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Correlations between pharmacological responses and structure of human lung parenchyma strips

John F. Bertram[1], Roy G. Goldie*, John M. Papadimitriou & James W. Paterson*

Departments of Pathology and Pharmacology*, University of Western Australia, Nedlands, Perth, 6009, Australia

1 Correlations were sought between responses of human lung parenchyma strip to 5-hydroxytryptamine (5-HT) and (−)-noradrenaline (NA) and the proportions of the three major, potentially contractile components within the strip, namely smooth muscle in airways proximal to alveolar ducts, vascular smooth muscle and contractile cells in alveolar septa.

2 After the isometric measurement of responses to 5-HT or to NA, lung strips were processed for stereological examination at the light microscopic level. On average, approximately 46% of the total volume of the lung strip was tissue and the remainder was air space. Tissue contained blood vessels (16.8%), airways proximal to alveolar ducts (4.8%) and alveolar parenchyma (78.4%).

3 Human lung parenchyma strips relaxed, contracted or failed to respond to 5-HT or NA. Results indicated that these agonists caused simultaneous contraction of blood vessels and relaxation of airways proximal to alveolar ducts. The size and type of responses to 5-HT or NA was significantly correlated with the ratio of the volume of blood vessels and larger airways.

4 Conversely, the proportion of alveolar tissue in lung strips was not significantly correlated with responses to 5-HT or NA.

Introduction

The lung parenchyma strip from the cat was developed as a new *in vitro* preparation of peripheral airways smooth muscle (Lulich, Mitchell & Sparrow, 1976). Since that time other workers have used lung parenchyma strips from the guinea-pig (Chand & De Roth, 1979; Siegl, Rossi & Orzechowski, 1979; Iakovidis, Malta, McPherson & Raper, 1980), rat (Burns & Doe, 1978; Lulich & Paterson, 1980; Frossard, Landry, Pauli & Ruckstuhl, 1981), cat (Chand, 1981), dog (Chand, De Roth & Eyre, 1979) and man (Ghelani, Holroyde & Sheard, 1980; Hanna, Bach, Pare & Schellenberg, 1981). It has been assumed that the drug-induced effect observed (increase or decrease in resting tension) was due solely to the response of the smooth muscle of airways proximal to alveoli (bronchioles, terminal bronchioles, respiratory bronchioles and alveolar ducts).

However, the status of the lung parenchyma strip as a preparation for assessing peripheral airways pharmacology has recently been challenged. Several authors have suggested that peripheral blood vessels may also contribute to drug-induced responses (Kleinstiver & Eyre, 1980; Mirbahar & Eyre, 1980; Evans & Adler, 1981; Goldie, Paterson & Wale, 1982). Considerable numbers of muscular blood vessels have been described in lung strips from several species (Lulich *et al.*, 1976; Drazen & Schneider, 1978; Mitchell & Denborough, 1979; Ghelani *et al.*, 1980; Evans & Adler, 1981). A third population of potentially contractile cells has also been implicated as contributing to drug-induced responses of lung parenchyma strips. Drazen & Schneider (1978) showed that ultra-thin preparations of guinea-pig lung parenchyma, which contained no conducting airways or blood vessels, contracted to histamine and carbachol. These authors concluded that alveolar 'contractile interstitial cells' (Kapanci, Assimacopoulos, Irle, Zwahlen & Gabbiani, 1974) and/or alveolar duct smooth muscle (Miller, 1921; Mitchell & Denborough, 1979) were responsible for these responses.

The contractile responses of human lung parenchyma strip to (−)-noradrenaline (NA) have also been attributed to stimulation of non-vascular α-

[1]Present address: Department of Biological Structure, School of Medicine, University of Washington, Seattle, 98195, U.S.A.

adrenoceptors, possibly in alveolar contractile cells (Black, Turner & Shaw, 1981). In human lung strips NA and 5-hydroxytryptamine (5-HT) caused contraction or relaxation or had little effect (Goldie *et al.*, 1982). Since both of these agonists relaxed the central airways and contracted the pulmonary arteries of man *in vitro*, (Goldie *et al.*, 1982), it was suggested that responses of human lung strips to these drugs were predominantly the nett results of opposing effects in peripheral airways and blood vessels. Thus, the magnitude and direction of responses may reflect the proportions and reactivities of airways and vascular smooth muscle in lung strips. For example, the nett response of the lung strip caused by an agonist producing similar contractile effects in two or more components, would be expected to be related to the sum of the proportions of those components. Conversely, for an agonist which had opposing effects in two or more contractile components, nett response would be related to the ratio of the proportions and reactivities of those components. The present study was restricted to analyses of the relationships between nett pharmacological responses and the proportions of the lung strip consisting of vascular tissue, airways tissue proximal to alveolar ducts or alveolar parenchyma.

Methods

Pharmacological studies

Thirty-eight strips (2 × 2 × 25 mm) of parenchyma were dissected from the margins of 12 separate macroscopically normal specimens of human lung obtained 4–14 h post mortem. Preparations were suspended under 0.5 g tension in Krebs–Henseleit solution maintained at 37°C and aerated with 5% CO_2 and 95% O_2. The composition of Krebs–Henseleit solution (mM) was NaCl 117.6, KCl 5.4, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.57, D-glucose 11.1 and $CaCl_2$ 2.5. Changes in isometric tension were measured using a Grass force-displacement transducer (FTO3C) coupled to a preamplifier and recorded on a Rikadenki pen-recorder (model 1328L). After a 2 h equilibrium period, during which the bathing solution was renewed twice, tissues were exposed to 5-HT (100 μM) or NA (100 μM) for 5 min. At this concentration, these agonists caused maximal changes in resting tension in human lung parenchyma strip, bronchus and pulmonary artery (Goldie *et al.*, 1982). To reduce the effect of the high concentration of the first agonist tested on the sensitivity of the lung strip to the second agonist, these amines were administered in random order with at least 3 changes of bath fluid and a rest period of 45 min before exposure to the second agonist. During the rest period, basal tone was restored and preparations were then exposed to the second agonist. After this test, tissues were washed and then prepared for histological examination. Experiments were performed double-blind so that the results of pharmacological studies were not revealed to histologists until their examination of all tissues was complete and *vice-versa*.

Drugs used were (−)-noradrenaline bitartrate (Sigma) and 5-hydroxytryptamine creatinine sulphate (Roche), which were prepared in 0.9% w/v NaCl solution, containing ascorbic acid (20 μg ml^{-1}) as an anti-oxidant.

Histological studies

Lung strips were fixed whole in buffered formol saline and processed in a standardized, conventional manner for embedding in paraffin. Serial sections of the preparations were made in the longitudinal axis at 6 μm and every 10th section was taken for stereological analysis. Sections were stained for elastin (Miller, 1971), using a van Gieson counterstain. This staining method clearly defined the 3 major structural features of the walls of blood vessels and airways proximal to alveolar ducts (collagen, red; smooth muscle, yellow; elastic fibres, black). The outer limit of blood vessel walls was defined as the external elastic lamina, while the external border of the smooth muscle layer defined the boundary of proximal airways. The section contiguous to every 10th section was stained with haematoxylin and eosin and these provided useful reference slides when a nuclear stain was required for the recognition of features.

Stereological analysis Stereological methods (Weibel, 1979; 1980) enable estimates of values for parameters describing a 3-dimensional body (for example lung strip) to be made from measurements of 2-dimensional images (tissue sections) of that body. In the present study, estimates of the area fractions (A_a) of lung strip sections, consisting of blood vessels with a sectioned area greater than approximately 1500 μm^2 (and therefore likely to contain smooth muscle) and airways (proximal to alveolar ducts), were converted to estimates of their volume proportions (V_v) in the intact lung strip (Weibel, 1979; 1980).

Sampling Slides for measurement were systematically selected. Fields on each slide were chosen using the weighted random sampling technique described by Miles & Davy (1976). Slides were randomly placed over a regular square grid on transparent plastic and fields overlying intersections of grid lines were selected for measurements.

Measurements In measuring the volume proportion (volume density) of tissue in the lung strip, V_v(T/S), sections were viewed with a Leitz light microscope employing a 16× objective lens. A regular 42 point graticule consisting of 21 line segments (Weibel, Kistler & Scherle, 1966) was located in a 12.5× eyepiece and V_v(T/S) was estimated using manual point counting. The remainder of the lung strip was air space. Points overlying free cellular elements in alveolar lumina, were also designated as air space. For all other measurements, images of sections were projected from the microscope onto the measuring tablet of a Zeiss MOP-1 image analysis system, interfaced to a PDP 11/03 computer. Sections were viewed with a 10× objective lens and the magnification of the projected image set at × 180. The following parameters were measured as volume densities (V_v) in the whole lung strip (S); blood vessels (as defined above), V_v(BV/S); blood vessel wall, V_v(BVwall/S); airways proximal to alveolar ducts, V_v(AWY/S); and airways wall, V_v(AWYwall/S). Volume densities for these features determined as a proportion of total tissue content (T) were defined as V_v(BV/T), V_v(BVwall/T), V_v(AWY/T) and V_v(AWYwall/T) respectively.

An estimate of the volume density of parenchymatous tissue in the whole lung strip, V_v(Paren/S) was derived as follows:

$$V_v(\text{Paren/S}) = V_v(\text{T/S}) - (V_v(\text{BV/S}) + V_v(\text{AWY/S})) \quad (1).$$

The volume density of parenchymatous tissue in total tissue, (V_v(Paren/T)) was determined as follows:

$$V_v(\text{Paren/T}) = V_v(\text{T/T}) - (V_v(\text{BV/T}) + V_v(\text{AWY/T})) \quad (2),$$

where V_v(T/T) = 1.
These equations define parenchymatous tissue as that tissue other than larger blood vessels and airways proximal to alveolar ducts.

Analysis of stereological data The statistical error in stereological estimates of volume densities of anatomical features bears an inverse relationship to the total number of points or total area measured for that feature. In the present study, a progressive mean technique (Burri, Giger, Gnägi & Weibel, 1968) was employed to determine the required number of points (manual) or area (MOP-1) measured to bring the mean value for volume density (V_v) permanently within 10% confidence limits. For estimates of V_v(T/S), approximately 1,700 points were counted for each specimen. For all other parameters (MOP-1) approximately 1.6×10^6 mm^2 of projected image (25–30 sections) were measured for each specimen. Values for all volume densities (V_v) are expressed as a mean ± s.e.mean %. Results were submitted to multiple regression analysis using the Statistical Package for the Social Sciences (S.P.S.S.). The probability of differences between mean values for both pharmacological and stereological parameters was determined using the Student's two-tailed, non-paired *t* test, and considered significant if $P < 0.05$.

Results

Pharmacological studies

Multiple regression analysis of results showed that no significant relationships existed between the size or direction (i.e. relaxation or contraction) of responses to agonists and the post-mortem age of the lung strips tested ($P > 0.05$). Variable responses were observed in lung strips to both 5-HT and NA. Of the 38 preparations exposed to 5-HT, 15 contracted (mean ± s.e.mean = 47.8 ± 10.3 mg), 17 relaxed (29.0 ± 3.2 mg) and the resting tension in 6 failed to alter by ±5 mg and were thus said to have not responded. Similarly, of 33 lung strips exposed to NA, 25 contracted (32.5 ± 4.3 mg), 7 relaxed (32.1 ± 8.0 mg) and one failed to respond. The sizes of contractions caused by 5-HT and by NA were not significantly different ($0.1 < P < 0.2$), nor were the sizes of relaxations caused by these two amines ($0.2 < P < 0.3$). Both 5-HT and NA sometimes caused contraction in one preparation and relaxation in another preparation from the same specimen of lung, while some lung strips relaxed to both agonists. Other response patterns seen were relaxation to 5-HT and contraction to NA, relaxation to both 5-HT and NA or contraction to both 5-HT and NA. However, contraction to 5-HT and relaxation to NA was never observed in the same preparation.

It was hoped that any differences in the structure of lung strips would be most clearly revealed following stereological analyses of those preparations which responded with the greatest increases or decreases in resting tension. Accordingly, 2 preparations from each of 6 lung specimens, 3 preparations from one specimen and one preparation from another specimen were selected. The pharmacological responses of these 16 preparations are summarised in Table 1. In 6 preparations, 5-HT caused an increase in resting tension (75.4 ± 19.2 mg) while 9 lung strips relaxed (33.6 ± 4.3 mg). One lung strip did not respond to 5-HT. Of 12 preparations exposed to NA, 8 contracted (43.4 ± 8.7 mg) and 4 relaxed (41.3 ± 11.7 mg).

Stereological studies

For the 16 lung strips analysed with stereological

Table 1 Extent of contraction (+) or relaxation (−) (mg tension) of human lung parenchyma strips to 5-hydroxytryptamine (5-HT) or noradrenaline (NA) (100 μM)

Lung strip	*5-HT*	*NA*
1(a)	+50.0	+67.5
(b)	−47.5	−70.0
2(a)	+152.5	
(b)	+62.5	+82.5
(c)	−25.0	
3(a)	+42.5	+45.0
(b)	+112.5	+62.5
4(a)	+32.5	+25.0
(b)	−32.5	+27.5
5(a)	−37.5	+20.0
(b)	−12.5	+27.5
6(a)	−22.5	−15.0
(b)	−30.0	−47.5
7(a)	−42.5	
(b)	0	
8(a)	−52.5	−32.5
Mean contraction	75.4	43.4
s.e.mean	19.2	8.7
n	6	8
Mean relaxation	33.6	41.3
s.e.mean	4.3	11.7
n	9	4

methods, slightly less than half (46.4 ± 1.6%) of the volume was comprised of tissue, V_v(T/S), the remainder being air space (Figure 1). Values for V_v(T/S) in the individual strips ranged from 30.2% to 56.4%. However, the amount of parenchymatous tissue as a proportion of total tissue, V_v(Paren/T) varied over a narrower range (68.8–85.7%), with a mean value of 78.4 ± 1.1%. All preparations contained a considerable volume of blood vessels (V_v(BV/T) = 16.8 ± 1.1%) and all but preparation 4a contained airways proximal to alveolar ducts (V_v(AWY/T) = 4.8 ± 0.9%). As might be expected, highly significant relationships existed between V_v(BV/T) and V_v(BVwall/T) and between V_v(AWY/T) and V_v(AWYwall/T) ($P < 0.001$).

Combined analysis of pharmacological and stereological data

5-Hydroxytryptamine Table 2 summarises the stereologically determined proportions of parenchyma, blood vessel and airway parameters in lung strips that either contracted or relaxed to 5-HT (100 μM). The volume of parenchyma as a proportion of total tissue, V_v(Paren/T), or as a proportion of total lung strip volume, V_v(Paren/S), in preparations that contracted to 5-HT was not significantly different from that in lung strips that relaxed ($0.2 < P < 0.3$). However, V_v(BVwall/T) was significantly greater and V_v(AWYwall/T) was significantly less in lung strips that contracted to 5-HT than in lung strips that relaxed to 5-HT. Furthermore, [V_v(BVwall/T)]/[V_v(AWYwall/T)] was significantly higher in lung strips that contracted to 5-HT, than in those that relaxed ($P < 0.001$). The results of regression analyses of values for stereological parameters against responses to 5-HT (±tension, mg) are presented in Table 3. Significant positive correlations were revealed between the size and direction of responses of human lung strip to 5-HT and V_v(BVwall/T). No significant relationship existed between responses to 5-HT and V_v(AWYwall/T). However, a highly significant positive correlation existed between responses to 5-HT and

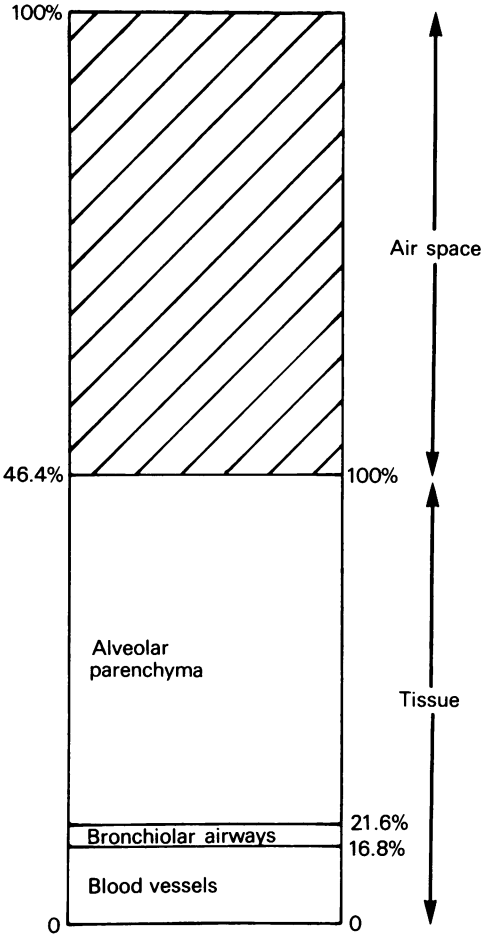

Figure 1 Schematic representation of human lung parenchyma strip showing the mean volume proportions (%) of alveolar parenchyma, peripheral airways and blood vessels in total tissue.

Table 2 Comparison of stereological parameters determined in human lung strips that either contracted or relaxed to 5-hydroxytryptamine (5-HT) and noradrenaline (NA) (100 μM)

5-HT	V_v(Paren/T)	V_v(BVwall/T)	V_v(AWYwall/T)	$\frac{V_v(BVwall/T)}{V_v(AWYwall/T)}$
contraction ($n=6$)	77.3±2.0	10.3±1.1	1.6±0.4	5.6±0.8
relaxation ($n=9$)	79.6±1.3^{NS}	7.2±0.5**	4.4±0.8*	2.0±0.3***
NA				
contraction ($n=8$)	76.9±1.5	9.4±1.0	3.3±1.1	3.5±0.9
relaxation ($n=4$)	80.2±1.7^{NS}	6.6±0.6^{NS}	3.6±0.6^{NS}	1.9±0.2^{NS}

Results are expressed as mean ± s.e.mean.
Significant difference between relaxation and contraction values; *$P<0.05$, **$P<0.02$, ***$P<0.001$.
NS no significant difference between relaxation and contraction values, $P>0.05$ (non-paired t test)

$[V_v(BVwall/T)]/[V_v(AWYwall/T)]$ ($r=0.87$, $P<0.01$). The line of best fit for this ratio against responses to 5-HT is shown in Figure 2.

Noradrenaline There was no significant difference in V_v(Paren/T), or in V_v(Paren/S), in preparations that either contracted or relaxed to NA ($0.2<P<0.3$) (Table 2). Mean values for V_v(BVwall/T) and $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$ were lower in preparations that relaxed to NA than in those that contracted to NA as was the case for 5-HT. However, the differences in these values with respect to NA responses did not reach statistical significance (Table 2). Conversely, regression analysis of the values for $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$ (Figure 3, Table 3) and for V_v(BV/S) against responses to NA showed significant positive correlations ($P<0.05$). No significant correlations existed between responses to NA and any other stereological parameter. Furthermore, there were no significant differences between preparations that contracted to NA or contracted to 5-HT with respect to any stereological parameter (Table 2). This was also true for preparations that relaxed to NA or relaxed to 5-HT.

Table 3 Correlation coefficients (r) and probabilities (P) from multiple regression analysis of relationships between responses of human lung parenchyma strips to 5-hydroxytryptamine (5-HT) or noradrenaline (NA) (±mg tension) and stereological parameters

	5-HT (n = 16)		*NA* (n = 12)	
	r	P	r	P
V_v(Paren/T)	−0.04	NS	−0.30	NS
V_v(BVwall/T)	+0.57	<0.05	+0.42	NS
V_v(AWYwall/T)	−0.45	NS	−0.27	NS
$\frac{V_v(BVwall/T)}{V_v(AWYwall/T)}$	+0.87	<0.01	+0.65	<0.05

NS, no significant relationship.

Discussion

Correlations were sought between pharmacological responses of human lung parenchyma strips and

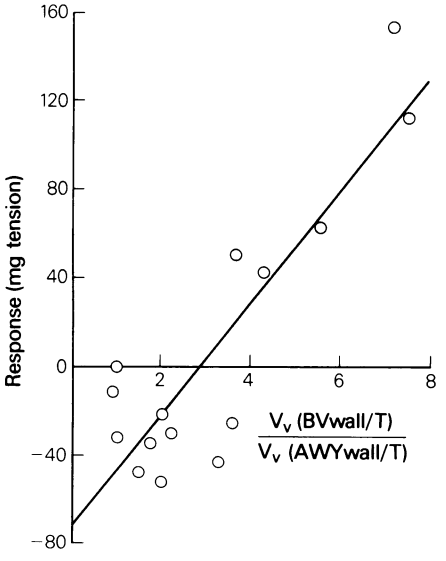

Figure 2 Regression analysis of the relationship between the ratio of the volume densities (V_v%) of blood vessel wall in tissue, to airways wall in tissue, $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$ and responses (+ or − mg tension) of human isolated lung parenchyma strips to 5-hydroxytryptamine (5-HT) (response = 25.3 (ratio) − 72.1; $r=0.87$, $P<0.01$).

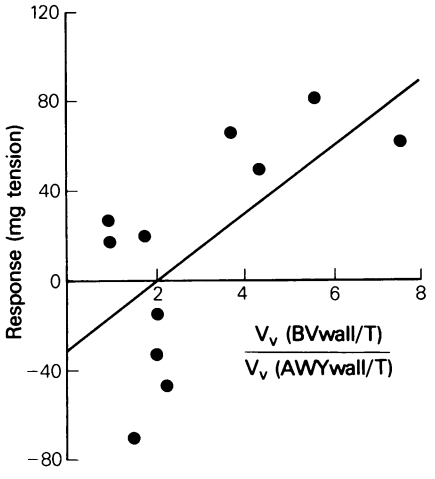

Figure 3 Regression analysis of the relationship between the ratio of the volume densities (V_v%) of blood vessel wall in tissue, to airways wall in tissue, $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$ and responses (+ or − mg tension) of human isolated lung parenchyma strips to noradrenaline/(NA) (response = 15.5 (ratio) − 31.1; $r = 0.65$, $P < 0.05$).

quantitative estimates of the proportions of the 3 major contractile components. While such correlations are not in themselves proof of causal links between pharmacological responses and structure, they may have greater significance in the light of other results. Both 5-HT and NA caused contraction, relaxation or had little effect in human lung strips, confirming previous findings (Goldie *et al.*, 1982). These results are consistent with the notion that different lung strips, from the same or from different specimens of lung, contained different proportions of at least two contractile components one of which contracted while the other relaxed to 5-HT and to NA. Since both 5-HT and NA caused contraction of human isolated pulmonary artery and relaxation of human isolated bronchial preparations, it has been suggested that both of these agonists may also have opposing effects in peripheral blood vessels and bronchioles in lung parenchyma strips (Goldie *et al.*, 1982). The size and direction of responses of human lung strips to 5-HT and NA may therefore be related to the relative volume densities (V_v%) of these contractile elements.

On average, human lung strips that contracted to 5-HT contained a significantly greater proportion of blood vessels and a significantly lesser proportion of larger airways than preparations that relaxed. However, all of these lung strips contained similar amounts of parenchymatous tissue. Importantly, a highly significant correlation existed between the size and direction of 5-HT-induced responses of lung strips and the ratio of the volume densities of blood vessel wall to airways wall in tissue, $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$. This relationship indicates that human lung strips containing greater than 3–4 times more vascular smooth muscle than airways smooth muscle will contract to 5-HT while preparations with lower ratios will relax. Of the 38 preparations exposed to 5-HT, 15 contracted. Presumably, these 15 preparations were responding to 5-HT as predominantly vascular preparations. Responses of contractile cells within alveolar parenchyma probably did not contribute significantly to 5-HT-induced responses. Indeed no direct evidence was obtained to suggest that 5-HT caused either relaxation or contraction of elements within alveolar tissue, although the possibility cannot be excluded. As with 5-HT, a significant positive correlation existed between NA-induced responses in human lung strips and the ratio of blood vessel wall to airways wall $[V_v(BVwall/T)]/[V_v(AWYwall/T)]$. These results suggest that NA caused contraction of peripheral blood vessels (α-adrenoceptor response) and concomitant relaxation of airways proximal to alveolar ducts (β-adrenoceptor response). The correlation indicates that NA will induce a nett contractile response in lung strips containing more than twice as much vascular smooth muscle as airways smooth muscle. Results with both 5-HT and NA suggest that human lung strips can be screened to separate those preparations which are most likely to behave as true airway preparations from those which may manifest primarily vascular responses to drugs.

The concept that most of the α-adrenoceptors in lung strips were vascular and thus morphologically separate from the majority of β-adrenoceptors (airways) is consistent with results in pig lung strips showing potentiation of β-adrenoceptor-mediated responses to isoprenaline following inhibition of catechol-*O*-methyl transferase (COMT) but little or no effect with respect to α-adrenoceptor mediated contractions (Goldie & Paterson, 1982). Black *et al.* (1981), suggested that the majority of α-adrenoceptors mediating contraction of human lung strips were probably located in parenchyma. However, no significant relationship existed between NA-induced responses and the proportion of parenchymatous tissue in lung strips V_v(Paren/S) or V_v(Paren/T). Furthermore, similar values for V_v(Paren/T) were determined in lung strips that either relaxed or contracted to NA. Similarly, the size and direction of responses to NA did not correlate significantly with the V_v(BVwall/T). However, it is possible that NA-induced contraction of peripheral blood vessels was modified by concomitant NA-induced stimulation of vascular β-adrenoceptors.

It is evident that pharmacological responses of

human lung strips do not necessarily reflect effects in peripheral airways only. The present work shows that different human lung parenchyma strips contain different mixtures of at least three potentially contractile components, namely blood vessels, airways proximal to alveolar ducts and alveolar contractile tissue. One or more of these components may contract or relax and contribute significantly to nett responses of lung strips. Contractile responses to 5-HT and to NA seem primarily to involve contractions of peripheral blood vessels while relaxation may primarily occur in airway structures. Although results from the present study do not exclude the possibility that alveolar cells responded by contracting to 5-HT and NA, they do suggest that the contractile and relaxant effects of these agonists in human lung strips are mediated mainly via receptors in other contractile components. It seems likely that this is also true for lung strip preparations from other species.

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