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The guinea-pig isolated bronchus for the *in vitro* study of small calibre airway reactivity

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1 Small calibre airway reactivity to different contractile and relaxant agents was tested *in vitro* using small segments (about 1 mm long and 0.2 mm in internal diameter) of guinea-pig isolated intralobular bronchi.

2 EC_{50} values of, and maximal contractile responses to contractile agents were as follows (mean ± s.e.mean, $n = 6$): acetylcholine 13.6 ± 2.6 μM and 1140 ± 80 mg; histamine 5.2 ± 0.7 μM and 1094 ± 95 mg; 5-hydroxytryptamine (5-HT) 0.7 ± 0.1 μM and 595 ± 61 mg; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 8.8 ± 1.2 μM and 1100 ± 88 mg; tetraethylammonium 2.9 ± 0.3 mM and 1055 ± 94 mg; KCl 14.6 ± 0.5 mM and 965 ± 81 mg.

3 EC_{50} values of, and maximal relaxant responses to β-adrenoceptor stimulants on preparations precontracted with acetylcholine (1.4×10^{-4}M) were: isoprenaline 0.40 ± 0.5 μM and 782 ± 65 mg, $n = 18$; salbutamol 0.19 ± 0.02 μM and 494 ± 55 mg, $n = 5$; terbutaline 0.87 ± 0.18 μM and 263 ± 40 mg $n = 5$; fenoterol 0.06 ± 0.02 μM and 722 ± 47 mg, $n = 5$; adrenaline 0.71 ± 0.13 μM and 653 ± 62 mg, $n = 5$; noradrenaline 10.8 ± 0.9 μM and 566 ± 97 mg, $n = 5$.

4 Differences in the maximal relaxant effects between the β-adrenoceptor stimulants showed that the preparation utilized is a relevant model for assessment of the intrinsic activity of these drugs.

5 The high ratio (about 180) of the EC_{50} for noradrenaline (β_1-adrenoceptor agonist) to that for fenoterol (β_2-adrenoceptor agonist), and the lack of effect of prenalterol (β_1-adrenoceptor agonist) suggested that β_2-adrenoceptors are preferentially involved in the relaxant activity of β-adrenoceptor stimulants in this preparation.

Introduction

Bronchial reactivity is usually investigated *in vitro* either on isolated tracheal strips (Patterson, 1958; Constantine, 1965) or on isolated parenchymal lung strips, which have been claimed to be more representative of distal airway reactivity (Lülich *et al.*, 1976; Chand & De Roth, 1979; Mitchell & Denborough, 1979; Siegl *et al.*, 1979).

Although these preparations are widely used for studying the physiological and/or pharmacological processes involved in regulation of smooth muscle contractility, they are open to criticism. With the guinea-pig isolated trachea, responses are limited to large proximal airways, the contribution of which to overall variations in pulmonary resistance is debatable (Siegl *et al.*, 1979). Attention has recently been drawn to the heterogeneity of the parenchymal lung strip model (Evans & Adler, 1981; Goldie *et al.*, 1982; Bertram *et al.*, 1983). This preparation contains several potentially contractile components, i.e. bronchiolar smooth muscle, vascular smooth muscle and alveolar interstitial myofibroblasts (Kapanci *et al.*, 1974), so that its overall pharmacological response may be due to either one or several of these components. Responses of the lung strip to α-adrenoceptor agonists (Goldie *et al.*, 1982; Bertram *et al.*, 1983; Advenier & Floch-Saint-Aubin, 1984) or to leukotriene D_4 (Weichmann *et al.*, 1983) involve a contribution from the vascular component. Furthermore, it is difficult to study bronchodilator effects on lung strips as the induced tone of this preparation is unstable (Finney *et al.*, 1983; Advenier & Floch-Saint-Aubin, 1984).

We have now applied to small bronchi a technique initially devised to study reactivity of mesenteric resistance arteries of the rat (Mulvany & Halpern, 1976; Freslon & Giudicelli, 1983). This paper describes the effects of drugs on isolated segments of guinea-pig intralobular bronchi with a mean diameter of 0.2 mm.

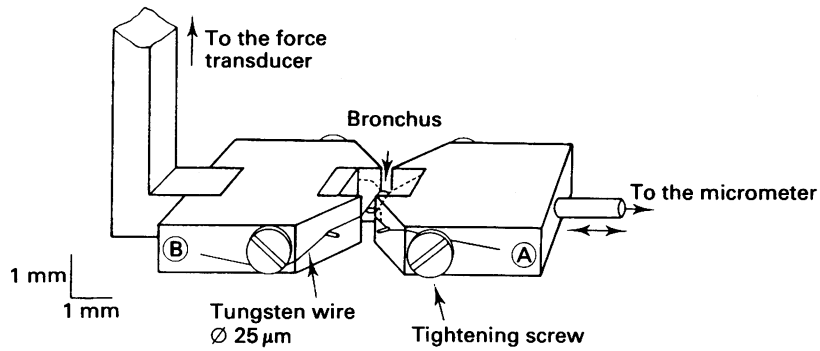

Figure 1 Diagrammatic representation of the device used for experimental studies on the isolated small bronchus (approximate length and internal diameter: 1 mm and 0.2 mm, respectively). Two Tungsten wires (external diameter 0.25 μm) are inserted into the bronchial lumen and secured under tension: A: support connected to a micrometer; B: support fixed on the arm of a force transducer.

Methods

Pharmacological study

Isolation of the bronchus Guinea-pigs of either sex, weighing 250 to 300 g, were anaesthetized with urethane 1.25 g kg^{-1} i.p., placed in dorsal decubitus and ventilated with a Loosco KR665 respirator at the rate of 80 ml min^{-1}. Thoracotomy was performed and the whole respiratory tract was removed under continuous ventilation. The main intralobular bronchus in the middle of the right median lobe was identified and dissected under a binocular microscope. A tungsten wire 25 μm in diameter was inserted into the lumen of the bronchus which was then extracted from the pulmonary lobe and cleared of all lung tissue.

Description of the myograph The myograph essentially consists of two supports equipped with jaws and connected one (A) to a micrometer (MR 50-4, Microcontrôle), the other (B) to a force transducer (BG 10, Kulite) (Figure 1).

A small segment of the bronchus (length 1 mm, internal diameter 0.2 mm) was then cut. The tungsten wire in its lumen was positioned on the jaw of support B and secured under tension with the two lateral screws. A second tungsten wire, also 25 μm in diameter was inserted into the lumen of the bronchus and fixed on support A in the same manner. Figure 1 shows the final configuration of the myograph with the bronchus positioned between the two supports.

This system enabled the preparation to be stretched laterally by increasing its internal diameter, using the micrometer connected to support A. Changes in parietal tension resulting from contractions or relaxations could be recorded through support B fixed on the arm of the force transducer.

The two supports of the myograph were immersed in a 4 ml bath containing Krebs solution gassed with 95% O_2, 5% CO_2 and kept at 37°C. The composition of the Krebs solution was as follows (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 23, KH_2PO_4 1.2, glucose 11.7.

Tension from the transducer was amplified (3629 CP Burr-Brown) and recorded on a potentiometric recorder (BD 40, Tracelab).

The myograph was placed under a microscope (G × 50, Earling Biosciences) to evaluate the internal diameter of the bronchus by measuring the distance between the two wires.

Experimental procedure After a 45 min equilibration period during which circulation of the Krebs solution was continued, the preparation was subjected to a mean static tension of 180 mg by racking support A of the myograph. This tension had been determined in a preliminary study by measuring the maximal contractile response of the bronchus to a constant concentration of acetylcholine (ACh) while the static tension was being increased by regular steps. When tension was stabilized and no spontaneous activity was observed, circulation of the Krebs solution was discontinued, and cumulative concentration-response curves were obtained for ACh, histamine, 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), methoxamine, tetraethylammonium (TEA) and KCl. Relaxant agents (isoprenaline, terbutaline, salbutamol, procaterol, fenoterol, adrenaline, noradrenaline, prenalterol and theophylline) were added to the bronchus precontracted with ACh. The response was measured after each addition of each drug, when a stable contraction or relaxation response had been obtained. After maximal concentration was reached, the preparation was washed by recirculating the Krebs solution. The preparation was allowed to rest for 45 min and a new concentration-response curve was

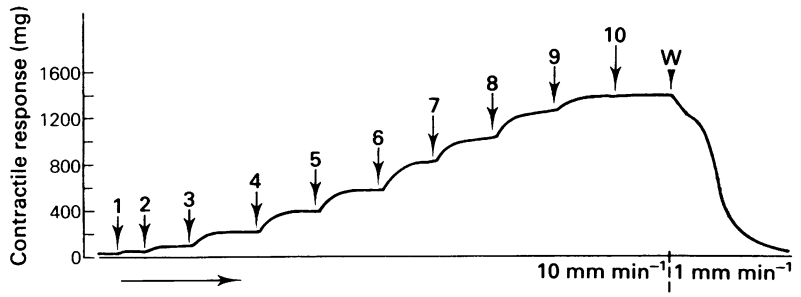

Figure 2 Effect of histamine on a small bronchus of guinea-pig. Concentrations were (M): 1, 4.2×10^{-7}; 2, 7.5×10^{-7}; 3, 1.4×10^{-6}; 4, 2.5×10^{-6}; 5, 4.2×10^{-6}; 6, 7.5×10^{-6}; 7, 1.4×10^{-5}; 8, 4.2×10^{-5}; 9, 1.4×10^{-4}; 10, 4.2×10^{-4}. W: Washout. (Note the reduction in paper speed).

attempted to confirm that no interaction had occurred between two drugs. We found that concentration-response curves could be reproduced at least 4 times in succession.

Calculations Concentrations producing 50% of maximal effect (EC_{50}) were determined by the log-probit method. Results are expressed as mean ±s.e.mean of 4 to 18 determinations on tissues from different guinea-pigs. Differences in means were determined by Student's t test; $P < 0.05$ was considered to be significant.

Drugs The following drugs were used: acetylcholine diHCl (Lematte & Boinot, Paris), histamine HCl (Prolabo, Paris), 5-hydroxytryptamine creatinine sulphate (Schucharht, München), calcium chloride (Prolabo, Paris), tetraethylammonium bromide (Sigma, St Louis, U.S.A.), $PGF_{2\alpha}$ (Chinon, Budapest), methoxamine HCl (Roussel, Paris), isoprenaline sulphate (Winthrop, Paris), salbutamol sulphate (Glaxo, Paris), terbutaline sulphate (Astra, Paris), procaterol HCl (Roussel, Paris), fenoterol HBr (Boehringer Ingelheim, Reims), adrenaline tartrate (Aguettant, Paris), noradrenaline bitartrate (Winthrop, Paris), prenalterol HCl (Astra, Paris), theophylline as sodium anisate (Bruneau, Paris). Acetylcholine isoprenaline (Isuprel), theophylline (Theophylline Bruneau), adrenaline and noradrenaline (Levophed) were used as proprietary injectable solutions. Dilutions were made with the incubation fluid.

Histological study

After the pharmacological study was completed, some bronchial segments were removed from the myograph and immersed in a fixation fluid (glutaraldehyde 1%, paraformaldehyde 4% in a 0.17 M phosphate buffer adjusted to pH 7.4), then post-fixed with 4% osmic acid in phosphate buffer and dehydrated in graded series of acetone. The segments were embedded in araldite, and transverse sections (0.5 μm) were obtained, with a glass knife microtome. Finally, the sections were stained with toluidine blue for microscopic examination and photography.

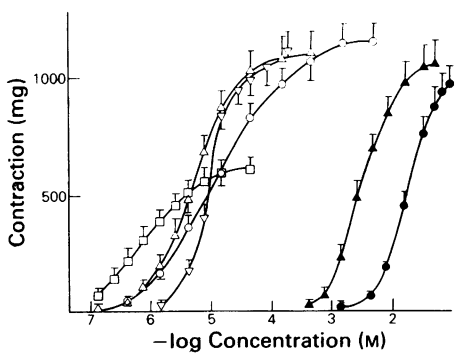

Figure 3 Concentration-response curves of the guinea-pig isolated bronchus preparation to acetylcholine (○), histamine (△), prostaglandin $F_{2\alpha}$ (▽), 5-hydroxytryptamine (□), tetraethylammonium (▲) and potassium chloride (●). Points show means and vertical lines represent s.e.means.

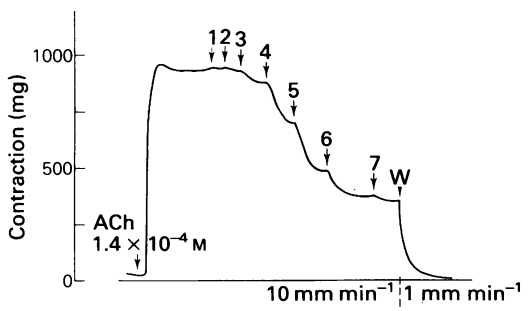

Figure 4 Effect of isoprenaline on a small bronchus contracted with acetylcholine (ACh; 1.4×10^{-4} M). Concentrations of isoprenaline were (M): 1, 1.4×10^{-8}; 2, 4.2×10^{-8}; 3, 1.4×10^{-7}; 4, 4.2×10^{-7}; 5, 1.4×10^{-6}; 6, 4.2×10^{-6}; 7, 1.4×10^{-5}. W: washout. (Note the reduction in paper speed.)

Table 1 Effective concentration (EC_{50}) and maximal contractile responses in the study of small calibre airway reactivity to contractile agents

	n	EC_{50}	*Maximal contractile response* (mg)
5-Hydroxytryptamine	6	0.7 ± 0.1 μM	595 ± 61
Histamine	8	5.2 ± 0.7 μM	1094 ± 95
Prostaglandin $F_{2\alpha}$	6	8.8 ± 1.2 μM	1100 ± 88
Acetylcholine	8	13.6 ± 2.6 μM	1140 ± 80
Tetraethylammonium	6	2.9 ± 0.3 μM	1055 ± 94
KCl	6	14.6 ± 0.5 μM	965 ± 81

Values show mean ± s.e.mean; *n* = number of experiments

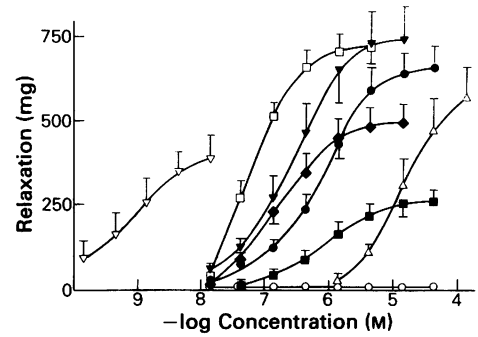

Figure 5 Concentration-response curves of the guinea-pig isolated bronchus preparation to isoprenaline (▼), adrenaline (●), noradrenaline (Δ), salbutamol (◆), terbutaline (■), fenoterol (□), procaterol (▽) and prenalterol (○). The extent of the relaxation is given in absolute values on the vertical axis. Points show means and vertical lines represent s.e.means.

Some bronchi removed from the lung but not subjected to pharmacological studies were also fixed, stained and cut into sections as described above, for comparison with treated bronchi.

Results

Pharmacological results

Contractile drugs Figure 2 is a typical example of an experimental tracing obtained, in this case, with histamine.

Figure 3 shows the dose-response curves of different contractile agents. Curves on the left were obtained with drugs possessing intrinsic contractile effects, and curves on the right with depolarizing agents – potassium chloride (KCl) or tetraethylammonium (TEA) – Foster *et al.*, 1983; Advenier *et al.*, 1984). It will be noted that the dose-response curve for ACh is the most extensive, ranging from 10^{-7} M to 10^{-3} M, and that the maximal contraction to 5-HT is half that of other drugs.

EC_{50} values, maximal contractile response to each of the agents and number of experiments are shown in Table 1.

Relaxant drugs Figure 4 shows the relaxant effects of isoprenaline, at various concentrations, on the contraction induced by ACh 1.4×10^{-4} M. Table 2 shows that these effects, expressed as mg, were the same with

Table 2 Response to isoprenaline (Iso) of the isolated small bronchus precontracted with acetylcholine (ACh) 1.4×10^{-5}M or 1.4×10^{-4}M

	ACh 1.4×10^{-5}M	*ACh* 1.4×10^{-4}M	*Significance*
Number of experiments	5	18	
Intensity (mg) of contraction induced by ACh	897 ± 98	1253 ± 73	$P < 0.05$
Intensity (mg) of maximal relaxation induced by Iso	708 ± 60	782 ± 65	ns
Intensity (%) of maximal relaxation induced by Iso	80 ± 3	61 ± 4	$P < 0.001$
EC_{50} of Iso (μM)	0.42 ± 0.04	0.40 ± 0.05	ns

Values show mean ± s.e.mean

Table 3 Response to different relaxing agents of the isolated small bronchus precontracted with acetylcholine 1.4×10^{-4}M

	n	EC_{50}(μM)	*Maximal relaxant response* (mg)
Isoprenaline	18	0. 40 ± 0. 05	782 ± 65
Adrenaline	5	0. 71 ± 0. 13	653 ± 62
Noradrenaline	5	10. 80 ± 0. 90	566 ± 97
Salbutamol	5	0. 19 ± 0. 02	494 ± 55
Terbutaline	5	0. 87 ± 0. 18	263 ± 40
Fenoterol	5	0. 06 ± 0. 02	722 ± 47
Procaterol	4	0.007 ± 0.003	384 ± 71
Theophylline	5	570 ± 70	746 ± 127

Values show mean ± s.e.mean; *n* = number of experiments.

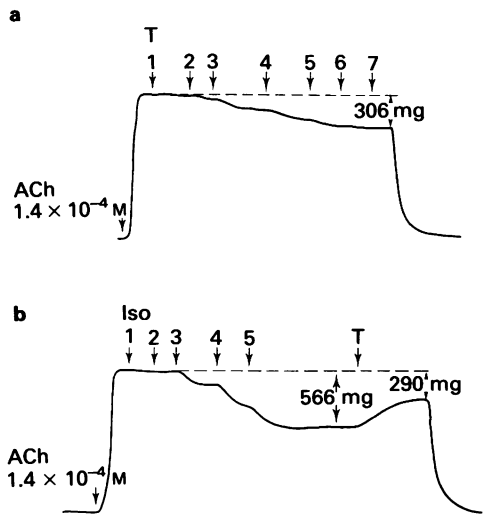

Figure 6 Demonstration of the reduced maximal response to terbutaline on the guinea-pig isolated small calibre bronchus contracted with acetylcholine (ACh 10^{-4} M). (a) Effect of terbutaline alone (T) added to the bath; concentrations were (M): 1, 10^{-7}; 2, 3×10^{-7}; 3, 10^{-6}; 4, 3×10^{-6}; 5, 10^{-5}; 6, 3×10^{-5}; 7, 10^{-4}. (b) Effect of terbutaline (T) (10^{-3} M) on the preparation first relaxed with isoprenaline (Iso); concentrations of isoprenaline were (M): 1, 10^{-7}; 2, 3×10^{-7}; 3, 10^{-6}; 4, 3×10^{-6}; 5, 10^{-5}.

ACh concentrations of 1.4×10^{-5} M and 1.4×10^{-4} M.

The concentration-response curves for adrenaline, noradrenaline, salbutamol, terbutaline, fenoterol, procaterol and the selective β_1-adrenoceptor agonist prenalterol against contractions induced by ACh (1.4×10^{-4}M) are shown in Figure 5 and quantified in Table 3. In these experiments fenoterol and procaterol were 6.6 and 57 times, respectively, more active than isoprenaline. Isoprenaline itself and fenoterol were 27 and 180 times, respectively, more active than noradrenaline.

Among selective the β_2-adrenoceptor agonists, only fenoterol produced the same sized maximal relaxation as isoprenaline, adrenaline and noradrenaline; the maximal relaxation responses obtained with salbutamol and terbutaline were less than that to isoprenaline. In the case of terbutaline, an example of inhibition of maximal response to isoprenaline by this drug is shown in Figure 6. The β_1-adrenoceptor agonist prenalterol was devoid of relaxant activity.

As shown in Table 3, the maximal relaxant effect of theophylline was the same as that of full agonists of β_2-adrenoceptors.

Histological results

Histological examination revealed that each bronchial section was composed of three concentric layers which were, from lumen to periphery: (1) a well-developed epithelium, (2) a thick ring of smooth muscle, and (3) disconnected arches of cartilaginous tissue. No abnormalities were observed. There were no structural differences between treated and untreated bronchi.

Discussion

Although numerous pharmacological studies of distal airways reactivity *in vitro* have been, and still are conducted on isolated parenchymal lung strips, some authors have advocated the use of other preparations. Thus, Hooker *et al.* (1977) have tested the effects of carbachol, histamine, KCl and isoprenaline on guinea-pig isolated bronchial segments 1.5 mm in internal diameter (i.d.). Aas & Helle (1982) have used a similar model to study the effects of neurotensin on right and left bronchial segments from rats (i.d. 1.6 mm or more), while Finney *et al.* (1983) have investigated the reactions of human bronchioles (i.d. 0.5 to 1.5 mm) to carbachol, histamine and adenosine. However, the tissue we used was distinctly smaller (i.d. 0.2 mm), although the presence of cartilaginous tissue at the periphery showed that we were dealing with bronchi (Tyler, 1983).

Our results in all experiments confirmed that intralobular bronchi are devoid of spontaneous activity, as already noted by Aas & Helle, (1982) working on rat isolated bronchi. In contrast, measurements of maximal contractile responses demonstrated that our bronchial segments could develop a mechanical tension of about 1100 mg, i.e. much higher than that of isolated parenchymal strips which contract to 300–400 mg in response to ACh and histamine (Siegl *et al.*, 1979; Mitchel & Denborough, 1979). In view of the small mass of the preparation (weight: 1 mg), this high degree of contractility is probably related to the thick circular smooth muscle observed on histological sections. In addition, the degree of contractility is undoubtedly better measured with our experimental device (Figure 1), which evaluates the parietal tension responsible for reduction in bronchial calibre, than with other preparations. Finally, it must be remembered that the contractility evaluated on isolated parenchymal lung strips results from tensions in the three dimensions exerted on tissue volume.

The results obtained with contractile agents showed that ACh, histamine and $PGF_{2\alpha}$ and the depolarizing agents tested (TEA, KCl) (Foster *et al.*, 1983; Advenier *et al.*, 1984) induced contractions of virtually equivalent intensities (Table 1). Only maximal response to 5-HT was 50% of that to other agents, as

previously observed by Aas & Helle (1982) on the rat isolated bronchus. Methoxamine was a special case. In apparent contradiction to the findings of Chahl & O'Donnell (1971), Black *et al.* (1981) and Advenier & Floch-Saint-Aubin (1984), which demonstrated that methoxamine causes spasm in guinea-pig isolated tracheas and/or parenchymal lung strips, this agent did not contract the bronchial preparation described in this paper, although the histology demonstrated extensive smooth muscle. This supports the hypothesis that the contraction of isolated lung strips induced by specific α_1-adrenoceptor agonists, such as methoxamine, is in fact due to these agents acting on α_1-adrenoceptors that are present in a large proportion of vascular smooth muscle (Bertram *et al.*, 1983; Advenier & Floch-Saint-Aubin, 1984). This finding may also be compared with that of Barnes *et al.* (1983) who determined the pulmonary distribution of α-adrenoceptors by autoradiographic localization of [^{3}H]-prazosin binding to frozen sections of ferret lung; although smooth muscle of small bronchi showed little labelling, that of bronchioles was heavily labelled; furthermore α-adrenoceptors were present in highest density in vascular smooth muscle.

The results with relaxant agents showed that the maximal relaxant effect of isoprenaline on small bronchi was the same as that of theophylline, as previously found by Karlsson & Persson (1981) in the guinea-pig isolated trachea. In addition, isoprenaline was proportionally less active when the preparation was contracted with high concentrations of ACh. This may be due to functional antagonism (Van den Brink, 1973), a phenomenon also observed with the guinea-pig isolated trachea model (O'Donnell & Wanstall, 1977; 1978; Karlsson & Persson, 1981; Torphy *et al.*, 1983).

In contrast to the isolated trachea, the bronchus was not relaxed by agonists for β_1-adrenoceptors, even though binding studies (Carlswell & Nahorski, 1983; Engle *et al.*, 1982), have demonstrated these receptors throughout the airways. Evidence for this was provided by the lack of activity of prenalterol, a specific β_1-adrenoceptor stimulant (Carlsson *et al.*, 1977), and by comparing the EC_{50} values for isoprenaline and fenoterol with that for noradrenaline (NA). Isoprenaline and fenoterol were 27 and 180 times, respectively, more active in the bronchus than NA, whereas in the isolated guinea-pig trachea, the isoprenaline: NA and fenoterol: NA ratios are 10 and 20 respectively (Carlswell & Nahorski, 1983). It should also be borne in mind that the effects of prenalterol (Johansson & Waldeck, 1981) or of the β_1-adrenoceptor agonist Ro 363 (Iakovidis *et al.*, 1980) on the guinea-pig trachea are very modest compared with those of β_2-adrenoceptor stimulants and/or appear only in the presence of 17-β-oestradiol (Johansson & Waldeck, 1981).

This study on the guinea-pig isolated bronchus clearly demonstrated that β_2-adrenoceptor agonists were either fully or partially active on bronchial smooth muscle and could be compared with each other in this respect. Only fenoterol exerted a full agonistic effect similar to that of isoprenaline, adrenaline and noradrenaline.

This new approach to bronchial activity, using a preparation of homogeneous structure, as opposed to the diverse components of parenchymal lung strips, shows that contraction and relaxation of the bronchial smooth muscle in response to various agents can now be studied on small calibre airways.

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References

AAS, P. & HELLE, K.B. (1982). Neurotensin receptors in the rat bronchi. *Regulatory Peptides*, **78**, 405–413.

ADVENIER, C., CERRINA, J., DUROUX, P., FLOCH, A. & RENIER, A. (1984). Effects of the different organic calcium antagonists on guinea-pig isolated trachea. *Br. J. Pharmac.*, **82**, 727–733.

ADVENIER, C. & FLOCH-SAINT-AUBIN, A. (1984). Bronchopulmonary effects of phenylephrine and methoxamine in the guinea-pig. Interaction with bronchoconstrictor drugs. *Eur. J. Pharmac.*, **100**, 59–69.

BARNES, P.J., BASBAUM, C.B., NADEL, J.A. & ROBERTS, J.M. (1983). Pulmonary alpha-adrenoceptors: autoradiographic localization using ^{3}H prazosin. *Eur. J. Pharmac.*, **88**, 57–62.

BERTRAM, J.F., GOLDIE, R.G., PAPADIMITRIOU, J.M. & PATERSON, J.W. (1983). Correlation between pharmacological responses and structure of human lung parenchyma strips. *Br. J. Pharmac.*, **80**, 107–114.

BLACK, J., TURNER, A. & SHOW, J. (1981). Alpha-adrenoceptors in human peripheral lung. *Eur. J. Pharmac.*, **72**, 83–86.

CARLSSON, E., DAHLÖF, C.G., HEDBERG, A., PERSSON, H. & TANGSTRAND, B. (1977). Differentiation of cardiac chronotropic and inotropic effects of beta-adrenoceptor agonists. *Naunyn-Schmiedebergs Arch. Pharmac.*, **300**, 101–105.

CARSWELL, H. & NAHORSKI, S.R. (1983). β-Adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies. *Br. J. Pharmac.*, **79**, 965–971.

CHAHL, L.A. & O'DONNELL, S.R. (1971). The effects on some receptors of guinea-pig trachea of a series of sympathetic amines. *Eur. J. Pharmac.*, **16**, 201–208.

CHAND, N. & DE ROTH, L. (1979). Responses of guinea-pig lung parenchymal strips to prostaglandins and some selected autacoids. *J. Pharm. Pharmac.*, **31**, 712–714.

CONSTANTINE, J.W. (1965). The spirally cut tracheal strip preparation. *J. Pharmac.*, **17**, 384–385.

ENGEL, G., HOYER, D. & BRETZ, U. (1982). Biochemical identification of subclasses of adrenoceptors in guinea-pig, rat and human lung. *Thérapie*, **37**, 541–547.

EVANS, J.N. & ADLER, K.B. (1981). The lung strip: evalution of a method to study contractility of pulmonary parenchyma. *Exp. Lung Res.*, **2**, 187–195.

FINNEY, M., KARLSSON, J.A. & PERSSON, C.G.A. (1983). An alternative human small airway preparation. *Br. J. Pharmac.*, **80**, 709P.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983). Evidence that the spamogenic action of tetraethylammonium in guinea-pig tracheas is both direct and dependant on the cellular influx of calcium ion. *Br. J. Pharmac.*, **79**, 255–263.

FRESLON, J.L. & GIUDICELLI, J.F. (1983). Compared myocardial and vascular effects of captopril and dihydralazine during hypertension development in spontaneously hypertensive rats. *Br. J. Pharmac.*, **80**, 533–543.

GOLDIE, R.G., PATERSON, J.W. & WALE, J.L. (1982). Pharmacological responses of human and porcine lung parenchyma, bronchus and pulmonary artery. *Br. J. Pharmac.*, **76**, 515–521.

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.

IAKOVIDIS, D., MALTA, E., McPHERSON, G.A. & RAPER, C. (1980). *In vitro* activity of Ro363, a β_1-adrenoceptor selective agonist. *Br. J. Pharmac.*, **68**, 677–685.

JOHANSSON, U. & WALDECK, B. (1981). Beta$_1$-adrenoceptors mediating relaxation of the guinea-pig trachea: experiments with prenalterol, a beta$_1$-selective adrenoceptor agonist. *J. Pharm. Pharmac.*, **33**, 353–356.

KAPANCI, Y., ASSIMACOPOULOS, A., IRLE, C., ZWAHLEN, A. & GABBIANI, G. (1974). Contractile interstitial cells in pulmonary alveolar septa: a possible regulator of ventilation/perfusion ratio? *J. cell Biol.*, **60**, 375–392.

KARLSSON, J.A. & PERSSON, C.G.A. (1981). Influence of tracheal contraction on relaxant effect *in vitro* of theophylline and isoprenaline. *Br. J. Pharmac.*, **74**, 73–79.

LÜLICH, K.M., MITCHELL, H.W. & SPARROW, M.P. (1976). The cat lung strip as an *in vitro* preparation of peripheral airways: a comparison of β-adrenoceptor agonists, autacoids and anaphylactic challenge on the lung strip and trachea. *Br. J. Pharmac.*, **58**, 71–79.

MITCHELL, H.W. & DENBOROUGH, M.A. (1979). Anaphylaxis in guinea-pig peripheral airways *in vitro*. *Eur. J. Pharmac.*, **54**, 69–78.

MULVANY, J.J. & HALPERN, W. (1976). Mechanical properties of vascular smooth muscle cells in situ. *Nature*, **260**, 617–619.

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-pigs. *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 beta-adrenoceptors in guinea pig trachea. *Eur. J. Pharmac.*, **47**, 333–340.

PATTERSON, R. (1958). The tracheal strip. Observations of the tracheal muscle. *J. Allergy*, **29**, 165–172.

SIEGL, P.K.S., ROSSI, G.V. & ORZECHOWSKI, R.F. (1979). Isolated lung strips of guinea-pigs: responses to beta-adrenergic agonists and antagonists. *Eur. J. clin. Pharmac.*, **54**, 1–7.

TORPHY, T.J., RINARD, G.A., RIETOW, M.G. & MAYER, S.E. (1983). Functional antagonism in canine tracheal smooth muscle: inhibition by methacholine of the mechanical and biochemical responses to isoproterenol. *J. Pharmac. exp. Ther.*, **227**, 694–698.

TYLER, W.S. (1983). Small airways and terminal units. Comparative subgross anatomy of lungs. Pleuras, intralobular septa and distal airways. *Am. Rev. resp. Dis.*, **128**, S32–S36.

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

WEICHMANN, B.M., MUCCITELLI, R.M., TUCKER, S.S. & WASSERMAN, M.A. (1983). Effect of calcium antagonists on leucotriene D_4-induced contraction of the guinea-pig trachea and lung parenchyma. *J. Pharmac. exp. Ther.*, **225**, 310–315.

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