High lung inflation increases mRNA levels of ECM components and growth factors in lung parenchyma

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High lung inflation increases mRNA levels of ECM components and growth factors in lung parenchyma. J. Appl. Physiol. 83(1): 120–128, 1997.—Remodeling of pulmonary capillaries occurs after chronic increases in capillary pressure (e.g., mitral stenosis). Also, remodeling of pulmonary arteries begins within 4 h of increased wall stress and is endothelium dependent. We have previously shown that high lung inflation increases wall stress in pulmonary capillaries. This study was designed to determine whether high lung inflation induces remodeling of the extracellular matrix (ECM) in lung parenchyma. Open-chest rabbits were ventilated for 4 h with 9 cm H2O positive end-expiratory pressure (PEEP) on one lung and 1 cm H2O PEEP on the other (High-PEEP group), or with 2 cm H2O PEEP on both lungs (Low-PEEP group). An additional untreated control group was also included. We found increased levels of mRNA in both lungs of High-PEEP rabbits (compared with both the Low-PEEP and untreated groups) for α1(III) and α2(I) procollagen, fibronectin, basic fibroblast growth factor, and transforming growth factor-β. In contrast, α2(I) procollagen and vascular endothelial growth factor mRNA levels were not changed. We conclude that high lung inflation for 4 h increases mRNA levels of ECM components and growth factors in lung parenchyma.

extracellular matrix; messenger ribonucleic acid; stress failure; wall tension; vascular remodeling; strength of capillaries

The magnitude of mechanical stress in a vessel wall is directly proportional to transmural blood pressure but inversely proportional to wall thickness. Because of the thinness of pulmonary capillary walls, it can be calculated that wall stresses in pulmonary capillaries during conditions of severe exercise are very high and similar to those in the normal human aorta (31). This suggests that the blood-gas barrier faces a serious bioengineering dilemma. It has long been appreciated that the capillary wall must be extremely thin to allow diffusive gas exchange. However, the capillary wall must also be immensely strong to withstand periods of very high wall stress.

There is evidence that the capillary wall is just strong enough to withstand the largest stresses encountered under normal physiological conditions. However, if capillary pressure becomes abnormally high, circumferential tension increases in the vessel wall, and stress failure of pulmonary capillaries occurs with high-permeability edema (29, 31). Exercise-induced pulmonary hemorrhage, high-altitude pulmonary edema, and neurogenic pulmonary edema are examples of conditions in which stress failure is caused by increased capillary transmural pressure. In addition, high lung inflation can cause stress failure of pulmonary capillaries by increasing longitudinal tension in tissue elements in the alveolar wall. Direct evidence that this occurs comes from morphometric studies where the appearance of breaks in the capillary wall after high lung inflation was quantified (9). Additionally, damage to pulmonary capillaries is prevented by encasing rabbits in whole body plaster casts to prevent expansion of the chest wall during high peak inspiratory pressure ventilation (10). The increased capillary permeability observed in some patients after mechanical ventilation at high lung volumes may also be attributed to stress failure of pulmonary capillaries (22).

The fact that the blood-gas barrier is just thick enough, and strong enough, to withstand the maximal wall stresses that develop under physiological conditions suggests that its structure is being continuously remodeled in response to capillary pressure. There is some evidence to support this hypothesis. For example, in mitral stenosis where capillary pressure is gradually raised over many months or years, alveolar capillaries show thickening of the endothelial cell basement membrane, with an increase in the number of cytoplasmic processes of pericytes (15). Similar changes are observed in pulmonary capillaries during pulmonary venoocclusive disease where capillary pressure is raised gradually over long periods of time (14). The importance of the basement membrane in providing strength to the capillary wall is supported by several observations including the fact that the thickness of the basement membrane in systemic capillaries increases as the hydrostatic pressure within these capillaries increases down the body (32), and glomerular capillaries, which are exposed to higher hydrostatic pressures than are pulmonary capillaries, have much thicker basement membranes (30).

The response of pulmonary capillaries and small parenchymal blood vessels to increased wall stress has not previously been evaluated at the molecular level. However, there has been extensive work on the response of large vessels to increased pressure in the pulmonary circulation. For example, Meyrick and Reid (20) used a hypoxic rat model to increase pulmonary arterial pressure for periods up to 52 days. After 2 days of hypoxia, they observed new smooth muscle in small pulmonary arteries, and by the third day they noted thickening of capillary endothelial cells. After 10 days of hypoxia, there was a doubling in thickness of the media and adventitia in the main pulmonary artery due to increased smooth muscle, collagen, elastin, and edema.

The molecular biology of the response of pulmonary arteries to increased wall stress has been studied by several groups. Mecham et al. (19) looked at the
response of pulmonary arteries to hypoxia in newborn calves. They observed a two- to fourfold increase in the number of medial smooth muscle cells and similar increases in elastin production and elastin mRNA level. Poliani et al. (24) exposed rats to hypoxia and also observed an increase in elastin synthesis in the pulmonary artery. In addition, they found increases in collagen synthesis and in the level of mRNA for \( \alpha_1(I) \) procollagen within 3 days of exposure to hypoxia. Tozzi et al. (28) applied mechanical tension to explant pulmonary artery rings from rats and showed increases in mRNA for \( \alpha_1(I) \) procollagen within 4 h that were endothelium dependent.

In this study, we postulated that vascular remodeling in the pulmonary artery in response to increased wall stress (as observed by Tozzi et al. (28)) represents a generalized property of the pulmonary vasculature, including pulmonary capillaries. Subjecting pulmonary capillaries and other vessels in the parenchyma to high lung inflation, and thus increased wall stress, may, therefore, initiate remodeling of the extracellular matrix (ECM) including basement membrane. To test this hypothesis, the right and left lungs of open-chest rabbits were independently cannulated so that lung volume in each lung could be selectively controlled by application of positive end-expiratory pressure (PEEP) in the expectation that the opposite lung would be an internal control. We restricted our study to parenchymal tissues because parenchyma has the greatest concentration of small blood vessels and we were specifically interested in the response of small pulmonary vessels to high lung inflation. We found increased levels of mRNA for ECM components and growth factors in lung parenchyma after ventilation at high lung volume. Unexpectedly, the response occurred on both sides of the lung.

**METHODS**

Experimental procedure. Twenty-one specific-pathogen-free female New Zealand White rabbits (Western Oregon Rabbitry, Beaverton, OR; body weight 3.9–4.5 kg) were used in this study. Before handling and while still in the cage, rabbits were sedated with intramuscular injection of ketamine-xylazine-atropine (25:7.5:0.25 mg/kg body wt), and the ear vein was cannulated (22-gauge Angiotech) for infusion of ketamine-xylazine-atropine (33:3:0.1 mg/kg body wt, respectively) sufficient for a 3.5-kg animal, then weighed and transported to the surgery room. Rabbits were then injected with the remainder of the dose (adjusted for measured body weight), and the ear vein was cannulated (22-gauge Angiotech) for infusion of ketamine-xylazine-atropine (25:7:5:0.25 mg/kg \( \times \) h, respectively). A tracheal tube was inserted for temporary ventilation of both lungs (20-ml tidal volume, 45 breaths/min), and the lungs were siphoned to open collapsed airways (peak airway pressure of 65 mm Hg throughout each experiment = 25–30 cm H2O). The right carotid artery was then cannulated for determination of blood gases and systemic blood pressure. Finally, ventilator tidal volume was reduced to 10 ml, the left chest was opened at the 4th intercostal space, and the left main bronchus was cannulated to allow independent ventilation of the left lung. The separate cannulations also allowed independent monitoring of expired \( \text{PO}_2 \), \( \text{PCO}_2 \), airway pressure, and lung volume in each side of the lung.

After cannulation, each lung was sighed again, and the rabbit was placed in a supine position. The bronchial cannula was positioned to allow ventilation of the animal with minimal peak airway pressure, and a PEEP of 1 cm H2O was applied to both sides of the lung. Blood gas measurements were then taken, and minor ventilatory adjustments of tidal volume and/or ventilation frequency were made to maintain the animal within normal physiological limits. Lung volumes were then determined by helium dilution for each side of the lung, both before and after the unilateral application of 0-cm H2O PEEP (High-PEEP group; 5 rabbits for RNA isolation and Northern analysis; 3 rabbits for transmission electron microscopy) or before and after the bilateral application of 2-cm H2O PEEP (Low-PEEP group; 5 and 3 rabbits for molecular and morphological analysis, respectively). Gas exchange on each side of the lung was calculated by multiplying the difference between inspired and expired \( \text{CO}_2 \) and the product of tidal volume and frequency of ventilation for that lung. All rabbits were ventilated with room air for 4 h, and their lungs were sighed, when necessary, to maintain patent airways. One-percent heparinized saline and sodium bicarbonate (pH 7.4) were injected into the carotid cannula as needed to maintain fluid balance and physiological blood pH. Preparation of the Low-PEEP group was identical to the High-PEEP group, except that the Low-PEEP animals required ventilation with a PEEP of 2 cm H2O to maintain adequate gas exchange and survived the 4-h period of ventilation. Samples of lung parenchyma were also collected for RNA isolation and Northern analysis from five unventilated rabbits that did not receive surgical intervention or induced anesthesia (Unventilated group).

RNA isolation and Northern analysis. After 4-h ventilation, the animals were euthanized by intravenous overdose of ketamine-xylazine-atropine, and the lungs were rapidly removed and rinsed in cold phosphate-buffered saline. Peripheral lung tissue (3- to 4-mm-wide strips from the edges of each lung) was then collected, weighed, and frozen in liquid nitrogen for subsequent isolation of RNA by using the method of Chomczynski and Sacchi (5). RNA preparations were quantitated by absorbance at 260 nm, and intactness of RNA was assessed by ethidium bromide staining after separation by electrophoresis in a 6.6% formaldehyde-1% agarose gel.

Fractionated RNA was transferred by Northern blot to a Zeta probe membrane (Bio-Rad, Hercules, CA) and ultraviolet cross-linked. After transfer, the blots were probed with oligolabeled \( [\alpha-3^P] \) dCTP cDNA probes, which have a specific activity of at least \( 1 \times 10^9 \) disintegrations·min·\(^{-1}\)·µg·\(^{-1}\) (Prime-It-II kit, Stratagene, La Jolla, CA). The membrane was then prehybridized and hybridized in 50% formamide 5× saline sodium citrate (SSC) (20× SSC is 0.3 M sodium chloride, 0.3 M sodium citrate), 10× Denhardt’s solution (100× Denhardt’s solution is 2% Ficoll, 2% polyvinyl pyrrolidone, 2% bovine serum albumin, Factor V), 50 mM NaH2PO4 (pH = 6.5), and 100–250 µg/ml salmon sperm at 37 or 42°C. Blots were washed with 2× SSC, 0.1% sodium dodecyl sulfate (SDS) at room temperature and with 0.1–0.5× SSC, 0.1% SDS at 50–65°C and were then exposed to X-ray film with the use of a Cronex Lightning Plus intensifier screen at ~70°C. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to ribosomal 18S RNA levels.

Source of recombinant plasmids. Rabbit \( \alpha_1(I) \) and \( \alpha_1(IV) \) procollagen cDNAs were provided by Y. Ninomiya (Okayama University Medical School, Okayama, Japan). Mouse \( \alpha_1(III) \) procollagen cDNA was cloned by Liu et al. (18). Rat fibronectin cDNA was cloned by Schwartzbauer et al. (27). Human basic fibroblast growth factor (bFGF) cDNA was cloned by Kurokawa et al. (16). Human transforming growth factor-\( \beta_1 \) (TGF-\( \beta_1 \)) cDNA was cloned by Qian et al. (25), and human vascular endothelial growth factor (VEGF) cDNA was cloned by Leung et al. (17).
Lung perfusion, tissue sampling, and transmission electron microscopy. After a 4-h ventilation, the animals were euthanized, and the lungs were briefly inflated to 25 cmH2O of positive pressure before being deflated to 5 cmH2O for perfusion with saline solution (11.06 g NaCl/l, 350 mosM and 1,000 U heparin/100 ml) after cannulation of the pulmonary artery (inflow) and left atrium (outflow), as previously described (9). Perfusion (capillary hydrostatic pressure = 22.5 cmH2O) was carried out for 3 min to remove blood from the pulmonary circulation, followed by fixative (phosphate-buffered 2.5% glutaraldehyde; total osmolality 500 mosM, pH 7.4) for 10 min. The upper level of the liquid in each reservoir was adjusted to maintain the preset perfusion pressure of 22.5 cmH2O throughout all perfusions. After fixation, the lungs were excised and stored in glutaraldehyde at 4°C.

Tissue preparation and morphometric procedures were identical to those used in previous studies (9). Briefly, one slab (~0.5 cm thick) was taken perpendicularly to the cranial-caudal axis at about one-third the distance from the bottom of either lower lobe in each animal. A thin vertical slice was then obtained from each slab and cut into smaller blocks (~1.5 x 1.5 x 2.0 mm), which were rinsed overnight in 0.1 M phosphate buffer adjusted to 350 mosM with NaCl (pH 7.4) and embedded in Araldite. Five tissue blocks were selected randomly from each vertical slice. Ultrathin sections (50–70 nm) were cut from each block with a LKB Ultratome III, contrasted with uranyl acetate and bismuth subnitrate (9), and micrographs for morphometry were taken on 70-mm films with a Zeiss 10 electron microscope. Micrographs of a carbon-grating replica (Fullam, Schenectady, NY) were recorded for calibration on each film.

For morphometry, we analyzed 12 micrographs from each block, yielding a total of 57–60 micrographs from each slab in each animal. Measurements were performed at a final magnification of x11,000 with a Videometric 150 image analyzer (American Innovision), after electronic positive reversal of the 70-mm negative films. As in previous studies (9), a print of each micrograph was also available and systematically examined during the measurements for unequivocal identification of small endothelial and epithelial disruptions as well as the presence (or absence) of basement membrane at all sites of rupture. The frequency of disruptions of the blood-gas barrier was quantified as the number of breaks per unit endothelial or epithelial boundary length in the sections after the contour of capillary (inner endothelial) and alveolar (outer epithelial) boundary segments in each field of view were traced and the number of endothelial and epithelial disruptions were counted. The presence, and extent, of interstitial edema was assessed by measuring the thickness (profile width) of each layer of the blood-gas barrier (endothelium, interstitium, and epithelium). One to five sites were systematically sampled in each micrograph (total 150–241 barrier sites/animal), and measurements were made at right angles to the barrier at random points, systematically determined by the image analyzer via electronically generated test lines intersecting the barrier, as described previously (9).

Statistical analysis. Statistical comparisons between groups were made by using one-way analysis of variance and the Student-Newman-Keuls test. Comparisons between the right and left lungs in the same rabbit were made by using a paired t-test. Values are expressed as means ± SE, and a value of P < 0.05 was considered significant.

RESULTS

Lung volumes and physiological measurements. End-expiratory lung volumes for the two groups of ventilated rabbits increased directly with the magnitude of PEEP. Within the High-PEEP group of rabbits used for molecular analysis, three rabbits received high lung inflation (9-cmH2O PEEP) on the right lung and two rabbits received high lung inflation on the left lung. This was necessary to control for possible differences in blood flow or ventilation, which might influence gene expression after application of high-PEEP ventilation. The opposite lung of each rabbit in the High-PEEP group received minimal lung inflation (1-cmH2O PEEP). Lung volume in High-PEEP rabbits increased from 11.5 ± 1.2 to 32.0 ± 3.7 ml (P < 0.05) before and after 9-cmH2O PEEP, respectively. Lung volume in the 1-cmH2O PEEP lung of these rabbits decreased from 10.0 ± 1.1 to 9.1 ± 0.5 ml after the application of 9-cmH2O PEEP to the paired lung, but this difference was not significant. The Low-PEEP rabbits were ventilated with 2-cmH2O PEEP on each lung, and the lung volumes of the five rabbits used for RNA analysis were 14.2 ± 0.8 and 14.4 ± 1.4 ml on the right and left lungs, respectively. Ventilation with 2-cmH2O PEEP produced a larger lung volume in these rabbits than ventilation with 1-cmH2O PEEP [14.3 ± 0.8 ml in the right and left lungs of Low-PEEP rabbits (n = 10 lungs) vs. 10.0 ± 1.1 ml on the 1-cmH2O-PEEP lung of High-PEEP rabbits before the application of 9-cmH2O PEEP (n = 5 lungs); P < 0.05].

Lung volume was also measured in the six rabbits used for morphological analysis. Mean lung volume for High-PEEP rabbits in this group increased from 10.7 ± 2.1 to 37.5 ± 9.9 ml before and after 9-cmH2O PEEP, respectively. Although the increase in lung volume was slightly greater in this group than in those High-PEEP rabbits used for RNA analysis, the difference was not statistically significant.

Blood gas parameters for all rabbits remained relatively stable during the 4-h period of ventilation. Mean values for blood pH ranged from 7.55 to 7.43, whereas values for mean arterial Po2 and Pco2 varied between 82.7–55.0 and 30.4–35.0 Torr, respectively, during 4-h ventilation. Mean arterial blood pressure decreased from 90.1 ± 4.9 mmHg (High-PEEP) and 84.9 ± 4.0 mmHg (Low-PEEP) at the beginning of ventilation to 50.9 ± 6.4 (P < 0.05 vs. time0) and 70.9 ± 4.9 mmHg. More than one-half of the loss in arterial blood pressure in High-PEEP rabbits occurred during the last hour of ventilation (3-h values for High-PEEP = 68.1 ± 4.7 mmHg and for Low-PEEP rabbits = 83.4 ± 4.4 mmHg). These values are significantly lower for High-PEEP rabbits and reflect the fact that 4 h were approaching the limit of time for ventilation of High-PEEP rabbits.

Peak airway pressures in the trachea and mainstem bronchus during the 4-h period of ventilation were similar in the 1-cmH2O-PEEP lung of High-PEEP rabbits and in each side of the lung of Low-PEEP rabbits (mean value for these 3 groups of lungs among the 16 ventilated rabbits = 15.8 ± 0.64 cmH2O, n = 24). Peak airway pressure in the 9-cmH2O-PEEP lung of High-PEEP rabbits increased to a mean value of 23.0 ± 1.7 cmH2O (n = 8 lungs) after application of 9-cmH2O PEEP.
PEEP (P < 0.05 vs. peak airway pressure in the other 3 groups of lungs).

During ventilation, the lungs were sighed to reopen collapsed airways and maintain minimal airway pressure. For the RNA group of rabbits, there were no differences in the total number of sighs required by either lung of Low-PEEP rabbits or the 9-cmH2O-PEEP lungs of High-PEEP rabbits (mean no. of sighs/4 h for these 3 groups of lungs: 4.7 ± 0.6, n = 24 lungs). In contrast, the 1-cmH2O-PEEP lung of High-PEEP rabbits in this group became compressed by the inflated contralateral side and required more frequent sighing to reopen alveoli and maintain gas exchange (mean no. of sighs/4 h on this side of the lung = 16.1 ± 1.9, n = 8 lungs). When expressed as frequency (no. of sighs/min), Low-PEEP rabbits and the high-PEEP lungs of High-PEEP rabbits averaged 1 sigh/51 min, whereas the 1-cmH2O-PEEP lungs of High-PEEP rabbits averaged 1 sigh/15 min.

Gas exchange in High-PEEP rabbits was similar on both sides of the lung after application of high-PEEP ventilation (10.6 ± 1.6 ml CO2/min on lungs ventilated with 9-cmH2O PEEP and 8.76 ± 1.0 ml CO2/min on lungs ventilated with 1-cmH2O PEEP). These values are not significantly different from each other and suggest that blood flow to the high-PEEP lung is sufficient to maintain gas exchange.

Transmission electron microscopy. Very few disruptions of the blood-gas barrier were observed, and their presence did not correlate with the magnitude of PEEP during ventilation (Table 1). Break frequencies of 1.6 ± 0.8 (endothelium, left lung, rabbit 2) and 0.4 ± 0.4 (e.g., epithelium, right lung, rabbit 3) correspond to 5 and 1 break out of the 58 and 60 micrographs examined in these samples, respectively. Overall, a total of 13 breaks (9 endothelial and 4 epithelial) out of 358 micrographs examined were found in the lungs of Low-PEEP rabbits, and 5 breaks (1 endothelial and 4 epithelial) out of 358 micrographs examined were found in the lungs of High-PEEP rabbits. In contrast, the thickness of the interstitium increased by 50% on the side of the lung receiving 9-cmH2O PEEP ventilation in High-PEEP rabbits, compared with the same side in Low-PEEP rabbits (P = 0.055; Table 1). No disruption of the basement membrane was found in any of the six rabbits, and the lungs showed no apparent increase in the number of inflammatory cells with high PEEP.

Gene expression of ECM components. In this study, gene expression of ECM components in peripheral lung parenchyma was quantified at the mRNA level by Northern analysis. We found that changes in the level of mRNA for each molecule analyzed were similar in both lungs of High-PEEP rabbits (Fig. 1). Values from the two lungs were, therefore, pooled for subsequent comparison with Low-PEEP rabbits to yield values from 10 lungs/group of five rabbits. The level of mRNA for α1(III) procollagen was threefold higher in the lungs of High-PEEP rabbits than in Low-PEEP rabbits, fibronectin mRNA was increased twofold, and α2(IV) procollagen mRNA was increased by 50% (Fig. 2, Northern blot analysis; and Fig. 3, densitometric analysis). Levels of mRNA for α2(I) and α1(III) procollagens and fibronectin in the lungs of Low-PEEP rabbits were also significantly increased compared with values in unventilated rabbits (Table 2, Unventilated group). However, the significance of these differences is unclear because

<table>
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Values are means ± SE; n, no. of rabbits. TEM, transmission electron microscopy; PEEP, positive end-expiratory pressure; LL, left lobe; RL, right lobe. *P = 0.055 vs. 2-cmH2O PEEP in RL.

Fig. 1. Densitometric analysis of Northern blots hybridized to 32P-oligolabeled DNAs specific for α2(I) procollagen [2(I)], α1(III) procollagen [1(III)], and α2(IV) procollagen [2(IV)] and fibronectin (FN) in parenchymal lung tissue from lungs of high-positive-end-expiratory-pressure (PEEP) rabbits that were ventilated for 4 h with 1-cmH2O PEEP on one side of lung (open bars) and 9-cmH2O on the other (solid bars). mRNA levels are expressed in arbitrary densitometric units normalized for loading. Values are means ± SE (n = 5 rabbits). No differences were statistically significant.
of uncontrolled variables (e.g., absence of both surgical intervention and 4-h infusion of anesthesia during ventilation in unventilated rabbits) that may contribute to the observed differences between the Low-PEEP and unventilated groups. For this reason, we have focused our analysis on the comparison between the Low and High-PEEP groups of rabbits and consider these differences to most accurately reflect the response of parenchymal blood vessels to high lung inflation.

Gene expression of growth factors. We also determined mRNA levels of growth factors that participate in vascular remodeling. Again, the observed changes in both lungs of High-PEEP rabbits were similar (Fig. 4). We found that mRNA levels of TGF-β1 were increased fourfold in the lungs of High-PEEP rabbits compared with Low-PEEP rabbits, whereas mRNA levels for bFGF increased by 60% (Fig. 5, Northern blot analysis; and Fig. 6, densitometric analysis). In contrast, the level of VEGF mRNA was not altered by high lung inflation. Levels of mRNA for bFGF were also higher in the lungs of Low-PEEP rabbits than in unventilated controls (Table 2).

Table 2. Values obtained with densitometric analysis of Northern blots hybridized to 32P-oligolabeled cDNAs

<table>
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<th>High-PEEP</th>
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<td>α2(I)</td>
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<td>VEGF</td>
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Values are means ± SE; n = 5 rabbits/group. 32P-oligolabeled cDNAs were specific for α2(I) procollagen [α2(I)], α1(III) procollagen [α1(III)], α2(IV) procollagen [α2(IV)], fibronectin (FN), transforming growth factor-β1 (TGF-β1), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF). Parenchymal tissue was collected from lungs of unventilated rabbits or from lungs of rabbits ventilated with 9-cmH2O PEEP on one lung and 1-cmH2O PEEP on the other (High-PEEP group) or with 2-cmH2O PEEP on both lungs (Low-PEEP group). *P < 0.05 vs. other group; †P < 0.01 vs. Low-PEEP; ‡P < 0.01 vs. High-PEEP group.

Fig. 4. Densitometric analysis of Northern blots hybridized to 32P-oligolabeled cDNAs specific for basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGF-β1), or vascular endothelial growth factor (VEGF) in parenchymal lung tissue from lungs of High-PEEP rabbits that were ventilated for 4 h with 1-cmH2O PEEP on one side of lung (open bars) and 9-cmH2O on the other (solid bars). mRNA levels are expressed in arbitrary densitometry units normalized for loading. Values represent means ± SE (n = 5 rabbits). No differences were statistically significant.
DISCUSSION

High lung inflation increases longitudinal tension in tissue elements of the alveolar wall and stretches both the blood-gas barrier and associated cells in the periphery of the lung. There is substantial literature on the response of cells to mechanical stretch (7). However, most studies have focused on the response of tissues such as bone, skeletal muscle, myocardium, systemic blood vessels and pulmonary arteries, and relatively few studies have focused on cells in peripheral lung parenchyma.

We have previously demonstrated that high lung inflation causes stress failure of pulmonary capillaries (9). In that study, we found that the mean number of breaks in pulmonary capillaries increased tenfold (from 0.7 to 7.1 breaks/mm in the endothelium and from 0.9 to 8.5 breaks/mm in the epithelium) when airway pressure was raised from 5 to 20 cmH₂O at a constant capillary transmural pressure of 32.5 cmH₂O (see Fig. 4 in Ref. 9). In addition, there was considerable edema formation (mean thickness of the capillary interstitium increased from 0.17 to 0.49 µm).

The present study was designed to determine whether high lung inflation induces vascular remodeling of pulmonary capillaries and small parenchymal blood vessels in vivo. Because our protocol required physiological maintenance of rabbits during 4-h ventilation, it was necessary to select PEEP levels sufficiently high to increase wall stress but not excessively high to cause lung injury or edema and compromise gas exchange. As shown in Table 1, capillaries in the two groups of rabbits were normal and exhibited no evidence of stress failure other than the development of mild interstitial thickening on the side of the lung in High-PEEP rabbits that received 9-cmH₂O PEEP ventilation. In addition, the basement membrane remained continuous along each of the few capillary breaks observed in all samples (9 breaks out of 358 micrographs in the Low-PEEP group; 1 break out of 357 micrographs in the High-PEEP group). These results, and the fact that all rabbits maintained gas exchange in both lungs during 4-h ventilation, suggest that stress failure of pulmonary capillaries did not occur. The observed interstitial thickening in capillaries of High-PEEP rabbits (Table 1) implies that high lung inflation caused stretching of the blood-gas barrier and fluid accumulation in the interstitial space. In total, the morphometric data suggest that 9-cmH₂O PEEP provided a modest increase in wall stress without causing gross lung injury. The lack of either gross injury or edema in the lungs of Low-PEEP rabbits, and in the 1-cmH₂O-PEEP lung of High-PEEP rabbits, further illustrates that wall stress remained low in these lungs during ventilation.

Vascular remodeling. Vascular remodeling is a dynamic process that involves both synthesis and degradation of collagen molecules (33). Functionally, types I, III, and IV collagen provide tensile strength to the vessel wall. Types I and III collagen are synthesized by fibroblasts, myofibroblasts, and smooth muscle cells and are distributed diffusely throughout the media and adventitia of small vessels and on the thick side of the capillary wall. In contrast, type IV collagen is synthesized by endothelial and epithelial cells and is found mainly in the basement membrane where it provides major support for the thin side of the blood-gas barrier (30).

Strong evidence that mechanical forces cause remodeling of the pulmonary vasculature comes from studies in which pulmonary artery rings were subjected to mechanical stretch. Tozzi et al. (28) applied static mechanical stretch to pulmonary artery segments for 4 h and observed increased synthesis of collagen and elastin at the protein level and increased gene expression of mRNA for α₁(1) procollagen and v-sis (a protooncogene encoding a peptide homologous to the B chain of platelet-derived growth factor (PDGF)). These changes occurred without a proliferative response as assayed by [³H]thymidine incorporation. Because the response was not present in arterial segments devoid of endothelium,
sis and secretion of type I and III procollagen and fibronectin (12). TGF-β1 is abundantly stored in the ECM and is released in an active form via both autocrine and paracrine pathways (1). Direct evidence that TGF-β1 participates in the regulation of vascular remodeling is provided by an in vivo study by Nabel et al. (21). These investigators transfected the human TGF-β1 gene into the iliofemoral artery of pigs and demonstrated that TGF-β1 gene expression is associated with hyperplasia of the vessel wall and increased synthesis of type I procollagen. They also found that the response is specific for TGF-β1, since transfer of the human PDGF-β gene did not alter procollagen levels in the arterial wall. Our results support these studies and imply that TGF-β1 participates in the early response of parenchymal cells to mechanically induced capillary remodeling.

Additional investigations are required to determine the cellular source of the changes in mRNA observed in the present study. Likely candidates are the fibroblast, myofibroblast, or pericyte. These cells are abundant in the small vessels and capillaries of the outer lung parenchyma. The myofibroblast, in particular, produces abundant amounts of fibronectin and is highly activated by TGF-β1 (8). In addition, several pathological conditions involving increased mechanical stress or tension correlate with the presence of myofibroblasts in the lung. For example, Kapanci et al. (13) observed activated myofibroblasts in patients with venoocclusive disease, postcapillary pulmonary hypertension secondary to heart failure, or mitral stenosis.

Mechanism of information transfer to the non-PEEP lung. A very interesting finding in this study is that the observed changes in mRNA were identical in both the 9-cmH₂O-PEEP and 1-cmH₂O-PEEP lungs of High-PEEP rabbits (Figs. 1 and 4). This observation suggests that a generalized organ-specific response occurred after the localized (unilateral) application of mechanical force. Although it is speculative, one possibility is that information is transferred via the circulation from the 9-cmH₂O-PEEP lung to the 1-cmH₂O-PEEP lung in High-PEEP rabbits. Precedents for signaling through the lung via the circulation come from transplantation studies by Hislop et al. (11). In that study, immature left lung was transplanted into an adult rat. Normally, compensatory growth in the adult rat lung after pneumonectomy occurs through an increase in size, rather than number, of alveoli (4). The alveoli in the remaining lung increase in size until the total lung volume is sufficient to replace the resected tissue. However, Hislop et al. (11) found that the presence of transplanted immature lung caused the contralateral adult lung to revert to an immature pattern of compensatory growth (i.e., growth occurred through an increase in number of alveoli rather than through an increase in size). Because all connections to the left lung were severed during transplantation, Hislop et al. concluded that the immature lung must release blood-borne factors that influence growth in the mature lung.

When one follows this line of reasoning, it is possible that activation of inflammatory cells (e.g., alveolar or interstitial macrophages), either in direct response to the authors conclude that endothelium-derived factors are involved in stretch-induced production of ECM proteins. Because of the increase in v-sis mRNA, they further proposed that PDGF (or a PDGF-like peptide) and, possibly, TGF-β and bFGF may be involved. Alternatively, work by others (1) suggests that cell interactions between endothelial cells and smooth muscle cells or pericytes may be required for growth factor activation.

Studies by Poiani et al. (24) provide additional in vivo evidence that remodeling of pulmonary arteries occurs after increased wall stress. These investigators exposed rats to normobaric hypoxia (10% oxygen) for up to 14 days and observed progressive increases in mean right ventricular pressure during the first 10 days of exposure. Increased amounts of collagen and elastin were also observed in the pulmonary artery during this time period, and these increases paralleled the development of pulmonary hypertension. Interestingly, levels of mRNA for α₁(I) procollagen increased in the pulmonary artery during hypoxia but not in the aorta, where blood pressure remained constant. In addition, collagen and elastin levels in the pulmonary artery returned to normal within 7 days after return of rats to normoxia. In total, these observations imply that increases in transmural pressure increase wall stress to alter gene expression of ECM proteins in the pulmonary artery.

In this study, we observed a threefold increase in α₁(III) procollagen mRNA after high lung inflation (Fig. 3). This observation is consistent with the possibility that parenchymal blood vessels adapt to increased wall stress by increasing type III collagen synthesis, thereby increasing the tensile strength of the vessel wall. We also observed a smaller (50%) increase in level of mRNA for α₂(IV) procollagen, which may suggest that vascular remodeling also occurs in the basement membrane of parenchymal blood vessels. In addition, fibronectin mRNA levels were twofold greater in High-PEEP rabbits than in Low-PEEP rabbits. Because fibronectin provides a scaffolding for cell attachment and plays an important role in relaying the transmission of force through the cytoskeleton, this observation provides further evidence that high lung inflation causes vascular remodeling. Thus, in our preparation, type III procollagen and fibronectin are the main ECM components expressed as a result of 4-h high lung inflation with smaller increases in α₂(IV) procollagen.

Role of growth factors. In this study, we also determined mRNA levels for several growth factors that have the potential to regulate remodeling in the capillary wall, either by increasing cell proliferation or by increasing ECM deposition. TGF-β1 mRNA levels were increased fourfold in response to high lung inflation, and levels of bFGF mRNA were increased by 61% (Fig. 6). TGF-β1 plays a central role in regulating the synthesis and degradation of ECM collagens and is produced by most cells, particularly macrophages, fibroblasts, endothelial cells, and platelets (26). bFGF also participates in regulation of these processes (6). In vitro studies have demonstrated that TGF-β1 elevates synthesis and secretion of type I and III procollagen and fibronectin (12). TGF-β1 is abundantly stored in the ECM and is released in an active form via both autocrine and paracrine pathways (1). Direct evidence that TGF-β1 participates in the regulation of vascular remodeling is provided by an in vivo study by Nabel et al. (21). These investigators transfected the human TGF-β1 gene into the iliofemoral artery of pigs and demonstrated that TGF-β1 gene expression is associated with hyperplasia of the vessel wall and increased synthesis of type I procollagen. They also found that the response is specific for TGF-β1, since transfer of the human PDGF-β gene did not alter procollagen levels in the arterial wall. Our results support these studies and imply that TGF-β1 participates in the early response of parenchymal cells to mechanically induced capillary remodeling.

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When one follows this line of reasoning, it is possible that activation of inflammatory cells (e.g., alveolar or interstitial macrophages), either in direct response to
strecth or on contact with exposed basement membranes after high lung inflation (9), stimulates the release of quick-acting circulatory mediators. In this regard, it has recently been demonstrated that ventilation of isolated perfused mouse lungs with end-expiratory lung volumes similar to those experienced by the High-PEEP rabbits in this study (2.5-fold increase) causes the release of prostacyclin, tumour necrosis factor-α, and interleukin-6 into the perfusate after 30-min ventilation (3). Alternatively, endothelial and epithelial cells as well as fibroblasts and myofibroblasts also respond to stretch and thus may play a role.

An additional possible factor in this study is that the frequency of sighs differed between groups of lungs. Both lungs of Low-PEEP rabbits and the 9-cmH₂O-PEEP lung of High-PEEP rabbits received, on average, 1 sigh/51 min, whereas the low-PEEP lung in High-PEEP rabbits required more frequent sighing (1 sigh every 15 min) to prevent compression of airways and maintain gas exchange. It is, therefore, possible that differences in the frequency of sighs, and not a blood-borne mediator, caused the observed increases in expression of mRNA in the 1-cmH₂O-PEEP lung of High-PEEP rabbits. Although this possibility cannot be ruled out, it seems unlikely that parenchymal blood vessels would selectively increase gene expression in response to sighs at 15-, but not at 51-min, intervals. Also, during normal ventilation, humans average 1 sigh/6 min when awake (2) and 1 sigh/36 min while sleeping, with considerable variation being observed between people (23). Data are not available on the frequency of sighs in rabbits. Thus, although data from humans suggest that the frequency of sighs used in this study may be within normal physiological limits, further study is required to resolve this question.

It is also possible that application of high PEEP narrows capillaries and increases vascular resistance to redistribute blood to the other lung. In this case, the increased blood flow to the lung receiving 1-cmH₂O PEEP ventilation would increase vascular pressure and wall stress that might cause vascular remodeling. Although this process may occur to some extent, the normal pulmonary circulation has such reserves that even directing the whole cardiac output through one lung causes only a small rise in vascular pressures. The increase in wall stress will, therefore, be small. In addition, gas exchange in the 9-cmH₂O-PEEP lung of High-PEEP rabbits was similar to gas exchange in the lung receiving 1-cmH₂O PEEP ventilation. This observation implies that blood flow was not abolished in the high PEEP lung during 4-h ventilation.

In summary, we found that high lung inflation for 4 h stimulates increased expression of a(1)(III) and a(2)(IV) procollagen mRNAs in lung parenchyma. On the assumption that these increases are translated into increased protein for these collagens, the result would be to strengthen the capillary wall. We also observed increases in fibronectin mRNA. A fibronectin-rich ECM allows increased cell attachment and transduction of forces from the extracellular environment through cytoskeletal elements. TGF-β₁ and bFGF mRNA levels were also significantly elevated after high lung inflation, but the level of VEGF mRNA did not change. In conclusion, our results show that mRNA levels of ECM components and growth factors increase in lung parenchyma after high lung inflation. Although further studies, using in situ hybridization techniques, are required to precisely identify the anatomical location of the observed changes, these observations are consistent with the possibility that increased wall stress initiates vascular remodeling in peripheral lung parenchyma.

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