Effects of β_2 -adrenoceptor agonists on anti-IgE-induced contraction and smooth muscle reactivity in human airways

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1 The β_2 -adrenoceptor agonists, salbutamol, salmeterol and RP 58802 relaxed basal tone of human isolated bronchial smooth muscle. Salmeterol- and RP 58802-induced relaxations persisted for more than 4 h when the medium was constantly renewed after treatment.

2 Salbutamol, salmeterol and RP 58802 reversed histamine-induced contractions in human airways (pD₂ values: 6.15 ± 0.21 , 6.00 ± 0.19 and 6.56 ± 0.12 , respectively).

3 Anti-IgE-induced contractions were significantly inhibited immediately after pretreatment of preparations with β_2 -adrenoceptor agonists (10 μ M). However, when tissues were treated with β_2 -agonists and then washed for a period of 4 h, salmeterol was the only agonist which significantly inhibited the anti-IgE response.

4 Histamine response curves were shifted to the right immediately after pretreatment of tissues with the β_2 -adrenoceptor agonists (10 μ M; 20 min), but maximal contractions were not affected. After a 4 h washing period, the histamine curves were not significantly different from controls. Concentration-effect curves to acetylcholine (ACh) or leukotriene C₄ (LTC₄) were not significantly modified after β_2 -agonist pretreatment.

5 These results suggest that β_2 -adrenoceptor agonists may prevent anti-IgE-induced contraction by inhibition of mediator release rather than alterations of those mechanisms involved in airway smooth muscle contraction.

Keywords: β_2 -Adrenoceptor agonist; salbutamol; salmeterol; RP 58802; anti-IgE; human airways; relaxation; contraction

Introduction

In addition to their bronchodilator activity, β_2 -adrenoceptor agonists have been reported to inhibit mediator release from inflammatory cells. Previous studies have shown that the immunological release of histamine from human chopped lung (Assem & Schild, 1969; Orange *et al.*, 1971; Butchers *et al.*, 1979) or from isolated lung mast cells (Church & Hiroi, 1987) can be reduced by prior treatment with β_2 -agonists. Furthermore, the production of leukotrienes induced by an allergenic challenge of sensitized lung parenchymal tissue was prevented by treatment with β_2 -agonists (Butchers *et al.*, 1991). A number of studies have demonstrated that antigen- and anti-IgE-induced contractions are dependent on the release of several spasmogenic factors (Schild *et al.*, 1951; Adams & Lichtenstein, 1979; Dahlgren *et al.*, 1983). A considerable number of studies have been performed to evaluate the bronchodilator actions of β_2 -agonists on basal or induced tone (Cerrina *et al.*, 1986; De Jongste *et al.*, 1987; Whicker *et al.*, 1988); however, few experiments have been performed to assess the inhibitory actions of β_2 -agonists against spasmogenic agents. Furthermore, a limited number of reports have been published to evaluate the inhibitory action of β_2 -agonists against antigen- or anti-IgE-induced contraction of airways (Buckner *et al.*, 1979).

The aim of the present study was to determine whether human bronchial smooth muscle responses to spasmogens such as histamine, acetylcholine (ACh) and leukotriene C₄ (LTC₄) are altered by β_2 -agonist pretreatment. In addition, an evaluation of the inhibitory effects of the β_2 -adrenoceptor agonists, salbutamol, salmeterol (Ullman & Svedmyr, 1988) and RP 58802 (Underwood *et al.*, 1992) on the functional response of human bronchial tissues to an anti-IgE challenge was carried out.

Methods

Bronchial tissues

Human bronchial tissues were obtained from patients who had undergone surgery for lung carcinoma. No subject had a history of asthma and baseline lung function and IgE titres were not assessed in these patients prior to surgery. Subsequent to pulmonary lobe resection, macroscopically normal bronchial tissues were dissected free of surrounding parenchyma, cut as rings and placed in Tyrode solution at 4°C. The composition of the Tyrode solution was (mM): NaCl 139.2, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.49, NaHCO₃ 11.9, NaH₂PO₄ 0.4 and glucose 5.5; pH 7.4. Tissues were used within 18 h after dissection.

Experimental procedure

Bronchial preparations from 3 to 5 mm in internal diameter were set up in 10 ml organ baths under initial loads of 2–3 g. The tissues were maintained at 37°C in Tyrode solution gassed with 5% CO₂ in O₂. Changes in force were recorded by isometric force transducers (Narco F-60) and Linseis physiographs. Tissues were equilibrated (90 min), during which period Tyrode solution was replaced every 10 min. Preparations were then contracted with histamine (50 μ M) and washed until basal tone was re-established.

Salbutamol, salmeterol and RP 58802 cumulative concentration-effect curves were constructed on precontracted tissues (histamine, 50 μ M) by adding incremental concentrations of relaxant agonist every 15 min until no further relaxation was achieved. In another series of experiments, the contracted preparations (histamine, 50 μ M) were relaxed with a single dose of agonist (10 μ M).

After addition of the β_2 -agonists (10 μ M; 20 min) to preparations at resting tone, contractions to either histamine, ACh, LTC₄ or anti-IgE were produced. In another series of

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experiments β_2 -agonists were added to the preparations at resting tone (20 min) and the tissues were washed for a period of 4 h before either anti-IgE or histamine curves were performed.

Drugs

The following drugs were used: histamine dihydrochloride and acetylcholine chloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), leukotriene C_4 (Bayer U.K. Ltd, Research Dept., Stoke Poges, England), ascorbic acid (Prolabo, France). Salbutamol, salmeterol, RP 58802 ($\alpha\{[(1H\text{-benzimidazolyl-1})\text{-3 methyl-1 propyl]amino-methyl}\}$ hydroxy-4 methoxy-3-benzene methanol dichlorhydrate, dihydrate) were synthesized at Rhône-Poulenc-Rorer and were kindly provided by Dr I. Cavero (Rhône-Poulenc-Rorer, Alfortville, France). The anti-human IgE (sheep anti-human IgE) serum; Nordic Immunological Laboratories, Tilburg, Netherlands) was directed against the Fc fraction of the IgE (i.e. the constant domains on the ϵ chain).

Histamine, ACh and salbutamol were dissolved in Tyrode's solution. Salmeterol, RP 58802 and LTC $_4$ were dissolved in dimethylsulphoxide (DMSO). Subsequent dilutions were made in Tyrode solution. The final dilution of DMSO was less than 1/10 000. This dilution did not affect either the resting tone or the maximal response to histamine. All β_2 -agonist solutions were prepared just prior to use and contained ascorbic acid (0.1 mM).

Data analysis

Histamine precontractions, effects of β_2 -adrenoceptor agonists on basal tone and anti-IgE responses are expressed in grams (g). Maximal relaxations ($E_{\max}$) to salbutamol, salmeterol and RP 58802 were expressed as a percentage reversal of the histamine response. ACh, histamine and LTC $_4$ concentration-effect curves are presented in figures as percentage of agonist maximal contraction. The $E_{\max}$ for these agonists are given in tables as percentage of initial histamine precontraction. Agonist potency was assessed by determination of the EC_{50} values, which were defined as the concentration of agonist which gave 50% of the maximal response. EC_{50} values were transformed to pD_2 values which are the negative logarithm of the EC_{50} values. Data are expressed as means $\pm$ s.e.mean of (n) experiments, where (n) represents the number of human lung samples. Statistical evaluation was performed using Student's t test for paired or unpaired variates where appropriate and a P value of less than 0.05 was considered to be significant.

Results

β_2 -Adrenoceptor agonist-induced relaxation

The β_2 -adrenoceptor agonists (10 μ M) all caused relaxation of basal tone (Figure 1). After a 4 h washing period, salbutamol-treated preparations returned to their control basal tone; in contrast, the baseline for salmeterol and RP 58802 at 4 h remained significantly lower than in control tissues (Figure 1). The relaxant effects of β_2 -agonists on human isolated bronchial muscle ring preparations contracted with histamine (50 μ M) are shown in Figure 2. Maximal responses obtained in the concentration-effect curves and potencies (pD_2 values) for these agonists are given in Table 1. Salmeterol produced a partial relaxation equivalent to approximately 50% reversal of histamine-induced tone. When the tissues were contracted with histamine (50 μ M) and then relaxed by the addition of a single concentration (10 μ M) of salbutamol, salmeterol or RP 58802, the relaxations obtained under these conditions were 75%, 49% or 80% reversal of the induced tone, respectively. The time necessary to attain these values was markedly different for

each agonist (salbutamol, 26.4 ± 10.5 min; salmeterol, 72.0 ± 13.2 min and RP 58802, 16.8 ± 5.0 min; $n = 3$).

Effects of β_2 -agonists on contractile responses

The immediate effects of β_2 -agonists (10 μ M; 20 min) on the anti-IgE-induced contraction and following a 4 h washing period are presented in Figure 3. β_2 -Agonist treatment significantly inhibited the anti-IgE-induced contraction. Salmeterol was the only relaxant agonist which exerted a prolonged inhibitory action on the anti-IgE-induced contraction.

The maximal contraction to histamine ($E_{\max}$) obtained from concentration-effect curves in controls was 5.49 ± 1.09 g and represented $127 \pm 10\%$ of the initial histamine (50 μ M) response. These values were not significantly different from the histamine response ($E_{\max}$) obtained in curves performed after the 4 h washing period ($123 \pm 12\%$ of histamine pre-contraction). Immediately after incubation with salbutamol, salmeterol or RP 58802, histamine curves were shifted to the

Figure 1 Human airway muscle tone in preparations incubated with either Tyrode solution (control; ●) or Tyrode solution containing 10 μ M salbutamol (○), salmeterol (▽) or RP58802 (□) followed by a 4 h washing period with Tyrode solution. The arrow indicates the beginning of the washing period (W). Values are means $\pm$ s.e.mean obtained from 18 lung samples. *Significantly different from data obtained in control tissues. ($P < 0.05$, Student's t test).

Figure 2 Relaxant effects of salbutamol (○), salmeterol (▽) and RP 58802 (□) in human airways contracted with histamine (50 μ M). Relaxant responses are expressed as percentage reversal of histamine-induced tone. Values are means $\pm$ s.e.mean obtained from 5 lung samples.

right without alteration in maximal response. However, after the washing period (4 h), the response and potency of histamine were not significantly different from data derived from control preparations (Figure 4, Table 2). Neither the $E_{\max}$ for ACh and LTC₄ nor the pD₂ values were modified after treatment with β_2 -agonists (Figure 5, Table 3).

Discussion

Salbutamol, salmeterol and RP 58802 relaxed basal tone and reversed histamine-induced contraction of human airways. Immediately following β_2 -adrenoceptor agonist pretreatments, anti-IgE-induced contractions were significantly blocked, an effect also observed with salmeterol at 4 h. After β_2 -agonist pretreatment, histamine curves were shifted to the right. This displacement was small and not observed after the

4 h washing period. In contrast, incubation with β_2 -agonists did not significantly alter ACh- and LTC₄-induced responses. These data suggest that while β_2 -agonists blocked IgE-mediated contractions, they did not modify human airway reactivity to several contractile agonists.

Human bronchial preparations *in vitro* relax when treated with antihistamines, leukotriene antagonists, TP-receptor antagonists or muscarinic antagonists (Norel *et al.*, 1991; Gorenne *et al.*, 1994; Ellis & Udem, 1994). These results suggest that resting tone in bronchial preparations, which is stable under control conditions during the experimental protocols, is maintained by the endogenous release of mediators. Therefore, the relaxation observed on basal tone with the β_2 -agonists may be the consequence of both a direct relaxant effect on smooth muscle and inhibition of spontaneous mediator release. Salbutamol and salmeterol were equipotent, while RP 58802 was slightly more potent in reversing

Table 1 β_2 -Adrenoceptor agonist relaxation on histamine-induced tone in human airways

	Histamine (g)	$E_{\max}$ (%)	pD ₂ values
Salbutamol	2.87 ± 0.32	81 ± 6	6.15 ± 0.21
Salmeterol	2.70 ± 0.72	49 ± 8	6.00 ± 0.19
RP 58802	2.14 ± 0.27	94 ± 7	6.56 ± 0.12

Bronchial preparations were contracted with histamine (50 μ M) and relaxed with cumulative concentrations of β_2 -adrenoceptor agonists. Histamine precontractions are expressed in g. Maximal relaxations to β_2 -adrenoceptor agonists ($E_{\max}$) are presented as percentage reversal of histamine contraction. Values are means $\pm$ s.e.mean obtained from 5 lung samples.

Figure 3 Inhibitory effects of β_2 -adrenoceptor agonists on the response to cumulative dilutions of anti-IgE in human airways. (a) Anti-IgE-induced contractions were obtained after incubation with Tyrode solution (control; solid columns) or Tyrode solution containing salbutamol (open columns), salmeterol (hatched columns) or RP 58802 (cross-hatched columns) (10 μ M; 20 min). (b) Anti-IgE-induced contractions were obtained after β_2 -agonists treatment followed by a 4 h washing period. Results are expressed in g. Values are means $\pm$ s.e.mean obtained from 4–6 lung samples. * P < 0.05.

Figure 4 Effects of β_2 -adrenoceptor agonists on histamine concentration-effect curves in human airways. (a) Curves were obtained after incubation (20 min) with Tyrode solution (●, control) or Tyrode solution containing 10 μ M salbutamol (○), salmeterol (▽) or RP 58802 (□). (b) Histamine curves obtained after the β_2 -agonists treatment followed by a 4 h washing period. Results are expressed as percentage of agonist maximal response. Values are means $\pm$ s.e.mean obtained from 3–4 lung samples.

Table 2 Effects of β_2 -adrenoceptor agonists on histamine concentration-effect curves in human airways

	Immediate		4 h	
	E_{max} (%)	pD_2 values	E_{max} (%)	pD_2 values
Control	127 $\pm$ 10	5.70 $\pm$ 0.11	123 $\pm$ 12	5.91 $\pm$ 0.14
Salbutamol	142 $\pm$ 29	5.16 $\pm$ 0.18*	107 $\pm$ 16	6.13 $\pm$ 0.12
Salmeterol	114 $\pm$ 15	5.20 $\pm$ 0.16*	115 $\pm$ 7	5.49 $\pm$ 0.12
RP 58802	104 $\pm$ 10	5.05 $\pm$ 0.20*	158 $\pm$ 4	6.08 $\pm$ 0.18

Bronchial preparations were initially precontracted with a single dose of histamine (50 μ M) and washed until basal tone was re-established. After preincubation with Tyrode solution or Tyrode solution containing the β_2 -adrenoceptor agonist (10 μ M; 20 min), histamine concentration-effect curves were performed either immediately after treatment (Immediate) or after treatment followed by a 4 h washing period. Maximal contractions (E_{max}) to histamine obtained from concentration-effect curves are expressed as percentage of initial histamine precontraction. Values are means $\pm$ s.e.mean obtained from 3–4 lung samples. *Significantly different from control ($P < 0.05$; Student's *t*-test).

Table 3 Effect of β_2 -adrenoceptor agonists on acetylcholine (ACh) or leukotriene C₄ (LTC₄) concentration-effect curves in human airways

	ACh response		LTC ₄ response	
	E_{max} (%)	pD_2 values	E_{max} (%)	pD_2 values
Control	134 $\pm$ 8	4.53 $\pm$ 0.25	139 $\pm$ 29	8.05 $\pm$ 0.10
Salbutamol	145 $\pm$ 10	4.07 $\pm$ 0.12	113 $\pm$ 20	7.49 $\pm$ 0.33
Salmeterol	124 $\pm$ 6	4.14 $\pm$ 0.13	150 $\pm$ 35	7.67 $\pm$ 0.27
RP 58802	154 $\pm$ 15	4.34 $\pm$ 0.32	110 $\pm$ 9	7.63 $\pm$ 0.18

Bronchial preparations were initially precontracted with histamine (50 μ M) and washed until basal tone was re-established. After incubation with Tyrode solution or Tyrode solution containing the β_2 -adrenoceptor agonist (10 μ M; 20 min), ACh or LTC₄ cumulative concentration-effect curves were produced. Maximal contractions (E_{max}) to ACh and LTC₄ obtained from concentration-effect curves are expressed as percentage of initial histamine precontraction. Values are means $\pm$ s.e.mean obtained from 5–8 lung samples.

histamine-induced tone. The pD_2 value for salmeterol was found to be different from that previously reported (Ball *et al.*, 1991; Nials *et al.*, 1993). An inverse relationship appears to exist between the degree of agonist-induced contraction and the ability of β_2 -agonists to elicit relaxation (Buckner & Saini, 1975; Torphy *et al.*, 1983; Van Amsterdam *et al.*, 1990). Hence, the variations in the pD_2 values observed with β_2 -adrenoceptor agonists may, in part, be due to the contraction of smooth muscle by different concentrations of contractile agents. Whether or not differences in potency of relaxant agonist may also be related to the methods used to establish the relaxations has not adequately been explored. A previous report (Dougall *et al.*, 1991) has shown that salmeterol is a partial agonist in guinea-pig airways, and is similar in potency to salbutamol (Lindén *et al.*, 1993). Data derived from human airways (present paper) confirm this observation, since salmeterol produced approximately 50% reversal of histamine contraction.

Previous reports have shown that anti-IgE-induced contraction in human airways produced a response which ranged between 59% to 83% of an acetylcholine (100 μ M; Björck & Dahlén, 1993) or methacholine (50 μ M; Jones *et al.*, 1989)-induced contraction. Gorenne and coworkers (1994) have reported that the response to anti-IgE was 46% to 68% of that to acetylcholine (100 μ M). In the present report the anti-IgE response was approximately 75% of that to histamine (50 μ M, 2.61 $\pm$ 0.48 g; immediate stimulation) and 81% of that to histamine (50 μ M, 2.32 $\pm$ 0.63 g; stimulation

Figure 5 Effects of β_2 -adrenoceptor agonists on concentration-effect curves to acetylcholine (ACh) (a) or leukotriene C₄ (LTC₄) (b) in human airways. All curves were obtained after incubation (20 min) with Tyrode solution (●, control) or Tyrode solution containing 10 μ M salbutamol (○), salmeterol (▽) or RP 58802 (□). Results are expressed as percentage of agonist maximal response. Values are means $\pm$ s.e. mean obtained from 5–8 lung samples.

at 4 h). These data suggest that the magnitude of the IgE-induced contraction is comparable with a contractile agonist response and is dependent upon inflammatory mediator release (Björck & Dahlén, 1993; Gorenne *et al.*, 1994).

However, few studies in airways have been performed where β_2 -agonists have been evaluated against immunological contractions. The present report shows a marked inhibition of anti-IgE-induced contractions in human airways. These results are consistent with previous reports where β_2 -mimetics inhibited the release of mediators during anaphylactic challenge in either isolated mast cells (Church & Hiroi, 1987), or human lung parenchymal tissues (Assem & Schild, 1969; Butchers *et al.*, 1979; 1991; Hughes *et al.*, 1983). The inhibition of mediator release was dependent on the β_2 subtype of adrenoceptors (Assem & Schild, 1969; Butchers *et al.*, 1980). Butchers *et al.* (1991) demonstrated *in vitro* that salmeterol

had a prolonged inhibitory effect on antigen-induced mediator release from human lung. However, measurements of mediator release during anti-IgE stimulation after treatment with β_2 -agonists in human bronchial preparations remain to be established. The anti-IgE-induced contractions in human airways were also blocked by salmeterol even after 4 h (present paper). Several *in vivo* studies (Ullman & Svedmyr, 1988; Twentyman *et al.*, 1990; Taylor *et al.*, 1992) demonstrated the prolonged inhibitory action of salmeterol on both the early and late phase bronchoconstriction observed in asthmatic patients after antigen inhalation.

Airway smooth muscle reactivity following β_2 -adrenoceptor agonists activity was examined by Hay and coworkers (1988) who reported that salbutamol treatment, in guinea-pig trachea, caused a small shift in carbachol dose-response curves without modifying the maximal response. In human airways, Advenier *et al.* (1991) demonstrated no inhibition of either the ACh or histamine contractions following treatment with salbutamol. The results presented in this paper show that histamine-induced contractions were significantly displaced to the right while ACh response curves were not altered.

Pretreatment of guinea-pig airway smooth muscle with high concentrations of salbutamol markedly inhibited the

contractions to LTC₄ and LTD₄ (Armour *et al.*, 1982), data which were confirmed by Hay and coworkers (1988). Thus the leukotrienes which are potent airway constrictors (Dahlén *et al.*, 1983) appear to be more susceptible to inhibition by β_2 -adrenoceptor agonists in guinea-pig airways. However, LTC₄-induced contractions in human isolated airways (present paper) were not modified even at β_2 -adrenoceptor agonist concentrations which were 10 fold higher than reported for guinea-pig tissues. The differences observed for LTD₄ inhibition induced by the β_2 -adrenoceptor agonists may be due to the species from which the airway muscle preparations are derived. The reason for this difference is not clear at present. While β_2 -adrenoceptor agonists are known to relax basal and induced tone of human airways (Whicker *et al.*, 1988; present paper), the reactivity to several contractile agonists appears not to be inhibited by the β_2 -agonists.

The data presented in the present paper using isolated human airways indirectly suggest that the mechanism by which β_2 -adrenoceptor agonists prevent IgE-mediated contraction may be due to inhibition of endogenous mediator release, as has been previously suggested (Butchers *et al.*, 1991), rather than to alterations of smooth muscle contraction.

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