 37344 is 3 orders of magnitude more potent than salbutamol (Arch et al., 1984). The fact that BRL 37344 was 4 orders of magnitude more potent than salbutamol in activating albumin transport strongly suggests the existence of the same, or simiar β3-adrenoceptors in tracheal epithelium. Further support for the presence of an atypical adrenoceptor comes from the observation that higher concentrations of ICI

118551 were required to inhibit the effects of BRL 37344 on albumin transport than those required to inhibit the salbutamol responses.

It was not feasible (practically or ethically) to determine pA_2 values for ICI 118551 in this preparation by Schild analysis, and the pA_2 values determined by MacKay's method have to be considered approximate. However, the difference obtained when using the two agonists (over 1.5 log units) lends further support to the notion that BRL 37344 stimulates albumin secretion via a different adrenoceptor from that stimulated by salbutamol. The combination of BRL 37344 plus ICI 118551 gave a pA_2 (5.55) that was almost identical (5.4) to that determined for brown adipocyte thermogenesis (Stock & Sudera, 1989). These values have to be compared with a pA_2 of 8.7 obtained for tracheal relaxation with the same combination of agonist and antagonist (Arch et al., 1984), which suggests that the adrenoceptor subtype involved in albumin secretion is more akin to the adipocyte β_3-adrenoceptor, than the tracheal smooth muscle β_2-adrenoceptor. The observation that 17 fold higher concentrations of ICI 118551 were required to produce 50% inhibition of the albumin response than the lysozyme reponse to salbutamol might suggest that activation of albumin transport by salbutamol may also involve an atypical receptor. The somewhat low pA_2 value (7.18) for ICI 118551 inhibition of the effects of salbutamol on albumin would be consistent with this.

Previous work (Webber & Widdicombe, 1989; Price et al.,

1990) has established that the appearance of albumin in the tracheal lumen is not due to passive transudation, but involves active transport across the epithelial cell layer. This is supported in the present study by the fact that the $EC_{100\%}$ concentrations of BRL 37344 and salbutamol both increased the concentration of albumin in the secreted mucus to a value up to twice that in the submucosal buffer. These results suggest strongly the presence of an active transport process. Mucus albumin probably has several protective functions (antioxidant, binding inflammatory mediators), apart from contributing to the general gel-forming and rheological properties of mucus (Webber & Widdicombe, 1989). Likewise, the serous cell secretion of lysozyme contributes to its bactericidal properties. It would appear that cholinergic stimulation of these two protective functions are independently modulated by adrenergic influences, with β_2-adrenoceptor activation causing an inhibition of lysozyme secretion and β_3-adrenoceptor activation causing a potentiation of albumin output. The therapeutic use of β_2-agonists could therefore, be considered potentially detrimental in terms of lysozyme secretion, whereas stimulation of albumin transport by a selective β_3-agonist, having little or no β_2-activity, might be of value in the prevention and/or treatment of inflammatory airway disease.

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