High vascular and airway pressures increase interstitial protein mRNA expression in isolated rat lungs

James C. Parker, Ellen C. Breen, and John B. West

High vascular and airway pressures increase interstitial protein mRNA expression in isolated rat lungs. J. Appl. Physiol. 83(5): 1697-1705, 1997.-We hypothesized that wall stresses produced by high peak airway (Paw) and venous (Ppv) pressures would increase mRNA levels for structural proteins of the interstitial matrix in isolated rat lungs. Groups of lungs (n = 6) were perfused for 4 h at a peak Paw of 35 cmH2O (HiPaw), cyclical Ppv of 28 cmH2O (HiPv), or baseline vascular and airway pressures (LoPress). In two separate groups, comparable peak pressures increased capillary filtration coefficient fourfold in each group. Northern blots were probed for mRNA of $\alpha_1(I)$, $\alpha_1(III)$, and $\alpha_2(IV)$ procollagen chains, laminin B chain, fibronectin, and transforming growth factor-$\beta_1$, and densities were normalized to 18S rRNA. mRNA was significantly higher in the HiPv group for type I (4.3-fold) and type III (3.8-fold) procollagen and laminin B chain (4.8-fold) and in the HiPaw group for type I (2.4-fold) and type IV (4.5-fold) procollagen and laminin B chain (2.3-fold) than in the LoPress group. Only fibronectin mRNA was significantly increased (3.9-fold) in the LoPress group relative to unperfused lungs. Estimated wall stresses were highest for alveolar septa in the HiPaw group and for capillaries in the HiPv group. The different patterns of mRNA expression are attributed to different regional stresses or extent of injury.

collagen; fibrosis; pulmonary hypertension; barotrauma; fibronectin; vascular permeability

AN INCREASED MECHANICAL load on parenchymal cells and connective tissues can initiate a process of adaptation to the higher stress. Increased collagen deposition and vascular cell proliferation have been demonstrated in response to high vascular distending pressures during pulmonary hypertension induced by a number of agents (6, 24). Increased amounts of collagen surrounding pulmonary arteries are adaptations to the high vascular pressures and contribute to the decreased pulmonary vascular distensibility (38). An adaptation to prolonged alveolar distension also occurs in the remaining lung after unilateral pneumonectomy and is accompanied by hyperplasia and parenchymal remodeling (31).

Most animal models of pulmonary hypertension induce remodeling of the larger pulmonary arteries, but there are few studies of microvascular adaptations to high pressures, or the time course for such changes. Pulmonary capillaries exposed to high vascular pressures during left heart failure often show increased wall thickening, which increases the apparent threshold for edema formation in patients and experimental animals exposed to chronic congestive heart failure (28, 37). Microscopic studies in patients with congestive heart failure suggest a thickening of the pulmonary microvascular basement membranes in response to chronic high pulmonary vascular pressures (20), but relatively little is known about the adaptation process of the alveolar-capillary barrier to the large distending stresses induced by high vascular or alveolar pressures (14, 27).

Several major structural proteins of the interstitium provide mechanical support for the lung and could be altered in any adaptation response. Type I collagen is the major structural collagen that provides the load-bearing structural framework of the lung parenchyma, support for alveolar ducts and alveolar openings, and tensile strength of vascular and bronchial sheaths (18). Type III collagen is the fibrillar collagen of the interstitial spaces, and type IV collagen is the major tensile component of basement membranes (18, 44). Approximately one-half of the capillary surface area is bordered by a true interstitial space containing type I and III collagen fibers, and one-half consists of very thin layers of epithelium and endothelium separated only by a fused basement membrane (36). Basement membrane is a structural complex of type IV collagen, laminin, entactin, heparin, fibronectin, and proteoglycans that contributes most of the tensile strength to the alveolar capillary wall (53). All these load-bearing structural proteins could be altered in response to higher levels of mechanical stress.

Acute increases in pulmonary vascular and airway pressures induce increases in capillary filtration coefficient ($K_t$) in dogs and rabbits (27, 32) due to the opening of transverse breaks in epithelium and endothelium that often extend through the basement membrane (9, 14, 42). Stress failure of the alveolar capillary basement membrane has been proposed as a mechanism for intrapulmonary bleeding in racehorses and the permeability pulmonary edemas of high altitude, head injury, and high-pressure mechanical ventilation (45, 47). An increased deposition of type IV collagen and laminin would be expected to increase the threshold pressure required for stress failure along the thin side of the interstitial space, whereas increased deposition of type I and type III collagen would provide increased support for the thick side of the alveolar capillary interstitium and the walls of larger vessels, airways, and alveolar septa (18, 46).

The purpose of the present study was to determine whether cyclical increases in pulmonary vascular and airway pressures would initiate an adaptation response by inducing increases in transcription of the mRNA for interstitial matrix proteins over a 4-h period in isolated, perfused rat lungs. Lungs were assayed for mRNA for type I, type III, and type IV collagen chains,
laminin B chain, fibronectin, and transforming growth factor-β (TGF-β), a cytokine involved in collagen synthesis. \( K_c \) values were measured in two separate groups at the peak pressures used in the high-pressure groups to determine whether the threshold pressure for microvascular injury had been attained. The different patterns of mRNA expression induced by the high airway and vascular pressures suggest responses to regional differences in stress or a response to different degrees of injury.

**METHODS**

**Rat Lung Preparation**

The isolated rat lung preparation has been previously described (15, 52). Briefly, male Charles River CD rats weighing 199–320 g (247 ± 8 g) were anesthetized with pentobarbital sodium (65 mg/kg ip), the trachea was cannulated, and the rats were ventilated with 20% \( O_2 \) and 5% \( CO_2 \) using a rodent ventilator (model 683, Harvard, S. Natick, MA) with a tidal volume of 2.5 ml and a positive end-expiratory pressure (PEEP) of 3 cmH\(_2\)O at 40 breaths/min. The chest was opened, and 300 U of heparin sodium were injected into the right ventricle. The pulmonary artery and left atrium were then cannulated, and the heart and lungs were excised en bloc. Lungs were perfused with 5% bovine albumin in Krebs bicarbonate buffer (37°C) at 6 ml/min using a roller pump (Minipuls 2, Gilson, Middleton, WI). Homologous blood (~10 ml) was obtained from a donor rat and added to the perfusate to obtain a hematocrit of ~10%. Arterial (Ppa), venous (Ppv), and airway (Paw) pressures were recorded using pressure transducers (Cobe, Lakewood, CO) and a polygraph (model 7, Grass, Quincy, MA). In two groups the lung weight was monitored using a force transducer to obtain weight gain. At the end of 4 h, perfusion was stopped, and the lung was weighed, randomly divided into pieces of ~0.3 g, which represented approximately one-half of one major lobe, and frozen in liquid nitrogen for Northern analysis.

Four groups of rat lungs were analyzed for mRNA expression. Peak pressures were chosen that we assumed would be near the threshold for injury but were actually pressures that induced increases in microvascular permeability. However, lungs were not grossly hemorrhagic, indicating that there was not extensive rupture of capillary basement membranes.

Unperfused control (UnPerf) group. Lungs (n = 5) were removed, weighed, divided into pieces (~0.3 g), and immediately frozen in liquid nitrogen for Northern analysis.

Low-pressure perfusion (LoPress) group. Lungs (n = 5) were isolated and perfused for 4 h as previously described at baseline vascular pressure and Paw.

High vascular pressure (HiPv) group. Lungs (n = 5) were isolated and prepared as described above. After a baseline period of 20 min, the venous outflow tubing was cycled occluded at a rate of 1/min using a solenoid occluder with the occlusion time adjusted to 15 s closed and 45 s open to obtain a peak Ppv of ~28–30 cmH\(_2\)O. Occlusions were timed using a darkroom timer (Gralab model 451, Dimco-Gray, Centerville, OH).

High airway pressure (HiPaw) group. Lungs (n = 5) were isolated and prepared as described above. After a baseline period of 20 min, the PEEP was increased to 10 cmH\(_2\)O and tidal volume was increased to obtain a peak inflation pressure (PIP) of ~35 cmH\(_2\)O.

Two additional groups of rat lungs were used to establish whether the threshold of injury was attained using the peak Paw and vascular pressure used in the preceding experiments.

High vascular pressure threshold (HiPvTh) group. Lungs (n = 5) were isolated and prepared as described above, except they were suspended from a force transducer to continuously monitor lung weight. After a baseline period of 20 min, a baseline \( K_c \) was measured as described below. Then the venous reservoir was raised to obtain a Ppv of 30 cmH\(_2\)O and maintained for 10–12 min, and the rate of weight gain was used to calculate \( K_c \).

High airway pressure threshold (HiPawTh) group. Lung weight was also continuously recorded in this group (n = 5). After a baseline period of 20 min, PIP was increased to 35 cmH\(_2\)O and PEEP was increased to 10 cmH\(_2\)O for 30 min, then \( K_c \) was measured.

The ratio of lung weight (LW) to body weight (BW) of unperfused controls (4.38 ± 0.13 g LW/kg BW) was used to calculate predicted lung weights for normalization of \( K_c \), where

\[
predicted \ LW \ g = 0.00438 \ BW \ g \tag{1}\]

**RNA Isolation and Northern Blot Analysis**

Rat lungs frozen in liquid nitrogen were stored at −70°C until analyzed. Total cellular RNA was isolated from each pellet by the method of Chomczynski and Sacchi (7). RNA preparations were quantitated by absorbance at 260 nm, and intactness was assessed by ethidium bromide staining after separation in 6.6% formaldehyde-1% agarose. Fractionated RNA was transferred by Northern blot to Zeta probe membranes (Bio-Rad, Hercules, CA). RNA was cross-linked to the membrane by ultraviolet irradiation for 1 min and stored at 4°C. The blots were then probed with oligolabeled (\( α-32P \)) dCTP cDNA probes that have a specific activity of at least 1 × 10\(^6\) disintegrations/min (dpm)/µg DNA (13). Specific cDNA probes for mRNA of matrix proteins were used for mouse \( α_2(IV) \) procollagen (19), rat fibronectin (34), mouse laminin B chain (3), rat \( α_1(III) \) procollagen (3), mouse \( α_1(III) \) procollagen (21), and rat TGF-β1 (29). Specific fibronectin isoforms could not be resolved with the probe used. Prehybridization and hybridizations were performed in 50% formamide, 5 × SSC (0.3 M sodium chloride, 0.3 M sodium citrate), 10 × Denhardt's solution (100 × Denhardt's solution is 2% Ficoll, 2% polyvinyl pyrrolidone), 50 mM sodium phosphate, pH 6.5, 1% sodium dodecyl sulfate (SDS), and 250 µg/ml salmon sperm DNA at 42°C. Blots were washed with 2 × SSC and 0.1% SDS at room temperature and 0.1 × SSC and 0.1 × SDS at 65°C. Blots were exposed to XAR-5 X-ray film (Eastman Kodak) using a Cronex Lightning Plus screen at 70°C. Autoradiographs were quantitated by densitometry within the linear range of signals, and densities in each lane were normalized to ribosomal 18S RNA levels to correct for loading differences. Densities of mRNA bands for the HiPaw and LoPress groups obtained from separate gels were compared by dividing by the mean values obtained for samples of the UnPerf lungs developed on the same gels.

**Capillary Filtration Coefficients**

Measurement of \( K_c \) in rat lungs has been previously described (15). Briefly, an isogravimetric state is obtained, then the venous reservoir is raised to obtain a Ppv of 10–15 cmH\(_2\)O and maintained for 20 min to obtain the rate of weight gain (ΔWt) over the last 2 min. The pulmonary capillary pressure (Ppc) is measured before and after the Ppv increase using the venous occlusion method and the difference (ΔPpc)
obtained. \( K_{fc} \) is calculated as follows

\[
K_{fc} = \frac{\Delta Wt}{\Delta Ppc}
\]

where \( K_{fc} \) is expressed as ml·min\(^{-1}\)·cmH\(_2\)O\(^{-1}\)·100 g predicted LW\(^{-1}\).

**Calculation of Wall Stresses**

The stresses produced at the high Paw and HiPv in the walls of capillaries and arteries were calculated as described by West and Mathieu-Costello (46). Wall stress in a vessel \( S_w \) is dependent on the radius of curvature \( (R) \), transmural pressure \( (P) \), and wall thickness \( (Th) \) as follows

\[
S_w = P \cdot \frac{R}{Th}
\]

Previous morphometric studies were used to obtain the diameters and wall thicknesses for capillaries (46) and small to large arteries (51) in rat lung and wall stresses calculated for the minimal and maximal pressure states. Transmural pressure for vessels was assumed to equal vascular pressure minus alveolar pressure for capillaries and vascular pressure minus perivascular pressure for larger vessels. This perivascular pressure was assumed to equal 2.3 cmH\(_2\)O at the highest PIP (35 cmH\(_2\)O) and 0 cmH\(_2\)O at lower Paw (36). The longitudinal vascular pressure gradient was assumed to be approximately linear under zone III conditions (36). Wall stresses could then be used to evaluate which structures received the greatest stress under the different pressure protocols and which structures would most likely be stimulated to induce gene expression (11).

**Statistics**

Values are means ± SE unless otherwise stated. The mRNA values were compared between groups using an analysis of variance with repeated measures and a Newman-Keuls post test. A logarithmic transform of density data before the analysis of variance was used because of a large range of densities in some groups. CRUNCH4 software and a Gateway 2000 digital computer were used for the analysis, with significance determined at \( P < 0.05 \).

**RESULTS**

**Hemodynamics**

Figure 1 shows polygraph tracings from isolated rat lung experiments using cyclical increases in airway and vascular pressures in the HiPaw and HiPv groups, respectively. Ppv, Ppa, and Paw are shown as a function of time.

Vascular and airway pressure values for the five perfused lung groups are summarized in Table 1. Minimal vascular pressures did not differ among the HiPv, HiPvTh, and LoPress groups, but mean Ppa was significantly higher in the HiPaw than in the LoPress group. Peak Ppv was significantly higher by 3.6 cmH\(_2\)O in the HiPvTh than in the HiPv group. PIP were not significantly different between HiPaw and HiPvTh groups, but pressures in both groups exceeded those in the HiPv, HiPvTh, and LoPress groups. The higher PIP and Ppa values in the HiPv and HiPaw groups than in the LoPress group indicate the interaction of vascular and airway pressures in these groups. Vascular pressures were not recorded in the rats of the UnPerf control group. Mean perfusate hematocrits for the five perfused groups ranged from 10.0 ± 0.6 to 9.2 ± 0.6%.

**Capillary Filtration Coefficients and Lung Weight Gain**

The \( K_{fc} \) values at baseline and high-pressure states for the HiPvTh and HiPawTh groups are shown in Fig. 2. \( K_{fc} \) was increased significantly to 4.5-fold baseline in the HiPvTh group after 12 min of a constant Ppv of 31.8 ± 0.6 cmH\(_2\)O and to 4.3-fold baseline in the HiPawTh group ventilated with 35.0 ± 0.6 cmH\(_2\)O PIP and 7.7 ± 1.1 cmH\(_2\)O PEEP for 30 min. There were no statistically significant differences between groups. Although the Ppv increase was sustained in the HiPvTh group compared with the cyclical increases in the HiPv group and the high PIP exposure time in the HiPawTh group was only 30 min rather than 4 h in the HiPaw group, the increases in \( K_{fc} \) in the two injury threshold groups (HiPvTh and HiPawTh) indicate that these peak pressures were sufficient to induce increased microvascular fluid conductances. However, the \( K_{fc} \) values do not represent the cumulative permeability changes in the HiPv and HiPaw groups.

The terminal lung weights for the UnPerf, LoPress, HiPv, and HiPaw groups were 0.96 ± 0.03, 1.07 ± 0.04, 1.46 ± 0.06, and 1.92 ± 0.05 g, respectively, and all groups were statistically different from each other, such that UnPerf < LoPress < HiPv < HiPaw. As a percentage of their predicted LW, the respective final LW of the LoPress, HiPv, and HiPaw groups increased by 7.0 ±
2.9, 42.9 ± 5.6, and 85.2 ± 4.6%. Terminal LW were not obtained for the HiPvTh and HiPawTh groups.

**Northern Blot Analysis and mRNA Levels**

The probe densities on the gels used for Northern analysis comparing lung samples from the UnPerf, LoPress, and HiPv groups are shown in Fig. 3. The increased amounts of radioactivity are clearly seen for procollagen chains \( \alpha_1(I) \) and \( \alpha_1(III) \), fibronectin, and laminin B. All mRNA values were normalized to the respective 18S rRNA values for that sample to correct for differences in gel loading.

Figure 4 shows the probe densities for gels comparing lung samples from the UnPerf group with those from the LoPress and HiPaw groups. The increased amounts of radioactivity are clearly seen for \( \alpha_1(I) \) and \( \alpha_2(IV) \) procollagen chains and fibronectin and to a lesser extent for laminin B and \( \alpha_1(III) \) procollagen. The LoPress and HiPaw were compared with the same UnPerf samples on separate gels but were not compared on the same gel. The HiPaw mRNA values were normalized to 18S rRNA and also to average UnPerf values.

**Table 1. Hemodynamic and ventilation parameters for isolated perfused lung groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PEEP, cmH(_2)O</th>
<th>PIP, cmH(_2)O</th>
<th>Minimal Ppa, cmH(_2)O</th>
<th>Ppv, cmH(_2)O</th>
<th>Peak Ppa, cmH(_2)O</th>
<th>Ppv, cmH(_2)O</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoPress</td>
<td>6</td>
<td>3.5 ± 0.3</td>
<td>9.3 ± 0.2</td>
<td>9.6 ± 0.4</td>
<td>4.2 ± 0.3</td>
<td>29.7 ± 1.5*</td>
<td>27.7 ± 1.3*</td>
</tr>
<tr>
<td>HiPv</td>
<td>6</td>
<td>3.3 ± 0.2</td>
<td>11.8 ± 1.0*</td>
<td>9.2 ± 0.3</td>
<td>5.3 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiPaw</td>
<td>6</td>
<td>11.4 ± 0.7*</td>
<td>35.8 ± 0.9†</td>
<td>18.0 ± 1.6†</td>
<td>4.2 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiPvTh</td>
<td>6</td>
<td>2.7 ± 0.1</td>
<td>11.0 ± 0.9*</td>
<td>8.8 ± 0.6</td>
<td>3.7 ± 0.1</td>
<td>36.2 ± 1.9†</td>
<td>31.3 ± 0.8†</td>
</tr>
<tr>
<td>HiPawTh</td>
<td>6</td>
<td>8.7 ± 0.6*</td>
<td>35.0 ± 0.5†</td>
<td>13.7 ± 1.3</td>
<td>3.7 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. LoPress, low-pressure perfusion; HiPv, high vascular pressure; HiPaw, high airway pressure; HiPvTh, high vascular pressure threshold; HiPawTh, high airway pressure threshold; Ppa, pulmonary arterial pressure; Ppv, pulmonary venous pressure; PEEP, positive end-expiratory pressure; PIP, peak inspiratory pressure. *P < 0.05 vs. LoPress group, †P < 0.05 vs. LoPress and HiPv groups.

Fig. 2. Capillary filtration coefficients (\( K_{fc} \)) measured at baseline and after increased pressures in high vascular pressure threshold (HiPvTh) and high airway pressure threshold (HiPawTh) groups. *P < 0.05 vs. baseline \( K_{fc} \).

Fig. 3. Northern blot analysis gels comparing lung samples from unperfused control (UnPerf), low-pressure perfusion (LoPress), and high vascular pressure (HiPv) groups. Radiograph of formaldehyde-agarose gel probed for mRNA for \( \alpha_1(I) \), \( \alpha_2(IV) \), and \( \alpha_1(III) \) procollagen, fibronectin, laminin B, and transforming growth factor-\( \beta_1 \) (TGF-\( \beta_1 \)), as well as ribosomal 18S rRNA, is shown.
The mRNA-to-18S rRNA ratios derived from the gels in Fig. 3 for the matrix proteins in the UnPerf, LoPress, and HiPv groups are shown in Fig. 5. The mRNA after 4 h of cyclical high vascular pressures (HiPv group) were significantly higher for type I and type III procollagen, fibronectin, laminin B, and TGF-β1 than the same mRNA in the UnPerf group and higher for type I and type III procollagen and laminin B than in either the LoPress or UnPerf group. Only fibronectin was significantly increased (3.9-fold) in the LoPress lungs above that in Unperf controls. The 91% increase in TGF-β1 mRNA in the LoPress group approached but did not attain significance using the Newman-Keuls post test. Relative to UnPerf controls, the HiPv group exhibited mRNA increases of 9.3-fold for type I and 5.8-fold for type III procollagen, 8.1-fold for fibronectin, and 8.6-fold for laminin B chain. There was a trend toward an increase in mRNA for type IV collagen (141% of that in unperfused lungs) that was not statistically significant.

Matrix protein mRNA-to-18S rRNA ratios derived from the gels in Fig. 4 for HiPaw and Unperf lung groups developed on the same gel are shown in Fig. 6. The density ratios for all mRNA values were significantly increased in the HiPaw group compared with the UnPerf group. Density units for type IV collagen were divided by 50 to fit the graph scale.

Figure 7 shows the mRNA for the HiPv, HiPaw, and LoPress groups divided by the mean mRNA values for the same unperfused control lungs (UnPerf) analyzed on each gel. mRNA was significantly higher in the HiPv group for type I (4.3-fold) and type III (3.8-fold) procollagen and laminin B chain (4.8-fold) and in the HiPaw group for type I (2.4-fold) and type IV (4.5-fold) procollagen.
gen and laminin B chain (2.3-fold) than in the LoPress group. mRNA for type III procollagen and laminin B chain was significantly higher for the HiPv group than for the HiPaw group, and type IV procollagen mRNA was higher in the HiPaw group than in the HiPv group. Fibronectin mRNA was four- to sixfold higher in all groups than in UnPerf lungs, but there were no differences between groups.

DISCUSSION

The most significant observations of the present study were that airway (HiPaw) and vascular (HiPv) distensions produce different patterns of matrix protein mRNA expression and that mRNA increases occurred in \(<4\) h. The pattern of differences may reflect differences in the regional distribution of mechanical stresses or differences in the degree of injury. Estimates of wall stresses calculated from rat lung morphometric data indicate that the peak vascular wall stresses produced by the HiPv protocol were similar in the capillaries and medium and larger arteries (Table 2). The peak circumferential wall stresses produced in alveolar walls are more difficult to estimate but are probably on the same order as those in the vascular walls at peak venous pressures. At lower PIP the alveolar wall stress is borne by the surface tension of the air-liquid interface with the alveolar walls folded in the corners (16). At total lung capacity, Gil et al. (16) found that only 42% of the lung recoil pressure was due to tissue forces, but approximately one-half of the tissue component can be attributed to the pleura at high lung volumes (17). Although the transpulmonary pressure is transmitted to all levels in a homogeneously expanding lung (25), the effective pressure on parenchymal tissue would be only \(\sim21-25\)% of the transpulmonary pressure. A reduced load on the alveolar tissues could partially compensate for the larger alveolar diameter and maintain alveolar wall stresses below levels that produce injury.

Fu et al. (14) counted the number of endothelial and epithelial breaks induced by high vascular pressures in rabbit lungs at high and low inflation pressures. They observed that increases in capillary transmural pressure from 12.5 to 32.5 cmH\(_2\)O at a transpulmonary pressure of 5 cmH\(_2\)O increased the number of transendothelial breaks by 10-fold. Likewise, an increase in transpulmonary pressure from 5 to 20 cmH\(_2\)O also increased the number of breaks by \(\sim10\)-fold. \(K_c\) in the HiPawTh group was 4.3-fold baseline after 30 min of 35.0 cmH\(_2\)O PIP with 7.7 cmH\(_2\)O PEEP and 4.5-fold baseline after 12 min of a constant increase in venous pressure averaging 31.8 cmH\(_2\)O in the HiPvTh group. Thus the alveolar septa and capillary walls in the present study should have obtained roughly equivalent degrees of stress, and the peak venous and airway pressures were sufficient to cause some degree of injury.

Previous investigators have reported an increased collagen mRNA expression and deposition at high pulmonary arterial pressures and high lung volumes, but these changes generally occurred over a longer time course than reported in the present study (30, 31). Our studies differ from models of sustained pulmonary hypertension, in that cyclical increases in venous pressure rather than sustained increases in arterial pressures were used. We found increases in mRNA for type I (4.3-fold) and type III (3.8-fold) procollagens and laminin B chain (4.8-fold) in the HiPv group relative to the LoPress group. Our observations support those of Tozzi et al. (39) of a 34% increase in incorporation of [\(^{14}\)C]proline into collagen in isolated central pulmonary arteries of rats exposed to a stress equivalent to 50 mmHg pressure for 4 h, indicative of increased collagen synthesis. In addition, mRNA levels for \(\alpha_1(I)\) procollagen in these vessel segments were increased by 53% and were endothelium dependent. Tozzi et al. (40) recently re-

Table 2. Estimated vascular wall stresses at minimal and maximal pressures

<table>
<thead>
<tr>
<th>Wall Stress, N/m(^2) (\times 10^3)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capillary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoPress</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>HiPv</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>HiPaw</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>32-(\mu)m-Diameter artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoPress</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HiPv</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>HiPaw</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>73-(\mu)m-Diameter artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoPress</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>HiPv</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>HiPaw</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>181-(\mu)m-Diameter artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoPress</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>HiPv</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>HiPaw</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>
ported an increased mitogen-activated protein kinase (MAPK) activity after only 30 min in rat pulmonary arteries stretched to simulate increased vascular pressure. MAPK activity then returned to control values at 4 h. This time course of MAPK activation is compatible with the mRNA increases observed in the present study after 4 h of cyclical pressure increases. In a rat coarctation model, Lipke et al. (22) also reported sustained increases in mRNA message and protein for laminin and fibronectin in the hypertensive aorta within 5 days. The mRNA for type IV collagen and perlecan increased without an accompanying increase in protein. In other rat lung studies, bleomycin-induced pulmonary hypertension also increased mRNA for \( \alpha_1(1) \) and \( \alpha_2(1) \) procollagen (54).

Hypertrophic lung growth and connective tissue remodeling in response to prolonged distension of the remaining lung has also been observed in response to unilateral pneumonectomy (31). When Zhang et al. (55) exposed young ferrets to 6 cmH2O PEEP for 2 wk, the remaining lung has also been observed in response to prolonged distension of the remaining lung has also been observed in response to unilateral pneumonectomy (31). When Zhang et al. (55) exposed young ferrets to 6 cmH2O PEEP for 2 wk, the lungs increased in weight and volume, but normalized pressure-volume curves using both air and saline were normal. This suggests that the chronic distension increased lung mass with a relatively normal architecture. In the present study we observed significant increases in mRNA in the HiPaw group for type I (2.4-fold) and type IV (4.5-fold) procollagen and laminin B chain (2.3-fold) compared with the LoPress group in only 4 h, suggesting a relatively rapid transduction mechanism. Whereas the signal transduction pathway cannot be determined from these data, it may involve cyclic nucleotides and activation of the MAPK cascade (10, 26, 33).

Whereas specific cell types were not identified in these studies, the different patterns of mRNA expression may relate to the cell types subjected to mechanical stress. Alveolar septal distortion could affect fibroblasts, myofibroblasts, pericytes, epithelial cells, and endothelial cells of the capillary sheet; whereas vascular distortion may cause a greater distortion of the smooth muscle of larger vessels as well as capillary endothelium. Studies of cultured vascular smooth muscle cells stimulated by cyclical stretching indicate an increased proliferation rate and increased production of type I and type III collagen, elastin, hyaluronan, and chondroitin sulfate (23, 50). In contrast, the same cyclical stretch decreased collagen production by endothelial cells (35), leading to postulation of a soluble, stress-related signaling factor from endothelium to other lung cells (31). Cultured fibroblasts also increase proliferation and production of collagen, fibronectin, and integrins under cyclical stretch (5). In addition, alveolar macrophages activated by injury may contribute to collagen production by secretion of inflammatory cytokines (41), and cultured alveolar epithelial cells increase their production of type I, type III, and type IV collagen under the influence of TGF-\( \beta_1 \) (12). Thus many cell types in the lung parenchyma may contribute to the increases in mRNA for collagens and other matrix proteins observed in the present study. Whether the mRNA expression is related to injury repair or remodeling for stress adaptation is unknown.

An increased deposition of collagen would be expected to confer increased tensile strength to the lung and result in vessels that are less easily ruptured by mechanical stress (44, 48). Type I collagen forms strong support fibers that weave through the alveolar septa, spiral through alveolar ducts, and supply strength to perivascular and peribronchial sheaths (36). Type III collagen is a fibrillar collagen of the interstitial matrix, so increases in type I and type III collagen would increase support of the thick side interstitium of capillaries and the surrounding adventitia of small and large vessels. Increases in type IV collagen and laminin are the major components of basement membrane, so support of the thin side of the capillary interstitium and newly formed cells in hyperplastic vessels would be increased (36). However, the amount of collagen or matrix proteins actually synthesized or deposited cannot be determined from the present study, and longer-term experiments are required.

Indirect evidence that an increased collagen may protect against microvascular damage due to high airway pressures is provided by studies of Adkins et al. (1), who compared the airway pressure injury thresholds of young and adult rabbit lungs. Adult rabbit lungs, which contain more collagen and had a lower lung compliance, were more resistant to high airway pressure-induced injury than the lungs of young rabbits (1, 27). Increases in capillary wall thickness would lead to an adaptation to higher vascular pressures, because an increased wall thickness returns wall stress toward normal at the higher vascular pressure and removes the stimulus for further hypertrophy (8). A thickened capillary wall would also increase the threshold for capillary rupture at intravascular pressure (46). Recently, Townsley et al. (37) reported a thickening of the alveolar-capillary barrier in dogs with pulmonary hypertension secondary to heart failure induced by 7 wk of cardiac pacing. Cellular layers and basement membrane contributed to thickness of the alveolar-capillary barrier, and the threshold vascular pressure for microvascular injury, as assessed by \( K_{\text{fp}} \), was increased in these lungs.

The observed increase in type IV procollagen mRNA in the HiPaw lungs of the present study differs from the findings of Berg et al. (4). They unilaterally ventilated lungs of intact rabbits using 9 cmH2O PEEP and 25 cmH2O PIP for 4 h. Significant increases were observed in parenchymal mRNA for \( \alpha_1(III) \) and \( \alpha_2(IV) \) procollagen, fibronectin, basic fibroblast growth factor, and TGF-\( \beta_1 \). Interestingly, the mRNA increases were bilateral, despite unilateral lung distension, and the mRNA for \( \alpha_2(1) \) and vascular endothelial growth factor were not significantly increased. The bilateral response suggests that circulating cytokines or growth factors may have an important role in matrix expression. The lack of a significant increase in mRNA for type I collagen chains in the study of Berg et al. may reflect a species difference, a smaller increase in lung distending volume due to volume restriction by chest wall, lower Ppa,
or longer response times in the intact rabbits than in isolated perfused lung preparations. Russo et al. (33) reported that similar increases in adenosine 3',5'-cyclic monophosphosphate and protein kinase activity that were observed at 24 h in the in situ rat lung after unilateral pneumonectomy occurred within only 20 min in isolated perfused rat lungs distended with the same positive pressure.

We were particularly interested in the response of type IV collagen to increased wall stress, because type IV collagen provides most of the tensile strength of the basement membrane, which is the major constituent of the thin side of the capillary wall in lungs (36). Welling et al. (44) showed that renal tubules exhibited the same pressure-volume curves with or without epithelium present and that basement membrane was the primary component responsible for tensile strength of the tubules. The extraordinary tensile strength of basement membrane is attributed to the type IV collagen and laminin, which self-assemble into strong polygonal sheets (53). Heparin, entactin, and fibronectin also contribute to basement membrane stability and cell attachment (53). Fibronectin mRNA was significantly increased in all perfused groups (HiPaw, HiPV, and LoPress) relative to the UnPerf group in the present study. Because fibronectin is a universal adhesive glycoprotein, an increased production may precede any tissue remodeling. However, separation of fibronectin types was not possible with the gel separation methods and mRNA probes used in the present study.

Various growth factors, and particularly TGF-β1, have been shown to promote collagen gene expression and deposition (2, 6). We observed significant increases in mRNA for TGF-β1 in the HiPaw and HiPV groups but not the LoPress group compared with the Unperf group. Although increased production of this cytokine has been linked to stimulation of collagen synthesis, release of preformed TGF-β1 can also occur and may influence collagen mRNA transcription before synthesis of new TGF-β1 (6). TGF-β1 is considered a primary controller of collagen synthesis (6), but other cytokines may be produced under cyclical strain or during injury that affect cell proliferation and matrix remodeling (5, 18, 41, 43, 49). The effect of lung distension on the contralateral undistended lung observed by Berg et al. (4) supports a role for cytokines in the process of matrix remodeling. Thus the observed mRNA expression may represent an adaptation response to stress and/or a repair response to injury. Multiple factors may influence the transduction of the mechanical stimulus, and different cell types may be stimulated to a different degree by the local stresses produced by specific regional pressures and stresses.

The authors appreciate the technical assistance of Sherri Martin. This research was supported by American Heart Association Grant 94013090 and National Institutes of Health Grant RO1-46910.

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Received 16 May 1997; accepted in final form 17 July 1997.


