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Characterization of the anatomical structures involved in the contractile response of the rat lung periphery

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1 When lung parenchymal strips are challenged with different smooth muscle agonists, the tensile and viscoelastic properties change. It is not clear, however, which of the different anatomical elements present in the parenchymal strip, i.e., small vessel, small airway or alveolar wall, contribute to the response.

2 Parenchymal lung strips from Sprague Dawley rats were suspended in an organ bath filled with Krebs solution (37°C, pH = 7.4) bubbled with 95%O_2/5%CO_2. Resting tension (T) was set at 1.1 g and sinusoidal oscillations of 2.5% resting length (L_o) at a frequency of 1 Hz were applied. Following 1 h of stress adaptation, measurements of length (L) and T were recorded under baseline conditions and after challenge with a variety of pharmacological agents, i.e., acetylcholine (ACh), noradrenaline (NA) and angiotensin II (AII). Elastance (E) and resistance (R) were calculated by fitting changes in T, L and $\Delta L/\Delta t$ to the equation of motion. Hysteresivity (η, the ratio of the energy dissipated to that conserved) was obtained from the equation $\eta = (R/E)2\pi f$.

3 In order to determine whether small airways or small vessels accounted for the responses to the different pharmacologic agents, further studies were carried out in lung explants. Excised lungs from Sprague Dawley rats were inflated with agarose. Transverse slices of lung (0.5-1.0 mm thick) were cultured overnight. By use of an inverted microscope and video camera, airway and vascular lumen area were measured with an image analysis system.

4 NA, ACh and AII constricted the parenchymal strips. Airways constricted after all agonists, vessels constricted only after AII. Atropine (Atr) pre-incubation decreased the explanted airway and vessel response to AII, but no difference was found in the parenchymal strip response.

5 Preincubation with the arginine analogue N^{ω}-nitro-L-arginine (L-NOARG) did not modify the response to ACh but mildly increased the oscillatory response to NA after co-preincubation with propranolol (Prop).

6 These results suggest that during ACh and NA challenge, small vessels do not contribute substantially to the parenchymal strip response. The discrepancy between results in airways, vessels and strips when Atr was administered prior to AII implicates a direct contractile response in the parenchymal strip.

Keywords: noradrenaline; acetylcholine; angiotensin II; viscoelastic; hysteresivity; lung parenchymal strip; lung explants

Introduction

It has recently been shown by a number of investigators that the pulmonary parenchyma plays a key role in constrictor responses of the lung (Tepper *et al.*, 1992; Ludwig *et al.*, 1987; Ingenito *et al.*, 1993; Nagase *et al.*, 1994a,b). Tissue resistance, the pressure drop in phase with flow between the alveolus and the pleura, is responsible for a substantial portion of the increase in lung resistance during both exogenous and endogenous constriction (Ludwig *et al.*, 1987, Nagase *et al.*, 1994a,b).

The lung parenchymal strip is a commonly used model for the study of the mechanical and pharmacological properties of the lung periphery (Lulich *et al.*, 1976; Drazen & Schneider, 1978; Clayton *et al.*, 1980; Bertram *et al.*, 1983; Goldie *et al.*, 1984; Fredberg *et al.*, 1993; Ludwig & Dallaire, 1994) and is considered a good proxy of the peripheral lung tissue. We and others (Fredberg *et al.*, 1993; Salerno *et al.*, 1995) have used the parenchymal strip as a means of investigating dynamic mechanical responses to constrictor challenge at the parenchymal level. However, the lung periphery is a complex system comprising alveolar wall, bronchioles and small vessels (Lulich *et al.*, 1976), and it is difficult to be certain of the precise elements that are responding to the contractile stimulus.

We have recently shown that the volume proportion of alveolar, bronchial and blood vessel wall does not correlate with oscillatory mechanics in subpleural parenchymal lung strips under baseline conditions (Ludwig & Dallaire, 1994). After induced constriction, the relationship is more complex and depends on the site from which the strip is excised. Strips from a more proximal location containing greater amounts of bronchial and blood vessel wall show a different structure-function relationship after acetylcholine-induced constriction than subpleural parenchymal strips (Salerno *et al.*, 1995). However, such an analysis gives only indirect information as to which structures are likely to be responding. The object of this investigation was to characterize further the peripheral structures contributing to the modification of elastic, resistive and hysteretic properties of the lung periphery during smooth muscle agonist challenge.

We chose to characterize the viscoelastic response in addition to the tensile response for the following reasons. The viscoelastic behaviour of the lung tissues, which can be measured during oscillation, contributes importantly to the work of breathing both under baseline conditions and after induced constriction (Ludwig *et al.*, 1987; Nagase *et al.*, 1994a). Energy losses occur during dynamic events which are reflected in the resistance and hysteresivity of the tissues. Hence dynamic measurements better reflect the physiological condition of tidal breathing. In addition, it has been demonstrated by Gunst and her colleagues (Gunst *et al.*, 1990; Tepper *et al.*, 1995) that oscillations can reduce airway

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smooth muscle contraction in both *in vivo* and *in vitro* models. Hence studying responses during oscillation is most pertinent to the *in vivo* state.

In addition to oscillating lung parenchymal strips, we investigated responses in lung explants in order to image directly airways and vessels and better define the precise anatomical structures involved in the contractile response. We exposed both preparations to a number of different pharmacological agonists with potentially different sites of action. Acetylcholine (ACh) was used as agents acting on cholinoceptors have been shown to activate lung parenchymal strips mechanically, both in isometric and oscillatory studies (Drazen & Schneider, 1978; Fredberg *et al.*, 1993; Salerno *et al.*, 1995). Noradrenaline (NA) was used in order to characterize the behaviour of the lung periphery during adrenoceptor stimulation, in which airway smooth muscle (ASM) activation could be accompanied by vascular smooth muscle activation (Bertram *et al.*, 1983). Finally, angiotensin II (AII) was used as a specific vascular smooth muscle agonist (Sardella & Ou, 1993) in order to address better the effects of pulmonary vascular activation on lung tissue mechanics.

Methods

Parenchymal strip preparation

Male Sprague Dawley rats weighing approximately 400 g were obtained from Charles River Inc. (St. Constant, Q.C, Canada) and housed in a conventional animal facility at McGill University. Each animal was anaesthetized with a peritoneal injection of sodium pentobarbitone (30 mg kg^{-1}). The thorax was opened and the animals were exsanguinated by severing the inferior vena cava. The heart, lungs and trachea were carefully resected *en bloc* ensuring that the lungs were not punctured. The trachea was cannulated with a polyethylene catheter (PE 260) and the lungs filled and rinsed to TLC in a modified Krebs solution (mM: NaCl 118, KCl 4.5, $NaHCO_3$ 25.5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 10: Sigma, St. Louis, MO, USA) at a pH of 7.40 and 6°C. Lung strips were cut and unloaded length (L_o) and wet weight (W_o) of each strip was recorded. The strips were kept in a recirculating bath of iced solution which was continuously bubbled with 95% O_2 and 5% CO_2. No more than three strips were cut from each animal and at least four animals were studied for each group.

Apparatus Metal clips were glued to either end of the tissue strip with cyanoacrylate. Steel music wires (diameter of 0.5 mm) were attached to the clips and the strip suspended vertically in an organ bath. A mercury bead was placed in the bottom of the organ bath, allowing the wire to pass through the bath but preventing the Krebs solution from leaking out. The bath was filled with 15 ml Krebs solution, maintained at 37°C and continuously bubbled with the 95%O_2/5%CO_2. One end of the strip was attached to a force transducer (model 400A, Cambridge Technologies, Watertown, MA, U.S.A.) which had an operating range of ± 10 g, resolution of ± 200 μg and compliance of 1 μm g^{-1}, while the other end was connected to a servo-controlled lever arm (model 300B, Cambridge Technologies, Watertown, MA, U.S.A.). The lever arm was capable of peak to peak length excursions of 8 mm and length resolution of 1 μm and was in turn connected to a function generator (model 3030, BK precision, Chicago, IL, U.S.A.) which controlled the frequency, amplitude and wave form of the oscillation. The resting tension (T) was set by movement of a screw thumb wheel system which effected slow vertical displacements of the force transducer. Length and force signals were low-pass filtered (8-pole Bessel 902LPF, Frequency Devices, Haverhill, MA, U.S.A.) with a corner frequency of 30 Hz, and converted from analog to digital with an analog to digital converter (DT2801-A, Data Translation Inc., Marlborough, MA, U.S.A.) and recorded on an A/T compatible computer.

The linearity and hysteresis of the system were tested by measuring the moduli of a steel spring of stiffness comparable with that of the tissue strip. The spring was suspended in the bath by music wire in the same manner as the strip. The frequency and amplitude-dependence of the system were assessed over a range of frequencies (0.1 – 10 Hz). The spring stiffness did not show any dependence upon oscillatory frequency below 5 Hz. The hysteresivity of the system was independent of frequency and had a value <0.003.

Explant preparation

Culture media Bicarbonate-buffered culture medium (BCM) was prepared from minimal essential medium (MEM) powder with Earle's salts and L-glutamine (GIBCO, Burlington, Ontario, Canada) supplemented with 2.2 g l^{-1} sodium bicarbonate (Fisher, Ottawa, Ontario, Canada), 20 ml l^{-1} of 50 × MEM amino acid solution (GIBCO), 10 ml l^{-1} sodium pyruvate (Sigma Chemical, St. Louis, MO, U.S.A.), 10 ml l^{-1} of 100 × vitamin solution (GIBCO), 0.1 μg ml^{-1} bovine insulin (Sigma Chemical), 0.1 μg ml^{-1} vitamin A (GIBCO), 0.1 μg ml^{-1} hydrocortisone (Sigma Chemical), and 50 μg ml^{-1} gentamicin (GIBCO). A BCM with double the amount of supplements was also prepared. The supplemented culture medium was adjusted to pH 7.2 and sterilized with a 0.22-μm filter (Millepore, Bedford, MA, U.S.A.), resulting in a BMC with a final pH of 7.3. N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid-(HEPES) buffered culture medium (HCM) was prepared in a manner identical to the BCM except that 5.96 g l^{-1} HEPES (Sigma Chemical) was substituted for sodium bicarbonate and the pH was adjusted to 7.2. Filter sterilization resulted in a final pH of 7.3.

Agarose type VII (Sigma Chemical) solutions (2% wt/vol) were prepared in 100 ml of BCM without supplements. The agarose solutions were autoclaved and stored at 4°C. All solutions were used within 1 week of preparation.

Preparation of explants Fifteen additional male Sprague Dawley rats weighing approximately 400 g were administered a lethal dose of pentobarbitone sodium (0.8 mg kg^{-1} ip), placed in a laminar flow hood, and washed with 70% ethanol. The rats were intubated by tracheotomy with a 9 cm length of sterile polyethylene tubing and exsanguinated by inferior vena cava section. The anterior chest wall was removed, and the heart and lungs were excised and placed in a sterile container with a hole in the lid allowing the tracheal tube to protrude.

The excised lungs were maintained at the desired lung inflation volume by inflation with agarose-BCM solution. Agarose in BCM (2.0% wt/vol) was melted at 65°C and cooled to 37°C. Equal volumes of the liquid 2.0% agarose solution at 37°C and BCM prepared with double supplements warmed to 37°C were mixed. The resulting 1.0% agarose-BCM solution was slowly inflated into the lung with a syringe until the desired lung inflation volume was obtained. A 1.0 ml bolus of air was then injected into the lung to clear the solution out of the conducting airways. The tracheal tube was clamped, and the preparation was cooled to 4°C for 30 min to allow the liquid solution to gel. Once cooled, the left lung was isolated and placed upright in a sterile 35 ml syringe from which the needle end had been cut off. A 4.0% agarose solution at 37°C was poured into the syringe and allowed to gel at 4°C for 30 min. The lung agarose block was then sectioned into 0.5 to 1.0 mm transverse slices with a hand held microtome blade (model 818, Cambridge Instruments, Buffalo, NY, U.S.A.) and placed in a 30 mm culture well insert (Millepore, Bedford, MA, U.S.A.) within a six-well plate (Costar, Cambridge, MA, U.S.A.) containing 2 ml of BCM. Lung explants were incubated overnight at 37°C in 5% CO_2 :95% air.

Image acquisition The culture dish inserts containing the lung explants were transferred to six-well plates (Costar) containing

2 ml of the HCM and placed on the stage of the inverted microscope. The airways and vessels were identified and imaged with a video camera (CDS, Sony, Nagano, Japan) and recorded with a video disk recorder (TQ2026F, Panasonic, Osaka, Japan). After baseline images were recorded, a 220 μl aliquot of the drug diluted in saline was added to the 2 ml of HCM medium outside the culture well insert in order to obtain the desired concentration.

Image analysis The stored images were digitized using an 80386 Intel-based microcomputer equipped with a frame-grabber board (PIP1024B, Matrox, Montreal, Quebec, Canada) and custom software. The digitized images were then transferred to a scientific work station (RS6000, IBM, Armonk, NY, U.S.A.), and measurements made with custom designed software. The lumen area was taken as the area enclosed by epithelial/endothelial luminal junction. Calibration was performed with a 0.01 mm graticule imaged with the same equipment at the same magnification. We assessed the intra-observer variability in the calculation of the lumen areas by randomly choosing 10 airway and 10 vessel images of comparable sizes, and measuring them twice. The intra-observer variability was 8.6% ± 5.1 (mean ± s.d.) for airway lumen area and 7.4% ± 6.7 for vessel lumen area. Airway and vessel lumen area after challenge as percentage of baseline was calculated for each sample. Maximal response was defined as the minimal area obtained and expressed as the percentage of complete closure {1 − (minimal area/baseline area)*100}.

Drugs

ACh was purchased from BDH, Inc. NA, N^{ω}-nitro-L-arginine (L-NOARG), propranolol (Prop) and AII were purchased from SIGMA Chemical Co. (St. Louis, MO, U.S.A.). Atr was purchased from MTS (Cambridge, Ont., Canada). The dose of ACh, AII and NA which resulted in a maximal response was identified in preliminary experiments and used to challenge the tissues.

Protocol

Lung parenchymal strips Strips were divided into 10 groups: (1) ACh challenge (1 mM) ($n = 18$); (2) ACh challenge (10 mM) after 20 min pre-incubation with L-NOARG (1 mM) ($n = 6$); (3) NA challenge (100 μM) ($n = 9$); (4) NA challenge (100 μM) after 20 min pre-incubation with Prop (10 μM) ($n = 6$); (5) AII challenge (100 μM) after 20 min pre-incubation with Prop (10 μM) and L-NOARG (1 mM) ($n = 6$); (6) AII challenge (10 μM) ($n = 9$); (7) AII challenge (10 μM) after 20 min pre-incubation with Atr (10 μM) ($n = 9$); (8) L-NOARG challenge (1 mM) ($n = 6$); (9) Atr challenge (10 μM) ($n = 8$) and (10) Prop challenge (10 μM) ($n = 6$).

Each strip was preconditioned by slowly cycling tension from 0 to 2 g. On the third cycle the strip was unloaded to a tension of 1.1 g and a sinusoidal length oscillation of 2.5% of L_o was applied at a frequency of 1 Hz. The sample underwent 60 min of stress adaptation with 2 changes of bath solution. Baseline data were recorded for 5 min, strips were challenged and measurements obtained for an additional 10 min. In all groups the average of the 10 s before the drug challenge was used as baseline. The peak value of each parameter was recorded.

Lung explants Lung explants were divided into the same ten groups as the lung parenchymal strips and challenged at the same dosages, with the exception that explants were pre-incubated with 0.1 μM Atr in addition to 10 μM. Explants were pre-incubated for 5 min prior to addition of agonists. Diameters of the unconstricted airways and vessels ranged from 0.05–0.30 mm. Images were recorded before challenge and every 5 s after challenge for at least 1 min. The peak of constriction was analysed and the corresponding decrease in lumen area calculated.

Measurement of strip mechanics

Elastance (E) and resistance (R) were estimated by applying the recursive least-square algorithm to the following equation of motion (Lauzon & Bates, 1991):

$$T = E\Delta l + R^{*}\Delta l/\Delta t + K \quad (1)$$

where l = length, $\Delta l/\Delta t$ is the length change per unit time. K, a constant reflecting resting tension, was also estimated by multiple linear regression. Results were standardized for strip size. The unstressed cross sectional area (A_o) of the strip was obtained from the formula:

$$A_o(cm^2) = W_o/(\rho \times L_o) \quad (2)$$

where ρ is the mass density of the tissue taken as 1.06 g cm^{-3}; W_o, the wet weight in grams and L_o the unloaded length in cm of the lung strips. Values of E and R were multiplied by L_o/A_o. A_o varied between 0.017 and 0.031 cm^2. Hysteresivity, η, a dimensionless variable coupling the dissipative and elastic behaviour, was calculated with the following equation (Fredberg & Stamenovic, 1989):

$$\eta = (R/E)^{*}2\pi f \quad (3)$$

where f is frequency.

Data analysis

Results before and after challenge were compared with paired two tailed t tests. Comparisons within ACh, NA and AII groups were done with one way analysis of variance (ANOVA). Fisher's LSD test was used for *post hoc* comparisons. Results were considered statistically significant at a probability level of 5%. Values are reported as mean ± standard error (s.e.).

Results

The baseline values of the dynamic mechanical parameters are presented in Table 1. There were no differences in baseline mechanics among groups.

Cholinoceptor response

Figure 1 shows responses in the parenchymal strips and the lung explants to pharmacological challenge with ACh (1 mM) alone and ACh after pre-incubation with L-NOARG (1 mM). ACh caused an increase in all the mechanical parameters which was not modified by inhibition of the nitric-oxide pathway. In the lung explant experiment, airways responded significantly to ACh while there was no response in the vessels. Pretreatment with L-NOARG did not affect the airway reaction.

Table 1 Physiological measurements at baseline

	Baseline value
T (10^{-3}N)	8.55 ± 0.82
E (10^{4}N m^{-1})	4.58 ± 1.55
R (10^{3}Ns m^{-2})	0.528 ± 0.166
η	0.074 ± 0.008

Values are means ± s.d.; T, tension; E, dynamic elastance; R, tissue resistance; η, hysteresivity.

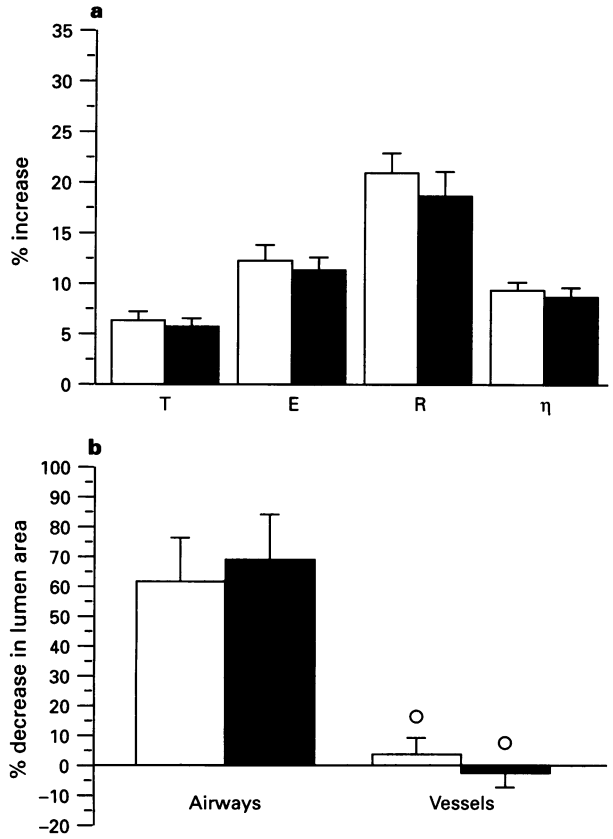

Figure 1 (a) Changes in tension (T), elastance (E), resistance (R) and hysteresivity (η) after challenge as percentage of baseline. Open columns, acetylcholine (ACh, 1 mM), ($n=18$); solid columns, ACh (1 mM) after preincubation with N^{ω}-nitro-L-arginine (L-NOARG, 1 mM), ($n=6$). (b) Changes in airway and vessel lumen area after challenge as percentage of baseline. Open columns, ACh (1 mM), ($n=7$ for airways and 11 for vessels); solid columns, ACh (1 mM) after preincubation with L-NOARG (1 mM), ($n=6$ for airways and 6 for vessels); ° not different from baseline.

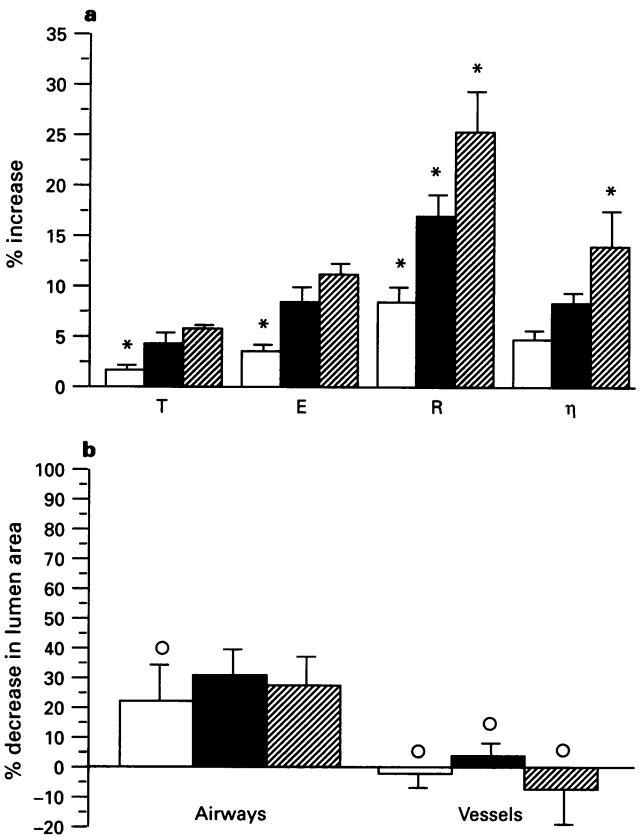

Figure 2 (a) Changes in tension (T), elastance (E), resistance (R) and hysteresivity (η) after challenge as percentage of baseline. Open columns, noradrenaline (NA, 100 μM), ($n=9$); solid columns, NA (1 mM) after preincubation with N^{ω}-nitro-L-arginine (L-NOARG 1 mM), ($n=6$); hatched columns, NA (100 μM) after preincubation with L-NOARG (1 mM) and propranolol (10 μM), ($n=6$). (b) Changes in airway and vessel lumen area after challenge as percentage of baseline. Open columns, NA (100 μM), ($n=7$ for airways and 9 for vessels); solid columns, NA (100 μM) after preincubation with L-NOARG (1 mM), ($n=15$ for airways and 15 for vessels); hatched columns, NA (100 μM) after preincubation with L-NOARG (1 mM) and propranolol (10 μM), ($n=11$ for airways and 9 for vessels); ° not different from baseline; *statistically different from the other two NA challenged groups (ANOVA).

Adrenoceptor response

As shown in Figure 2, NA (100 μM) caused increases in parenchymal strip mechanics. The responses of T, E and R were enhanced when the strips were pre-incubated with Prop (10 μM). The increase in R and η was further enhanced by inhibition of the nitric-oxide pathway. In the explant preparation, NA caused significant constriction of the small airways only when the β blocker, propranolol, was present. Pre-incubation with L-NOARG (1 mM) did not further modify the response. The small vessels did not respond to adrenoceptor stimulation. (As this response was somewhat surprising, in an additional 5 vessels unresponsive to NA we tested the viability of the preparation by administering AII, which provoked reactions in 4/5 vessels).

Angiotensin II response

AII (10 μM) caused increases in the different mechanical parameters which were not significantly affected by pre-incubation with Atr (10 μM). AII significantly constricted the small airways and vessels in the explant preparation. The response to AII was completely ablated in the explant preparation by pre-incubation with Atr (10 μM) (Figure 3). Incubation with a lower concentration of Atr (0.1 μM) resulted in a significant reduction in the response to AII in airways ($P=0.02$) and a borderline significant reduction in vessels ($P=0.054$).

Response to inhibitors alone

L-NOARG had no effect on parenchymal strip mechanics nor on airways and vessels in the explant preparation. Atr significantly decreased T, E and R in the parenchymal strip ($-3.1\% \pm 0.7$, $-5.6\% \pm 1.3$, $-12.0\% \pm 3.3$ of baseline, respectively) but did not modify airway and vessel lumen area in the explant preparation. Prop caused a modest but significant increase in strip E ($0.8\% \pm 0.1$ of baseline, $P<0.05$), but had no significant effect on airway or vessel lumen area.

Discussion

The object of this study was to address specifically the question of which structures respond in the lung periphery during challenge with smooth muscle agonists, and whether these structures are able to modify the tensile and viscoelastic behaviour of the lung periphery. The lung periphery is a heterogeneous tissue comprising alveolar walls, small vessels and small airways (Lulich *et al.*, 1976). Airways and pulmonary vessels have the potential to increase isometric tension of lung parenchymal strips during constriction (Lulich *et al.*, 1976; Bertram *et al.*, 1983). The contribution of these structures to

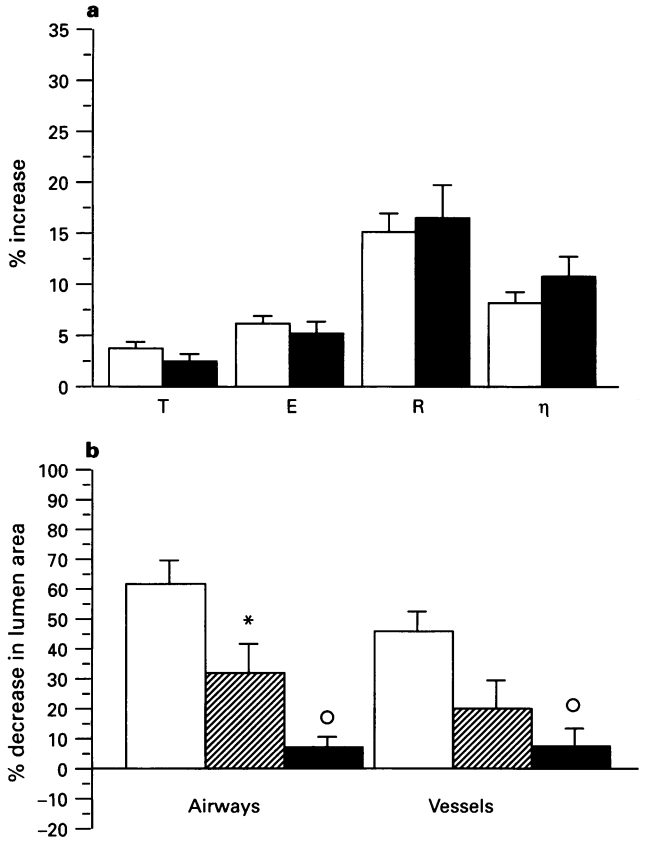

Figure 3 (a) Changes in tension (T), elastance (E), resistance (R) and hysteresivity (η) after challenge as percentage of baseline. Open columns, angiotensin II (AII, 10 μM), ($n=9$); solid columns, AII (10 μM) after preincubation with atropine (Atr, 10 μM), ($n=9$). (b) Changes in airway and vessel lumen area after challenge as percentage of baseline. Open columns, AII (10 μM), ($n=13$ for airways and 18 for vessels); solid columns, AII (10 μM) after preincubation with Atr (10 μM), ($n=12$ for airways and 12 for vessels); hatched columns, AII (10 μM) after preincubation with Atr (0.1 μM), ($n=12$ for airways and 13 for vessels). ° Not different from baseline; *statistically different from AII alone (ANOVA).

modification of the viscoelastic properties of the lung parenchyma is not known. Insofar as the viscoelastic properties best reflect energy dissipation during tidal breathing, measurement of these parameters during oscillation is especially pertinent. Finally, contractile elements in the alveolar wall (Kapanci *et al.*, 1974) could also play a role in the parenchymal strip response.

In a previous study (Salerno *et al.*, 1995) we assessed the relationship between dynamic oscillatory behaviour and the fractional proportion of the different anatomical constituents which comprise parenchymal lung strips, i.e. alveolar, blood vessel and bronchial wall. The correlation between structure and function during induced constriction was somewhat complex. Whereas there was no correlation between the response of the different mechanical parameters and the amount of bronchial, small vessel and alveolar wall in subpleural parenchymal strips, when more proximal strips containing greater amounts of blood vessel and bronchial wall were examined, correlations between changes in function and structure were found.

In the current experiment, in order to identify more precisely the anatomical structures responsible for the contractile response, we examined responses not only in parenchymal lung strips but also in the lung explant preparation which permits direct visualization of both small airways and vessels. Furthermore, we exposed both preparations to a series of different smooth muscle agonists with the potential to stimulate differentially airways and vessels. The lung explant has been shown to be a good model for the study of airway and vessel reactivity (Dandurand *et al.*, 1993; Shi *et al.*, 1994), and provided us with a means to assess directly airway and vessel response. It is important to consider, however, whether information from parenchymal strips and lung explants are directly comparable. Some of the airways and vessels we analysed in the explant preparation were slightly larger in size than those present in the parenchymal strips: 0.05–0.30 mm calibre in the explanted preparations vs <0.2 mm in the parenchymal strips. The smaller size of the structures studied as compared with previous lung explant studies (Dandurand *et al.*, 1993; Shi *et al.*, 1994) resulted in somewhat larger relative errors in lumen area measurements. Finally, it was difficult in the explants to measure airway or vessel dilatation, whereas in the parenchymal strips it was more straightforward to measure decreases in baseline tension. We believe however, that the overall direction and magnitude of the response were maintained, and hence reflect the behaviour of the small conducting airways and vessels present within the strip preparation.

Cholinoceptor responses

In previous studies cholinoceptor stimulation has been shown to increase dynamic elastance, tissue resistance and hysteresivity in both *in vivo* and *in vitro* experiments (Ludwig *et al.*, 1987; Fredberg *et al.*, 1993; Salerno *et al.*, 1995). Muscarinic receptors have been identified at the level of the alveolar wall as well as in airway smooth muscle and presumably provide the mechanism of activation (Mak *et al.*, 1992; Roffel *et al.*, 1993). In the present study, acetylcholine constricted both the lung parenchymal strips and the airways of the lung explant preparation. As reported previously (Leach *et al.*, 1992) the peripheral vascular compartment was insensitive to this drug. The reactions of the parenchymal strip and the airways and vessels in the explant preparation were not modified by preincubation with a nitric-oxide blocker. These data suggest that vessels do not contribute to the mechanical response of the parenchymal strip to agents acting on cholinoceptors and that endothelial nitric-oxide production does not play an important role in modifying the response at the level of the lung periphery.

Adrenoceptor responses

In the current study, the lung parenchymal strips reacted modestly to NA. When the preparations were pre-incubated with Prop the reaction was enhanced in the strip preparation; in the explant preparation the response of the airways became statistically significant. The blockade of nitric oxide with L-NOARG enhanced the response to NA and Prop in two of the mechanical parameters measured in the parenchymal strips, R and η, but not in the others. The significance of this partial effect is unclear. The failure of NA to constrict the pulmonary vasculature was not related to the production of nitric-oxide as pre-incubation with the nitric-oxide blocker, L-NOARG, did not affect the response. These results suggest that at the peripheral level adrenoceptor stimulation affects the lung periphery by airway smooth muscle activation. Small airways as well as alveolar ducts and contractile elements in the alveolar walls (Sata *et al.*, 1995) could all potentially contribute to constriction-induced modifications in the viscoelastic properties of the peripheral lung tissues.

There are conflicting reports in the literature regarding the effects of adrenoceptor stimulation on the airways and pulmonary vasculature. Bertram *et al.* (1983) assessed the isometric response of human lung strips to histamine and NA. They found a relationship between histological makeup (vessel proportion vs. airway proportion) and tension developed, and suggested that the vascular compartment was responsible for the NA response. Leach *et al.* (1992) characterized the pharmacological and mechanical properties of small pulmonary arteries (100–300 μm) in the rat and found a very modest

response to NA in arteries of this size. Black *et al.*, (1981) observed contraction following challenge with NA in human lung strips which they attributed to a distinct population of α-adrenoceptors in the peripheral lung. Finally, Sata *et al.* (1995) have recently reported NA-induced contraction of a hamster lung parenchymal preparation which consisted primarily of alveolar walls. The results of the current study support the conclusion that the vasculature is not the responding structure, at least at the level of the peripheral lung. α-Adrenoceptors in the small airways or even the alveolar wall itself (Keeney & Oelberg, 1993) probably account for the response to noradrenaline.

Angiotensin II response

In order to study the effect of vascular activation in our model, AII was used. AII has been shown to be a dose-dependent constrictor of the pulmonary vasculature in man (Lipworth & Dagg, 1994) as well as in the rat (Sardella & Ou, 1993). The strip preparation responded with an increase in all the mechanical parameters. When we tested this drug on the lung explant both airways and vessels constricted. AII has been shown in guinea-pigs to cause mild contraction of tracheal smooth muscle (Mizrahi *et al.*, 1982), and in rabbits to potentiate prejunctionally neurally mediated ASM contraction via activation of AII receptors on cholinergic nerve terminals (Yamawaki *et al.*, 1992). In the current study, contraction was partially blocked by Atr at lower concentrations (0.1 μM) and completely blocked at higher concentrations (10 μM) in both the airways and vessels in the lung explant preparation. The additional modification of the contractile response at the higher concentration (10 μM) may be related to a non specific inhibitory effect (Bowman & Rand, 1980). The more modest inhibition at the lower concentration, is probably explained by a specific anti-cholinoceptor effect. (Of note, the effect of 0.1 μM of Atr on vessels did not quite achieve statistical significance). In the airway the mechanism could be that described above (Yamawaki *et al.*, 1992). In the vessels the mechanism is more difficult to explain as ACh alone did not cause vessel constriction. It is possible that the vascular effect required a synergistic action between the two mediators. Alternatively the effect of Atr may have been nonspecific even at the lower concentration. The parenchymal strip response, on the other hand, was not significantly altered by cholinoceptor blockade. The discrepancy between the response in airways and vessels vs parenchymal strips suggests the presence of smooth muscle elements in the parenchymal strip that have pharmacological properties distinct from those of small airways or vessels. Myoepithelial cells in the alveolar wall could represent such a structure. It is also interesting to consider the observation of Gasc *et al.* (1994) who described the presence of AII type 1 receptors in rat pulmonary parenchyma that were not found in rat airways or vasculature.

In conclusion we have shown that during adrenoceptor and cholinoceptor stimulation of the lung periphery in the Sprague Dawley rat, the pulmonary vasculature probably does not contribute to the mechanical response. Rather the increase in R, E, and η in the parenchymal strip must be attributed to either small airways or possibly 'alveolar' constriction. It is difficult to determine whether isolated vascular constriction can singly alter parenchymal strip mechanics, as our specific vascular agonist, AII, also contracted small peripheral airways. It is likely that AII also affected the pulmonary parenchyma more directly as shown by the dissociation between the effects of atropine pre-incubation on the AII response in explanted airways and vessels vs parenchymal strips. Changes in parenchymal strip mechanics with agonist challenge generally reflect the integrated response of the different anatomical elements which comprise this complex structure.

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References

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

BLACK, J., TURNER, A. & SHAW, J. (1981). α-adrenoceptors in human peripheral lung. *Eur. J. Pharmacol.*, **72,** 83–86.

BOWMAN, W.C. & RAND, M.J. (1980). *Textbook of Pharmacology* (second edition). pp. 39.25. Oxford: Blackwell.

CLAYTON, D.E., BUSSE, W.W. & BUCKNER, C.K. (1980). Contribution of vascular smooth muscle to contractile responses of guinea-pigs isolated lung parenchymal strips. *Eur. J. Pharmacol.*, **70,** 311–320.

DANDURAND, R.J., WANG, C.G., PHILLIS, N.C. & EIDELMAN, D.H. (1993). Responsiveness of individual airways to methacholine in adult rat lung explants. *J. Appl. Physiol.*, **75,** 364–372.

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,** 1441–1447.

FREDBERG, J.J., BUNK, D., INGENITO, E. & SHORE, S.A. (1993). Tissue resistance and the contractile state of lung parenchyma. *J. Appl. Physiol.*, **74,** 1387–1397.

FREDBERG, J.J. & STAMENOVIC, D. (1989). On the imperfect elasticity of lung tissue. *J. Appl. Physiol.*, **67,** 2408–2419.

GASC, J-M., SHANMUGAM, S., SIBONY, M. & CORVOL, P. (1994). Tissue-specific expression of type 1 angiotensin II receptor subtypes. *Hypertension*, **24,** 531–537.

GOLDIE, R.G., BERTRAM, J.F., PAPADIMITRIOU, J.M. & PATERSON, J.W. (1984). The lung parenchyma strip. *Trends Pharmacol. Sci.*, **5,** 7–9.

GUNST, S.J., STROPP, J.Q. & SERVICE, J. (1990). Mechanical modulation of pressure-volume characteristics of contracted canine airways *in vitro*. *J. Appl. Physiol.*, **68,** 2223–2229.

INGENITO, E.P., DAVISON, B. & FREDBERG, J.J. (1993). Tissue resistance in the guinea pig at baseline and during methacoline constriction. *J. Appl. Physiol.*, **75,** 2541–2548.

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.

KEENEY, S.E. & OELBERG, D.G. (1993). Alpha 1-adrenergic and muscarinic receptors in adult and neonatal rat type II pneumocytes. *Lung*, **17,** 355–366.

LAUZON, A.M. & BATES, J.H.T. (1991). Estimation of time-varying respiratory mechanical parameters by recursive least squares. *J. Appl. Physiol.*, **71,** 1159–1165.

LEACH, R.M., TWORT, C.H.C., CAMERON, I.R. & WARD, J.P.T. (1992). A comparison of the pharmacological and mechanical properties in vitro of large and small pulmonary arteries of the rat. *Clin. Sci.*, **82,** 55–62.

LIPWORTH, B.J. & DAGG, K.D. (1994). Vasoconstrictor effects of Angiotensin II on the pulmonary vascular bed. *Chest*, **105,** 1360–1364.

LUDWIG, M.S. & DALLAIRE, M. (1994). Structural composition of lung parenchymal strip and mechanical behaviour during sinusoidal oscillation. *J. Appl. Physiol.*, **77,** 2029–2035.

LUDWIG, M.S., DRESHAY, I., SOLWAY, J., MUNOZ, A. & INGRAM, JR R.H. (1987). Partitioning of pulmonary resistance during constriction in the dog: effects of volume history. *J. Appl. Physiol.*, **62,** 807–815.

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 beta-adrenoreceptor agonists, autacoids and anaphylactic challenge on the lung strip and trachea. *Br. J. Pharmacol.*, **56,** 71–79.

MAK, J.C.W., BARANIUK, J.N. & BARNES, P.J. (1992). Localization of muscarinic receptor subtype mRNAs in human lung. *Am. J. Respir. Cell Mol. Biol.*, **7**, 344–348.

MIZRAHI, J., COUTURE, R., CARANIKAS, S. & REGOLI, D. (1982). Pharmacological effects of peptides on tracheal smooth muscle. *Pharmacology*, **25**, 39–50.

NAGASE, T., DALLAIRE, M.J. & LUDWIG, M.S. (1994a). Airway and tissue responses during hyperpnoea-induced constriction in guinea pigs. *Am. J. Respir. Crit. Care Med.*, **149**, 1342–1347.

NAGASE, T., MORETTO, A., DALLAIRE, M.J., EIDELMAN, D.H., MARTIN, J.G. & LUDWIG, M.S. (1994b). Airway and tissue responses to antigen challenge in sensitized Brown Norway rats. *Am. J. Respir. Crit. Care Med.*, **150**, 218–226.

ROFFEL, A.F., ELZINGA, C.R. & ZAAGSMA, J. (1993). Cholinergic contraction of the guinea pig lung strip is mediated by muscarinic M2-like receptors. *Eur. J. Pharmacol.*, **250**, 267–279.

SALERNO, F.G., DALLAIRE, M. & LUDWIG, M.S. (1995). Does the anatomic makeup of parenchymal lung strips affect oscillatory mechanics during induced constriction? *J. Appl. Physiol.*, **79**, 66–72.

SARDELLA, G.L. & OU, L.C. (1993). Chronically instrumented rat model for hemodynamic studies of both pulmonary and systemic circulation. *J. Appl. Physiol.*, **74**, 849–852.

SATA, M.K., TAKAHASHI, K., SATO, S. & TOMOIKE, H. (1995). Structural and functional characteristics of peripheral pulmonary parenchyma in golden hamsters. *J. Appl. Physiol.*, **78**, 239–246.

SHI, W., WANG, C.G., DANDURAND, R.J., EIDELMAN, D.H. & MICHEL, R.P. (1994). Responses of pulmonary vessels in lung explants to vasoactive agents. *Am. J. Respir. Crit. Care Med.* (Abstract), **149**, A435.

TEPPER, R., SATO, J., SUKI, B., MARTIN, J.G. & BATES, J.H.T. (1992). Low-frequency pulmonary impedance in rabbits and its response to inhaled methacholine. *J. Appl. Physiol.*, **73**, 290–295.

TEPPER, R., SHEN, X., BAKAN, E. & GUNST, S.J. (1995). Maximal airway response in mature and immature rabbits during tidal ventilation. *J. Appl. Physiol.*, **79**, 1190–1198.

YAMAWAKI, I., TAMAOKI, F., YAMAUCHI, F. & KONNO, K. (1992). Angiotensin II potentiates neurally mediated contraction of rabbit airway smooth muscle. *Resp. Physiol.*, **89**, 239–247.

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