

## Long- and short-acting $\beta_2$ adrenoceptor agonists: interactions in human contracted bronchi

M. Molimard<sup>\*,\*\*</sup>, E. Naline<sup>\*\*</sup>, Y. Zhang<sup>\*\*</sup>, V. Le Gros<sup>†</sup>, B. Begaud<sup>\*</sup>, C. Advenier<sup>\*\*</sup>

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**ABSTRACT:** The aim of this study was to systematically compare the interaction of the long-acting  $\beta_2$ -adrenoceptor agonists formoterol and salmeterol with short-acting  $\beta_2$ -adrenoceptor agonists in contracted human bronchi.

Human bronchi were obtained at thoracotomy from patients with lung cancer. Formoterol or salmeterol at concentrations inducing up to 92 and 94% of their maximal relaxant effect, respectively, were added to bronchial rings contracted with carbachol ( $10^{-6}$  M). After a time period of 30 min, concentration-response curves for the short-acting  $\beta_2$ -adrenoceptor agonists, salbutamol, terbutaline, isoprenaline and fenoterol were recorded.

Administration of equieffective concentrations of salmeterol and formoterol, resulted in only salmeterol inducing a shift to the right of isoprenaline, terbutaline, fenoterol and salbutamol concentration-response curves. The rank order of shift was salbutamol > fenoterol > terbutaline > isoprenaline. Formoterol, up to concentrations of  $3 \times 10^{-9}$  M induced submaximal relaxation resulting in no shift in short-acting  $\beta_2$ -adrenoceptor agonist concentration-response curves.

Salmeterol but not formoterol appears to antagonize the relaxation of human contracted bronchi induced by short-acting  $\beta_2$ -agonists. These results obtained *in vitro* cannot be translated in clinical terms. This study, however, highlights the need for clinical studies on the interaction of long-acting and short-acting  $\beta_2$ -adrenoceptor agonists in acute severe asthma.

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\*Dépt de Pharmacologie, CHU Pellegrin-Carreire, Bordeaux, France. \*\*Laboratoire de Pharmacologie, Faculté de Médecine Paris-Ouest, Paris, France. †Laboratoire Novartis Pharma, Rueil-Malmaison, France.

Correspondence: M. Molimard  
Département de Pharmacologie  
CHU Pellegrin-Carreire  
33076 Bordeaux Cedex  
France  
Fax: 33 556245889

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Inhaled  $\beta_2$ -agonists are the most effective bronchodilators in current use and are the most widely prescribed symptomatic antiasthma therapy in the world. The introduction of long-acting inhaled  $\beta_2$ -agonists, formoterol and salmeterol, has been an important advance in asthma therapy. Current guidelines recommend that short-acting  $\beta_2$ -agonists should be used only as relief medication, whereas long-acting  $\beta_2$ -agonists should be introduced for regular use in patients with asthma already receiving inhaled steroid therapy [1].

The clinical efficacy and long duration of action of formoterol and salmeterol appears to be very similar [2–4] despite the fact that salmeterol has a longer duration of action than formoterol *in vitro* [3, 5]. There are, however, some interesting differences between the two compounds: 1) the onset of action of salmeterol appears to be slower than that observed with formoterol, which does not seem to differ from short-acting compounds both *in vitro* and *in vivo* [5–7]; and 2) salmeterol is a partial agonist and formoterol a nearly full agonist in airway smooth muscle, including that of human bronchi [5, 8–10]. This difference of efficacy may be enhanced by increased bronchial tone. Indeed, we have demonstrated previously that the efficacy of partial  $\beta_2$ -agonists is reduced compared to full  $\beta_2$ -agonists in precontracted human airways [5]. According to the basic principles for agonist/antagonist interaction, a partial agonist has to occupy more receptors than a full agonist to induce the same effect, and behaves as an antag-

onist in the presence of an agonist with higher efficacy acting on the same receptor [11]. This may suggest that a long-acting  $\beta_2$ -adrenoceptor partial agonist could be have as an antagonist of the  $\beta_2$ -agonist relief medication, especially in cases of increased bronchial tone or inflammation. This may lead to heterologous desensitization and subsequent reduction in the number of functional  $\beta_2$ -receptors [12, 13]. An antagonistic effect of salmeterol was demonstrated towards various short-acting  $\beta_2$ -adrenoceptor agonists inducing relaxation on guinea-pig precontracted trachea [9, 10], towards adrenaline and formoterol in human precontracted bronchi [5, 10] and towards  $\beta_2$ -adrenoceptors mediating inhibition of respiratory burst in guinea-pig eosinophils [14].

The aim of the present study was to systematically compare the antagonistic effect of formoterol and salmeterol on currently prescribed short-acting  $\beta_2$ -adrenoceptor agonists in precontracted human isolated bronchi and to classify the short-acting  $\beta_2$ -adrenoceptor agonists with regards to this antagonism.

### Materials and methods

#### *Human bronchial tissue preparation*

Bronchial tissues were removed from 29 patients (mean age 61.6 (range 42–85) yrs) during surgery for lung cancer.

All were exsmokers. None was asthmatic. Immediately after resection, segments of bronchi with an inner diameter of 1–3 mm were taken from areas as far removed from the malignancy as possible. They were placed in oxygenated Krebs-Henseleit solution (NaCl, 119; KCl, 5.4; CaCl<sub>2</sub>, 2.5; KH<sub>2</sub>PO<sub>4</sub>, 0.6; MgSO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25; glucose, 11.7 mM) and stored overnight at 4°C. After removal of the adhering fat and connective tissues, four to eight rings of the same bronchus were prepared. Each set of bronchial rings was suspended under an initial tension of 2 g in Krebs-Henseleit solution, bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37°C. The force of contraction was measured isometrically with UF1 strain gauges (Pioden, Buckingham, UK), amplifiers and an I.O.S.-Moise 3 recorder system (EMKA Technologies, Mitry Mory, France).

In all experiments, human bronchi were first contracted maximally with acetylcholine (ACh) (3×10<sup>-3</sup> M), and then relaxed with theophylline (3×10<sup>-3</sup> M). In agreement with MITCHELL *et al.* [15], preconditioning of the bronchial tissue did not alter the subsequent response to carbachol (10<sup>-6</sup> M). During the next 60 min, the tissues were washed every 15 min and were equilibrated before beginning the experimental procedure. Experiments were conducted on parallel groups of four to eight rings, one ring serving as a control.

#### Experimental procedures

In order to assess the effect of airway contraction on β<sub>2</sub>-adrenoceptor agonist-induced relaxation, experiments were performed on tissue either precontracted with carbachol (10<sup>-6</sup> M) or with no precontraction, and noncumulative concentration-response curves to formoterol (10<sup>-11</sup>–10<sup>-7</sup> M) or salmeterol (10<sup>-9</sup>–10<sup>-3</sup> M) or cumulative concentration-response curves to the short acting β<sub>2</sub>-adrenoceptor agonists, salbutamol (10<sup>-9</sup>–3×10<sup>-4</sup> M), terbutaline (10<sup>-8</sup>–3×10<sup>-4</sup> M), isoprenaline (10<sup>-8</sup>–3×10<sup>-4</sup> M) and fenoterol (10<sup>-9</sup>–3×10<sup>-4</sup> M) were recorded.

For interaction studies, the bronchial rings were contracted with carbachol (10<sup>-6</sup> M), giving 63±3% of maximal contraction (n=11). After the contraction plateau was reached, bronchial rings were incubated for 30 min with Krebs' solution (control) or one concentration of formoterol (3×10<sup>-11</sup>, 10<sup>-10</sup>, 3×10<sup>-10</sup>, 10<sup>-9</sup>, or 3×10<sup>-9</sup> M) or salmeterol (10<sup>-9</sup>, 10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup> M) inducing 13–92% and 22–94% of formoterol and salmeterol maximal relaxation, respectively. After a time period of 30 min, concentration-response curves to the short-acting β<sub>2</sub>-adrenoceptor agonists, salbutamol, terbutaline, isoprenaline and fenoterol, were recorded by administering increasing concentrations of drugs at 5–15 min intervals. After the maximal effect of each β<sub>2</sub>-adrenoceptor agonist was obtained, theophylline (3×10<sup>-3</sup> M) was added to the bath in order to determine the maximal relaxation. When the preparations were contracted with carbachol (10<sup>-6</sup> M), the spontaneous decrease of tension was about 5±2% for 1 h (n=11).

Only one concentration-response curve to β<sub>2</sub>-adrenoceptor agonist was recorded in each ring.

#### Expression and statistical analysis of results

The maximal relaxant effect (E<sub>max</sub>) of each β<sub>2</sub>-adrenoceptor agonist, was expressed as a percentage of the action

of theophylline (3×10<sup>-3</sup> M). The concentration of β<sub>2</sub>-adrenoceptor agonist which induced a relaxation equal to 50% of that induced by theophylline (3×10<sup>-3</sup> M) (-logEC<sub>50</sub>) was determined. Intrinsic activity was determined in comparison to isoprenaline: E<sub>max</sub> (drug)/E<sub>max</sub> (isoprenaline).

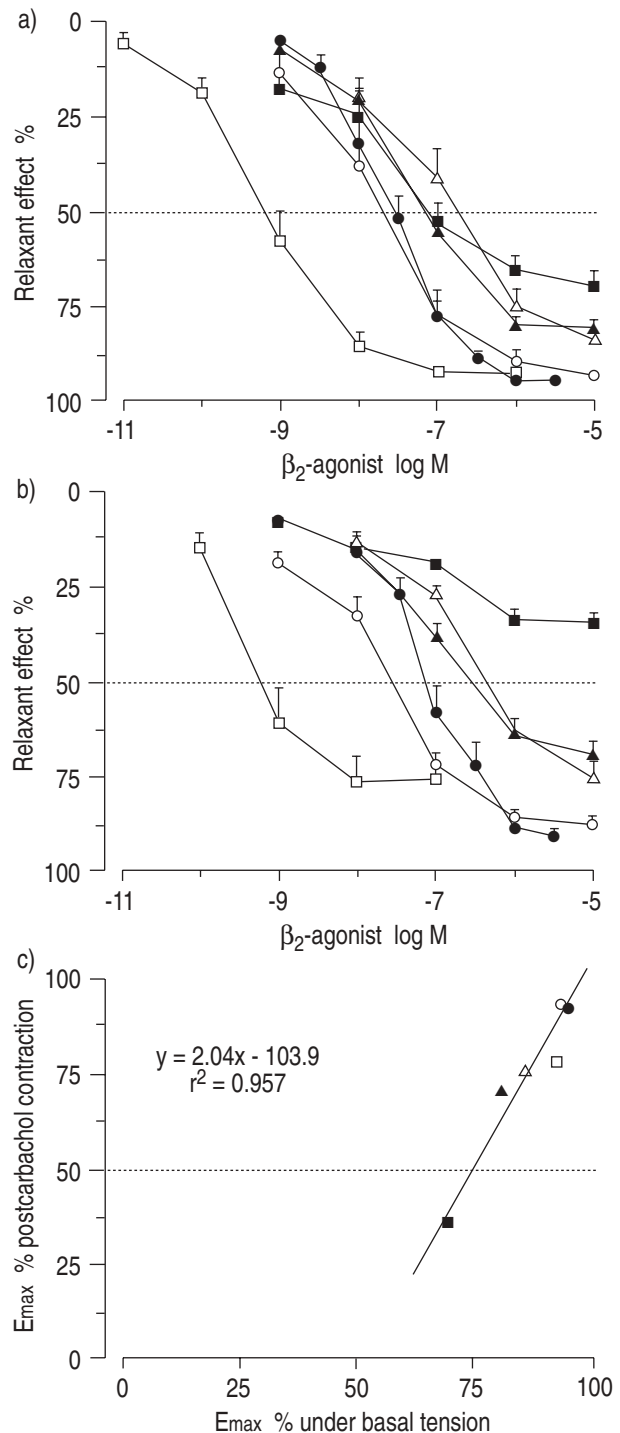


Fig. 1. – Comparison of the concentration response curves of the β<sub>2</sub>-adrenoceptor agonists: isoprenaline (●); fenoterol (○); salbutamol; (▲); terbutaline (△); formoterol (□); and salmeterol (■) on human bronchi: a) under basal tension; b) after contraction with carbachol (10<sup>-6</sup> M); and c) the relationship between maximal relaxant effects (E<sub>max</sub>) of the aforementioned β<sub>2</sub>-adrenoceptor agonists on human bronchi under basal tension and after contraction by carbachol (10<sup>-6</sup> M). Relaxant effect is expressed as a percentage of the action of theophylline (3×10<sup>-3</sup> M).

Table 1. – Potency (-logEC<sub>50</sub>) and efficacy (E<sub>max</sub>) of some β<sub>2</sub>-adrenoceptor agonists on the isolated human bronchus at resting tone or after contraction by carbachol 10<sup>-6</sup> M

	Resting tone				Carbachol 10 <sup>-6</sup> M			
	n	-logEC <sub>50</sub>	E <sub>max</sub>	IA	n	-logEC <sub>50</sub>	E <sub>max</sub>	IA
Fenoterol	9	7.70±0.22	94±1	1.00	8	7.44±0.09*	92±2	1.02
Isoprenaline	16	7.57±0.11	95±1	1.00	9	7.09±0.10 <sup>++</sup>	91±2	1
Salbutamol	16	7.11±0.11*	81±2***	0.85	13	6.43±0.21*, <sup>++</sup>	69±3***, <sup>+</sup>	0.78
Terbutaline	8	6.81±0.18**	86±3**	0.90	8	6.34±0.07***, <sup>+</sup>	75±4***, <sup>+</sup>	0.82
Formoterol	16	9.20±0.16***	93±1	0.97	5	9.11±0.17***	77±6* <sup>+</sup>	0.84
Salmeterol	16	7.30±0.21	70±4***	0.73	19	-	35±2***, <sup>+++</sup>	0.36

Values are presented as absolute number, and mean±SEM. n: number of experiments; -logEC<sub>50</sub>: log of the concentration of drug which induces 50% of the relaxation induced by theophylline (3×10<sup>-3</sup> M); E<sub>max</sub>: maximal relaxant effect expressed as a percentage of the maximal relaxant effect induced by theophylline (3×10<sup>-3</sup> M); IA: intrinsic activity compared to isoprenaline, E<sub>max</sub> (drug)/E<sub>max</sub> (isoprenaline). \*, \*\*, \*\*\*: p<0.05, p<0.01, p<0.001, compared to isoprenaline. +, ++, +++: p<0.05, p<0.01, p<0.001, compared to resting tone.

Statistical analysis of the results was performed using analysis of variance and Student's t-test for unpaired data. All values are expressed as mean±SEM. A p-value of less than 0.05 was considered statistically significant.

**Drugs**

The drugs used were: salmeterol (Ciba-Geigy, Basle, Switzerland), formoterol fumarate (Ciba-Geigy), terbutaline sulphate (Astra, Rueil-Malmaison, France) fenoterol HBr (Boehringer Ingelheim, Reims, France), isoprenaline HCl, salbutamol HCl, carbachol (carbamylcholine chloride) (Sigma, St. Louis, MO, 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 (10<sup>-5</sup> M) and then diluted in Krebs' solution.

**Results**

*Effect of human bronchi contraction on response to β<sub>2</sub>-agonists*

Figure 1 shows that human airway contraction reduced both the efficacy and potency of the β<sub>2</sub>-adrenoceptor agonists. Indeed, human airway contraction by carbachol (10<sup>-6</sup> M) reduced the E<sub>max</sub> induced by β<sub>2</sub>-adrenoceptor agonists (fig. 1, table 1). The efficacy of each β<sub>2</sub>-adrenoceptor agonist on human contracted bronchi was ranked fenoterol > isoprenaline > formoterol > terbutaline > salbutamol > salmeterol. The reduction of efficacy after the contraction of human bronchi was correlated with the efficacy under basal tension. The more partial the agonist under basal tension, the greater the reduction in efficacy (fig. 1c). Thus, the E<sub>max</sub> of salmeterol (10<sup>-5</sup> M) and formoterol (10<sup>-8</sup> M) on contracted human bronchi were 35±2 and 77±6% of theophylline (3×10<sup>-3</sup> M) induced relaxation, respectively.

Furthermore, human airway contraction by carbachol (10<sup>-6</sup> M) induced a shift to the right of the β<sub>2</sub>-adrenoceptor agonist concentration-response curves, with a significant decrease in -logEC<sub>50</sub> for isoprenaline, salbutamol

and terbutaline. The potency of each β<sub>2</sub>-adrenoceptor agonists on contracted human bronchi was ranked formoterol > fenoterol > isoprenaline > salbutamol > terbutaline (table 1).

*Interaction between salmeterol and short-acting β<sub>2</sub>-agonists on human contracted bronchi*

Salmeterol at concentrations inducing 22–94% of its own E<sub>max</sub> (10<sup>-9</sup>–10<sup>-6</sup> M) induced a dose-dependent shift to the right for all the short-acting β<sub>2</sub>-agonist concentration-response curves studied (fig. 2). In the presence of salmeterol (10<sup>-8</sup> M), the shifts to the right were 0.9, 0.7, 0.7, and 0.4 log units for salbutamol, terbutaline, fenoterol and isoprenaline concentration-response curves, respectively. This demonstrates that the concentrations of each agonist needs to be increased eight, five, five and two times, respectively, to obtain a similar effect. With salmeterol (10<sup>-7</sup> and 10<sup>-6</sup>) those factors were: 178 and 603 for salbutamol; 66 and 575 for fenoterol; 55 and 214 for terbutaline; and 6 and 21 for isoprenaline. Except for fenoterol, the E<sub>max</sub> of the β<sub>2</sub>-adrenoceptor agonists were not reached even at very high concentrations (3×10<sup>-4</sup> M) in the presence of salmeterol (10<sup>-7</sup> and 10<sup>-6</sup> M).

*Interaction between formoterol and short-acting β<sub>2</sub>-agonists on human contracted bronchi*

Formoterol at concentrations inducing 13–92% of its own E<sub>max</sub> (3×10<sup>-11</sup>–3×10<sup>-9</sup> M) had no antagonistic effect toward any of the short-acting β<sub>2</sub>-adrenoceptor agonists studied (fig. 3). Fenoterol and isoprenaline, with each having an E<sub>max</sub> higher than the relaxant effect induced by formoterol (3×10<sup>-9</sup> M) (71% of theophylline (3×10<sup>-3</sup> M) induced relaxation), could further relax the bronchi, after formoterol induced relaxation, without any shift in the concentration response curves. The E<sub>max</sub> of the β<sub>2</sub>-adrenoceptor agonists, except for salbutamol, were unaffected by formoterol pretreatment. The effects of very high doses of salbutamol (10<sup>-5</sup> and 10<sup>-4</sup> M) were reduced at the very high concentrations of formoterol studied.

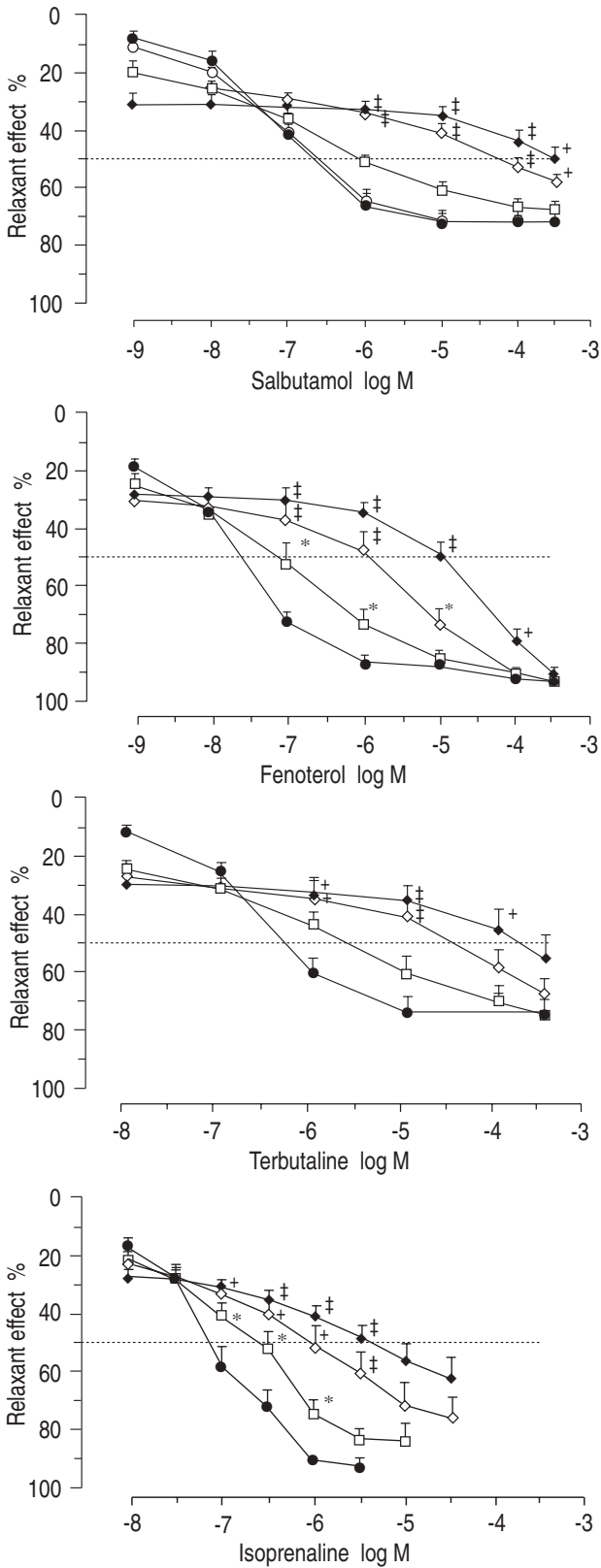


Fig. 2. — Concentration response curves of isoprenaline ( $10^{-8}$ – $3 \times 10^{-5}$  M) (n=8–9), fenoterol ( $10^{-9}$ – $3 \times 10^{-4}$  M) (n=7–8), terbutaline ( $10^{-8}$ – $3 \times 10^{-4}$  M) (n=7–8) and salbutamol ( $10^{-9}$ – $10^{-4}$  M) (n=5–9) on human isolated bronchi contracted by carbachol in the absence (control: ●) or the presence of salmeterol ( $10^{-9}$  M: ○;  $10^{-8}$  M: □;  $10^{-7}$  M: ◇;  $10^{-6}$  M: ◆). Relaxant effect is expressed as a percentage of the action of theophylline ( $3 \times 10^{-3}$  M). \*, †, ‡: p<0.05, p<0.01, p<0.001, compared to control.

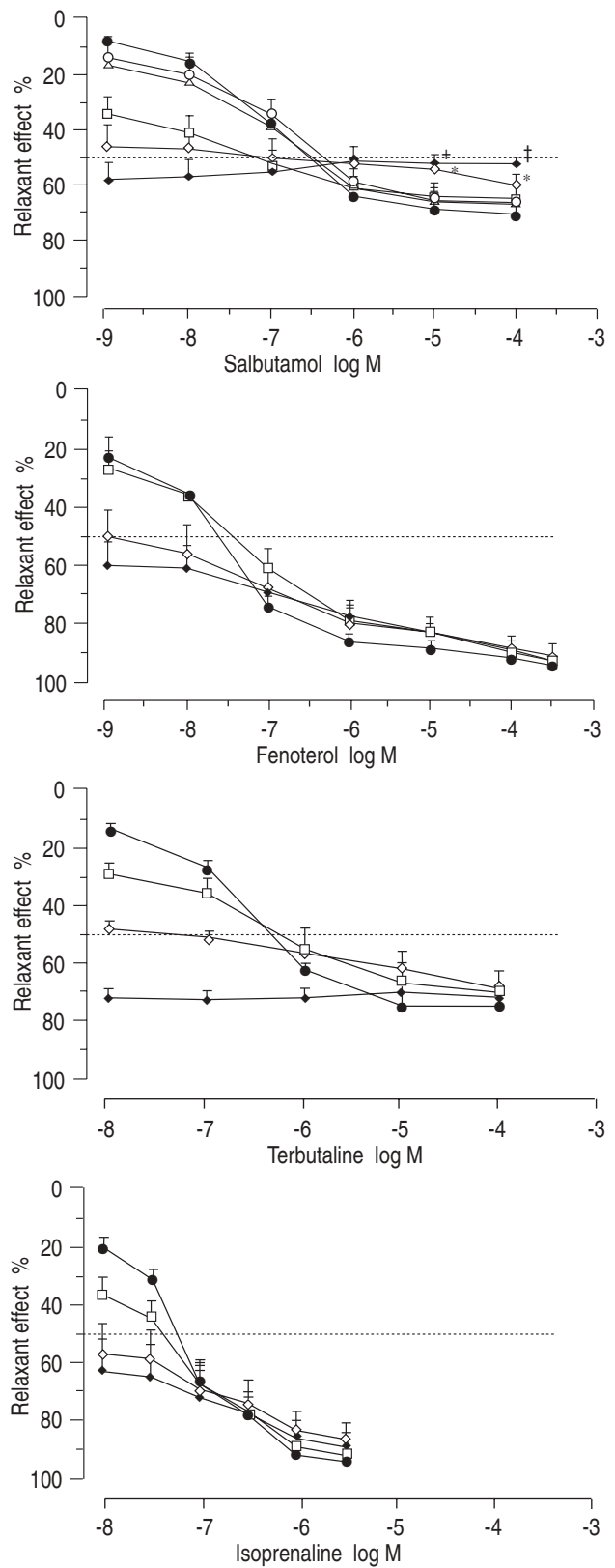


Fig. 3. — Concentration response curves of isoprenaline ( $10^{-8}$ – $3 \times 10^{-6}$  M) (n=7), fenoterol ( $10^{-9}$ – $3 \times 10^{-4}$  M) (n=7), terbutaline ( $10^{-8}$ – $10^{-4}$  M) (n=7–8) and salbutamol ( $10^{-9}$ – $3 \times 10^{-4}$  M) (n=7–10) on human isolated bronchi contracted by carbachol in the absence (control: ●) or the presence of formoterol ( $3 \times 10^{-11}$  M: ○;  $10^{-10}$  M: △;  $3 \times 10^{-10}$  M: □;  $10^{-9}$  M: ◇ or  $3 \times 10^{-9}$  M: ◆). Relaxant effect is expressed as a percentage of the action of theophylline ( $3 \times 10^{-3}$  M). \*, †, ‡: p<0.05, p<0.01, p<0.001, compared to control.

## Discussion

Precontraction of human bronchi by carbachol reduced the relaxant efficacy of all  $\beta_2$ -adrenoceptor agonists tested, including salmeterol and formoterol. This is in agreement with the functional antagonism between muscarinic agonists and  $\beta_2$ -adrenoceptor agonists, notably in human airways [5, 16, 17]. This phenomenon may be linked to  $\beta_2$ -adrenoceptor or Gs protein phosphorylation by protein kinase C or even to the inhibition of adenylate cyclase by the muscarinic  $M_2$  receptor mediated Gi protein [13], this latter component being discussed elsewhere [18]. We have recently demonstrated that in the presence of Ach the intrinsic activity of the  $\beta_2$ -adrenoceptor agonists is reduced more for partial agonists than for full agonists [5]. In the present study, it has been demonstrated that the magnitude of reduction of the efficacy of  $\beta_2$ -agonists due to contraction by carbachol is correlated with their respective efficacy at basal tension. These results are in agreement with receptor theories suggesting that the need for relatively greater receptor occupancies by partial agonists creates a situation in which functional receptor number becomes limiting in terms of obtaining a maximal response [19].

Such a functional antagonism between muscarinic and  $\beta_2$ -adrenoceptor agonists could be of particular interest in patients treated with long-acting  $\beta_2$ -adrenoceptor agonists with increased bronchial tension, especially in cases of acute severe asthma. Indeed, long-lasting receptor occupancy by a long-acting  $\beta_2$ -adrenoceptor agonist, having reduced intrinsic activity as a result of airway contraction, could possibly result in an antagonism of the short-acting  $\beta_2$ -adrenoceptor agonist used for asthma rescue. We therefore performed our comparative study on precontracted airways.

The clinical relaxant effect of salmeterol 50  $\mu\text{g}$  is almost comparable with that observed with formoterol 12  $\mu\text{g}$  [2–4], but the relative concentrations of formoterol and salmeterol are not known at the smooth muscle level. The diffusion parameters of the two compounds are likely to be different due to their different lipophilicity [3]. Since the relaxant efficacy of salmeterol and formoterol are clinically comparable, we initially considered *in vitro* concentrations of formoterol and salmeterol with comparable relaxant efficacy. As no antagonistic effect with formoterol was observed at these concentrations, concentrations were increased up to concentrations inducing submaximal relaxation.

Our results show that in human precontracted bronchi, salmeterol behaves as an antagonist to all  $\beta_2$ -adrenoceptor agonists studied *i.e.* isoprenaline, fenoterol, terbutaline and salbutamol. This antagonism appears to be more pronounced toward salbutamol. Indeed, from a concentration of salmeterol ( $10^{-8}$  M) inducing 33% relaxation, an eight-fold increase in salbutamol concentration is necessary to achieve 50% of its own  $E_{\text{max}}$ . By comparison only a two-fold dose increase is required for isoprenaline. The rank order of shift magnitude was salbutamol  $\dot{S}$  fenoterol  $\dot{S}$  terbutaline  $>$  isoprenaline. These results are in agreement with earlier studies performed on guinea-pig trachea [9, 10] and on human bronchi [5, 10]. This is in agreement with the basic principles for agonist/antagonist interaction, according to which a partial agonist behaves as an antagonist for an agonist acting on the same receptor [11].

For relaxations comparable to those obtained with salmeterol, formoterol had no antagonistic effect toward any of the  $\beta_2$ -adrenoceptor agonists tested. Furthermore, there was no significant shift in  $-\log EC_{50}$  in any of the  $\beta_2$ -adrenoceptor agonists tested, even at the sub-maximal relaxation induced by formoterol ( $3 \times 10^{-9}$  M). This demonstrates its lack of antagonist effect in this range of concentration. The maximal relaxation induced by salbutamol (the most partial of the short-acting  $\beta_2$ -adrenoceptor agonists studied) was reduced by formoterol pretreatment. This could be due to a higher affinity of salbutamol and the need for partial agonists to occupy all the receptors to induce  $E_{\text{max}}$  [19]. This effect is specific to salbutamol, since no similar effect with any other of the short acting  $\beta_2$ -adrenoceptor agonists studied was found.

In a recent clinical study, no interaction was observed between salmeterol or formoterol pretreatment and airway response to fenoterol [20], but the doses of salmeterol were half those currently prescribed. In another study, no interaction was found between salbutamol and salmeterol at doses up to 200  $\mu\text{g}$  [21]. These studies were performed in patients with an almost normal forced expiratory volume in one second (FEV<sub>1</sub>). As such, these results were not reproduced in the clinical situation of acute severe asthma, where short acting inhaled  $\beta_2$ -agonists are needed in addition to long-acting  $\beta_2$ -agonist pretreatment, and where the number of functional  $\beta_2$ -adrenoceptor agonists may be reduced. Thus, the power of these studies, in the search for differences between the two long-acting agonists is limited by the lack of prior bronchoconstriction. The results of the present study cannot be translated absolutely in clinical terms. The *in vitro* airway smooth muscle concentrations of the  $\beta_2$ -agonists obtained after inhalation are not known. It is, however, suggested that as a result of the present study further clinical investigation may be of interest in patients with a higher degree of bronchial tone, in the search for long- and short-acting  $\beta_2$ -adrenoceptor agonist interactions.

In conclusion our results demonstrate that salmeterol but not formoterol behaves as an antagonist of all other  $\beta_2$ -agonists studied on human precontracted bronchi, and that formoterol interacts with salbutamol but not with terbutaline-, fenoterol- or isoprenaline-induced relaxation. The clinical relevance of these results are difficult to determine as conditions obtained *in vitro* are difficult to translate in clinical terms. Further clinical studies are needed to search for long- and short-acting  $\beta_2$ -adrenoceptor agonist interactions in acute severe asthma.

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