Relaxant effects and durations of action of formoterol and salmeterol on the isolated human bronchus

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ABSTRACT: The objective of this study was to evaluate the potency and efficacy (intrinsic activity) of formoterol and salmeterol and their duration of action in comparison with other β-adrenoceptor agonists in isolated human bronchi.

Human bronchi were obtained at thoracotomy from patients with lung cancer. Potency (-log of the concentration of drug inducing 50% of maximal relaxation (-log EC_{50})) and efficacy (maximal effect (Emax), % of response to theophylline 3×10^{-3} $mol \cdot l^{-1}$) were determined by analysis of cumulative isometric concentration-response curves to $β_2$-adrenoceptor agonists in bronchial rings at resting tone or contracted maximally with acetylcholine 10^{-3} $mol \cdot l^{-1}$ to induce functional antagonism. The onset and duration of action of β-adrenoceptor agonists were measured by assessing the relaxant activity of drugs on the basal tone of isolated bronchi.

In terms of potency, the rank order of the substances studied was formoterol > fenoterol ≥ salmeterol ≥ isoprenaline ≥ salbutamol ≥ adrenaline ≥ terbutaline. Formoterol was 150–200 times more potent than isoprenaline. On preparations contracted with acetylcholine 10^{-3} $mol \cdot l^{-1}$ the intrinsic activity (IA) of salbutamol, terbutaline and salmeterol compared with that of isoprenaline ranged 0.62–0.66. Intrinsic activity was higher with formoterol (0.84) and fenoterol (0.75). The onset of action of formoterol (2.14±0.55 min, n=11) was not significantly different from that of salbutamol (1.90±0.24 min, n=8) but shorter than that of salmeterol (6.40±1.40 min, n=10). The duration of action of formoterol was approximatively 4.5 fold longer than that of salbutamol; but, under similar conditions, salmeterol was more than 24 fold longer acting than salbutamol.

Formoterol appears to be a very potent and long-acting β-adrenoceptor agonist, with fast onset of effect on the isolated human bronchus model. However, its duration of action, *in vitro*, was found to be shorter than that of salmeterol.
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$β_2$-adrenoceptor agonists constitute an established treatment for asthma. They are effective bronchodilators and protect against various constrictor challenges, such as exercise, methacholine, cold air and histamine. The major drawback of currently available inhaled drugs is their short duration of action, *i.e.* less than 6 h.

Formoterol and salmeterol are new, highly potent $β_2$-selective adrenoceptor agonists with a long duration of action in human subjects [1–8]. Extensive preclinical studies have been performed in animals, especially in the guinea-pig [9–11].

The purpose of this study was firstly to evaluate the potency and maximum relaxant effect of formoterol and salmeterol, on the isolated human bronchus, in comparison with those of salbutamol and four other β–adrenoceptor agonists. Studies were performed on preparation at basal tone or precontracted with acetylcholine (ACh) in order to investigate differences in functional antago-

nism [12]. We also studied the influence of epithelium on the concentration-response curve for formoterol and salmeterol. Finally, we evaluated the onset of action and duration of effect of these β-adrenoceptor agonists on the isolated human bronchus at basal tone.

Material and methods

Human bronchial preparations

Human bronchial tissue (usually with an inner diameter of 1–3 mm) was obtained from patients undergoing surgery for lung cancer (squamous cell carcinomas, oat cell carcinomas and adenocarcinomas) but taken at a distance from the malignancy. Bronchial strips were dissected free from parenchyma and transported to the

laboratory in Krebs' solution, previously aerated with a mixture of 95% O_2 and 5% CO_2 (pH=7.40). The tissue was stored overnight at 4°C, and the experiment was performed on the next day.

Previous experience in this laboratory and published data have demonstrated that overnight storage of tissue does not alter its reactivity. Rings from a segmental bronchus were prepared and suspended in Krebs' solution under an initial tension of 2.0 g. When required, the epithelium was removed by gently and repeatedly rubbing the luminal surface with a cotton-tipped applicator, as described previously [13, 14]. After 1.5 h of equilibration, with washing every 15 min, the resting tension was between 2 and 3 g. Under these conditions, responses to agonists were reproducible. Tensions were measured isometrically with strain gauges (UF1), amplifiers, and I.O.S. Moise 3 recorder system (EMKA-Technologies, Paris, France). The composition of the Krebs' solution was (mmol·l^{-1}): NaCl 118; KCl 5.4; $CaCl_2$ 2.5; KH_2PO_4 1.1; $MgSO_4$ 0.6; $NaHCO_3$ 25; and glucose 11.7. In all experiments, human bronchi were first contracted to maximal tension with acetylcholine 10^{-3} mol·l^{-1} and then relaxed with theophylline 3×10^{-3} mol·l^{-1}. Under these conditions, we were able to establish that after addition of theophylline there was, in relation to resting tension, a reserve of dilatation ranging from 0.40 to 3.20 g. The tissues were then allowed to rest for a period of 1–1.5 h before beginning the experimental procedures.

Experimental procedures

Concentration-response curves obtained with β-stimulants. Following the resting period, the bronchial rings, with or without epithelium, were either left at resting tone or contracted with ACh 10^{-3} mol·l^{-1}. After the contraction plateau was reached, concentration-response curves were established by cumulative additions of β–adrenoceptor agonists at intervals of 5–60 min to obtain a relaxation plateau. After the maximal effect of each β–adrenoceptor agonist was obtained, theophylline 3×10^{-3} mol·l^{-1} was added to the bath in order to determine the maximal relaxation. When the preparations were contracted by Ach 10^{-3} mol·l^{-1}, the spontaneous decrease of tension was about 8.8±3.3% for 3 h (n=12).

Only one concentration-response curve to any of the β-adrenoceptor agonists was recorded in each ring, the same compound being tested on parallel rings (with or without epithelium).

Influence of formoterol and salmeterol on concentration-response curves to adrenaline. Following the resting period, bronchial rings were incubated for 1 h with Krebs' solution (control), adrenaline (10^{-5} mol·l^{-1}) or one concentration of formoterol (10^{-9} or 10^{-8} mol·l^{-1}) or salmeterol (10^{-8}, 10^{-7} or 10^{-6} mol·l^{-1}). Thirty minutes after repeated washing of the preparations, ACh 10^{-5} mol·l^{-1}, giving 63±4% of maximal contraction (n=6), was added to the bath, and after an equilibrium plateau was reached concentration-response curves to adrenaline were obtained by cumulative addition. At the end of the experiment,

theophylline 3×10^{-3} mol·l^{-1} was added in order to determine the maximal relaxation. Only one concentration-response curve to adrenaline was recorded in each ring.

Duration of residual action of isoprenaline, salbutamol and formoterol. Following the resting period, β-adrenoceptor agonists at concentration giving approximatively 80% of maximal response on bronchus at basal tone were added to the bath. When maximal response was stabilized (isoprenaline 3–5 min, salmeterol 20 min, other drugs 15 min) the bronchi were washed and then allowed to return to basal tone. The time from addition to the bath to attainment of 50% of maximal relaxation by the compound ($t_{E/2}$onset) and the time to return, after washing, from maximal relaxation to 50% basal tone ($t_{E/2}$ recovery) were calculated. Responses were compared to theophylline used in similar conditions at the beginning of experiments and the compound/theophylline ratios were determined.

Expression and statistical analysis of the results. The maximal relaxant effects (Emax) of β-adrenoceptor agonists are expressed as a percentage of the action of theophylline 3×10^{-3} mol·l^{-1}. The concentrations of β–adrenoceptor agonist which induced a relaxation equal to 50% of that induced by theophylline 3×10^{-3} mol·l^{-1} (-log EC_{50}) was determined. PA_2 value is the negative logarithm of the molar concentration of antagonist in the presence of which the potency of the agonist is reduced by 2 times.

Statistical analysis of the results was performed using analysis of variance and Student's t-test for paired or unpaired data. All values in the text and table are expressed as mean±standard error of the mean. Values of p<0.05 were considered to be significant.

Drugs

The drugs used were: salmeterol (base salt), formoterol fumarate (Ciba-Geigy, Basle, Switzerland), terbutaline sulphate (Astra, France), fenoterol HBr (Boehringer Ingelheim, Reims, France), isoprenaline HCl, adrenaline bitartrate, salbutamol HCl (Sigma, St. Louis, USA), acetylcholine HCl (Pharmacie Centrale des Hôpitaux, Paris, France). Theophylline sodium anisate was used as proprietary injectable solution (Theophylline Bruneau, Paris, France).

Salmeterol was dissolved in distilled water in the presence of HCl 0.01 M and then diluted in Krebs' solution. All other drugs were dissolved daily in distilled water and diluted in Krebs' solution.

Results

Activity of the β–adrenoceptor agonists tested on the human isolated bronchus

The concentration-response curves to the relaxant effects of isoprenaline, salbutamol, formoterol and salmeterol on bronchi in resting tone or precontracted with ACh
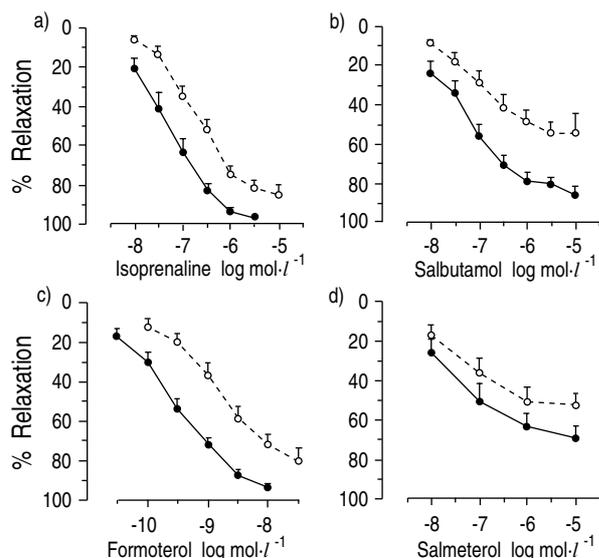
Fig. 1. – Concentration-response (relaxation) curves of: a) isoprenaline; b) salbutamol; c) formoterol; and d) salmeterol on isolated human bronchi, expressed as a % of maximal theophylline relaxation (3×10^{-3} mol·l^{-1}). Experiments were performed on preparations at resting tone (●) or precontracted with acetylcholine 10^{-3} mol·l^{-1} (○). Values are mean±SEM. Number of experiments is indicated in table 1.
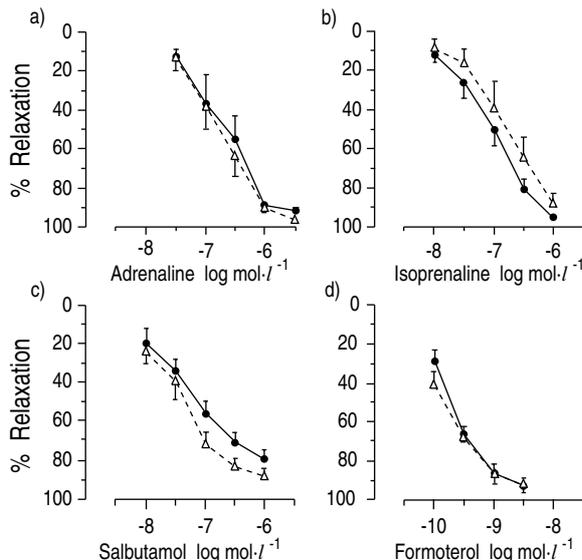

Fig. 2. – Influence of epithelium removal on the concentration-response (relaxation) curves of: a) adrenaline; b) isoprenaline; c) salbutamol; and d) formoterol on the isolated human bronchus at resting tone expressed as a % of maximal theophylline relaxation (3×10^{-3} mol·l^{-1}). ● : control (with epithelium). Δ : after epithelium removal. Values are mean±SEM. Number of experiments 6–8.

10^{-3} mol·l^{-1} are shown in figure 1. The potency and efficacy of these β-adrenoceptor agonists were calculated from these curves by determining the -log EC_{50} values and the maximal effects (Emax) (table 1).

Other concentration-response curves (not represented) were used to investigate the effects of adrenaline, terbutaline and fenoterol.

Table 1 shows that in bronchi at resting tone the rank order of potency of the β–adrenoceptor agonists was formoterol > fenoterol ≥ salmeterol ≥ isoprenaline ≥ salbutamol ≥ adrenaline > terbutaline. The ratios of activity in relation to isoprenaline were 208, 5.1, 1.4, 1.0 (isoprenaline), 0.64, 0.34 and 0.14, respectively. In terms of efficacy, only salbutamol and salmetetol were significantly less active than isoprenaline, thereby showing a partial agonistic effect.

Under maximal contraction induced by ACh 10^{-3} mol·l^{-1}, the concentration-response curves of the β-adrenoceptor agonists were displaced to the right (fig. 1). The -log EC_{50} of adrenaline, isoprenaline, formoterol and fenoterol

were reduced by 0.56, 0.75, 0.89 and 1.06 log units, respectively, thus showing losses of potency of 3.63, 5.62, 7.76 and 11.48, respectively. The rank order of potency of these β-adrenoceptor agonists in relation to isoprenaline remained the same as that observed in resting tone situation, *i.e.* formoterol > fenoterol > isoprenaline ≥ adrenaline, with ratios of activity in relation to isoprenaline of 151, 2.5, 1.0 and 0.54. The intrinsic activity of terbutaline, salbutamol and salmeterol was reduced to 0.62 to 0.66; those of fenoterol and formoterol were reduced less (to 0.75 and 0.84, respectively).

Figure 2 shows that in these experiments the effects of adrenaline, isoprenaline, salbutamol and formoterol were not significantly modified by removal of the epithelium.

Interaction between formoterol and salmeterol with adrenaline

Figure 3 shows that after a 1 h incubation of human bronchi with adrenaline 10^{-5} mol·l^{-1} followed by a 30 min washing

Table 1. – -log EC_{50} (potency) and Emax (efficacy) of some β-adrenoceptor agonists on the isolated human bronchus at resting tone or contracted with acetylcholine (ACh) 10^{-3} mol·l^{-1}

	Resting Tone				ACh 10^{-3} M			
	n	-log EC_{50}	Emax	IA	n	-log EC_{50}	Emax	IA
Isoprenaline	12	7.31±0.12	98±1.0	1.00	12	6.56±0.13++	85±3.7++	1.00
Adrenaline	11	6.85±0.12	95±1.1	0.97	11	6.29±0.10++	87±3.5+	1.02
Salbutamol	10	7.12±0.17	83±2.4***	0.85	11	-	55±6.5***+++	0.64
Terbutaline	12	6.48±0.10	94±2.2	0.96	12	-	56±8**+++	0.66
Fenoterol	12	8.02±0.16	97±2.1	0.99	12	6.96±0.14++	64±5**+++	0.75
Formoterol	12	9.63±0.11	94±1.2*	0.96	12	8.74±0.19++	71±6.9++	0.84
Salmeterol	10	7.60±0.19	70±6.0 ***	0.71	15	-	53±5.0***+	0.62

n: number of experiments; -log EC_{50}: -log of the concentration of drug which induces 50% of the relaxation induced by theophylline 3×10^{-3} mol·l^{-1}; Emax: maximal effect as percentage of maximal effect induced by theophylline 3×10^{-3} mol·l^{-1}; IA: intrinsic activity compared to isoprenaline: Emax (drug)/Emax (isoprenaline). Significantly different from isoprenaline: *: p<0.05; **: p<0.01; ***: p<0.001. Significantly different from resting tone: +: p<0.05; ++:p<0.01; +++: p<0.001.
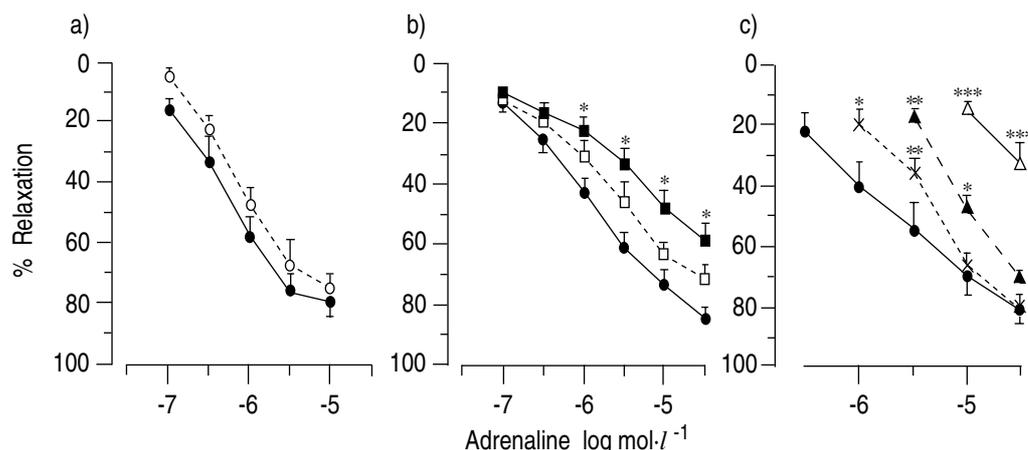
Fig. 3. – Influence of incubation of isolated human bronchi with: a) adrenaline; b) formoterol; and c) salmeterol on the concentration-response curves of adrenaline on the human isolated bronchus precontracted with acetylcholine 10^{-5} mol·l^{-1}. Concentration-response curves of adrenaline were performed before (control) or after 1 h incubation with adrenaline, formoterol or salmeterol and 0.5 h washing. Values are expressed as a % of the maximal theophylline relaxation (3×10^{-3}mol·l^{-1}) and given as mean±SEM. Number of experiments: 6–8. Significant differences from control are: *: p<0.05; **: p<0.01; ***: p<0.001. —●—: control; - -○- -: adrenaline 10^{-5} mol·l^{-1}; - -□- -: formoterol 10^{-9} mol·l^{-1}; —■—: formoterol 10^{-8} mol·l^{-1}; - -×- -: salmeterol 10^{-8} mol·l^{-1}; - -▲- -: salmeterol 10^{-7} mol·l^{-1}; —△—: salmeterol 10^{-6} mol·l^{-1}.

period the concentration-response curve of adrenaline was slightly (0.3 log unit) but not significantly displaced, suggesting that under these conditions desensitization and down-regulation were not marked. When human bronchi were incubated in the presence of formoterol under similar conditions, a significant (1 log unit) displacement of the concentration-response curves to adrenaline was observed with formoterol 10^{-8} mol·l^{-1} i.e. at a concentration giving a maximal effect (fig. 1). With salmeterol, the concentration-response curves were displaced by 0.6 log unit at a concentration of 10^{-8} mol·l^{-1} producing 17–26% of the relaxant effect (fig. 1). This displacement thereafter increased to reach 1.7 log unit with salmeterol 10^{-6} mol·l^{-1} (60% of the relaxant effect). A study of curve displacement using the method of ARUNLAKSHANA and SCHILD [15] demonstrated a noncompetitive antagonism (slope 0.60; apparent pA$_2$ 8.75).

Duration of action

Table 2 shows that isoprenaline and adrenaline acted most rapidly (t$_{E/2}$ onset of 0.65±0.19 min and 1.16±0.37 min, respectively, n=6 and 7). It also shows that the time

to onset of action was about the same with terbutaline, fenoterol and formoterol, whereas the time to onset of action of salmeterol was significantly longer than that of isoprenaline or salbutamol (6.40±1.40 min, n=10). Concerning the duration of action (measured by the time taken to return to the tone corresponding to half the maximal relaxation in relation to basal tone), it appears clear that the duration of action of salbutamol, terbutaline or fenoterol was only 1.7–2.2 times longer than that of isoprenaline. The duration of action of formoterol was approximately 4.5 times longer than that of salbutamol. Salmeterol was very long-acting, since in 5 of the 10 bronchi tested its effect remained maximal 2.5 h after washing out, and on the remaining five bronchi the duration of effect was 24 times longer than that of salbutamol.

Discussion

Potency and efficacy of β-adrenoceptor agonists

The results of this study on the human isolated bronchus show that formoterol is a very potent β-adrenoceptor

Table 2. – Onset and duration of action of some β-adrenoceptor agonists on the isolated human bronchus

	Onset of action			Offset of action		
	n	t$_{E/2}$ onset min	Compound/theophylline ratio	n	t$_{E/2}$ recovery min	Compound/theophylline ratio
Theophylline 10^{-4}M	11	2.91±0.47	1	11	4.78±0.48	1
Isoprenaline 3×10^{-7}M	6	0.65±0.19++	0.28±0.05++	6	4.22±0.63	0.82±0.09+
Adrenaline 10^{-7}M	7	1.16±0.37	0.47±0.08+	7	2.15±0.54*+	0.43±0.08**+++
Salbutamol 3×10^{-7}M	8	1.90±0.24**	0.91±0.16**	8	7.59±2.30	1.55±0.23*
Terbutaline 10^{-6}M	6	1.33±0.29	0.63±0.08**	6	9.44±1.90*	2.21±0.25***
Fenoterol 3×10^{-8}M	6	3.52±0.80**	1.10±0.18***	6	7.21±0.88*	1.36±0.16*
Formoterol 3×10^{-9}M	11	2.14±0.55*	0.73±0.08***	11	33.9±4.9***+++	7.76±1.11***+++
Salmeterol 3×10^{-7}M	10	6.40±1.40**++	2.67±0.39***+++	5	102.2±24.7**++	24.5±5.5***+++
				5	t$_{E/2}$>150	>31

Values are mean±SEM. t$_{E/2}$ onset: calculated as the time from administration of an agonist to attainment of 50% maximal relaxation; t$_{E/2}$ recovery: calculated as the time from washing the preparation to attainment of 50% recovery of basal tone; n: number of experiments. Significantly different from isoprenaline: *: p<0.05; **: p<0.01; ***: p<0.001. Significantly different from salbutamol: +: p<0.05; ++: p<0.01; +++: p<0.001.

agonist, since it was found to be 209, 107 and 325 times more potent than isoprenaline, salmeterol and salbutamol, respectively. These figures are distinctly higher than those reported in previous studies, where formoterol was said to be about 10 times more potent than isoprenaline and 100 times more potent than salbutamol on the guinea-pig isolated trachea [10, 11, 16, 17]. Our results also show that in the presence of ACh 10^{-3} mol·l^{-1} the effects of formoterol, like those of other β-stimulants, are reduced both in potency and efficacy (or intrinsic activity), due to a functional antagonism [10, 12, 18, 19]. In terms of intrinsic activity (IA) the β-stimulants that we studied seemed to be full agonists on preparations at resting tone, whereas in the presence of acetylcholine in high concentrations their intrinsic activity was reduced to varying degrees: considerably for salbutamol, terbutaline and salmeterol (IA between 0.62–0.66), much less for formoterol (IA 0.84), and intermediately for fenoterol (IA 0.75), in relation to isoprenaline. This suggests that terbutaline, salbutamol and salmeterol are weaker β-adrenoceptor agonists than formoterol and fenoterol, in agreement with the results of LEMOINE and OVERLACK [10] and LINDÉN et al. [11] on the guinea-pig isolated trachea.

The mechanism of functional antagonism between acetylcholine and β-stimulants has recently been studied on human bronchi by VAN AMSTERDAM et al. [20]. These authors demonstrated a significant correlation between phosphoinositide (PI) production induced by methacholine and histamine and the reduction of isoprenaline -log EC_{50} and Emax values, and they proposed two hypotheses to account for the relationship between the levels of PI metabolism and the degree of β-adrenoceptor function in human airway smooth muscle: 1) competition of the two transduction mechanisms involving adenylate cyclase activation and PI metabolism for the functional response; and 2) interference of the PI with the β-adrenoceptor-mediated activation of adenylate cyclase. The second possibility was supported by the observation that the reduction in potency of isoproterenol to relax tracheal smooth muscle was paralleled by a reduced accumulation of cyclic adenosine monophosphate (cAMP) [21]. It has been suggested that protein kinase C (PKC), which is activated via PI turnover or diacyglycerol [22], plays an important role in the inactivation of β-adrenoceptors. Observations in bovine trachea have indicated that carbachol as well as phorbol 12-myristate 13-acetate, a direct activator of PKC, could induce a reduced β-adrenoceptor number and a reduced cAMP response to isoproterenol [23]. MEURS and co-workers [22, 24, 25] have shown that activation of PKC may also lead to a reduced β-adrenoceptor function in human lymphocytes, because of uncoupling of the receptor from the Gs-protein. In addition to PI metabolism, muscarinic agonists have been reported to stimulate the guanine nucleotide-dependent regulatory protein (Gi), leading to adenylate cyclase inhibition. However, the role of Gi protein stimulation in functional antagonism remains to be further studied.

The fact that formoterol has a greater intrinsic activity than salbutamol, salmeterol and terbutaline might be explained by its greater ability to interact with the high-affinity state of the receptor [10, 26]. In 1980, KENT et

al. [27] had already demonstrated a significant correlation between intrinsic activity and the activation of adenylate cyclase in the frog erythrocyte membrane. This suggestion is supported by the studies of ROUX et al. [17], who showed that in the guinea-pig isolated trachea model formoterol induces a stronger formation of the high-affinity states of the β-adrenoceptor than salmeterol, and that this is related to the intrinsic activity of these drugs.

Differences in intrinsic activity may also account for the nature of the interaction of salmeterol and formoterol versus adrenaline. The fact that salmeterol strongly reduces the effects of adrenaline is in agreement with its weaker intrinsic activity, and the partial agonistic effect in accordance with the studies of LINDÉN et al. [11], and of RABE et al. [28], demonstrating an antagonistic effect of salmeterol at β-adrenoceptors mediating inhibition of respiratory burst in guinea-pig eosinophils.

Influence of epithelium on the effects of β-adrenoceptor agonists

We studied the influence of epithelium on the effects of isoprenaline, adrenaline, salbutamol and formoterol. The airway epithelium is known to modify the effects of numerous bronchoconstrictors or bronchodilators, and various theories have been put forward in an attempt to explain its modulatory action. The epithelium may act as a diffusion barrier [29]. It may also release a relaxant factor, which might be a prostanoid derived from the arachidonic acid cascade (prostaglandin E_2 (PGE_2)) [30], and/or it may release a substance of another nature that relaxes the rat aorta denuded of its endothelium [31].

Contradictory results have been reported concerning bronchodilators. Several authors have shown that abrasion of the epithelium could reduce the effects of β-adrenoceptor agonists (isoprenaline) on the trachea of dog [32, 33], guinea-pig [34], and rat [35], whilst other authors have observed an increased response of the bovine [36] or guinea-pig [37, 38] trachea. AIZAWA et al. [39], working on human bronchi, found no changes in response to isoprenaline. Our results showing no effect of epithelium removal were similar to those of these authors, which might suggest differences between species.

Duration of action of formoterol

Our results first showed that the onset of action of formoterol on the human isolated bronchus is shorter than that observed with salmeterol, in agreement with experiments on the guinea-pig isolated trachea [11, 16, 40–42], or on asthmatics [43].

Our results clearly showed that after washing of the preparations formoterol had a more prolonged relaxant effect on the human bronchus than salbutamol, since its duration of action was approximatively 4.5 fold longer. These results are in agreement with previous experiments performed on the guinea-pig isolated trachea. Indeed, in one study, the remaining β–stimulating effect on the

guinea-pig isolated trachea after wash-out and continuous flushing of the organ baths for 1 h was only 9±4% for salbutamol 10^{-7} mol·l^{-1}, as against 78±7% for formoterol 10^{-8} mol·l^{-1} [44]. In addition, our results have demonstrated the very long action of salmeterol, since in one half of the bronchi tested relaxant responses to salmeterol were maintained without loss for periods of at least 150 min, and in the other half the duration of action of salmeterol was about 14 times longer than that of salbutamol. However, COLEMAN et al. [45], who also worked on the isolated human bronchus, observed a formoterol to salbutamol ratio of duration of action (1.48) that was lower than that reported in the present study; these authors also noted that salmeterol had a very long action, similar to our figures. The difference between our results and those of COLEMAN et al. [45] can probably be explained by the fact that these authors used a superfusion system for washing their preparations, whereas we washed ours by renewing the baths.

The mechanism of this long duration of action has not yet been clearly elucidated. In the case of salmeterol, it has been hypothesized that the lipophilic side-chain binds to a structure located near the active site of the β-adrenoceptor [42]. This hypothesis was supported: 1) by the observation that sotalol, a β-adrenoceptor antagonist, was able to temporarily antagonize salmeterol-induced relaxation of the airway smooth muscle, but relaxation was "reasserted" when sotalol was washed from the tissue [42, 46]; and 2) by studies on the binding of the receptor showing an apparent irreversibility of the binding of salmeterol on β-adrenoceptors of the guinea-pig trachea [42].

However, the reassertion effect after wash-out of small molecular weight β-adrenoceptor antagonists is a property common to lipophilic β-adrenoceptor antagonists, and has also been described for formoterol [47]. ANDERSON [9], LÖFDAHL and CHUNG [7] and JEPSSON et al. [16] have suggested that lipophilicity may also be related to the long duration of the bronchorelaxation induced by these drugs. Indeed, recent studies [48, 49] have found that formoterol and salmeterol are retained within the lipid bilayer of synthetic membranes, in correlation with the lipophilicity, membrane affinity of salmeterol being 30 fold higher than that of formoterol. Thus, within this hypothesis, it could be suggested that after inhalation, drugs enter the membrane, from where they gradually re-equilibrate with the aqueous biophase to interact with the β₂-adrenoceptors. This theory implies a concentration-dependent duration of action. This is the case for formoterol, but not for salmeterol [50]. For this latter drug, other mechanisms, such as irreversible binding on receptor, are probably involved [42, 50].

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