

12. Clausen JL. Prediction of normal values. In: Clausen JL, ed. Pulmonary function testing and controversies. New York, N.Y.: Grune & Stratton, 1982:49-59.
13. Pirofsky B. Intravenous immunoglobulin therapy in hypogammaglobulinemia. *Am J Med* 1984;76:53-60.
14. Kohler P. Pulmonary manifestations and management of antibody deficiency in adults. *Chest* 86(suppl):24-8.
15. Sorensen RU, Polmar SH. Efficacy and safety of high-dose intravenous immune globulin therapy for antibody deficiency syndromes. *Am J Med* 1984;76(suppl):83-90.
16. White WB, Desbonnet CR, Ballow M. Immunoregulatory effects of intravenous immune serum globulin therapy in common variable hypogammaglobulinemia. *Am J Med* 1987; 83:431-6.
17. Cunningham-Rundles C, Siegal FP, Smithwick EM, et al. Efficacy of intravenous immunoglobulin in primary humoral immunodeficiency disease. *Ann Intern Med* 1984;101:435-9.
18. Ammann AJ, Ashman RF, Buckley RH, et al. Use of intravenous gamma globulin in antibody immunodeficiency: results of a multicenter controlled trial. *Clin Immunol Immunopathol* 1982;22:60-7.
19. Roifman CN, Lederman HM, Lavi S, Stein LD, Levison H, Gelfand EW. Benefit of intravenous IgG replacement in hypogammaglobulinemia patients with chronic sinopulmonary disease. *Am J Med* 1985;79:171-4.

The effect of cetirizine on the human isolated bronchus: Interaction with salbutamol

C. Advenier, MD, PhD,* M-L. Candenas, PhD,*** E. Naline, PhD,*
C. De Vos, PhD** Paris, France, and Braine-l'Alleud, Belgium

The effects of cetirizine (C), a new generation, nonsedative, H₁-antihistamine drug, were studied on human isolated bronchi. C is a potent antagonist of the bronchial muscle contraction induced by histamine, irrespective of whether C is administered by cumulative addition or prophylactically. In the former case, this effect of C was significant at a concentration of 3×10^{-8} mol/L and was maximal at a concentration of 10^{-5} mol/L. The $-\log$ of concentration of C causing 50% of the maximal effect induced was 7.30 ± 0.05 ($n = 6$), and the effect produced was not significantly modified by bronchial epithelium removal. When C was administered prophylactically, the concentration-response curves to histamine were displaced to the right, but the reduction of maximum histamine response suggests a noncompetitive type of antagonism. C is devoid of notable anticholinergic effects. At concentrations of 10^{-8} to 10^{-7} mol/L, C proved capable of enhancing the relaxant effect produced by salbutamol (10^{-7} to 3×10^{-7} mol/L) on human isolated bronchi that had been contracted by either histamine or acetylcholine (ACh). The synergy appeared to be additive or potentiating, depending on salbutamol (SAL) concentrations. Under similar conditions, mepyramine does not potentiate the effects of SAL against histamine. Finally, at concentrations of 10^{-8} to 10^{-6} mol/L, C reduced the functional antagonism observed between SAL and ACh, as can be observed by the increase, in the presence of C, of the maximal relaxant effect of SAL on contractions produced by 10^{-3} mol/L of ACh. We may, therefore, conclude that C appears to be a specific antihistamine on human isolated bronchi and that it appears to potentiate the bronchodilator effect of SAL on this preparation. (J ALLERGY CLIN IMMUNOL 1991;88:104-13.)

Key words: Histamine antagonists, adrenergic β -receptor agonists, cetirizine, salbutamol, mepyramine, acetylcholine, human isolated bronchi, human airways

From the *Faculté de Médecine Paris Ouest, Laboratoire de Pharmacologie, Paris, France, and **UCB, Pharmaceutical sector, Braine-L'Alleud, Belgium.

Received for publication June 28, 1990.

Revised Dec. 17, 1990.

Accepted for publication March 12, 1991.

Reprint requests: C. Advenier, MD, Faculté de Médecine Paris Ouest, Laboratoire de Pharmacologie, 15, rue de l'école de Médecine, F-75270, Paris Cedex 06, France.

***M-L. Candenas, PhD, is the recipient of a fellowship of the Ministerio de Educacion y Cencia (España).

1/1/29411

Early H_1 -receptor antagonists were found to have a moderate bronchodilator action, but this action was insufficient to be therapeutically useful in asthma.^{1,2} Furthermore, anticholinergic properties indicated that other pharmacologic effects contributed significantly to the bronchodilator action.^{3,4} Critical assessment of the influence of effective H_1 -receptor blockade in asthma was also confused by sedation at large doses.² The new generation of potent and more selective histamine H_1 -receptor antagonists, which had the additional advantage of being nonsedative, enabled further evaluation of any bronchodilator action. Thus, terfenadine or astemizole was reported to produce some improvement in asthma symptom scores, β -adrenoreceptor agonist use, and peak expiratory flow measurement,^{2,5,6} but although results reached statistical significance, the effects of these drugs were of marginal clinical significance. In addition, the demonstration of new properties, over and above the antihistamine activity, could prove potentially interesting for the treatment of allergic phenomena and/or asthma.

In the case of C, which is a new generation H_1 -antihistamine virtually free from central nervous system sedation,⁷ the inhibition of eosinophil migration induced by an allergen or by platelet aggregating factor has been described in the skin and in atopic subjects.⁸⁻¹⁰ Because eosinophils appear to play an important role in the inflammatory process associated with allergic skin or bronchial reactions, this property of C could potentially prove to be interesting in the treatment of allergic bronchial hyperreactivity.¹¹

The aim of this study was to continue to assess the effects produced by C by use of human isolated bronchi to search for a possible interaction between this substance and the β -adrenergic stimulants, an interaction that, if it proves positive, could have a potential interest in the treatment of asthma. The study on the human isolated bronchus consisted of (1) quantifying the antihistaminic and anticholinergic properties of C, (2) characterising the type of antagonism implicated in the antihistaminic activity, (3) determining the role of the bronchial epithelium in this latter effect, and (4) studying the characteristics of the synergy of the C-SAL association with regard to the contraction produced by histamine and ACh, especially within the framework of the functional antagonism observed between β -adrenoceptor agonists and ACh.¹²

MATERIAL AND METHODS

Human bronchi preparations

Human bronchial tissue (usually with an inner diameter of 3 to 6 mm) was obtained from patients undergoing surgery for lung cancer (squamous cell carcinomas, oat cell carci-

Abbreviations used

ACh:	Acetylcholine
C:	Cetirizine
SAL:	Salbutamol
$-\log EC_{50}$:	$-\log$ of concentration of histamine or acetylcholine eliciting 50% of the maximal effect of acetylcholine, 10^{-3} mol/L
pD_2 :	$-\log$ of concentration of cetirizine or salbutamol eliciting 50% of the maximal effect induced by the drug
E_{max} :	Maximal relaxation induced by salbutamol (percent versus theophylline, 3×10^{-3} mol/L)

nomas, 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. Some published data have demonstrated that overnight storage of tissue does not alter its reactivity.^{13,14} Rings from a segmental bronchus were prepared and suspended in Krebs' solution under an initial tension of 2.0 g. After 1½ hours of equilibration with washing every 15 minutes, 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 2 recorder system (Celaster, Celle l'Evescault, Lusignan, France). The composition of the Krebs' solution was (millimolars per liter) 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 ACh, 10^{-3} mol/L, and then relaxed with theophylline, 3×10^{-3} mol/L. These concentrations did not alter responsiveness of the tissue. A period of 1½ hours of rest was observed before the beginning of the experimental procedure.

Experimental procedures

Specific activity of C on the basal tone and antagonism toward histamine and ACh: Study by cumulative addition. The experiments were performed on groups of six bronchi separated into three series of two bronchi each mounted in parallel. After a rest period, the first series was left to find its own basal tone, whereas the second and the third series were contracted by either 10^{-5} mol/L of histamine or 10^{-4} mol/L of ACh to reach a contraction of 50% to 70% of the maximum contraction produced by 10^{-3} mol/L of ACh.

In each of these series, and after reaching a contraction plateau for the bronchi contracted by histamine or by ACh, increasing doses of C in the range of 3×10^{-8} to 10^{-5} mol/L, or the equivalent quantity in volume of Krebs' solution, was added to the bath. A 20- to 40-minute interval

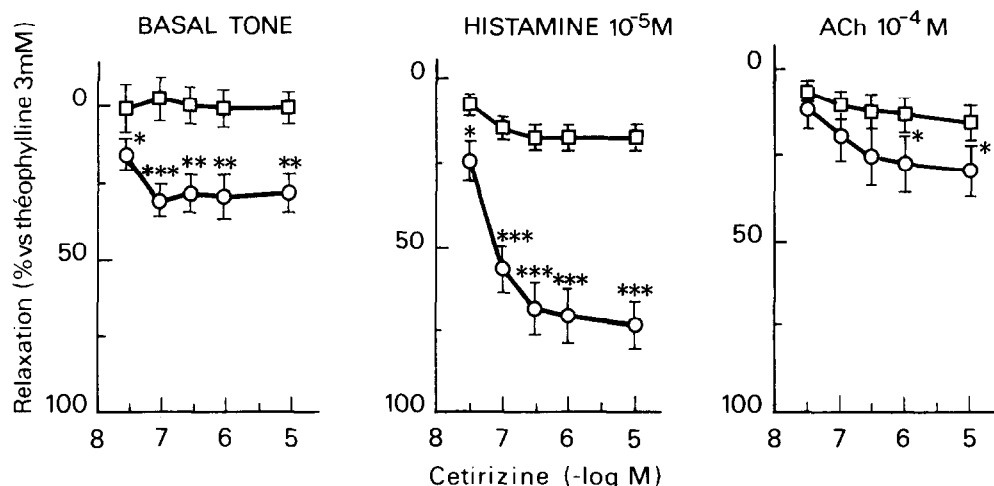


FIG. 1. Influence of the Krebs' solution (□) or C (○) when either is added in a cumulative manner on human bronchi in a state of basal tone or contracted by 10^{-5} mol/L of histamine or by 10^{-4} mol/L of ACh. Experiments were performed on a series of bronchi mounted in parallel. Values are means $\pm$ SEM ($n = 6$). Differences between the Krebs' solution and C are significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's paired t test).

was observed between each addition until equilibrium was achieved. At the end of the experiment, 3×10^{-3} mol/L of theophylline was added to the bath to determine the maximum relaxation. The effects produced by C were expressed as a percentage of the effects produced by this dose of theophylline. Only one concentration-response curve for C was established for each bronchial segment.

Influence of epithelium abrasion on the antihistaminic effect of C. The experiments were performed on two rings from the same bronchus mounted in parallel. One ring was used as control. On the other ring, the epithelium was removed by gently and repeatedly rubbing the luminal surface with a cotton-tipped applicator, as previously described.^{15,16} After the first contact with 10^{-3} mol/L of ACh and a rest period of 1½ hours, the effect produced by C was tested comparatively on both preparations relative to the contraction induced by 10^{-5} mol/L of histamine in accordance with the terms of the study by cumulative addition previously described.

Study according to a preventive protocol. The experiments were conducted on series of eight segments of bronchi mounted in parallel, some, to test the antihistaminic effect, and the other preparations for the anticholinergic effect. After the 1½-hour rest period, six of the preparations were incubated with a given concentration of C, each (10^{-8} , 3×10^{-8} , and 10^{-7} mol/L, when antihistamine action was studied, or 10^{-8} , 10^{-7} , and 10^{-6} mol/L, when anticholinergic action was studied) for 1 hour, the bath being renewed after 30 minutes, and the last two preparations in each series serving as controls to assess the effect produced by either histamine or ACh.

Afterward, always in the presence of C when the therapeutic effect was being considered, a concentration-response curve was established for both histamine and ACh in accordance with a cumulative addition proto-

col, a dose 10 times higher being added to the bath every 15 to 30 minutes once the equilibration plateau had been reached. In the case of histamine, at the end of the scale, a 10^{-3} mol/L dose of ACh was added to the bath to obtain a 100% concentration.

The effect produced was expressed as a percentage of the ACh-induced contraction. Only one concentration-response curve was established for ACh and for histamine per preparation. Similar experiments were conducted in the same conditions to investigate the antagonism exerted by mepyramine versus histamine.

Study of the C or mepyramine-SAL interaction

Influence of SAL and C or mepyramine when these agents are added separately or together to the concentration-response curves for histamine and ACh. The experiments were performed on series of preparations mounted in parallel, some, to study the effect produced by histamine, and the other preparations to study ACh both in the presence and in the absence of treatment.

After the 1½-hour rest period, one preparation served as control; the other preparations were treated with C (10^{-8} or 10^{-7} mol/L), with mepyramine (10^{-9} , 10^{-8} , and 10^{-7} mol/L), or with SAL (10^{-7} or 3×10^{-7} mol/L), added separately or together, for 1 hour, the bath being renewed after 30 minutes. Afterward, always in the presence of the drugs when the treatment effect was being considered, a concentration-response curve was established for both histamine and ACh in accordance with a cumulative addition protocol.

Concentration-response curves for SAL versus 0 to 10^{-3} mol/L of ACh: Influence of C (10^{-6} to 10^{-8} mol/L) on the functional antagonism observed between SAL and ACh. The

experiments were performed on series of eight bronchial rings mounted in parallel. After the 1½-hour rest period, three segments were treated for 1 hour with 10^{-8} , 10^{-7} , and 10^{-6} mol/L of C, the bath being renewed after 30 minutes, then treated with 10^{-3} mol/L of ACh. At this point, four other segments were treated with 10^{-6} to 10^{-3} mol/L of ACh, the eighth segment remaining in a state of basal tone (control or ACh, 0). Once the effect produced by ACh had reached a plateau, concentration-response curves were established for SAL at the same time for the eight segments in accordance with a cumulative addition protocol, additions being made every 10 to 20 minutes so that the effect could reach and maintain a plateau. At the end of the concentration-response curve establishment, a 3×10^{-3} mol/L dose of theophylline was added to the bath to determine the 100% relaxation. The effect produced by SAL was determined as a percentage of the maximal effect of theophylline. Only one concentration-response curve was established per preparation.

Expression of results and calculation

The results are expressed as a percentage of the action of theophylline, 3 mmol/L for relaxation, and as a percentage of the action of ACh, 1 mmol/L for contraction. Concentration-response curves were determined visually from a plot of log concentration versus percent of maximal response produced by each substance in individual experiments. Potency and efficacy were evaluated, respectively, in terms of pD_2 (C and SAL) or $-\log EC_{50}$ (histamine and ACh) and E_{max} ; pD_2 is the negative logarithm of the drug concentration (moles per liter) that induced 50% of the maximal effect induced by this drug; $-\log EC_{50}$ is the negative logarithm of the drug concentration (moles per liter) that induces 50% of the maximal effect of ACh, 10^{-3} mol/L. E_{max} is the tension or relaxation generated by a maximally effective concentration of the drug and is expressed in percentage of the maximal effect of theophylline, 3×10^{-3} mol/L. Shifts of concentration responses were calculated from individual experiments as the difference between $-\log EC_{50}$. Values in the text are mean $\pm$ SEM. Paired Student's *t* tests were used for the comparison of the ($-\log EC_{50}$) curves to the control, and Student's *t* tests of significance of the mean were used to test the interaction between two substances. Two two-way analysis of variance (groups and bronchi) were used to analyze the antagonism between SAL and ACh and the influence of C on this antagonism.

The treatment effect is divided into three contrasts: a treatment effect, the four (or three for the second case) doses of ACh versus control, a linear component calculated from the four doses of ACh (the three doses of C for the second case), the control being excluded, and a quadratic component calculated from the four doses of ACh (the three doses of C in the second case), the control being excluded. All the tests were two-tailed, and the level of significance was 5%.

Substances

ACh, di HCl (Pharmacie Centrale des Hôpitaux, Paris, France), histamine, di HCl, SAL sulfate (Sigma Chemical

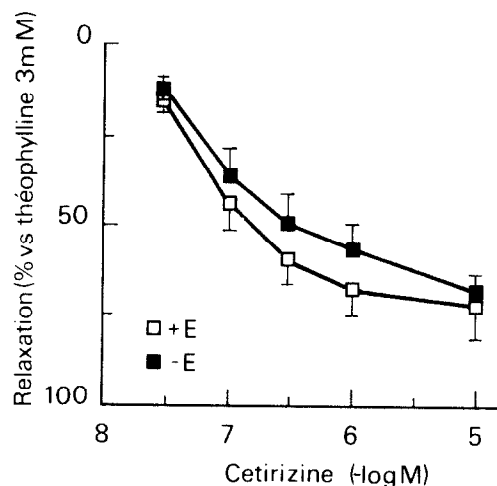


FIG. 2. Action of C when it is added by cumulative addition on human bronchi mounted in parallel and contracted by 10^{-5} mol/L of histamine in the presence ($\square$) or absence ($\blacksquare$) of epithelium. Values are means $\pm$ SEM ($n = 5$).

Co., St. Louis, Mo.), C dihydrochloride (UCB, Braine-l'Alleud, Belgium), and theophylline anisate (Bruneau, Paris, France) were used in these experiments. Theophylline was used as proprietary injectable solution and diluted in Krebs' solution. All other drugs were dissolved in distilled water and diluted in Krebs' solution.

RESULTS

Effect produced by C on the basal tone of isolated bronchi: Antagonism to histamine and ACh

Cumulative addition of C. C brought about a modest, but significant, relaxation of human isolated bronchi at basal tone or when bronchi were contracted by 10^{-4} mol/L of ACh (Fig. 1). Relative to the effect produced by 3×10^{-3} mol/L of theophylline, the maximum effect produced by C was $31\% \pm 5\%$ ($n = 6$; $p < 0.001$) (10^{-7} mol/L) as regards basal tone, and $21\% \pm 3\%$ ($n = 6$; $p < 0.001$) (10^{-5} mol/L) for ACh. In contrast, C produced an important effect where histamine was concerned. This effect was significant with a 3×10^{-8} mol/L dose and maximum at a 10^{-5} mol/L dose, with a relationship of $77\% \pm 7\%$ relative to the effect produced by 3×10^{-3} mol/L of theophylline ($p < 0.001$; $n = 6$). According to the concentration used, this effect achieved equilibrium after 15 to 30 minutes of contact. The pD_2 obtained for C with these conditions was 7.30 ± 0.05 ($n = 6$). The antihistaminic activity of C was not significantly modified by abrasion of the bronchial epithelium (Fig. 2). The pD_2 of C was 7.04 ± 0.06 when epithelium was absent, and 7.13 ± 0.07 (not significant; $n = 5$) when epithelium was present.

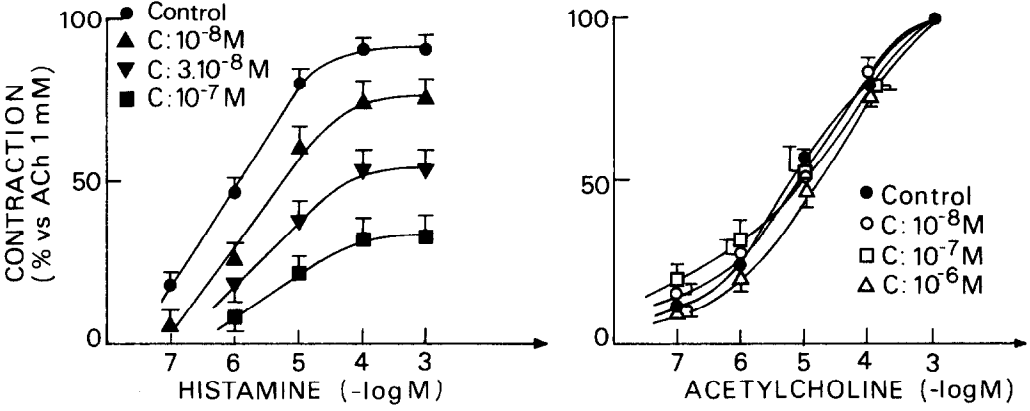


FIG. 3. Concentration-response curves for histamine or ACh on human isolated bronchi in the presence or absence of C. Values are means $\pm$ SEM ($n = 4$ to 6).

TABLE I. $-\log EC_{50}$ for histamine in the presence or in the absence of C and/or SAL on human isolated bronchi

Drugs (mol/L)	n	$-\log EC_{50}$	Reduction in $-\log EC_{50}$ (log unit) relative to the control	Additional shift in the $-\log EC_{50}$ for the combinations (log unit)
		Histamine		
Control	7	5.86 ± 0.12	—	—
C 10^{-8}	7	5.35 ± 0.11	$0.51 \pm 0.17^*$	—
SAL 10^{-7}	7	5.61 ± 0.16	0.25 ± 0.22	—
C 10^{-8} Plus SAL 10^{-7}	7	4.73 ± 0.28	$1.13 \pm 0.36^*$	$0.37 \pm 0.08^*$
Control	6	5.84 ± 0.14	—	—
C 10^{-8}	6	5.31 ± 0.12	$0.55 \pm 0.21^*$	—
SAL 3×10^{-7}	6	5.32 ± 0.16	0.51 ± 0.24	—
C 10^{-8} Plus SAL 3×10^{-7}	6	4.50 ± 0.59	1.32 ± 0.69	0.27 ± 0.39

n , Number of experiments.
The values presented are mean $\pm$ SEM.
*Significant variation, $p < 0.05$ (Student's t test).

Preventive addition of C. When C was added to the bath 1 hour before the start of the concentration-response curves to histamine and ACh, it inhibited the effect produced by histamine but did not modify the action of ACh (Fig. 3).
In the case of histamine, the concentration-response curves were displaced to the right, but the maximum response of this mediator was not maintained (Fig. 3), suggesting the existence of a noncompetitive antagonism.¹⁷

The C-SAL interaction

Influence of C and/or SAL on the concentration-response curves for histamine and ACh. Both C and SAL, or their combination, induced a displacement of the concentration-response curves for histamine

(Fig. 4). In Table I, the $-\log EC_{50}$ values for histamine under the various experimental conditions and those of the displacements of the concentration-response curves are presented. The displacements were significant when C was used alone ($\Delta -\log EC_{50}$, 0.51 ± 0.17 log unit, $n = 7$; $p < 0.05$, or 0.55 ± 0.21 log unit, $n = 6$; $p < 0.05$), but this was not the case with SAL.
The 10^{-8} mol/L of C plus 10^{-7} mol/L or 3×10^{-7} mol/L of SAL combination brought about a greater displacement than did the sum of the displacements induced by each substance added separately. The additional displacement was significant for the 10^{-7} mol/L of SAL concentration only (0.37 ± 0.08 log unit, $n = 7$; $p < 0.05$), suggesting, in this case, a potentiating type of synergy.

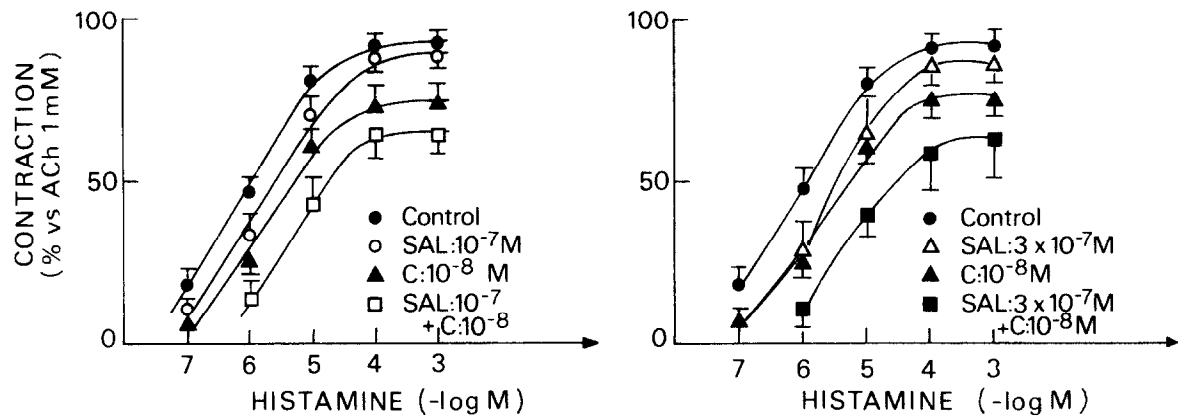


FIG. 4. Concentration-response curves for histamine on human isolated bronchi in the absence (●) or presence (▲) of 10^{-8} mol/L of C, 10^{-7} mol/L (○), or 3×10^{-7} mol/L (△) of SAL, or the 10^{-8} mol/L of C plus 10^{-7} mol/L of SAL (□) or 3×10^{-7} mol/L SAL combinations (■). Values are means $\pm$ SEM ($n = 6$ or 7 ; compare with Table I).

TABLE II. $-\log EC_{50}$ for histamine in the presence or absence of mepyramine and/or SAL on human isolated bronchi

Drugs (mol/L)	$-\log EC_{50}$	Reduction in $-\log EC_{50}$ (log unit) relative to the control	Additional shift in the $-\log EC_{50}$ for the combinations (log unit)
	Histamine		
Control	5.88 ± 0.08	—	—
Mepyramine 10^{-9}	5.58 ± 0.11	0.30 ± 0.08	—
Mepyramine 10^{-8}	5.23 ± 0.08	0.65 ± 0.05	—
Mepyramine 10^{-7}	4.37 ± 0.06	1.52 ± 0.10	—
SAL 10^{-7}	5.49 ± 0.16	0.39 ± 0.12	—
Mepyramine 10^{-9} plus SAL 10^{-7}	5.55 ± 0.17	0.33 ± 0.22	-0.33 ± 0.22
Mepyramine 10^{-8} plus SAL 10^{-7}	5.15 ± 0.18	0.75 ± 0.21	-0.28 ± 0.17

The values presented are mean $\pm$ SEM ($n = 6$).

In the case of the 10^{-8} mol/L of C plus 3×10^{-7} mol/L of SAL combination, the additional displacement (0.27 ± 0.39 log unit) was not significant, thus suggesting an additive effect. Under similar conditions, mepyramine did not induce a significant synergism (Table II).

As regards the concentration-response curves for ACh (Fig. 5; Table III), these curves were not significantly displaced by C (10^{-8} or 10^{-7} mol/L) or by SAL (3×10^{-7} mol/L) when they were added separately. When the two substances were combined, an additional displacement was observed that was significant for the 3×10^{-7} mol/L of SAL plus 10^{-7} mol/L C combination, suggesting, again, a potentiating type of synergy.

In the case of the C-SAL interaction versus ACh,

only the 3×10^{-7} mol/L concentration of SAL was studied because of the lower potency of this β -stimulant against ACh than against histamine.

Influence of ACh on the concentration-response curves for SAL: Role played by C on the ACh-SAL interaction. Under the influence of increasing concentrations of ACh increasing the level of contraction of isolated bronchi, the concentration-response curves for SAL were progressively displaced to the right, and the maximum effect produced by this substance was reduced, demonstrating a functional antagonism between SAL and ACh¹² (Fig. 6, A). The pD_2 and the E_{max} for SAL were significantly reduced ($p < 0.05$) for the 10^{-3} mol/L concentration of ACh (Student's t test) (Table IV). A significant effect ($p < 0.05$) of the four doses of ACh on the bronchi and a significant

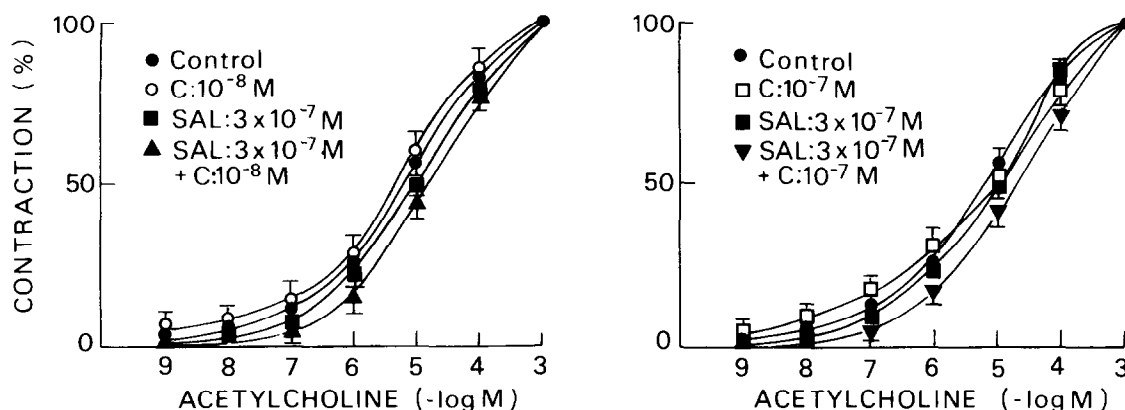


FIG. 5. Concentration-response curves for ACh on human isolated bronchi in the absence (●) or presence of 10^{-8} mol/L (○) or 10^{-7} mol/L (□) of C, 3×10^{-7} mol/L of SAL (■), or 3×10^{-7} mol/L of SAL plus 10^{-8} mol/L (▲) or 10^{-7} mol/L (▼) of C. Values are means $\pm$ SEM ($n = 6$ or 8; compare with Table III).

TABLE III. $-\log EC_{50}$ for ACh in the presence or absence of C and/or SAL on human isolated bronchi

Drugs (mol/L)	n	$-\log EC_{50}$	Reduction in $-\log EC_{50}$ (log unit) relative to the control	Additional shift in the $-\log EC_{50}$ for the combinations (log unit)
		ACh		
Control	6	5.01 ± 0.23	—	—
C 10^{-8}	6	5.27 ± 0.27	-0.26 ± 0.33	—
SAL 3×10^{-7}	6	5.05 ± 0.17	-0.02 ± 0.14	—
C 10^{-8}	6	4.90 ± 0.18	0.11 ± 0.18	0.39 ± 0.31
plus SAL 3×10^{-7}				
Control	8	4.95 ± 0.18	—	—
C 10^{-7}	8	5.05 ± 0.22	-0.10 ± 0.20	—
SAL 3×10^{-7}	8	5.01 ± 0.13	-0.06 ± 0.11	—
C 10^{-7} plus SAL 3×10^{-7}	8	4.64 ± 0.12	0.31 ± 0.20	$0.46 \pm 0.13^*$

n, Number of experiments.

The values presented are mean $\pm$ SEM.

*Significant variation, $p < 0.05$ (Student's *t* test).

linear dose/effect of ACh on the pD_2 ($p < 0.001$) and on E_{max} ($p < 0.01$) for SAL were demonstrated by means of a two-way analysis of variance.

In contrast, in the presence of C, the concentration-response curves for SAL against 10^{-3} mol/L of ACh were progressively displaced to the left with an increase in the maximum effect of SAL (Fig. 6, B; Table IV).

The two-way analysis of variance demonstrated a significant linear dose/effect of the addition of C on the E_{max} ($p < 0.05$) for SAL.

DISCUSSION

In the first part of this study, we analyzed the antihistaminic effect of C on human isolated bronchi and

the effect produced by this substance versus ACh. The results obtained clearly demonstrate the antihistamine activity of C on the human isolated bronchus, which is in accordance with the results obtained with in vitro animal models.¹⁸ In man, C was demonstrated to provide a significant degree of protection against the bronchospasm induced by histamine. Given as single oral dose of 5, 10, or 20 mg to 10 subjects with mild asthma, it provided a dose-dependent protective effect >25 mg of hydroxyzine.¹⁹ In a recent study on 10 patients with mild asthma, a single oral dose of C, 15 mg, produced a 74-fold increase over the placebo value of the provocative dose of histamine causing a fall of 20% in FEV_1 .²⁰ To compare our in vitro results with in vivo experiments in man in terms of concen-

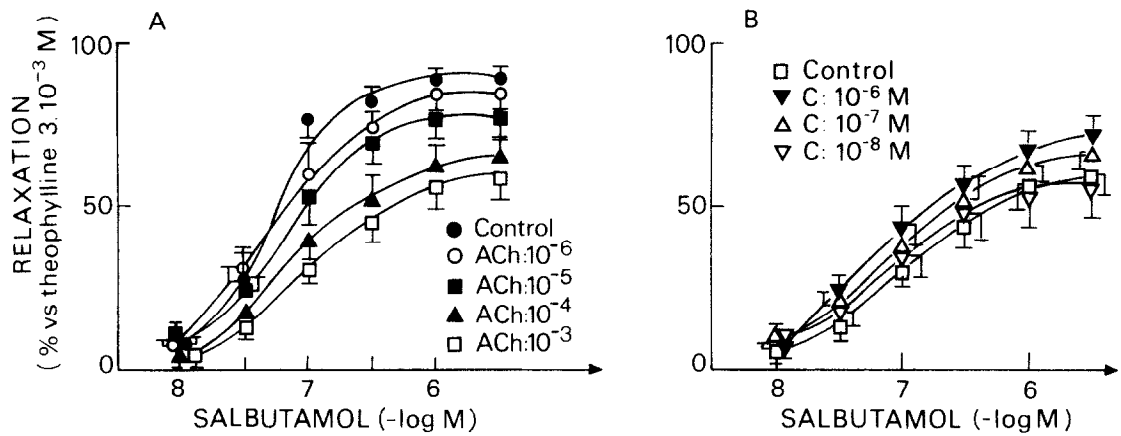


FIG. 6. A, Influence of the level of tension induced by 10⁻⁶ to 10⁻³ mol/L of ACh on the concentration-response SAL curves. B, Influence of 10⁻⁶ to 10⁻⁸ mol/L of C on the concentration-response SAL curves on preparations contracted by 10⁻³ mol/L of ACh. Values are means $\pm$ SEM (n = 8).

TABLE IV. pD₂ and maximum relaxant effect (E_{max}) produced by SAL relative to the increasing levels of contraction induced by 10⁻⁶ to 10⁻³ mol/L of ACh and the influence of 10⁻⁶ to 10⁻⁸ mol/L of C on the SAL-ACh combination

Drugs (mol/L)	ACh-Induced contraction (% ACh 10 ⁻³ mol/L)	pD ₂ SAL	E _{max} SAL (% vs theophylline 3 mmol/L)
Control	0	7.33 $\pm$ 0.07	83.2 $\pm$ 6.4
ACh 10 ⁻⁶	17 $\pm$ 4	7.30 $\pm$ 0.11	84.7 $\pm$ 2.9
ACh 10 ⁻⁵	61 $\pm$ 10	7.33 $\pm$ 0.10	70.7 $\pm$ 8.6
ACh 10 ⁻⁴	72 $\pm$ 14	7.14 $\pm$ 0.07	65.6 $\pm$ 5.8
ACh 10 ⁻³	100	7.02 $\pm$ 0.06‡	59.8 $\pm$ 6.4‡
ACh 10 ⁻³ Plus C 10 ⁻⁸	—	7.15 $\pm$ 0.06	57.4 $\pm$ 8.4‡
ACh 10 ⁻³ Plus C 10 ⁻⁷	—	7.12 $\pm$ 0.06‡	66.9 $\pm$ 5.6
ACh 10 ⁻³ Plus C 10 ⁻⁶	—	7.03 $\pm$ 0.12‡	71.8 $\pm$ 4.6

Values presented are mean $\pm$ SEM (N = 8).
Two-way analysis of variance:
*Global effect of the tested concentrations versus control, $p < 0.05$.
†Linear component of the tested concentrations, $p < 0.05$.
‡Significant differences from control (Student's t test), $p < 0.05$ (NS, not significant).

tration of drug, C plasma concentration after an oral administration of 10 mg are 359 μ g/L (0.78 μ mol/L) at 1 hour (maximum concentration) and 211 μ g/L (0.46 μ mol/L) at 3 hours, in agreement with the concentration range used in our experiments.²¹
In vitro, our results demonstrate that the action of C is the same irrespective of whether the product is studied by cumulative or preventive addition. Moreover, the antihistaminic action of C appears to be of a noncompetitive type in preparations of human bronchi. The therapeutic value of a noncompetitive antagonism has been underlined, because the binding of

noncompetitive antihistamines to the H₁-histamine receptors is only slowly reversible, and an excess of histamine produces little or no displacement of the antihistamine. Antagonists of this type might thus be expected to provide a potentially stronger and more prolonged protection against the effects of histamine than competitive antagonists.^{22, 23}
It was interesting to study the results of abrasing the bronchial epithelium on the antihistaminic action of C, since epithelial lesions are regarded as being one of the possible factors underlying bronchial hyperactivity.¹¹ Our results demonstrate that neither the

potency nor the efficacy (maximum effect) of C is altered by epithelium removal. These results are comparable to results previously obtained with theophylline or papaverine on isolated guinea pig trachea.^{24, 25} With this preparation, however, abrasion of the epithelium reduces the efficacy but not the potency of theophylline.^{26, 27}

Finally, this study demonstrates that C can exert a significant, although it is very modest, effect on human isolated bronchi in the basal tone and has no anticholinergic activity. The mild activity observed in our experiments (Fig. 1) could come from the effect observed on bronchi in the basal tone. An in vitro study with the receptor-binding technique on guinea pig brain membranes has demonstrated that C does not displace the cholinergic muscarinic ligand quinuclidinyl benzilate (inhibitory concentration of 50%, $>10^{-6}$ mol/L equals 4.61 μ g/ml).²⁸ On the guinea pig isolated trachea, carbachol-induced contraction is not inhibited by high concentration of C (up to 147 μ g/ml).¹⁸ In an in vivo model of induced bronchospasm in guinea pig, C was inactive on ACh-induced spasms.²⁹ Furthermore, in 14 subjects with asthma, a 2-week therapy with C, 10 mg twice daily, during a period of stable nonspecific bronchial reactivity, did not cause any significant change in methacholine reactivity.³⁰

In the second part of this study, we studied the interactions between C and SAL. We demonstrated that, when the bronchi were pretreated with 10^{-8} mol/L of C and/or 10^{-7} mol/L or 3×10^{-7} mol/L of SAL, the concentration-response curves for histamine were displaced to the right and that C and SAL acted synergistically; the nature of this synergy depended on the concentration of SAL, being potentiating at a small dose and additive at a large dose. However, no clear explanation for this SAL dose-related difference is available. Under similar conditions, mepyramine does not potentiate the effects of SAL, which suggests that the action of C is not related to its antihistaminic activity.

As regards ACh, analysis of the SAL plus C combination was carried out only with a high (3×10^{-7} mol/L) concentration of SAL, since the effect produced by this combination requires higher concentrations of β -stimulants than is necessary with histamine.³¹

Our experiments have demonstrated that 10^{-8} and 10^{-7} mol/L of C and 3×10^{-7} mol/L of SAL do not have any effect by themselves on the concentration-response curves for ACh, but that for the high 10^{-7} mol/L concentration of C, a significant synergy becomes apparent.

The permissive action of C on the effect produced by SAL has also been demonstrated by the partial reversion of the functional antagonism that exists between ACh and SAL. The functional antagonism phenomenon consists of reduction in efficacy (maximum effect) and in potency ($-\log EC_{50}$) of the relaxant effect of a β -stimulant when the smooth muscle has been contracted by increasing concentrations of the contracting agent.¹² It is said to be functional because ACh and β -stimulants act on different receptors, and it appears to be based, biochemically, on inhibition by ACh of the protein kinase that is dependent on the amount of cyclic adenosine monophosphate stimulated by the β -stimulants.^{32, 33} Our results demonstrate that C, even though devoid of any anticholinergic effect, is capable of increasing the efficacy of SAL, when this efficacy is reduced by the highest concentration of ACh, and that this effect is significantly dose related. A similar effect on the potency of a β -stimulant has also been described for the ipratropium-fenoterol combination on human isolated bronchi.³⁴

The potentiation by C of SAL effects might prove of interest if it were observed in patients with asthma. Other antihistamines have been studied in subjects with asthma to observe their effect produced on the use of β -stimulants.^{1, 2, 5, 6} Thus, Taytard et al.⁶ have found that terfenadine (120 mg twice daily) reduced the use of SAL in subjects with asthma, the figures given being 2.1 ± 0.4 puffs per day in the terfenadine-treated group and 2.9 ± 0.5 in the placebo-treated group. This effect, although it is statistically significant, nevertheless, was only of marginal clinical interest. Holgate and Finnerty² have demonstrated a greater reduction (40%) in the use of β_2 -adrenergic stimulant but only with very large doses of terfenadine (180 mg three times a day). If the potentiating action exerted by C on the bronchodilator effect of SAL is independent of its antihistaminic activity, as revealed by the absence of a similar effect with mepyramine, then a beneficial effect could be expected in some subjects with asthma during maintenance therapy with combined β_2 -agonists and C.

REFERENCES

1. Simons FER. H_1 -receptor antagonists: clinical pharmacology and therapeutics. *J ALLERGY CLIN IMMUNOL* 1989;84:845-61.
2. Holgate ST, Finnerty JP. Antihistamine in asthma [Symposium]. *J ALLERGY CLIN IMMUNOL* 1989;83:537-47.
3. Bowman WC, Rand MJ. Local hormones and antacids: biogenic amines, purines, kinins, and prostaglandins. In: Bowman WC, Rand MJ, eds. *Textbook of pharmacology*. Oxford: Blackwell, chap 12, 1980:12.2-16.
4. Nimegeers CJE, Awouters FHL, Janssen PAJ. The in vivo

- pharmacological profile of histamine H₁-antagonists in the rat. *Drug Develop Res* 1982;2:559-66.
5. Holgate ST, Emanuel MB, Howarth PH. Astemizole and other H₁-antihistamine drug treatment of asthma. *J ALLERGY CLIN IMMUNOL* 1985;76:375-80.
6. Taytard A, Beaumont D, Pujet JC, Sapene M, Lewis PS. Treatment of bronchial asthma with terfenadine: a randomized controlled trial. *Br J Clin Pharmacol* 1987;24:743-6.
7. Pechadre JC, Vernay D, Trolese JF, Bloom M, Dupont P, Rihoux JP. Comparison of the central and peripheral effects of cetirizine and terfenadine. *Eur J Clin Pharmacol* 1988; 35:255-9.
8. Fadel R, Herpin-Richard N, Henocq E, Rihoux JP. Inhibitory effect of cetirizine 2 HCl on eosinophil migration in vivo. *Clin Allergy* 1987;7:373-9.
9. Michel L, De Vos C, Rihoux J-P, Burtin C, Benveniste J, Dubertret L. Inhibitory effect of oral cetirizine on in vivo antigen-induced histamine and PAF-acether release and eosinophil recruitment in human skin. *J ALLERGY CLIN IMMUNOL* 1988;82:101-9.
10. Charlesworth EN, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Effect of cetirizine on mast cell-mediator release and cellular traffic during the cutaneous late-phase reaction. *J ALLERGY CLIN IMMUNOL* 1989;83:905-12.
11. Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J ALLERGY CLIN IMMUNOL* 1989;83:1013-26.
12. Van den Brink FG. The model of functional interaction. II. Experimental verification of a new model: the antagonism of beta-adrenoceptor stimulants and other antagonists. *Eur J Pharmacol* 1973;22:279-86.
13. Brink G, Grimaud C, Guillot C, Orehek J. The interaction between indomethacin and contractile agents on human isolated airway muscle. *Br J Pharmacol* 1980;69:383-8.
14. Advenier C, Naline E, Renier A. Effects of Bay K 8644 on contraction of the human isolated bronchus and guinea pig isolated trachea. *Br J Pharmacol* 1986;88:33-9.
15. Devillier P, Advenier C, Drapeau G, Marsac J, Regoli D. Comparison of the effects of epithelium removal and of an enkephalinase inhibitor on the neurokinin-induced contractions of guinea pig isolated trachea. *Br J Pharmacol* 1988;94:675-84.
16. Naline E, Devillier P, Drapeau G, Toly L, Bakdach H, Regoli D, Advenier C. Characterization of neurokinin effects and receptor selectivity in human isolated bronchi. *Am Rev Respir Dis* 1989;140:679-86.
17. Arunlakshana O, Schild MO. Some quantitative use of drug antagonists. *Br J Pharmacol Chemother* 1959;14:48-58.
18. De Vos C, Maleux MR, Gobert J. In vitro pharmacologic profile of cetirizine 2 HCl, a new non sedating antiallergic drug. *Allergol Immunol Clin* 1987;2:82.
19. Brik A, Tashkin DP, Gong H, Dauphinee B, Lee E. Effect of cetirizine, a new histamine H₁ antagonist, on airway dynamics and responsiveness to inhaled histamine in mild asthma. *J ALLERGY CLIN IMMUNOL* 1987;80:51-6.
20. Ghosh SK, De Vos C, Mc Ilroy I, Patel KR. Effect of cetirizine on histamine and leukotriene LTD₄-induced bronchoconstriction in mild atopic asthmatics. *Clin Exp Allergy* 1990;20:39.
21. Wood SG, John BA, Chasseaud LF, Yeh J, Chung M. The metabolism and pharmacokinetics of ¹⁴C-cetirizine in humans. *Ann Allergy* 1987;59:31-4.
22. Reinhardt D, Borchard U. H₁-receptor antagonists: comparative pharmacology and clinical use. *Klin Wochenschr* 1982; 60:983-90.
23. Trzeciakowski JP, Levi R. Antihistamines. In: Middleton E Jr, Reed CE, Ellis EF, eds. *Allergy: principles and practice*, vol 1. 2nd ed. St Louis: CV Mosby, 1983:575-92.
24. Farmer SG, Fedan JS, Hay DWP, Raeburn D. The effects of epithelium removal on the selectivity of guinea pig isolated trachealis to bronchodilator drugs. *Br J Pharmacol* 1986; 89:407-14.
25. Candenas ML, Naline E, D'Ocon MP, Cortes D, Advenier C. Effects of cularine and other isoquinoline alkaloids on guinea pig trachea and human bronchus. *J Pharm Pharmacol* 1990; 42:102-7.
26. Goldie RG, Papadimitriou JM, Paterson JW, Rigby PJ, Self HM, Spina D. Influence of the epithelium on responsiveness of guinea pig isolated trachea to contractile and relaxant agonists. *Br J Pharmacol* 1986;87:5-14.
27. Lundblad Kal, Persson CGA. The epithelium and the pharmacology of guinea pig tracheal tone in vitro. *Br J Pharmacol* 1988;93:909-17.
28. Snyder SH, Snowman AM. Receptor effects of cetirizine. *Ann Allergy* 1987;59:4-8.
29. De Vos C, Maleux MR, Gobert J. Antiallergic properties of cetirizine 2 HCl in various models of anaphylaxis in animals. *Allergol Immunol Clin* 1987;2:81.
30. Finnerty JP, Holgate ST, Rihoux JP. The effects of two weeks treatment with cetirizine on bronchial reactivity to methacholine in asthma. *Br J Clin Pharmacol* 1990;29:79-84.
31. Advenier C, Cerrina J, Duroux P, Floch A, Renier A. Effects of five different organic calcium antagonists on guinea pig isolated trachea. *Br J Pharmacol* 1984;82:727-33.
32. Torphy TJ, Rinard GA, Rietow MG, Mayer SE. Functional antagonism in canine tracheal smooth muscle: inhibition by methacholine of the mechanical and biochemical responses to isoproterenol. *J Pharmacol Exp Ther* 1983;227:694-9.
33. Torphy TJ, Zheng C, Peterson SM, Fiscus RR, Rinard GA, Mayer SE. Inhibitory effect of methacholine on drug-induced relaxation, cyclic AMP accumulation, and cyclic AMP-dependent protein kinase activation in canine tracheal smooth muscle. *J Pharmacol Exp Ther* 1985;233:409-17.
34. Advenier C, Naline E, Matran R, Toly L, Bakdach H. Interaction between fenoterol, ipratropium, and acetylcholine on human isolated bronchus. *J ALLERGY CLIN IMMUNOL* 1988; 82:40-6.