Br. J. Pharmac. (1981), **74**, 73–79

INFLUENCE OF TRACHEAL CONTRACTION ON RELAXANT EFFECTS *in vitro* OF THEOPHYLLINE AND ISOPRENALINE

J.-A. KARLSSON & C.G.A. PERSSON

Research and Development Laboratories, AB Draco, Box 1707, S-221 01 Lund, Sweden

1 Relaxation by (−)-isoprenaline (Iso) and theophylline (Theo) was measured in guinea-pig isolated trachea, in the presence or absence of carbachol.

2 With basal tone or with carbachol at a concentration of 5.4×10^{-7} M, causing 70% maximal contraction, Iso and Theo relaxed the trachea to the same extent.

3 With carbachol concentrations of 5.4×10^{-6} M and 5.4×10^{-5} M (96% and 100% maximal contractions) Iso caused no more than 63% and 34%, respectively, of the maximum relaxation to Theo.

4 When calculated at 25% of the maximum Theo relaxation, the Iso/Theo potency ratio was gradually reduced from 14,160 when evaluated at basal tone to 1,560 at the highest carbachol concentration.

5 In combination, at their maximally effective concentrations, Theo and Iso produced no larger a relaxation than did Theo alone.

6 At the two highest concentrations of carbachol, concentration-response curves to Theo were virtually superimposable whether determined in the absence or the presence of Iso at its maximally effective concentration.

7 It is concluded that Theo causes a greater relaxation of highly contracted tracheal muscle than Iso.

Introduction

It is generally held that relaxation of bronchial smooth muscle is the most important anti-asthmatic effect of β-adrenoceptor stimulants as well as of methylxanthines (Kahn, 1907; Trendelenburg, 1912; Paterson, Woolcock & Shenfield, 1979). Theoretically, both types of drugs may act by increasing the intracellular level of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), through stimulation of adenylate cyclase and inhibition of phosphodiesterase, respectively. However, alternative mechanisms must be considered in particular with theophylline (Kolbeck, Speir, Carrier & Bransome, 1979; Bergstrand, 1980).

One way of examining differences between methylxanthines and β-receptor agonists is to study relaxant properties in isolated bronchi contracted to a varying extent by mediators of asthma. It is well established that the potency and degree of relaxation induced by β-adrenoceptor agonists are gradually reduced in the presence of increasing concentrations of a muscarinic agent such as carbachol (van den Brink, 1973; Buckner & Saini, 1975; O'Donnell & Wanstall, 1977). However, it is still debatable as to what extent the effects of xanthines may depend on the tonus of the airway smooth muscle (cf. Jones, Hamilton & Lefcoe, 1974; Olsson & Persson, 1976).

In the present study the relaxant effect of theophylline (Theo) was examined and compared with isoprenaline (Iso) in guinea-pig isolated tracheal preparations. The effects have been evaluated on basal tone as well as in the presence of different carbachol concentrations. In addition, some aspects of the combined drug actions have been studied.

These results have been presented in part at the Joint Meeting of the Scandinavian and German Pharmacological Societies (Karlsson & Persson, 1980).

Methods

Guinea-pigs of either sex weighing 200–400 g were used in the study. They were killed by a blow on the head and the trachea was dissected out. Two consecutive tracheal rings were carefully cut out and a thread tied to the cartilage on each side of the muscle. Rings were cut from different portions of the trachea and two to four preparations were obtained from each animal. After the cartilage had been cut between the threads, the open ring preparation was mounted in a jacketed, temperature controlled (37°C), 50 ml organ bath with Krebs solution of the following composition (mM): NaCl 118.0,

0007-1188/81/09 0073-07 $01.00

KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, glucose 5.5, with a pH of 7.4. The Krebs solution was gassed with 95% O_2 and 5% CO_2.

Changes in muscle tension were measured isometrically via strain-gauge transducers (Grass FT03). The preparations were adjusted to an initial tension of about 0.6 g. They were allowed to rest for 30–60 min before any drugs were added. During the resting period the muscle tonus mostly increased until a stable tension was attained which varied between 0.4 and 1.2 g. Some preparations exhibited spontaneous activity but this had always ceased before the resting period was over.

The drugs were added by injection to the organ bath in a cumulative geometric progression of concentrations, until no further effect was obtained. On a total of 72 preparations, 142 concentration-response (C/R) evaluations were made. As a rule, each specific drug evaluation was done on two different rings from the same animal yielding a mean result which was used for statistical calculations. Each C/R line that is illustrated is calculated from results obtained in six to nine animals. Mean values ± s.e.mean were calculated for each concentration of the relaxant drugs. Regression analysis was made of the linear portion of the C/R lines. Differences were examined by Students' *t* test for unpaired observations.

Substances used were: carbamylcholine chloride (Ph. Nord.), (−)-isoprenaline hydrochloride (Sigma), theophylline, anhydrous (Knoll). Stock solutions were made up daily, theophylline with an additional equivalent of 0.5 M NaOH and (−)-isoprenaline with 0.1 mg/ml ascorbic acid.

Results

Contraction by carbachol

Cumulative C/R curves for carbachol were evaluated on 6 preparations (Figure 1). The calculated pD_2 value was 6.70 ± 0.11, well in accordance with values from other types of guinea-pig tracheal preparations (Buckner & Saini, 1975; Spilker & Minatoya, 1975; Hooker, Calkins & Fleisch, 1977). Three different concentrations of carbachol, 5.4×10^{-7} M, 5.4×10^{-6} M and 5.4×10^{-5} M, were selected, corresponding to 70%, 96% and 100% of the maximum tension produced by carbachol.

Relaxation by theophylline (Theo)

Cumulative C/R curves for Theo are shown in Figure 2. With each increase in carbachol concentration the

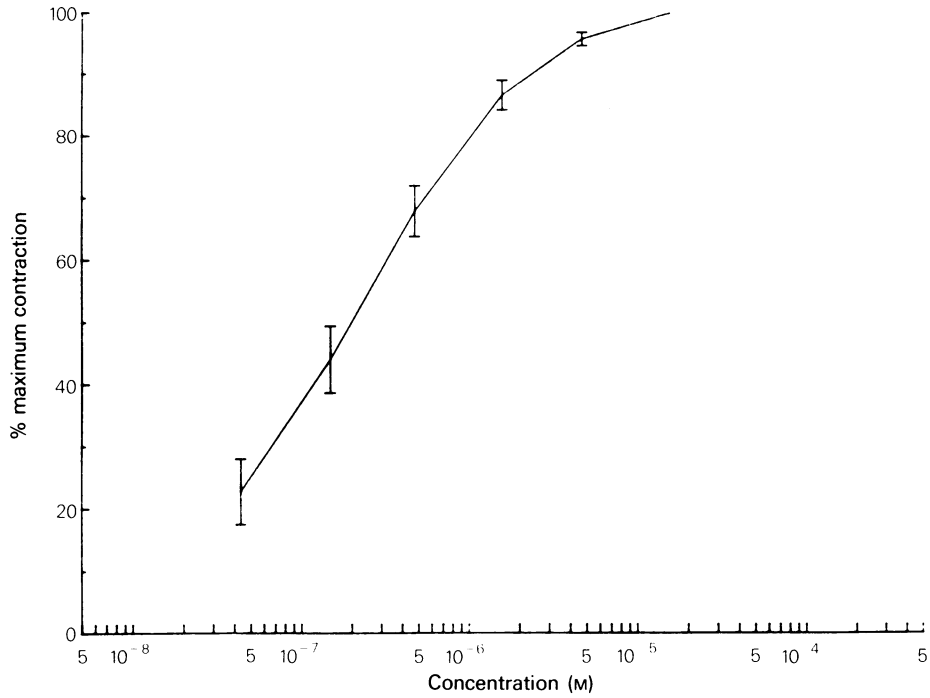

Figure 1 Cumulative concentration-response (C/R) curves for carbachol-induced contraction of guinea-pig isolated trachea. On the ordinate scale, contraction is expressed as a percentage of its own maximum. Mean results of 6 experiments are shown; vertical bars indicate s.e.mean.

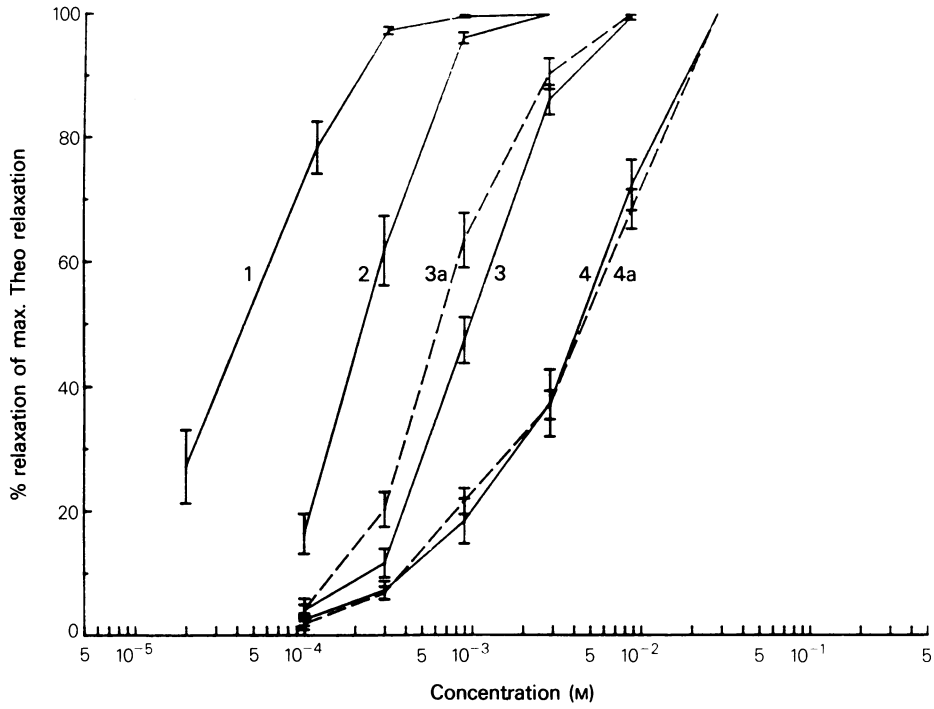

Figure 2 The complete lines illustrate cumulative concentration-response (C/R) curves for theophylline (Theo) on guinea-pig isolated tracheal muscle. The muscles were either spontaneously contracted (1) or contracted by carbachol: 5.4×10^{-7} M (2), 5.4×10^{-6} M (3) and 5.4×10^{-5} M (4). The broken C/R curves represent the effects of Theo when evaluated in the presence of a maximally effective isoprenaline (Iso) concentration. These two sets of tracheas were contracted by carbachol: 5.4×10^{-6} M (3a) and 5.4×10^{-5} M (4a). Mean results are shown; vertical bars indicate s.e.mean.

curves were shifted in parallel to the right, without any tendency of the shift to reach a maximum (Figure 2). When measured from each new level of tone, the amplitude of maximum relaxation to Theo became smaller. This change has been expressed by measuring the ratio of the size of the subsequent maximal Theo-induced relaxation to the size of the carbachol-induced contraction (see Table 1). The ratio fell from 2.0 to 1.0 as the carbachol concentration was increased from 5.4×10^{-7} M to 5.4×10^{-5} M. Addition of Iso after a maximal Theo relaxation did not produce any further relaxation at any carbachol concentration.

Relaxation by isoprenaline (Iso)

Cumulative C/R curves to Iso are shown in Figure 3. Also, in the presence of a maximally effective concentration of Iso, the C/R to Theo was evaluated. When expressing the Iso effect as a percentage of the maximum Theo relaxation (Figure 3), the curves were shifted to the right, the maximum depressed and the slopes flattened as the carbachol concentration was increased.

Iso relaxed, to the same extent as Theo, tracheal preparations with spontaneous tone and also those incubated in the low concentration of carbachol. In the presence of higher concentrations of carbachol (5.4×10^{-6} M and 5.4×10^{-5} M) Iso was less effective, its maximal effect being 63 and 34%, respectively, that of Theo. When the effect of Iso was calculated as a percentage of its own maximum, the rightward shift of the C/R lines reached a maximum at a carbachol concentration of 5.4×10^{-6} M (cf. EC_{50} values for Iso at normal pH shown in Table 3).

When measured from each new level of tone, the amplitude of maximum relaxation to Iso became smaller. The ratio, measured as for Theo described earlier, decreased from 2.0 to 0.3 as the carbachol concentration was increased from 5.4×10^{-7} M to 5.4×10^{-5} M (see Table 1).

Combined actions of theophylline and isoprenaline

In the presence of the two highest carbachol concentrations, where Theo was the more effective relaxant, the combined actions of Iso and Theo were evaluated. In preparations already relaxed by a maxi-

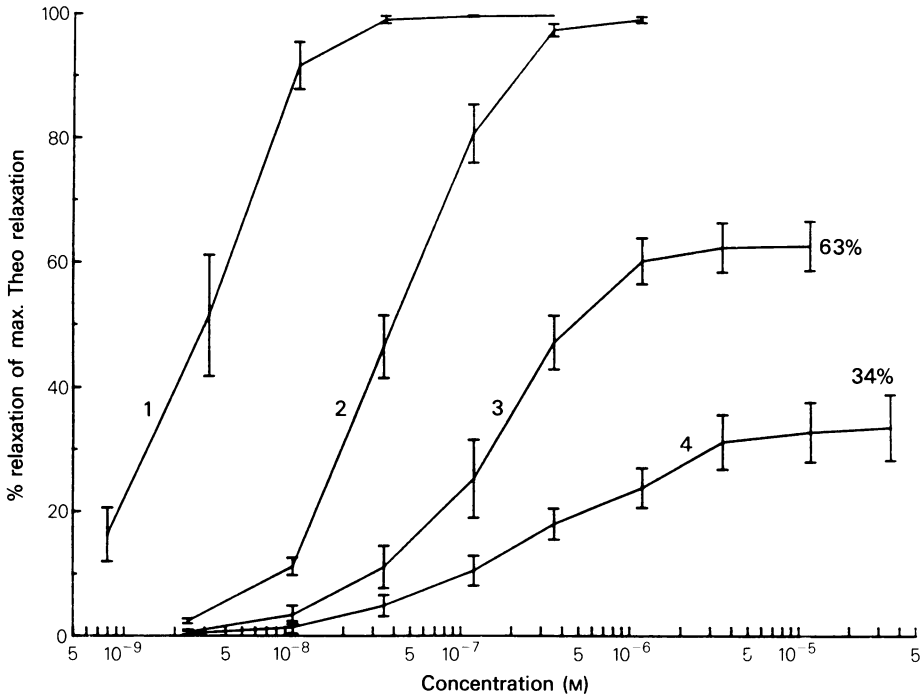

Figure 3 Cumulative concentration-response (C/R) curves for isoprenaline (Iso)-induced relaxation of guinea-pig isolated trachea. On the ordinate scale, relaxation is expressed as a percentage of the maximum theophylline effect. The effect of Iso was evaluated on either spontaneously contracted trachea (1) or on trachea contracted by different concentrations of carbachol: 5.4×10^{-7} M (2), 5.4×10^{-6} M (3) and 5.4×10^{-5} M (4). Mean results are shown; vertical bars indicate s.e.mean.

mally effective concentration of Iso, further relaxation occurred with cumulatively added Theo and the curves were almost superimposable on those caused by Theo alone (Figure 2). In the presence of the medium carbachol concentration, the C/R line for the additional relaxation by Theo was shifted leftwards 1.4 times compared to that obtained with Theo alone (confidence limits 1.08–1.7, $P<0.01$). No shift was found with the highest carbachol concentration. The maximum relaxation with Theo and Iso combined was slightly larger than that produced by Theo alone at the medium carbachol concentration (Table 1), whereas at the highest carbachol concentration no difference was seen.

Potency ratios between isoprenaline and theophylline

The potency ratios between Iso and Theo in different contractile states were calculated, based on concentrations of each drug producing 25% of the maximum Theo-relaxation. These ratios thus defined potency differences between Iso and Theo on the linear por-

Table 1 The ratio of the size of the maximum relaxation (g) produced by isoprenaline (Iso), theophylline (Theo) or their combination with respect to the size of the carbachol-induced contraction (g)

Carbachol (M)	*Iso*	n	*Theo*	n	*Iso + Theo*	n
5.4×10^{-7}	2.0±0.2*	6	2.0±0.4	6		
5.4×10^{-6}	1.0±0.1	8	1.4±0.2	8	1.6±0.2	8
5.4×10^{-5}	0.3±0.1	6	1.0±0.1	6	1.0±0	5

Mean results ± s.e.mean are given.
*A value of 1 equals complete inhibition of the contraction by carbachol. A value > 1 means that the trachea was relaxed below the basal tone that existed before the addition of carbachol. Conversely, a value < 1 indicates that the drug could not fully relax the tissue to the level of basal tone.

Table 2 Negative log molar EC_{25} values for theophylline (Theo) and isoprenaline (Iso) on tracheas spontaneously contracted or contracted by different concentrations of carbachol

	Carbachol concentration (M)			
	0	5.4×10^{-7}	5.4×10^{-6}	5.4×10^{-5}
Theo EC_{25}	4.66 ± 0.06	3.90 ± 0.03	3.34 ± 0.05	2.84 ± 0.09
n	8	7	9	8
Iso EC_{25}	8.81 ± 0.09	7.79 ± 0.07	6.92 ± 0.18	6.04 ± 0.17
n	9	7	9	5
Potency ratio	14125	7762	3802	1585

Concentrations are expressed as −log M (±s.e.mean). EC_{25} values calculated from the drug concentration yielding 25% of the maximum effect produced by Theo.

tion of the C/R lines to both drugs and for each carbachol concentration, at the same degree of relaxation.

The Iso/Theo potency ratio was gradually reduced from about 15000 at basal tone to about 1500 at the highest carbachol concentration (Table 2).

Influence of pH on the relaxation

Theo had to be dissolved as the sodium-salt and the addition of high concentrations of Theo produced a rise in pH of the Krebs solution (Table 3). Alkalosis under *in vitro* and *in vivo* conditions has earlier been shown to affect only slightly the relaxant and contractile effects of drugs in bronchial smooth muscle (Stephens, Meyers & Cherniack, 1968; Baïsset, Montastruc & Tran, 1971; Duckles, Rayner & Nadel, 1974). It was still of interest to examine whether the increased pH influenced the present comparison between Iso and Theo.

Control experiments were thus performed with Iso in a Krebs solution where pH was elevated by addition of 0.5 M NaOH (Table 3).

There was no sign of breakdown of Iso during the alkalosis, i.e. no colour change occurred and normal, sustained relaxations were produced by Iso. The carbachol-induced contraction was not affected by the alkalosis and the C/R curves for Iso were identical with those obtained under normal conditions (Table 3, cf. Figure 3). It was concluded that the pH-changes in the organ bath did not affect the present comparison between Iso and Theo.

Discussion

β-Adrenoceptor stimulants and methylxanthines are about equally widely used in the therapy of obstructive lung disease. In studies on drug actions, it seems that attention has been focused mainly on various aspects of β-receptor agonists. This is reflected in the abundance of studies dealing with relaxant characteristics of β-receptor agonists on bronchi (van den Brink, 1973; Raper & Malta, 1973; Buckner & Saini, 1975; O'Donnell & Wanstall, 1977). Some comparisons between β-stimulants and methylxanthines have previously been made in isolated airway smooth muscle preparations. These studies have shown that in tracheal preparations moderately contracted by various agents, β-agonists and Theo are about equally effective, with relative potencies that are proportional to the potencies found clinically (Dungan & Lish, 1961; Olsson & Persson, 1976). In addition, β-receptor stimulation did not produce a further relax-

Table 3 Negative log molar EC_{50} (± s.e.mean) values of theopylline and isoprenaline in relaxing carbachol-contracted tracheal muscle and corresponding pH values

Carbachol (M)	*pH*	*Theophylline* $-\log EC_{50}$	n	*pH* (a)	*Isoprenaline* $-\log EC_{50}$	n	*pH* (b)	$-\log EC_{50}$	n
0	7.5	4.30 ± 0.05	8	7.4	8.52 ± 0.09	9			
5.4×10^{-7}	7.6	3.64 ± 0.04	7	7.4	7.41 ± 0.06	7	7.9	7.49 ± 0.02	4
5.4×10^{-6}	7.6	3.02 ± 0.03	9	7.4	6.85 ± 0.13	9	8.2	6.93 ± 0.06	4
5.4×10^{-5}	7.8	2.42 ± 0.08	8	7.4	6.65 ± 0.22	6	9.0	6.25 ± 0.55	2

Theo pH values were measured with the corresponding concentration of Theo in Krebs solution. With Iso, EC_{50} values were determined (a) in normal Krebs solution at pH 7.4 and (b) in Krebs solution with sufficient NaOH added to adjust pH as shown.

ant effect when added to human isolated large and small bronchi that had been relaxed by maximally effective Theo concentrations (Persson, 1980). Other workers comparing β-agonists and Theo in guinea-pig tracheas have suggested that carbachol may affect relative potency but not the degree of relaxation (Jones *et al.*, 1974). This is at variance with findings on bovine trachea where Theo fully (100%) and Iso partially (approx. 75%) relaxed the tension induced by carbachol, 4×10^{-7} M (Andersson, Kövesi & Ericsson, 1978). Working with cat isolated trachea, Mitchell & Denborough (1979) found that a high concentration of Theo (10 mM) caused a flattening of the dose-response curve to acetylcholine while a 100,000 times lower concentration of Iso (0.1 μM) only displaced the curve to the right. Thus in earlier literature there was some information suggesting that in contracted tracheas the mechanical response to β-agonists might differ from that to Theo.

Because of its use in the earlier studies with β-agonists (Buckner & Saini, 1975) it was natural to select carbachol as the contractile agent in the present study. This drug would also show the contraction characteristics of acetylcholine which may be the key mediator among all the possible non-allergic and allergic asthma mediators. The major importance of acetylcholine in asthma is indicated by observations that atropine often is clinically effective although it antagonizes this mediator only (cf. Crompton, 1968; Simonsson, 1977). The use of tracheal smooth muscle from guinea-pig was justified by findings that it may react similarly to more peripheral airways as well as to human isolated bronchi (Persson & Ekman, 1976).

At basal tone and in the presence of the low carbachol concentration, Iso and Theo were found to relax the tracheas to the same extent, Iso being about 10,000 times more potent than Theo. This is what can be expected in moderately contracted preparations (Dungan & Lish, 1961; Olsson & Persson, 1976). However, by increasing the concentration of carbachol above 5.4×10^{-7} M, the degree of relaxation by Iso, relative to that caused by Theo, was gradually reduced. At the highest carbachol concentration (5.4×10^{-5} M) Iso produced one third of the maximal relaxant response to Theo. Additionally, when evaluated at spontaneous tone and at the progressively increasing carbachol concentrations, potency ratios between the two relaxants were gradually reduced (from about 15,000 to 1,500).

Among the β-receptor agonists, Iso is the most effective relaxant of highly contracted tracheal preparations (Raper & Malta, 1973), and a possible clinical importance of differences in effectiveness among β-agonists has been suggested (O'Donnell & Wanstall, 1978). The present findings related to Iso and carbachol are in agreement with earlier observations (Buckner & Saini, 1975). The relaxant properties of Theo were also affected by carbachol but, as discussed above, less so than Iso. The results thus showed Theo to be a more efficient relaxant of highly contracted tracheal muscle than Iso. The importance of this difference is difficult to assess. It is for instance not known if the results can be confirmed *in vivo*. Furthermore, relaxation of bronchial smooth muscle may be just one of many pulmonary effects that can be produced by β-agonists and methylxanthines. Comparisons of effectiveness of Theo and β-receptor agonists should also be of interest concerning e.g. effects on mediator-induced oedema (Persson, Ekman & Erjefält, 1979) and anaphylactic reactions in the lung (Andersson, 1980).

It seems likely that Iso may initiate relaxation of tracheal smooth muscle by stimulating adenylate cyclase and thus raising the level of cyclic AMP (Andersson, 1972; Andersson *et al.*, 1978). Theo, being a phosphodiesterase inhibitor, might also act by increasing the intracellular level of cyclic AMP but the importance of this mechanism has been seriously questioned. For instance, Kolbeck and co-workers (1979) working with guinea-pig and canine tracheal tissues showed that theophylline-induced relaxant effects were not associated with changes in the intracellular level of cyclic AMP. Our finding, that the sensitivity of the tracheal muscle to the action of Theo was unaffected whether in the presence or absence of Iso, a drug that effectively stimulates adenylate cyclase, seems to agree with the view that mechanisms of action other than phosphodiesterase inhibition are involved in the relaxant effects of Theo.

References

ANDERSSON, P. (1980). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: anti-anaphylactic effects of sodium cromoglycate and aminophylline. *Br. J. Pharmac.*, **69**, 467–472.

ANDERSSON, R. (1972). Cyclic AMP and calcium ions in mechanical and metabolic responses of smooth muscles; influence of some hormones and drugs. *Acta Physiol. scand., Suppl.*, **382**, 1–59.

ANDERSSON, R.G.G., KÖVESI, G. & ERICSSON, E. (1978). Beta-adrenoceptor stimulation and cyclic AMP levels in bovine tracheal muscle of old and young animals. *Acta pharmac. tox.*, **43**, 323–327.

BAÏSSET, A., MONTASTRUC, P. & TRAN, M.-A. (1971). Étude expérimentale de l'influence des variations du pH sanguin sur les effets bronchiques de divers médicaments. *Phat.-Biol.*, **19**, 1061–1067.

BERGSTRAND, H. (1980). Phosphodiesterase inhibition and theophylline. *Eur. J. Resp. Dis. Suppl.*, **109, 61,** 37–44.

BRINK VAN DEN, F.G. (1973). The model of functional interaction II. Experimental verification of a new model: the antagonism of β-adrenoceptor stimulants and other agonists. *Eur. J. Pharmac.*, **22,** 279–286.

BUCKNER, C.K. & SAINI, R.K. (1975). On the use of functional antagonism to estimate dissociation constants for beta-adrenergic receptor agonists in isolated guinea-pig trachea. *J. Pharmac. exp. Ther.*, **194,** 565–574.

CROMPTON, G.K. (1968). A comparison of responses to bronchodilator drugs in chronic bronchitis and chronic asthma. *Thorax*, **23,** 45–55.

DUCKLES, S.P., RAYNER, M.D. & NADEL, J.A. (1974). Effects of CO_2 and pH on drug-induced contractions of airway smooth muscle. *J. Pharmac. exp. Ther.*, **190,** 472–481.

DUNGAN, K.W. & LISH, P.M. (1961). Bronchodilators: some qualitative and quantitative pharmacologic correlates in the guinea-pig. *J. Allergy*, **32,** 139–151.

HOOKER, C.S., CALKINS, P.J. & FLEISCH, J.H. (1977). On the measurement of vascular and respiratory smooth muscle responses in vitro. *Blood Vessels*, **14,** 1–11.

JONES, T.R., HAMILTON, J.T. & LEFCOE, N.M. (1974). Variations in the relative potencies of bronchodilators in the presence and absence of carbachol-induced tone. *Can. J. Physiol. Pharmac.*, **52,** 358–361.

KAHN, R.H. (1907). Zur Physiologie der Trachea. *Arch. Anat. Physiol.*, 398–418.

KARLSSON, J.-A. & PERSSON, C.G.A. (1980). Theophylline relaxes the trachealis muscle to a greater extent than isoprenaline. *Naunyn Schmiedebergs Arch. Pharmac.*, **313** (R47).

KOLBECK, R.C., SPEIR, JR., W.A., CARRIER, G.O. & BRANSOME, JR. E.D.C. 1979). Apparent irrelevance of cyclic nucleotides to the relaxation of tracheal smooth muscle induced by theophylline. *Lung*, **156,** 173–183.

MITCHELL, H.W. & DENBOROUGH, M.A. (1979). Drug interactions in cat isolated tracheal smooth muscle. *Clin. exp. Pharmac. Physiol.*, **6,** 249–257.

O'DONNELL, S.R. & WANSTALL, J.C. (1977). The use of functional antagonism to determine whether β-adrenoceptor agonists must have a lower efficacy than isoprenaline to be trachea-atria selective in vitro in guinea-pig. *Br. J. Pharmac.*, **60,** 255–262.

O'DONNELL, S.R. & WANSTALL, J.C. (1978). Evidence that the efficacy (intrinsic activity) of fenoterol is higher than that of salbutamol on β-adrenoceptors in guinea-pig trachea. *Eur. J. Pharmac.*, **47,** 333–340.

OLSSON, O.A.T. & PERSSON, C.G.A. (1976). Effect of β-adrenoceptor stimulants, papaverine and theophylline on guinea-pig trachea contracted by different drugs. *Acta pharmac. tox.*, **38,** 281–283.

PATERSON, J.W., WOOLCOCK, A.J. & SHENFIELD, G.M. (1979). Bronchodilator drugs. *Am. Rev. Resp. Dis.*, **120,** 1149–1188.

PERSSON, C.G.A. (1980). Some pharmacological aspects on xanthines in asthma. *Eur. J. Resp. Dis. Suppl.* 109, **61,** 7–16.

PERSSON, C.G.A. & EKMAN, M. (1976). Contractile effects of histamine in large and small respiratory airways. *Agents & Actions*, **6,** 389–393.

PERSSON, C.G.A., EKMAN, M. & ERJEFÄLT, I. (1979). Vascular anti-permeability effects of β-receptor agonists and theophylline in the lung. *Acta pharmac. tox.*, **44,** 216–220.

RAPER, C. & MALTA, E. (1973). Salbutamol: agonistic and antagonistic activity at β-adrenoceptor sites. *J. Pharm. Pharmac.*, **25,** 661–663.

SIMONSSON, B.G. (1977). Anticholinergic drugs – a review. *Scand. J. Resp. Dis.*, **101,** 147–153.

SPILKER, B. & MINATOYA, H. (1975). The role of bronchoconstrictors in evaluating smooth muscle relaxant activity. *Archs Int. Pharmacodyn.*, **217,** 201–217.

STEPHENS, N.L., MEYERS, J.L. & CHERNIACK, R.M. (1968). Oxygen, carbon dioxide, H^+ ion and bronchial length-tension relationships. *J. appl. Physiol.*, **25,** 376–383.

TRENDELENBURG, P. (1912). Physiologische und pharmakologische Untersuchungen an der isolierten Bronchialmuskulatur. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **69,** 79–107.

(Received November 24, 1980.
Revised March 17, 1981.)