Pharmacological and histological examinations of regional differences of guinea-pig lung: a role of pleural surface smooth muscle in lung strip contraction

¹Wai-shiu Fred Wong, ²Shari L. Bloomquist, Alison M. Bendele & Jerome H. Fleisch

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, U.S.A.

1 Parenchymal lung strip preparations have been widely used as an *in vitro* model of peripheral airway smooth muscle. The present study examined functional responses of 4 consecutive guinea-pig lung parenchymal strips isolated from the central region (segment 1) to the distal edge (segment 4) of the lower lung lobe. The middle two segments were designated as segments 2 and 3.

2 Lung segments 1 and 4 exhibited significantly greater contraction than the other 2 segments to KCl when responses were expressed as mg force per mg tissue weight. Contractile responses to bronchospastic agents including histamine, carbachol, endothelin-1, leukotrienes (LT) B₄ and D₄, and the thromboxane A₂-mimetic U46619 demonstrated no significant difference in EC₅₀ values among the 4 lung segments.

3 Contractile responses of segments 1 and 4 to antigen-challenge (ovalbumin), ionophore A23187 and substance P were significantly greater than the other 2 segments with respect to either sensitivity or maximum responsiveness.

4 U46619-induced contractions of the 4 lung segments were relaxed in similar manner by papaverine and theophylline up to 100%, salbutamol up to 80%, and sodium nitroprusside by only 20%. In contrast, sodium nitroprusside markedly reversed U46619-induced contraction of pulmonary arterial rings and bronchial rings.

5 Histological studies identified 2–4 layers of smooth muscle cells underlying the lung pleural surface. Mast cells were prominent in this area. Moreover, morphometric studies showed that segment 4 possessed the least amount of smooth muscle structures from bronchial/bronchiolar wall and vasculatures as compared to the other 3 segments, and a significant difference in this respect was evident between segment 1 and segment 4.

6 Since lung segments 1 and 4 are covered with larger surface area of lung pleura, the present results suggest that the significantly greater intrinsic contractile responses of segments 1 and 4 are associated with the presence of increased lung pleural surface possibly together with more mast cells. Thus, a primary contribution to the net contraction of the lung parenchymal strips may be smooth muscle from the lung pleura, alveolar ducts and interstitial contractile cells rather than from bronchi/bronchioles and microvasculatures.

Keywords: Peripheral airway smooth muscle; lung pleural surface; ovalbumin; A23187; eicosanoids; thromboxane A₂ mimetic; sodium nitroprusside

Introduction

Isolated lung parenchymal strip preparations were developed by Lulich *et al.* (1976), and Drazen & Schneider (1978) to study the function of peripheral airway smooth muscle. This *in vitro* model of the peripheral lung contracts to broncho-spastic substances thought to be associated with airway hypersensitivity reactions including various eicosanoids, neurohormones, and peptides (Lulich *et al.*, 1976; Gerard, 1987; Burka, 1988). Antigen-induced contractions of immunologically sensitized parenchymal lung strips have provided further understanding of the pathogenic mechanisms of allergic asthma and have aided in development of potential anti-asthmatic agents (Chand & Eyre, 1978; Fleisch *et al.*, 1981; 1985).

Controversies exist regarding the validity of the lung parenchymal strip as a true representation of peripheral airway smooth muscle. The lung parenchymal strip is a complex tissue consisting of many cell types including smooth muscle from bronchial and bronchiolar wall, pulmonary vasculature, alveolar ducts, and interstitial contractile cells (Kapanai *et al.*, 1974; Drazen & Schneider, 1978; Evans & Alder, 1981; Goldie *et al.*, 1984). Moreover, Halonen *et al.* (1990) have recently demonstrated the significance of smooth muscle

layers underlying guinea-pig lung pleura as the primary site of action of platelet activating factor (PAF)-induced parenchymal lung strip contraction. All of these components are potential elements for the net contraction of lung strips. Systematic regional study of the peripheral lung has not been performed to determine which segment should be isolated to study contractile and relaxant responses to various pharmacological agonists, to ionophore, or to anaphylactic substances. The present investigation evaluated the regional differences in contractile and relaxant responses of 4 consecutive longitudinal parenchymal segments in order to establish the contribution of lung pleural smooth muscle to guinea-pig lung strip contractions.

Methods

Tissue preparation

Male Hartley guinea-pigs (Charles River, Portage, MI, U.S.A.) weighing 350–500 g were killed by decapitation under CO₂ narcosis. After thoracotomy, the heart and lungs were excised *en bloc* and perfused with 50 ml Krebs-bicarbonate solution via the pulmonary artery. Four longitudinal segments of similar size (3 mm × 20 mm) were cut from the right lower lobe of the lung and were designated from central to peripheral regions as segments 1, 2, 3 and 4, respectively (Figure 1).

¹ Author for correspondence.

² Present address: Purdue University, West Lafayette, Indiana, U.S.A.

Figure 1 Diagram illustrating the isolation of 4 consecutive longitudinal parenchymal strips from the lower right lobe of guinea-pig lung. Lung strips possessed both the anterior and posterior pleural surfaces (shaded area).

The thickness of these lung strips varied from 2–3 mm for segments 1 and 4, and was 3–5 mm for the other two segments. All lung strips possessed both anterior and posterior pleural surfaces which accounted for approximately 75% and 46% of the total surface area of segments 1 and 4, and segments 2 and 3, respectively.

Lung strips were suspended isometrically under the previously determined optimum resting tension of 1 g (see below) in organ baths containing 10 ml Krebs-bicarbonate solution aerated with 95% O₂ and 5% CO₂ at 37°C. In some experiments, ring segments (~3 mm in length) from the main pulmonary artery and the right bronchi were isolated and mounted under the same conditions as the lung strips, except for the resting tension being 2 g. Composition of the Krebs-bicarbonate solution in mmol l⁻¹ was: NaCl 118.2, KCl 4.6, NaHCO₃ 24.8, CaCl₂ · 2H₂O 2.5, KH₂PO₄ 1.2, MgSO₄ · 7H₂O 1.2 and dextrose 10.0. Contractile responses were monitored with a force displacement transducer (Grass FT-03) coupled to a Grass Model 7D polygraph recorder (Grass Instrument Co., Quincy, MA). A 60 min equilibration period was allowed before any experimentation was begun, during which time the bath fluid was changed every 10–15 min.

Sensitization procedures

In the antigen-induced contraction study (Schultz Dale reaction; Schultz, 1910; Dale, 1913; Chand & Eyre, 1978), parenchymal strips were isolated from guinea-pigs passively sensitized by a single intraperitoneal (i.p.) injection of 1 mg kg⁻¹ rabbit IgG antibody against chicken egg albumin (Organon Teknica Corp, West Chester, PA, U.S.A.). The animals were killed 2 days post injection (see tissue preparation).

Optimum resting tension determination

To determine the passive force required to produce maximum contractile responses of the 4 different lung segments, a series of length-tension studies was conducted. An initial resting force of 250 mg was applied to the parenchyma strips followed by a 60 min equilibration period before contractions were induced by 30 mM KCl. After repeated washings and returning to baseline tension, the resting force applied to the lung strips was increased stepwise by 250 mg after each KCl-induced contraction until contractile responses were weaker than preceding ones. At the end of the experiment, parenchymal strips were blotted and weighed on a Mettler H31 balance. Contractile responses were expressed as percentage of maximal contractions evoked by 30 mM KCl and some data were illustrated as mg force per mg weight of the tissue strips (mg F mg⁻¹ wt).

Contractile studies

After the 60 min equilibration period, parenchymal strips were contracted by addition of single concentrations of 30 mM and 60 mM KCl. Since preliminary data indicated that contractile responses to a single dose of 60 mM KCl were consistently greater than those produced by a single dose of 160 mM KCl, the former was used as the 100% standard control response for all subsequent agonist-evoked contraction. Except for the concentration-response curves to histamine and carbachol, which were obtained on the same parenchymal strips, individual lung strips were used to produce a single concentration-response curve to a specific agonist. Whereas increasing concentrations of KCl (5–160 mM), histamine (0.01–100 µM), carbachol (0.01–100 µM), A23187 (0.1–100 µM), ovalbumin (OA, 0.1–3000 ng ml⁻¹), leukotriene D₄ (LTD₄, 0.1–100 nM), endothelin-1 (0.1–300 nM), or U46619 (a thromboxane A₂-mimetic, 0.1–3000 nM) were administered cumulatively into the organ baths, substance P (0.01–10 µM) and LTB₄ (2–3000 nM) were added sequentially to avoid potential development of tachyphylaxis. To prevent enzymatic degradation of the peptide agonists peptides, endothelin-1- and substance P-induced contractions were elicited in the presence of 3 × 10⁻⁶ M thiorphan, an inhibitor of neutral endopeptidase (Gerard, 1987), which was added to the organ baths for 20 min before the administration of the peptides.

Relaxation studies

To determine if the 4 parenchymal segments exhibited a similar relaxation pattern to various smooth muscle relaxants, a series of spasmolytic experiments was performed. Histamine (1 × 10⁻⁴ M, a dose that produced maximum contractile response to this agonist) and carbachol (2 × 10⁻⁵ M, a dose that produced at least 80% of the maximum contractile response to this agonist) were first chosen to pre-contrast parenchymal strips. However, contractions elicited by these agonists waned within 20 min. In contrast, 3 × 10⁻⁶ M U46619, a concentration that evoked maximal responses of the lung strips to this agonist, generated sustained contractions for at least 2 h. As a result, all parenchyma were pre-contracted with U46619 for 20 min (time required to reach plateau contraction) before one of the four relaxing agents including papaverine (0.001–300 µM), theophylline (0.01–100 µM), salbutamol (0.01–100 µM), or sodium nitroprusside (SNP, 0.01–100 µM) was added cumulatively to the organ baths. Concentration-relaxation responses to these agonists were obtained by injecting the next higher dose into the baths when the reduction of contractile force by the preceding dose was less than 25 mg in 5 min. Only one curve to a specific relaxant was generated from each lung strip. The relaxant effect was expressed as percentage reduction of the U46619-induced peak contraction of the parenchymal strips. In the salbutamol-mediated relaxation study, 1 × 10⁻⁶ M phentolamine was preincubated with the lung tissues for 20 min before the β-adrenoceptor agonist was added to prevent potential interaction of salbutamol (at high doses) with α-adrenoceptors in the lung strips. To differentiate between the contributory role of vascular and bronchial smooth muscle in lung parenchymal contraction, the relaxant effects of SNP (0.001–100 µM) on pulmonary arterial and bronchial rings pre-contracted with 3 × 10⁻⁶ M U46619 were studied and compared to that on the lung strips.

Histological studies

Lungs were removed and infused tracheally with either 10% neutral buffered formalin (3 animals) or Carnoy's fixative (2 animals), cut into strips, as described above, and prepared for routine paraffin embedding. A 6 µm tissue section was cut sagittally from the midpoint (~1.5 mm) of the thickness of each lung segment. Formalin fixed tissues were stained with haematoxylin and eosin or Masson's trichrome stain. Carnoy's fixed

tissue was stained with toluidine blue. Haematoxylin and eosin stained sections from each of the 4 lung segments of the left and right lower lobes of 3 animals were evaluated for number of bronchi (with cartilaginous rings), number of bronchioles (without cartilaginous rings) containing 3 or more layers of smooth muscle cells and number of blood vessels containing at least 3 layers of myocytes. Since smooth muscle structures in each lung segment from both left and right lung lobes were essentially the same, data were pooled together for statistical analysis. The total area for each section was determined by use of a Bioquant Image Analysis system and numbers of each structure per cm² of tissue determined. The mean values for each of the four strips were calculated by adding the numbers obtained from each of the six sections (right and left lung of 3 animals). Trichrome stained sections were evaluated for distribution and amount of smooth muscle. Toluidine blue stained sections were examined for distribution of mast cells.

Data analysis

All data are presented as mean $\pm$ s.e.mean. Sensitivities (EC_{50}) of the tissues were determined at 50% maximum response to a stimulant or a relaxant using a 4-parameter logistic model of nonlinear regression analysis. Comparisons of mean contractile or relaxant responses, mean EC_{50} values, and mean numbers of smooth muscle structures per cm² of tissue section were performed by one-way analysis of variance followed by either Tukey's honestly significant difference test (HSD) or Dunnett's two-tail t test. Significance level was set at $P < 0.05$.

Drugs and chemicals

Histamine dihydrochloride, carbamylcholine chloride (carbachol), substance P, ovalbumin, salbutamol hemisulphate, sodium nitroprusside and DL-thiorphan were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), endothelin-1 (human, porcine) from Peninsula Lab. Inc. (Belmont, CA, U.S.A.), IgG fraction rabbit anti-ovalbumin from Organon Teknica Corp. (West Chester, PA, U.S.A.), U46619 (11 α , 9 α -epoxymethano-prostaglandin H₂) from Upjohn Co. (Kalamazoo, MI, U.S.A.) and phentolamine hydrochloride from Ciba Pharmaceutical Co. (Summit, N.J., U.S.A.). LTB₄, LTD₄, A23187, papaverine hydrochloride and theophylline were obtained from Eli Lilly and Co. (Indianapolis, IN, U.S.A. and Windlesham, U.K.). Leukotrienes B₄ and D₄ were stored in ethanol, U46619 in methyl acetate and IgG antibody in sterile saline at -20°C , and prepared freshly by serial dilution in distilled H₂O. A23187 and DL-thiorphan were dissolved in dimethyl sulphoxide, theophylline in hot (60–70 $^{\circ}\text{C}$) distilled H₂O, and all other drugs were prepared in distilled H₂O.

Results

Optimum resting tension determination

The 4 longitudinal lung segments rapidly contracted to 30 mM KCl, a dose previously shown to elicit near maximum contractile responses of guinea-pig lung parenchymal strips under a resting tension of 500 mg (Fleisch *et al.*, 1982). As the amount of resting force applied to the lung strips was increased from an initial tension of 250 mg, the magnitude of KCl-induced contractile responses increased correspondingly (Figure 2a). Maximum contractions for lung segments 3 and 4 were achieved with 1 g resting tension while those for segments 1 and 2 were attained with 1.25 g and 1.5 g resting force, respectively. To avoid excessive stretching of the parenchymal strips, an optimum resting force of 1 g was chosen for all lung segments to assure near maximum active contractile responses ($\sim 90\%$) in all subsequent experiments. When contractions of

Figure 2 (a) Percent active contractile force developed in lung segments 1 ($\square$), 2 ($\blacksquare$), 3 ($\circ$) and 4 ($\bullet$) in response to 30 mM KCl at a stepwise increase in resting tension applied to the tissues. Each point represents the mean (s.e.mean shown by vertical bars) of 4 experiments. (b) Contractile responses of the 4 lung segments to 30 mM KCl at 1 g resting tension were expressed as mg active force generated per mg weight of the tissues (mg F mg⁻¹ wt). Each column represents mean (s.e.mean shown by vertical bars) of 4 experiments.

the parenchymal strips were expressed as mg force produced per mg weight of each lung strip at 1 g resting tension, values for segments 1 and 4 were 3.06 ± 0.33 and 3.35 ± 0.28 respectively, both of which were significantly greater ($P < 0.004$ and $P < 0.001$) than those for segments 2 and 3, being 1.96 ± 0.11 and 1.50 ± 0.16 (Figure 2b).

Contractile studies

Concentration-response relationships of the 4 parenchymal segments to KCl, histamine, carbachol and substance P are depicted in Figure 3. Although the lung strips exhibited similar sensitivity to increasing concentrations of KCl with $-\log$ molar EC_{50} values ranging from 1.71 ± 0.02 to 1.58 ± 0.03 , and reached maximum contractile responses at 160 mM KCl, a significant leftward shift ($P < 0.05$, 1.35 fold) of the concentration-effect curve for segment 4 from that for segment 3 was noted (Figure 3a). However, the pharmacological and physiological significance of this small shift is questionable. The 4 parenchymal segments also demonstrated equivalent sensitivities to either histamine or carbachol with $-\log$ molar EC_{50} values ranging from 6.10 ± 0.12 to 5.73 ± 0.16 for the former and from 5.66 ± 0.08 to 5.43 ± 0.09 for the latter. Detectable contractions were observed at 1×10^{-8} M for histamine and at 1×10^{-7} M for carbachol, and peak contraction was achieved at 1×10^{-4} M for both agonists (Figure 3b, c). The maximum contractile response of the 4 lung segments produced by histamine, being 100% of the 60 mM KCl control response, was considerably higher than those induced by carbachol, the latter being only 70% of the KCl control. There was no significant difference in sensitivity among the 4 lung segments to substance P with $-\log$ molar EC_{50} values ranging from 6.91 ± 0.12 to 6.69 ± 0.16 ; however, the magnitude of contractions of segments 1 and 4 to this agonist was significantly ($P < 0.05$) higher than that

Figure 3 Log concentration-response curves of lung segments 1 ($\square$), 2 ($\blacksquare$), 3 ($\circ$), and 4 ($\bullet$) to (a) KCl (5–160 mM), (b) histamine (0.01–100 μ M), (c) carbachol (0.01–100 μ M), and (d) substance P (0.01–10 μ M). Results are expressed as percentage of contractile responses to 60 mM KCl. Each point represents the mean of 5–12 experiments; vertical bars show s.e.mean. * indicates $P < 0.05$.

produced by the other 2 strips throughout the entire range of concentrations (Figure 3d).

In tissue from sensitized animals, the ovalbumin concentration-response curve for lung segment 1 was significantly shifted ($P < 0.05$) to the left as compared to the other 3 segments which exhibited similar sensitivity to increasing concentrations of ovalbumin (Figure 4a). The $-\log \text{ g ml}^{-1} \text{ EC}_{50}$ value of ovalbumin for segment 1 was 8.67 ± 0.18 as compared to that for the other 3 segments ranging from 8.13 ± 0.05 to 7.80 ± 0.18 , which represents a 4–7 fold increase in sensitivity. Moreover, the magnitude of contractile responses of segment 1 was significantly greater than that of the other 3 segments at concentrations of ovalbumin between $3 \times 10^{-10} \text{ M}$ and $1 \times 10^{-6} \text{ M}$. The maximum contractile response produced by ovalbumin was about 120% of the KCl control response.

Figure 4 Log concentration-response curves of lung segments 1 ($\square$), 2 ($\blacksquare$), 3 ($\circ$), and 4 ($\bullet$) to (a) ovalbumin (10^{-10} – $3 \times 10^{-6} \text{ g ml}^{-1}$) and (b) A23187 (0.1–100 μ M). Results are expressed as percentage of contractile responses to 60 mM KCl. Each point represents the mean of 5–6 experiments; vertical bars show s.e.mean. * indicates $P < 0.05$.

The concentration-response curves of segments 1 and 4 to A23187 were significantly shifted ($P < 0.05$) to the left with significantly higher maximum contractile responses ($P < 0.05$) as compared to the curves for the other two segments (Figure 4b). The $-\log \text{ molar EC}_{50}$ values of A23187 for segments 1 and 4 were 6.72 ± 0.06 and 6.64 ± 0.07 , whereas those for segments 2 and 3 were 6.34 ± 0.12 and 6.01 ± 0.12 respectively.

Table 1 summarizes the potencies ($-\log \text{ molar EC}_{50}$) and % maximal responses of LTB₄, LTD₄, U46619 and endothelin-1 on the 4 lung segments. There was no significant difference ($P > 0.05$) in $-\log \text{ molar EC}_{50}$ to these agonists among the 4 lung parenchymal strips.

Relaxation studies

All lung segments contracted strongly to a single dose of $3 \times 10^{-6} \text{ M}$ U46619, and maximum contractions were achieved at 20 min and lasted for at least 2 h with negligible decreases in tension. Papaverine and theophylline produced similar concentration-dependent relaxation of all lung segments with very steep slopes within a narrow range of concentrations. Complete reversal (100% decrease) of U46619-induced contraction was obtained at $3 \times 10^{-4} \text{ M}$ for papaverine and at $3 \times 10^{-3} \text{ M}$ for theophylline. Theophylline exhibited equivalent potency ($P > 0.05$) in relaxing the 4 lung strips, whereas significant differences in sensitivities to papaverine ($P < 0.05$, 15–19 fold) were observed among lung segment 2 and segments 1 and 4 (Table 2). Salbutamol, $1 \times 10^{-4} \text{ M}$, elicited approximately 65% maximum reduction of contraction for lung segments 1 and 2, and at $3 \times 10^{-5} \text{ M}$, about 85% maximum reversal of contraction for lung segments 3 and 4 was observed. No further relaxation was observed in any lung segments beyond these concentrations. There was no significant difference ($P < 0.05$) in the potency of salbutamol among the 4 lung strips (Table 2).

In contrast to the above mentioned smooth muscle relaxants, sodium nitroprusside produced only a moderate relaxant effect on U46619-precontracted lung strips with a maximum

Table 1 Potencies and % maximal responses of various agonists on 4 lung segments

Agonists	Lung segments ^a			
	1	2	3	4
LTB ₄	7.9 ± 0.1 ^{b, c} (68.4 ± 3.5) ^d	7.9 ± 0.1 (59.0 ± 6.8)	7.8 ± 0.0 (53.7 ± 5.7)	7.7 ± 0.0 (69.4 ± 10.3)
LTD ₄	8.0 ± 0.3 (111.0 ± 7.6)	7.4 ± 0.2 (102.0 ± 4.3)	7.3 ± 0.2 (91.7 ± 4.8)	7.9 ± 0.2 (105.4 ± 4.3)
U46619	8.2 ± 0.8 (96.6 ± 7.2)	7.7 ± 0.9 (92.3 ± 10.9)	7.1 ± 0.8 (79.1 ± 8.4)	7.9 ± 0.7 (95.2 ± 7.6)
Endothelin	7.3 ± 0.1 (81.5 ± 7.1)	7.1 ± 0.1 (78.1 ± 3.1)	7.1 ± 0.1 (80.5 ± 2.1)	7.2 ± 0.1 (75.0 ± 3.2)

^a Lung parenchymal strips were isolated longitudinally from the central to the peripheral region of the lower lung lobe and were designated as lung segments 1, 2, 3, and 4 respectively (see Methods).

^b Each value represents the mean ± s.e.mean of 5–6 experiments.

^c –log molar EC₅₀.

^d % response to 60 mM KCl.

LTB₄, LTD₄: leukotrienes B₄ and D₄.

relaxation of 20–25% at 3×10^{-5} M (Figure 5a). In contrast, in pulmonary arterial and bronchial rings pre-contracted with 3×10^{-6} M U46619, sodium nitroprusside totally relaxed the pulmonary artery contraction at 3×10^{-6} M with –log molar EC₅₀ value of 7.45 ± 0.05 and reversed the bronchial smooth muscle contraction by 90% with a –log molar EC₅₀ value of 5.87 ± 0.08 (Figure 5b).

Histological studies

Morphometric analyses of the 4 lung segments are summarized in Table 3. Lung segment 2 contained significantly more bronchi ($P < 0.05$) than the other 3 segments, whereas

lung segment 1 had more bronchioles ($P < 0.05$) than the other 3 segments. There were no significant differences in the distribution of pulmonary vasculature among the 4 segments. Despite the similarity in contractile responses observed between segments 1 and 4, the latter segment possessed significantly ($P < 0.05$) less total numbers of bronchi, bronchioles, and blood vessels than the former. On the other hand, 2–4 layers of smooth muscle cells with oblique orientation were identified to be associated with the visceral pleura surrounding the parenchyma (Figure 6). In toluidine blue-stained sections, mast cells were found not only in the connective tissue surrounding airways and vasculature, but also in that along the pleural surface.

Discussion

The present investigation demonstrated that parenchymal strip preparations isolated from different regions of guinea-pig lung responded in a qualitatively similar but quantitatively distinct manner to various pharmacological agents. When active tension was expressed as mg force produced per mg weight of tissue, lung segments 1 and 4 generated equivalent contractile responses to KCl which were significantly greater than those produced by the other two segments. The greater magnitude of contractile responses observed in segments 1 and 4 could not be attributed to additional smooth muscle structures including bronchi, bronchioles, or pulmonary vasculatures located in these lung strips. Morphometric analyses revealed that segment 4 consisted of significantly fewer ($P < 0.05$) contractile structures per cm² section area

Figure 5 Log concentration-relaxation curves of (a) lung segments 1 (□), 2 (■), 3 (○), and 4 (●) and (b) pulmonary arterial ring segments (△) and right bronchial smooth muscle ring segments (▲) pre-contracted with 3×10^{-6} M U46619 to sodium nitroprusside (SNP) (0.01–100 μM). Results are expressed as percentage decreases in U46619-evoked maximum response of the respective tissues. Each point represents the mean of 5–6 experiments; vertical bars show s.e.mean.

Figure 6 Photomicrograph of guinea-pig lung showing mesothelium (arrow head) with underlying smooth muscle cells (arrows). Haematoxylin and eosin, bar = 25 μm.

Table 2 Potencies and % maximal relaxation of various relaxants on 3×10^{-6} M U46619-precontracted lung segments

Relaxants	Lung segments ^a			
	1	2	3	4
Papaverine	4.6 ± 0.2 ^{b,c,*} (100.0 ± 0.0) ^d	3.4 ± 0.5 (100.0 ± 0.0)	3.9 ± 0.2 (100.0 ± 0.0)	4.7 ± 0.2* (100.0 ± 0.0)
Theophylline	3.0 ± 0.1 (100 ± 0.0)	3.1 ± 0.2 (100.0 ± 0.0)	3.1 ± 0.1 (100.0 ± 0.0)	3.4 ± 0.2 (100.0 ± 0.0)
Salbutamol	6.5 ± 0.0 (62.8 ± 2.0)	6.6 ± 0.1 (65.7 ± 6.2)	6.7 ± 0.1 (87.9 ± 4.6)	6.6 ± 0.1 (79.3 ± 5.4)

^a Lung parenchymal strips were isolated longitudinally from the central to the peripheral region of the lower lung lobe and were designated as lung segments 1, 2, 3, and 4 respectively (see Methods).

^b Each value represents the mean ± s.e.mean of 5–6 experiments.

^c –log molar EC₅₀.

^d % relaxation to 3×10^{-6} M U46619-induced precontraction of the lung segments.

* $P < 0.05$ indicates significant difference from lung segment 2.

than segment 1. The larger contractions produced by segments 1 and 4 appeared to be associated with pleural surface smooth muscle since both segments 1 and 4 had a considerably larger pleural surface area (75%) than segments 2 and 3 (46%), a finding that supports a recent study by Halonen *et al.* (1990). Contractile responses to various bronchospastic substances including histamine, carbachol, endothelin-1, LTB₄, LTD₄, or U46619 demonstrated no significant difference in sensitivity or maximum responsiveness among the 4 parenchymal lung strips. This homogeneity among the lung segments is probably due to the normalization procedure of transforming contractile response as percent maximum KCl control response.

The Schultz-Dale reaction describes an immunologically-mediated contraction of smooth muscle, in this case, parenchymal lung strips, due to the release of anaphylactic substances from sensitized effector cells such as the mast cell (Schultz, 1910; Dale, 1913; Chand & Eyre, 1978; Fleisch *et al.*, 1982). A23187, a divalent cationic ionophore, has been shown to release nonimmunologically anaphylactic mediators from lung with subsequent smooth muscle contraction (Piper & Seale, 1979; Fleisch *et al.*, 1982; Burka, 1983; Cheng *et al.*, 1987). In addition, A23187 can directly activate smooth muscle contraction by increasing cytoplasmic free Ca²⁺ concentration via enhancement of transmembrane Ca²⁺ influx and intracellular Ca²⁺ release (Kondo, 1989). The concentration-response curve for IgG-sensitized lung segment 1 to ovalbumin was to the left of those for the other lung segments. Moreover, concentration-response relationships for segments 1 and 4 to A23187 were significantly ($P < 0.05$) more sensitive with maximum responsiveness substantially greater than segments 2 and 3. Thus, it appears that the increased sensitivity and maximum responsiveness of segment 1 to ovalbumin, and segments 1 and 4 to A23187 were probably associated with more mast cell degranulation upon challenge and, in addition, enhanced availability of Ca²⁺ to the smooth muscle in the pleural surface in response to A23187. Histological observations from the present study were reinforced by an additional report from our laboratory (Wong *et al.*, 1991) indicating that there is a widespread distribution of mast cells beneath the pleural surface. Similarly, concentration-response curves for segments 1 and 4 exhibited significantly higher

maximum responses to substance P, which may be linked to the ability of this peptide to degranulate mast cells (Foreman, 1987).

All 4 parenchymal lung segments developed sustained tonic contractile responses to 3×10^{-6} M U46619, a thromboxane A₂-mimetic. This permitted evaluation of smooth muscle relaxants as a function of the various lung segments. The precontracted lung segments exhibited similar patterns of concentration-dependent relaxation to papaverine and theophylline with 100% reversal achieved at 3×10^{-4} M for the former and 3×10^{-3} M for the latter. Papaverine-induced relaxation of smooth muscle is mainly due to inhibition of both Ca²⁺ influx and of internal Ca²⁺ release (Fujioka, 1984; D'ocon, 1989); whereas theophylline-mediated relaxation is highly correlated with its inhibitory action against phosphodiesterase activity subsequent to an increase in adenosine 3':5'-cyclic monophosphate (cyclic AMP) level (Obianime *et al.*, 1989; Brackett *et al.*, 1990). Salbutamol also caused a relatively uniform degree of relaxation of the 4 lung segments. However, this β -adrenoceptor agonist elicited only 60–80% maximum relaxation of the lung segments even at high concentrations between 1×10^{-4} M and 3×10^{-4} M.

Unexpectedly, sodium nitroprusside, which relaxes vascular smooth muscle by activating guanylate cyclase and subsequently increasing cyclic GMP levels (Murad *et al.*, 1985), barely reversed the U46619-induced contraction of the lung strips (by no more than 25%) at a concentration as high as 3×10^{-5} M. In contrast, near complete relaxation of pulmonary artery rings and bronchial rings contracted by U46619 was obtained by the same compound at 3×10^{-6} M for the artery and 1×10^{-4} M for the bronchi. These data indicate that sodium nitroprusside had the lowest efficacy in relaxing lung strips as compared to papaverine, theophylline, and salbutamol, and exhibited differential relaxing actions on lung parenchyma, pulmonary vascular smooth muscle and airway smooth muscle. Similarly, Dale & Obianime (1985) reported that sodium nitroprusside, at a concentration of 1×10^{-5} M, produced only minimal inhibitory effects on histamine-induced contraction of guinea-pig parenchymal lung strips and it actually increased the contraction of lung strips induced by phorbol ester. In contrast, sodium nitroprusside at the same concentration produced marked relax-

Table 3 Morphometric analysis of number of profiles of smooth muscle structures per cm² section area^a of 4 lung segments

Segments ^b	No. of bronchi	No. of bronchioles	No. of vessels	Total number
1	2.4 ± 1.0**	35.9 ± 2.0	36.9 ± 2.3	75.1 ± 3.8
2	8.7 ± 1.2	23.9 ± 1.2***	33.4 ± 2.7	65.9 ± 3.4
3	3.6 ± 1.8*	26.7 ± 3.5*	28.9 ± 3.7	59.2 ± 4.9
4	0.4 ± 0.4***	25.4 ± 4.2*	29.8 ± 4.9	55.6 ± 7.6*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$: indicate significant difference from the largest value in each column.

^a Mean section areas (cm²) of segments 1, 2, 3 and 4 were 0.56 ± 0.05 , 0.56 ± 0.06 , 0.56 ± 0.04 and 0.39 ± 0.02 , respectively.

^b 6 sections per segment group.

ation of bovine tracheal smooth muscle pre-contracted with carbachol (Katsuki & Murad, 1977). These results suggest that either the responsiveness of the pulmonary artery and bronchus to sodium nitroprusside is quite different from that of the pulmonary microvasculature and bronchioles in the parenchymal strips, or the pulmonary microvasculature and bronchioles play a minor role in lung parenchymal strip contraction. Szarek & Evans (1988) demonstrated differential responses between parenchymal strips and bronchioles in that LTC₄ contracted only the parenchymal strips but not the bronchioles. Taken together, these data suggest that U46619-mediated parenchymal lung strip contraction is primarily due to force development by smooth muscle from the alveolar ducts, interstitial contractile cells and pleural surface rather than from the bronchioles and microvasculature. Recent observations by Halonen *et al.* (1990) showed that smooth muscle underlying the visceral pleura is the primary contractile element for PAF-induced contraction of guinea-pig peripheral lung strips. In these experiments, removal of the pleural surface rendered the lung strips unresponsive to PAF.

References

- BRACKETT, L.E., SHAMIN, M.T. & DALY, J.W. (1990). Activities of caffeine, theophylline, and enprofylline analogs as tracheal relaxants. *Biochem. Pharmacol.*, **39**, 1897–1904.
- BURKA, J.F. (1983). Studies on the role of arachidonic acid metabolites in airways contraction induced in vitro by antigen and calcium ionophore A23187. *Agent Actions*, **13**, 318–326.
- BURKA, J.F. (1988). Role of eicosanoids in airway smooth muscle tone. In *Mechanisms in Asthma: Pharmacology, Physiology, and Management*. ed. Armour, C.L. & Black, J.L. pp. 35–46. New York: Alan R. Liss, Inc.
- CHAND, N. & EYRE, P. (1978). The Schultz-Dale reaction: a review. *Agent Actions*, **8**, 171–184.
- CHENG, J.B., ESKRE, J.D. & PILLAR, J. (1987). Comparison of antigen and Ca²⁺ ionophore-induced peptidoleukotriene release from guinea pig lung preparations using high-performance liquid chromatography. *J. Pharmacol. Exp. Ther.*, **241**, 786–792.
- DALE, H.H. (1913). The anaphylactic reaction of plain muscle in guinea pig. *J. Pharmacol. Exp. Ther.*, **4**, 167–223.
- DALE, M.M. & OBIANIME, A.W. (1985). Phorbol myristate acetate causes in guinea-pig lung parenchymal strip a maintained spasm which is relatively resistant to isoprenaline. *FEBS Letters*, **190**, 6–10.
- D'OCON, M.P. (1989). Different effects of papaverine, isoprenaline and caffeine on rat uterine contractility in Ca-free medium. *Arch. Int. Pharmacodyn.*, **302**, 268–279.
- 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.
- EVANS, J.N. & ALDER, K.B. (1981). The lung strip: Evaluation of a method to study contractility of pulmonary parenchyma. *Exp. Lung Res.*, **2**, 187–195.
- FLEISCH, J.H., HAISCH, K.D. & SPAETHE, S.M. (1982). Slow reacting substance of anaphylaxis (SRS-A) release from guinea pig lung parenchyma during antigen- or ionophore-induced contraction. *J. Pharmacol. Exp. Ther.*, **221**, 146–151.
- FLEISCH, J.H., RINKEMA, L.E., HAISCH, K.D., SWANSON-BEAN, D., GOODSON, T., HO, P.P.K. & MARSHALL, W.S. (1985). LY171883, 1-(2-hydroxy-3-propyl-4-(1H-tetrazol-5-yl) butoxy) phenyl > ethanone, an orally active leukotriene D₄ antagonist. *J. Pharmacol. Exp. Ther.*, **233**, 148–157.
- FOREMAN, J.C. (1987). Substance P and calcitonin gene-related peptide: effects on mast cells and in human skin. *Int. Arch. Allergy Appl. Immunol.*, **82**, 366–371.
- FUJIOKA, M. (1984). Lack of a causal relationship between the vasodilator effect of papaverine and cyclic AMP production in the dog basilar artery. *Br. J. Pharmacol.*, **83**, 113–124.
- GERARD, N.P. (1987). Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung. *J. Clin. Invest.*, **79**, 1819–1825.
- GOLDIE, R.G., BERTRAM, J.F., PAPADIMITRIOU, J.H. & PETERSON, J.W. (1984). The lung parenchyma strip. *Trends Pharmacol. Sci.*, **5**, 7–9.
- HALONEN, M., DUNN, A.M., PALMER, J.D. & MCMANUS, L.M. (1990). Anatomic basis for species differences in peripheral lung strip contraction to PAF. *Am. J. Physiol.*, **259**, L81–L86.
- 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.
- KATSUKI, S. & MURAD, F. (1977). Regulation of adenosine cyclic 3',5'-monophosphate and guanosine cyclic 3',5'-monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.*, **13**, 330–341.
- KONDO, N. (1989). Voltage-dependence of ryanodine-sensitive component of contraction in A23187- and isoprenaline-treated cardiac muscles. *Br. J. Pharmacol.*, **98**, 51–60.
- 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, autocooids and anaphylactic challenge on the lung strips and trachea. *Br. J. Pharmacol.*, **58**, 71–79.
- MURAD, F., RAPOPORT, R.M. & R. FISCUS, R. (1985). Role of cyclic-GMP in relaxations of vascular smooth muscle. *J. Cardiovasc. Pharmacol.*, **7** (Suppl. 3), S111–S118.
- OBIANIME, A.W., HIRST, S.J. & DALE, M.M. (1989). The effect of smooth muscle relaxants working through different transduction mechanisms on the phorbol dibutyrate-induced contraction of the guinea-pig lung parenchymal strip: Possible relevance for asthma. *Pulm. Pharmacol.*, **2**, 191–200.
- PIPER, P.J. & SEALE, J.P. (1979). Non-immunological release of slow-reacting substance from guinea-pig lungs. *Br. J. Pharmacol.*, **67**, 67–72.
- SCHULTZ, W.H. (1910). Physiological studies in anaphylaxis. I. The reaction of smooth muscle of the guinea pig sensitized with horse serum. *J. Pharmacol. Exp. Ther.*, **1**, 549–567.
- SZAREK, J.L. & EVANS, J.N. (1988). Pharmacologic responsiveness of rat parenchymal strips, bronchi, and bronchioles. *Exp. Lung Res.*, **14**, 575–585.
- WONG, W.S.F., BENDELE, A.M. & FLEISCH, J.H. (1991). Lung pleural smooth muscle as the main contributor to A23187- and ovalbumin-induced guinea pig lung parenchymal strips contraction. *Pharmacologist*, **33**, 155.

(Received June 14, 1991)

Revised November 5, 1991

Accepted November 18, 1991)