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PHARMACOLOGICAL RESPONSES OF HUMAN AND PORCINE LUNG PARENCHYMA, BRONCHUS AND PULMONARY ARTERY

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1 Responses of preparations of human and porcine isolated bronchus and pulmonary artery to carbachol (CCh), methacholine, histamine, 5-hydroxytryptamine (5-HT), (−)-noradrenaline (NA), (−)adrenaline (Adr) and (±)-isoprenaline (Iso) were compared with responses to the same agonists in isolated lung parenchyma strips.

2 All preparations from both human and porcine lung contracted in response to histamine and all, except preparations of porcine pulmonary artery, contracted in response to CCh. Human and porcine pulmonary artery and parenchyma strip contracted in response to NA while bronchial preparations invariably relaxed. Iso caused relaxation of human and porcine bronchus and parenchyma strip. Although 5-HT was completely inactive in tissues isolated from pig lung, this amine was a powerful spasmogen in human pulmonary artery, relaxed human bronchus and caused variable responses in human parenchyma.

3 Results indicate that the pharmacological characteristics of human and porcine parenchyma strips may be explained in terms of responses of vascular or airways smooth muscle.

Introduction

It has recently been shown that bovine lung parenchyma strip has pharmacological characteristics in common with both pulmonary vascular and airways smooth muscle (Mirbahar & Eyre, 1980). Lung parenchyma strips contain several potentially contractile components. These include bronchiolar smooth muscle (Lulich, Mitchell & Sparrow, 1976; Colebatch & Mitchell, 1981) alveolar interstitial myofibroblasts (Kapanci, Assimacopoulos, Isle, Zwahlen & Gabbiani, 1974) and vascular smooth muscle (Lulich *et al.*, 1976; Burns & Doe, 1978; Drazen & Schneider, 1978; Mitchell & Denborough, 1979; Ghelani, Holroyd & Sheard, 1980). Thus pharmacological responses of lung strips may result from responses of one or more of these components. Information concerning the pharmacological characteristics of human isolated lung tissues is sparse. Most previous investigations used tissues obtained during surgery (Kapanci *et al.*, 1974; Ghelani *et al.*, 1980; Lulich & Paterson, 1980; Mathé, Åström & Persson, 1981). In the present study, post-mortem specimens of healthy human lung were used, thus reducing the possibility of drug or disease-induced abnormalities. Pig lung was also used since fresh, healthy tissue could be obtained readily from animals of approximately the same size and weight as the adult human. We documented the responses of human and porcine isolated lung parenchyma strips to various agonists and compared these responses with those of human and porcine isolated bronchus and pulmonary artery in order to assess the relative importance of vascular and airways smooth muscle to pharmacological responses of the lung parenchyma strip.

Methods

Bronchi (2–3 mm, i.d.) and their accompanying pulmonary arteries as well as strips of peripheral parenchyma were dissected from lungs of freshly slaughtered pigs or from macroscopically normal specimens of human lung obtained 4–12 h post mortem. Pulmonary arteries were prepared as described for sheep coronary arteries by Brine, Cornish & Miller (1979). Bronchi were dissected free of all peripheral lung tissue, including visible blood vessels and cut into spirals. Lung parenchyma was cut into strips approximately 25 × 2 × 2 mm distal to all bronchi and blood vessels visible to the naked eye. With the exception of some parenchyma strips which were taken from histological examination, preparations were suspended under 0.5 g tension in Krebs solution maintained at 37°C and aerated with 5% CO_2 in O_2. After equilibrating for 60–90 min, tissues were exposed to single or cumulative concentrations of various agonists. Changes in tension were measured with a Grass force-displacement transducer (FTO3C) coupled to a pre-amplifier and recorded on a Rikadenki pen-recorder (model 1328 L).

Since pig bronchial preparations developed little tension spontaneously, tone was induced by carbachol (CCh). The sensitivity of pig bronchi to CCh

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increased rapidly and so preparations were first exposed to separate, successively lower concentrations of spasmogen (200, 100, 40 ng/ml) administered between washouts at intervals of 30 min. After this pretreatment, a further challenge using CCh (40 ng/ml; 2.2×10^{-7} M) produced a contraction of similar magnitude to that produced by the preceding 40 ng/ml dose. This concentration of CCh caused a mean (±s.e.mean) contraction = 28.3 ± 4% E_{max} CCh ($n = 10$). Cumulatively administered concentrations of noradrenaline (NA), adrenaline (Adr) or isoprenaline (Iso) caused a complete relaxation of this induced tone. Higher concentrations of CCh caused contractions which were not always completely relaxed by these catecholamines.

All tissue responses were expressed as a % of the maximal response ($E_{max} = 100\%$) to the agonist. The mean EC_{50} value from at least two consecutive control concentration-effect curves was taken as a measure of agonist potency. In some cases, additional concentration-effect curves were produced following equilibration of tissues in the presence of various receptor antagonists for 60 min.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (±)-isoprenaline hydrochloride, carbamylcholine chloride, methacholine chloride, histamine diphosphate (Sigma); mepyramine maleate (M & B); metiamide (SKF); propranolol hydrochloride (ICI); phentolamine mesylate (Ciba); 5-hydroxytryptamine (serotonin) creatinine sulphate (Roche); 1,1-dimethyl-4-phenyl piperazinium (DMPP) (ICN.K & K Laboratories Inc.). Drug solutions were freshly prepared in saline (0.9% w/v NaCl solution). Solutions of catecholamines were stabilized with ascorbic acid (20 μg/ml).

Results

Carbachol, methacholine (MeCh) and 1,1-dimethyl-4-phenylpiperazinium (DMPP)

Both CCh and MeCh caused concentration-dependent contractions of human and porcine bronchus and parenchyma and of human pulmonary artery (Table 1, Figure 1). The order of sensitivity to CCh was porcine bronchus > human bronchus > porcine parenchyma > human pulmonary artery > human parenchyma. Neither CCh nor MeCh altered the resting tension in porcine pulmonary artery.

CCh and MeCh caused similar mean (±s.e.mean) maximal increases in resting tension (Tn_{max}) in the porcine bronchus (3558 ± 244 and 3200 ± 970 mg, respectively) and parenchyma (94 ± 4 and 160 ± 24 mg, respectively). CCh was 5–6 times more potent than MeCh in the porcine bronchus but had a similar potency in parenchyma. DMPP

Table 1 EC_{50} values and developed tension maxima (Tn_{max}) for histamine, carbachol, noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in tissues isolated from human and porcine lung

		Histamine	*Carbachol*	*NA*	*5-HT*
Man					
Parenchyma	EC_{50} (μM)	12.5 ± 3.9	9.2 ± 2.4	4.9 ± 1.1	6.1 ± 4.7
	Tn_{max} (mg)	112 ± 5	93 ± 19	54 ± 10	76 ± 12
		(6)	(6)	(6)	(4)
Bronchus	EC_{50} (μM)	0.8 ± 0.2	1.3 ± 5.0	—	—
	Tn_{max} (mg)	915 ± 240	703 ± 273		
		(6)	(7)		
Pulmonary artery	EC_{50} (μM)	1.43 ± 0.3	3.8 ± 0.7	0.3 ± 0.2	1.3 ± 0.8
	Tn_{max} (mg)	604 ± 133	298 ± 119	345 ± 64	370 ± 75
		(5)	(6)	(5)	(6)
Pig					
Parenchyma	EC_{50} (μM)	9.4 ± 2.2	3.6 ± 0.6	3.0 ± 0.5	—
	Tn_{max} (mg)	158 ± 16	94 ± 4	105 ± 8	
		(6)	(4)	(15)	
Bronchus	EC_{50} (μM)	21.7 ± 4.8	0.6 ± 0.1	—	—
	Tn_{max} (mg)	1218 ± 77	3558 ± 244		
		(10)	(18)		
Pulmonary artery	EC_{50} (μM)	3.9 ± 0.7	—	0.4 ± 0.1	—
	Tn_{max} (mg)	1205 ± 82		870 ± 75	
		(14)		(5)	

EC_{50} and Tn_{max} values expressed as mean ± s.e.mean.
Numbers in parentheses represent number of observations.

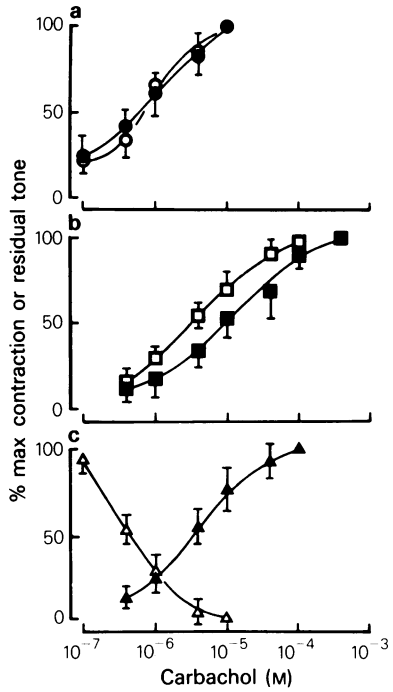

Figure 1 Mean, cumulative, $\log_{10}$ concentration-effect curves to carbachol in preparations of (a) isolated human (●, $n = 7$) and porcine (○, $n = 18$) bronchus, (b) human (■, $n = 6$) and porcine (□, $n = 4$) parenchyma and (c) human (▲, $n = 6$) and porcine (△, $n = 4$) pulmonary artery. Carbachol-induced relaxation of porcine pulmonary artery was only observed in preparations pre-contracted with adrenaline (2.0×10^{-7} M). All results were expressed as % maximum contraction or residual tone. Points represent means and vertical lines show s.e.means of n observations.

(1.0×10^{-7}–5.1×10^{-5} M) did not alter either the resting or CCh or MeCh-induced tension in preparations of human or porcine bronchus, parenchyma or pulmonary artery.

Noradrenaline, adrenaline and isoprenaline

Both NA and Adr invariably caused a concentration-dependent increase in resting tension in porcine parenchyma and porcine and human pulmonary artery (Table 1; Figure 2). In human parenchyma, these amines usually caused a concentration-dependent contraction. However, in some preparations of human parenchyma, Adr and NA (1×10^{-4} M) caused only weak and poorly sustained contractions, or even relaxation (Figure 3). In pulmonary artery and parenchyma from both pig and man, where these amines caused contractions, Adr was 2–3 times more potent than NA. The order of sensitivity to both NA and Adr was human artery > porcine artery > porcine parenchyma > human parenchyma. Human artery was 16 times more sensitive than human parenchyma to either amine.

Iso caused concentration-dependent decreases in resting tension in parenchymal strips and was marginally more potent in porcine than in human tissue (Figure 4). Iso (1.0×10^{-7}–3.2×10^{-6} M) did not alter resting tension in either human or porcine pulmonary artery; it failed to reduce Adr (2.0×10^{-7} M)-induced tone in porcine pulmonary artery, although small concentration-related relaxations were observed in 2 of 6 human arteries tested (Iso $EC_{50} = 2.5 \times 10^{-7}$ M; $n = 2$).

In porcine parenchyma and pulmonary artery, concentration-effect curves to NA and Adr were unaffected ($P > 0.05$) in the presence of propranolol (5.0×10^{-7} M). However, responses were markedly inhibited when phentolamine (1.5×10^{-6} M) was also added (Table 2). In parenchyma, the K_B value for phentolamine (NA as agonist) was 6.5×10^{-8} M

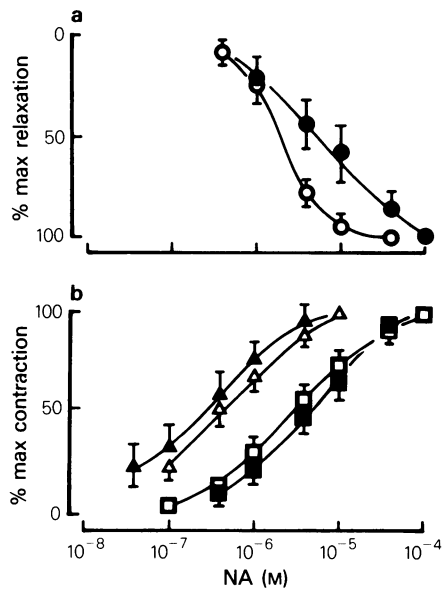

Figure 2 Mean, cumulative, $\log_{10}$ concentration-effect curves to noradrenaline (NA) in preparations of (a) isolated human (●, $n = 8$) and porcine (○, $n = 25$) bronchus (relaxations) and (b) human (■, $n = 6$) and porcine (□, $n = 15$) parenchyma and human (▲, $n = 5$) and porcine (△, $n = 5$) pulmonary artery (contractions). NA-induced relaxation of porcine bronchus was only observed in preparations pre-contracted with carbachol (2.0×10^{-7} M) and in preparations of human bronchus which developed tone spontaneously. All results are expressed as % maximum contraction or relaxation. Points indicate mean and vertical lines show s.e.means of n observations.

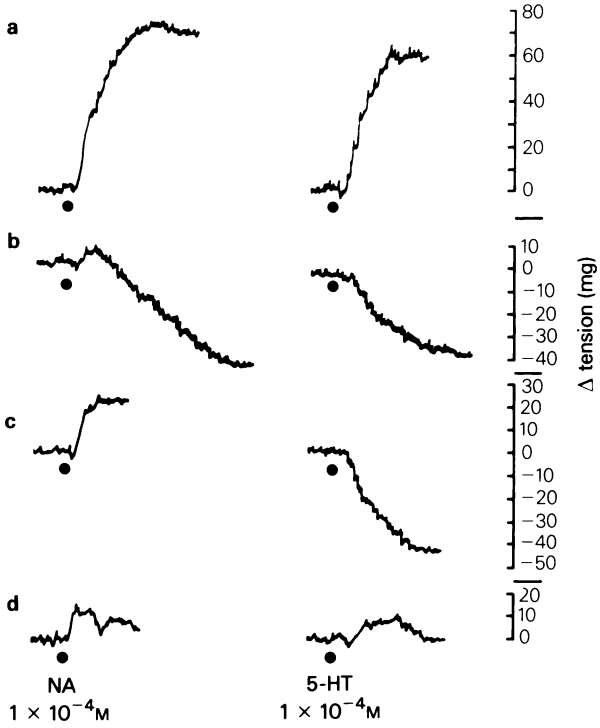

Figure 3 Responses to noradrenaline (NA) or 5-hydroxytryptamine (5-HT) (each 1.0×10^{-4} M) in 4 separate preparations of human lung parenchyma obtained from 4 different specimens of lung. Preparations (a), (b), (c) and (d) were mounted in organ baths 10, 5, 10 and 8.5 h respectively, after known times of death.

($n = 4$) and in pulmonary artery this value was 3.2×10^{-8} M ($n = 5$).

Catecholamines never caused contraction of bronchial preparations. Iso always caused a concentration-dependent decrease in spontaneously developed tension in human bronchus (Iso $EC_{50} = 2.28 \pm 0.60 \times 10^{-8}$ M; $n = 11$) and of CCh-induced (40 ng/ml; 2.2×10^{-7} M) tone in porcine bronchus (Iso $EC_{50} = 3.65 \pm 0.23 \times 10^{-7}$ M; $n = 15$) (Figure 4a). In the presence of this concentration of CCh, all three catecholamines NA, Adr and Iso, caused complete relaxation of preparations of porcine bronchus. When the concentration of CCh used was 80 ng/ml, the mean Iso EC_{50} value was significantly increased ($P < 0.001$) to $6.80 \pm 0.76 \times 10^{-7}$ M ($n = 5$), but when the concentration was reduced from 40 to 20 ng/ml, the mean Iso EC_{50} value was not significantly changed ($P > 0.01$). Iso was about 16 times more potent in human than in porcine bronchus. Relaxations in both types of bronchial preparation were inhibited by propranolol (2.5×10^{-7} M).

5-Hydroxytryptamine

In tissues from porcine lung, 5-HT failed to alter resting tension. However, when tone was raised in porcine pulmonary artery by Adr (2.0×10^{-7} M), 5-HT caused small relaxations. 5-HT was completely inactive in preparations of parenchyma and bronchus pre-contracted with CCh (40 ng/ml; 2.2×10^{-7} M).

In contrast, in human tissues, 5-HT always caused a concentration-dependent increase in resting tension in pulmonary arteries and a decrease in spontaneously developed tension in bronchus, while in preparations of parenchyma, it either had no effect or caused relaxations or contractions (Table 1; Figures 3 and 5).

Table 2 Effect of propranolol and phentolamine on the sensitivity (EC_{50}) of preparations of porcine parenchyma and pulmonary artery to the contractile effects of noradrenaline (NA) and adrenaline (Adr)

		$EC_{50} \times 10^{-6}$ M		
		Control	*Propranolol* (5×10^{-7} M)	*Propranolol + phentolamine* (5×10^{-7} M) (1.5×10^{-6} M)
Parenchyma	NA	3.0 ± 0.5	4.20 ± 0.30*	99.8 ± 13.9
		(15)	(4)	(4)
	Adr	1.1 ± 0.2	1.6 ± 0.2*	79.3 ± 23.5
		(6)	(5)	(5)
Pulmonary artery	NA	0.4 ± 0.1	0.3 ± 0.1*	14.0 ± 4.4
		(5)	(4)	(4)
	Adr	0.1 ± 0.02	0.2 ± 0.1*	12.8 ± 4.8
		(7)	(4)	(4)

Numbers in parentheses indicate number of observations.
In porcine parenchyma strips, control cumulative concentration-effect curves were produced for NA or Adr ($1 \times 10^{-7} - 1 \times 10^{-4}$ M) and in porcine pulmonary artery for NA or Adr ($5 \times 10^{-8} - 1 \times 10^{-5}$ M).
*Mean EC_{50} value not significantly different from control ($P > 0.05$; non-paired t test).

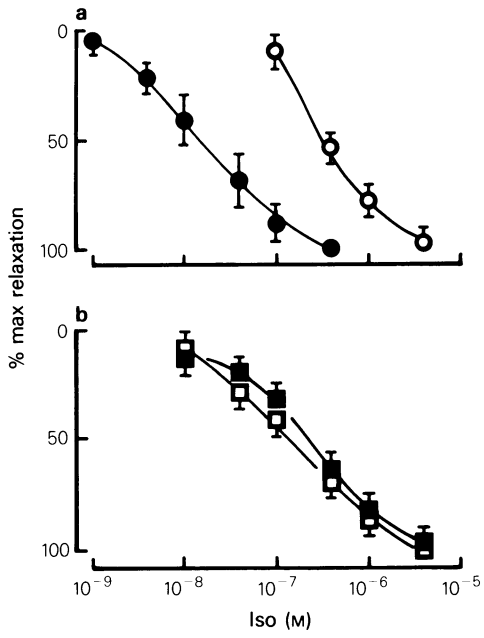

Figure 4 Mean, cumulative, $\log_{10}$ concentration-effect curves to the relaxant effects of isoprenaline (Iso) in preparations of (a) isolated human (●, $n = 11$) and porcine (○, $n = 15$) bronchus and (b) human (■, $n = 13$) and porcine (□, $n = 15$) parenchyma. Results are expressed as % maximum relaxation. Points represent means and vertical lines show s.e.means of n observations. Preparations of porcine bronchus were precontracted with carbachol (40 ng/ml). All other tissues developed tone spontaneously.

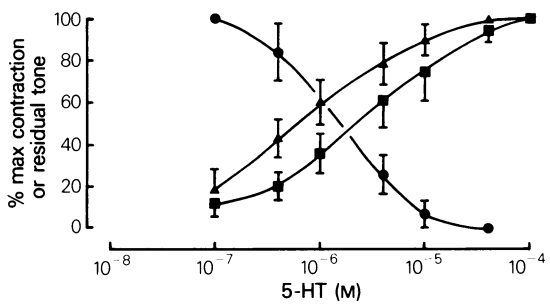

Figure 5 Mean, cumulative, $\log_{10}$ concentration-effect curves to the contractile effects of 5-hydroxytryptamine (5-HT) in preparations of human isolated parenchyma (■, $n = 4$) and pulmonary artery (▲, $n = 6$) and the relaxant effects of 5-HT in human bronchus (●, $n = 4$). Results are expressed as % maximum contraction or residual tone. Points represent means and vertical lines show s.e.means of n observations.

Histamine

Histamine caused concentration-dependent contractions of both human and porcine parenchyma, bronchus and pulmonary artery (Table 1; Figure 6). In preparations from pig lung, responses to histamine were markedly inhibited by mepyramine ($2.0 \times 10^{-8} - 1.0 \times 10^{-7}$ M), while metiamide ($1.0 \times 10^{-4} - 1.0 \times 10^{-3}$ M) had no effect. The order of decreasing sensitivity was human bronchus > human pulmonary artery > porcine pulmonary artery > porcine parenchyma > human parenchyma > porcine bronchus. Histamine was 27 times more potent in human than porcine bronchus. This agonist caused significantly greater mean maximal increases in tension (Tn_{max}) than any of the other spasmogens tested in human and porcine pulmonary artery and porcine parenchyma ($P < 0.001$, non-paired t test) (Table 1). In human parenchyma and bronchus, Tn_{max} was approximately equivalent for histamine and CCh, while Tn_{max} for CCh was about 3 times greater than for histamine in porcine bronchus.

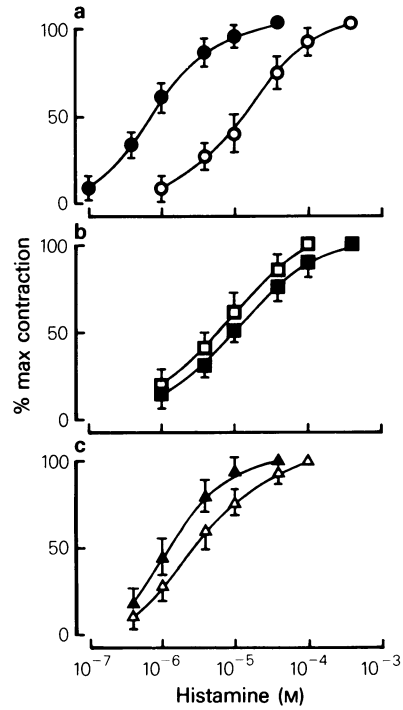

Figure 6 Mean, cumulative, $\log_{10}$ concentration-effect curves to the contractile effects of histamine in isolated preparations of (a) human (●, $n = 6$) or porcine (○, $n = 10$) bronchus, (b) human (■, $n = 6$) or porcine (□, $n = 6$) parenchyma or (c) human (▲, $n = 5$) or porcine (△, $n = 14$) pulmonary artery. Results are expressed as % maximum contraction. Points represent means and vertical lines show s.e.means of n observations.

Discussion

If the pharmacological responses of large and small pulmonary blood vessels are similar, it would be expected that responses of isolated preparations of pulmonary artery to drugs would reflect the effects of these agents on vascular components of parenchyma lung tissue. Similarly, responses of bronchial smooth muscle to drugs would indicate the likely responses of the peripheral bronchiolar component of parenchyma tissue. Results of the present study largely support this concept of a two component model of the lung parenchyma strip (Mirbahar & Eyre, 1980).

Responses of porcine lung parenchyma strip to Iso and CCh were consistent with the responses of porcine central airways smooth muscle. Porcine pulmonary artery was apparently devoid of β-adrenoceptors since Iso (5×10^{-8}–6.4×10^{-6} M) had no effect on either resting or adrenaline-induced tone. Furthermore, propranolol failed to increase significantly the α-adrenoceptor potency of Adr or NA in arteries (Table 2). In contrast, the contractile effects of NA and Adr in porcine parenchyma strip and pulmonary artery suggested that responses of the small pulmonary blood vessels within the lung strips also contribute to tension changes in these preparations. NA and Adr were 5–10 times less potent in lung parenchyma than in pulmonary artery. This may be due to a relative scarcity of a α-adrenoceptors, reflecting the sparsity of vascular smooth muscle in lung strips, or due to a reduction in nett α effect caused by stimulation of airways β-adrenoceptors. In contrast, histamine was a spasmogen in both pulmonary artery and bronchus from the pig. The contractile effect of histamine in porcine parenchyma strip can thus be attributed to stimulation of histamine receptors in either airways or vascular smooth muscle or both. These receptors were of the H_1 sub-type since responses were blocked by mepyramine but were unaffected by metiamide. 5-HT was inactive in porcine bronchial, arterial and therefore also in parenchyma preparations.

The data from human lung can be interpreted in the same way. Thus Iso-induced relaxation of human lung parenchyma strip was apparently mainly due to stimulation of β-adrenoceptors in bronchiolar smooth muscle, while the contractile effects of histamine and CCh might have involved airways or vascular smooth muscle or both. Therefore, a two component model consisting of vascular and bronchiolar smooth muscle does allow prediction of response patterns of the lung parenchyma strip. Furthermore, this model provides an explanation for the mixture of responses observed in human lung strip to both NA and 5-HT. The responses of human bronchus and pulmonary artery would lead one to predict that both NA and 5-HT would either contract or relax the lung strip. However, in one preparation, 5-HT (1×10^{-4} M) caused relaxation while NA (1×10^{-4} M) caused contraction and in another preparation, little if any effect was seen with either agent (Figure 3). In another lung strip, both NA and 5-HT caused contraction while both agonists relaxed a fourth preparation. These results suggest differences in the relative proportions of airways and vascular smooth muscle in different human parenchyma strips. Studies to determine the relationship between pharmacological response and the volume proportions of blood vessels and bronchioles in lung parenchyma strips are underway in our laboratories.

An alternative possibility is that a homogeneous population of contractile cells exists in lung parenchyma which has pharmacological characteristics in common with both vascular and bronchiolar smooth muscle. Kapanci *et al.* (1974) attributed Adr-induced contraction of parenchyma strip to stimulation of alveolar interstitial myofibroblasts. However, it has not been established that these cells which contain actin fibrils contract or relax in response to applied agonists. Many non-contractile cells contain actin fibrils (Pollard & Weihing, 1974). Furthermore, the fact that different lung strips may contract or relax to NA and 5-HT, indicates the presence of different proportions of at least two types of contractile cells in different human lung strips. Further studies are needed to determine the extent and pharmacological nature of alveolar myofibroblast contractility.

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References

BRINE, F., CORNISH, E.J. & MILLER, R.C. (1979). Effects of uptake inhibitors on responses of sheep coronary arteries to catecholamines and sympathetic nerve stimulation. *Br. J. Pharmac.*, **67**, 553–561.

BURNS, J.W. & DOE, J.E. (1978). A comparison of drug-induced responses on rat tracheal, bronchial and lung strip *in vitro* preparations. *Br. J. Pharmac.*, **64**, 71–74.

COLEBATCH, H.J.H. & MITCHELL, C.A. (1971). Constriction of isolated living liquid filled dog and cat lungs with histamine. *J. appl. Physiol.*, **30**, 691–702.

DRAZEN, J.M. & SCHNEIDER, M.W. (1978). Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol *in vitro*. *J. clin. Invest.*, **61**, 1441–1448.

GHELANI, A.M., HOLROYDE, M.C. & SHEARD, P. (1980). Response of human isolated bronchial and lung paren-

chymal strips to SRS-A and other mediators of asthmatic bronchospasm. *Br. J. Pharmac.*, **71,** 107–112.

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.

LULICH, 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.

LULICH, K.M. & PATERSON, J.W. (1980). An *in vitro* study of various drugs on central and peripheral airways of the rat: a comparison with human airways. *Br. J. Pharmac.*, **68,** 633–636.

MATHÉ, A.A., ÅSTRÖM, A. & PERSSON, N.-A. (1971). Some bronchoconstricting and bronchodilating responses of human isolated bronchi: evidence for the existence of α-adrenoceptors. *J. Pharm. Pharmac.*, **23,** 905–910.

MIRBAHAR, K.B. & EYRE, P. (1980). Bovine lung parenchyma strip has both airway and vascular characteristics. (Pharmacological comparisons with bronchus, pulmonary artery, and vein). *Res. Commun. Chem. Path. Pharmac.*, **29,** 15–25.

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

POLLARD, T.D. & WEIHING, R.R. (1974). Actin and myosin and cell movement. *C.R.C. Crit. Rev. Biochem.*, **2,** 1–66.

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