Remodeling of lung interstitium but not resistance vessels in canine pacing-induced heart failure

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Methods.

Rapid ventricular pacing model. Heart worm-negative, conditioned mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg iv). Under sterile operating conditions, a bipolar pacing electrode (Medtronic) was introduced into the right ventricle via the right jugular vein. A small subcutaneous pocket was created anterior to the first rib for insertion of the pacemaker generator (custom SX-5985 or 8329, Medtronic), with the lead tunneled subcutaneously to the pocket and attached to the generator. After the skin incisions were closed, anesthesia was discontinued. During the recovery phase, the implanted pacemaker was not active. Cefazolin sodium (1 g im) was given at the completion of the surgical procedure. The dogs were maintained on the antibiotic orally (500 mg twice daily) for 5 days postoperatively. After recovery from surgery (1–2 days), the generator was set to deliver 240–245 impulses/min (1, 30, 34, 35) by means of an external programmer (model 9710, Medtronic). Pacing was maintained for ~1 mo or until left ventricular shortening fraction (LVFS), measured via echocardiography in sinus rhythm, had fallen to ~50% of the baseline prepace value.

Isolation of lung lobes. Paced animals (n = 10) and unpaced controls (n = 6) were anesthetized to a surgical plane of anesthesia for the terminal experiment with pentobarbital sodium (~15 mg/kg iv in the paced group vs. 30 mg/kg iv in controls), supplemented with intravenous α-chloralose as needed, then intubated and mechanically ventilated with room air. Subsequently, 10,000 U of heparin were adminis-
tered intravenously. After a left thoracotomy, tissue aliquots (1–2 g) were resected from the left cranial lobe of all six control and seven paced animals for measurement of interstitial matrix composition, as discussed below. These samples were briefly rinsed in cold saline, blotted, weighed, and finally frozen (−35°C) until measurements could be completed. Blood-free extravascular lung water (EVLW), residual blood, and blood-free dry weight were measured using the remain-
der of the left cranial lobe, together with the left middle lobe, as reported previously (35). Right caudal lobes from 5 control dogs and all 10 paced dogs were then isolated for fixation. The lobar bronchus was cannulated, and the lobe was suspended vertically via the cannula above a large container. Buffered 10% Formalin (Sigma Chemical) was immediately instilled into the airway to a pressure of 20 cmH2O, and the lobe was then immersed in additional fixative. The lobe was left in this position for 24 h to ensure complete fixation.

Measurement of interstitial fluid pressure. In separate control (n = 13) and paced (n = 6) animals, 10 min after heparin administration, the left caudal lobe was removed for ex vivo perfusion and measurement of interstitial fluid pres-
sure (Pi). The excised lobes were cannulated, ventilated with 30% O2 and 5% CO2, and perfused with autologous blood, as previously described (30, 34, 35). Pulmonary arterial and venous (Pv) pressures and lobe weight were con-
tinually monitored with a polygraph (model 7, Grass). P v was set at 4–5 cmH2O, and blood flow was set at a maximal value that kept the lobe isogravimetric, i.e., the lobe neither gained nor lost weight. Pi was measured using the wick-in-needle tech-
nique (7). A 3-mm-long hole was bored into the side of a 23-gauge needle, 0.5 cm from the bevel, then the needle was threaded with filamentous nylon strands to fill the needle bore. Before use, the prepared wick-in-needle was soaked in 0.9% saline overnight. To measure Pi, the needle was inserted into the tissue cuff lateral and parallel to the lobar vein, until ≈1 cm of the needle tip was covered with lung parenchyma. The wick-in-needle was connected to a pressure transducer via polyethylene tubing filled with saline. The experiment was discarded if any leakage or bleeding occurred at the site of the needle insertion or in the cuff where the needle was present. In all cases, phasic cycling of Pi was observed in concert with ventilation-induced swings in airway pressure, as one would expect because of the interdependence of lung parenchyma and vascular dimensions (24). After the lobe had stabilized, baseline measurements of Pi were made. Plasma proteins were then diluted by replacing 150 mL of blood in the venous reservoir with the same volume of saline, and Pi was increased to 20 cmH2O. Together, these changes promoted edema formation (10). After 1 h, final measurements of Pi were completed. At the end of the experiment, blood-free EVLW was measured in each perfused lobe, as previously described (35). The final Pi and EVLW in the perfused lobe were compared with the baseline Pi in the same lobe. Baseline EVLW was taken from that in the left cranial and middle lobes of the same animal.

Tissue matrix analysis. Hyaluronan, uronic acid (as a measure of total uronic acid-containing glycosaminoglycans), and total collagen contents were determined in lung parenchyma. Tissue samples were freeze-dried to constant weight. The difference between wet and dry weight, normalized to the dry weight, yielded a measure of total tissue water (TTW). Hyaluronan and total glycosaminoglycans were measured in tissue extracts after hydrolysis, as previously described (26, 36). Hyaluronan was measured using a specific radioassay (HA Test 50, Pharmacia) that detects hyaluronan in the nanogram range without interference from fibronectin, chon-
droitin sulfate, or keratin sulfate. Total glycosaminoglycans were measured colorimetrically as uronic acid after reactions of tissue hydrolysate with 0.025 M sodium tetraborate in sulfuric acid and 0.125% carbazol. For measures of lung collagen, separate aliquots of freeze-dried tissue were hydro-
lized in 6 N HCl for 16 h at 125°C. The analysis of collagen in the hydrolysate was based on a colorimetric reaction with perchorlic acid and p-dimethylaminobenzaldehyde to detect hydroxyproline, then collagen mass was calculated with the assumption of a content of 0.91 μmol hydroxyproline per 1 mg collagen (26). Total noncollagen proteins were measured in separate aliquots of freeze-dried tissue. 1 mL of 0.2 N NaOH was added to 10 mg of freeze-dried tissue and incubated at 60°C overnight. Protein concentration in the resultant clear solution was measured using the Bradford assay (Bio-Rad). As a negative control, 10 mg of collagen were similarly hydrolyzed, but no protein was detected in the assay. The mass of matrix components in tissue was normalized per gram of dry tissue weight and per milligram of total noncollagen protein in control and paced groups.

Morphometry. Initially, fixed lobes were embedded in gelatin and then sectioned into 1-cm slabs with a random starting orientation. Point counting and the Cavalieri method were used to define the total lung volume as well as the volume fractions for coarse and fine lung parenchyma (22). For this purpose, coarse parenchyma was arbitrarily defined as vessels or airways with an external diameter >2 mm, so that, by subtraction, the remainder of the lung volume was composed of fine parenchyma. Next, the fractionator technique was used to choose four to seven tissue cubes at random for subsequent processing (9, 22). Four to six 5-μm frozen sections were cut from each block and transferred to glass slides for processing. Intervening sections were discarded. Sections were stained with Masson’s trichrome to highlight smooth muscle and collagen fibers and examined using light micros-
copy. Images were captured with an on-line color videocam-
era. Vessels to be analyzed were chosen using systematic random sampling; each section was examined from a rotating start point, and then one arterial and one venous vessel in each size bin (see below) were analyzed until all size bins were filled or until the slide had been completely surveyed. For each vessel examined, the intersection of a grid on the camera monitor and the vessel diameter was marked. Images were captured at ×100 magnification using a ×10 objective lens and a ×10.220.33.5 on April 11, 2017 http://jap.physiology.org/ Downloaded from

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Fig. 1. Schematic illustration of morphometric measurements. First, short-axis diameter was measured. Then, wall thickness (arrow labeled "wall") was measured at right angles to lumen surface at 4 points around vessel wall, marked by intersection of wall with grid crosshairs. Next, length of total wall from intimal to adventitial surface (arrow labeled T) along crosshair and length occupied by media (arrow labeled M), collagen, and fluid cuff were measured. Volume fractions were then calculated by linear integration, since volume fraction is equivalent to length fraction (9, 22). Thickness of media + intima layer was calculated as product of total wall thickness and length fraction for media + intima.

Table 1. Matrix content in canine lung after pacing-induced heart failure

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Pace (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobar blood-free dry wt, g</td>
<td>3.3 ± 0.4</td>
<td>5.8 ± 0.6*</td>
</tr>
<tr>
<td>Lobar residual blood, ml/g dry wt</td>
<td>2.5 ± 0.2</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Total protein, mg/g dry wt</td>
<td>341.2 ± 31.0</td>
<td>455.6 ± 26.9*</td>
</tr>
<tr>
<td>Hyaluronan mg/g dry wt</td>
<td>0.62 ± 0.08</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>µg/mg protein</td>
<td>1.90 ± 0.36</td>
<td>1.45 ± 0.13</td>
</tr>
<tr>
<td>Uronic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/g dry wt</td>
<td>11.1 ± 0.5</td>
<td>11.0 ± 0.6</td>
</tr>
<tr>
<td>µg/mg protein</td>
<td>33.5 ± 3.0</td>
<td>24.5 ± 1.6*</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/g dry wt</td>
<td>163.7 ± 11.4</td>
<td>145.5 ± 11.3</td>
</tr>
<tr>
<td>µg/mg protein</td>
<td>0.50 ± 0.07</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>TTW, g/g dry wt</td>
<td>3.74 ± 0.06</td>
<td>4.61 ± 0.12*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of lobes. TTW, total tissue water. Measure of total protein excluded collagen (see METHODS). *P < 0.05 vs. control.
ing intima), and fluid cuff were remarkably similar in the two groups. Medial (including intima) thickness and the ratio of media (including intima) to the lumen perimeter for each size bin are shown in Fig. 4. Although the media in arteries and veins >200 µm tended to be thicker in the paced group, this was significant only for arteries ≥401 µm. The tendency toward larger ratios of media + intima to lumen perimeter, although not statistically significant, does suggest a larger degree of vascular tone in the paced group, reflective of the increased resistance seen at the whole lobe level (30, 35). Finally, occluded microvessels suggestive of rarefaction were extremely rare and then seen only in sepal corner vessels. No such vessels were observed in the control group, and only two were found in all sections of lung examined from paced animals.

Changes in the P_i vs. EVLW relationship. In contrast to the morphometric analysis, the shift in the relationship between P_i and EVLW (Fig. 5) was suggestive of significant interstitial remodeling. In the paced group the average P_i was normal, i.e., subatmospheric, despite the elevation in baseline EVLW. In fact, in one paced lobe with a baseline EVLW of 5.43 ml/g, P_i was –6.5 cmH_2O. In both groups, when further hydration was induced, P_i increased similarly to near 0 cmH_2O. Although EVLW does reflect cell water in addition to interstitial water, the increment in EVLW with hydrostatic and colloid osmotic pressure-induced hydration should reflect an increment in interstitial fluid volume. Thus one can infer that lung interstitial compliance (i.e., the change in interstitial volume induced by a given increment in interstitial fluid volume, shown by the slope of the P_i vs. EVLW relationship) is similar in both groups.

DISCUSSION

During congestive heart failure, the pulmonary vasculature is chronically exposed to high P_v at rest, with further increases up to 37 mmHg during exercise (13, 31). In an experimental model of heart failure induced by rapid ventricular pacing in dogs, we found similar
pulmonary venous hypertension, accompanied by an almost threefold increase in pulmonary vascular resistance, an 

\[ \frac{50\%}{50\%} \] fall in pulmonary vascular compliance, and significant pulmonary edema compared with controls \(30, 34, 35\). The hemodynamic changes seen in the paced group are not likely due to the pulmonary edema per se, since Michel et al. \(23\) found that acute formation of substantial interstitial edema does not compress pulmonary vessels. This left open the possibility that vascular structure was directly modified. In addition to the alterations in hemodynamics in lobes from paced dogs, we found significant enhancements in the pulmonary vasoconstrictor responses to ANG II \(30\) and norepinephrine \(35\). The enhanced response to norepinephrine was maintained at high \(P_v\). Because pressor responses in normal lung are attenuated when \(P_v\) is acutely elevated \(3, 12\), this observation was also suggestive of structural modification. Our working hypothesis has been that, as a consequence of the chronic venous hypertension and/or the endocrine sequelae to heart failure, the lung vasculature and interstitium are remodeled in an adaptive response.

### Table 2. Demographics of morphometric measurements in the lung microvasculature

<table>
<thead>
<tr>
<th>Size Bin</th>
<th>No. of Vessels</th>
<th>No. of Measurements</th>
<th>Short Axis, µm</th>
<th>Long-to-Short Axis Ratio</th>
<th>Wall Thickness, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artery</td>
<td>Vein</td>
<td>Artery</td>
<td>Vein</td>
<td>Artery</td>
</tr>
<tr>
<td>21–50 µm</td>
<td>Control</td>
<td>34</td>
<td>44</td>
<td>122</td>
<td>148</td>
</tr>
<tr>
<td>Pace</td>
<td>81</td>
<td>103</td>
<td>301</td>
<td>377</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>51–100 µm</td>
<td>Control</td>
<td>21</td>
<td>33</td>
<td>72</td>
<td>112</td>
</tr>
<tr>
<td>Pace</td>
<td>52</td>
<td>74</td>
<td>188</td>
<td>270</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>101–200 µm</td>
<td>Control</td>
<td>17</td>
<td>23</td>
<td>59</td>
<td>81</td>
</tr>
<tr>
<td>Pace</td>
<td>32</td>
<td>49</td>
<td>102</td>
<td>176</td>
<td>147 ± 7</td>
</tr>
<tr>
<td>201–400 µm</td>
<td>Control</td>
<td>16</td>
<td>5</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>Pace</td>
<td>31</td>
<td>29</td>
<td>93</td>
<td>96</td>
<td>259 ± 12</td>
</tr>
<tr>
<td>&gt;401 µm</td>
<td>Control</td>
<td>12</td>
<td>3</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>Pace</td>
<td>13</td>
<td>12</td>
<td>39</td>
<td>37</td>
<td>697 ± 95</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses represent number of arteries and veins for which long axis could be measured in each bin. *\(P < 0.05\) vs. arteries within the same group.

Fig. 3. Volume fractions for media + intima, collagen, and fluid cuff grouped into bins according to lumen short-axis diameter for pulmonary microvessels in control and paced dogs. †\(P < 0.05\) vs. artery in same group.

Fig. 4. Thickness of media + intima layer and ratio of this thickness to lumen perimeter of vessels in each size bin for control and paced groups. *\(P < 0.05\) vs. control for same vessel type; †\(P < 0.05\) vs. artery in same group.
thickening of the media vascular wall collagen volume fraction in any size bin. Acid content increases acutely in the overhydrated evidenced by our previous observation that lung uronic detect changes in tissue matrix content in our hands is that the methods used in the present study can indeed residual blood content is similar in the two groups. Differences are not due to extravasated blood, since the lobar content and blood-free dry weight in the paced group the data were normalized per gram of dry lung weight. No demonstrable changes in lung matrix content when the dogs were paced to failure over 1 mo, there were marked changes in vascular structure was due to the fact that the lung lobes were not fixed at constant pressure. However, because medial thickness tension per se. This notion is supported by the study of LaBourene et al. (16). They reported that 6 wk of pulmonary vein banding resulted in no medial hypertrophy or altered matrix deposition in pulmonary arteries, despite significant pulmonary arterial hypertension and right ventricular hypertrophy. Mild intimal thickening and an increase in fractional collagen content were found in large pulmonary veins, although again no medial hypertrophy was observed. Jones and Reid (14) suggested that the pattern of structural changes in pulmonary hypertension is dependent on the initial target of injury. Because the baseline pulmonary microvascular permeability remains normal in the pacing model of venous hypertension, the lack of any initiating endothelial injury and/or the lack of an attendant inflammatory response may contribute to the apparent protracted time course for remodeling of the resistance vasculature. That finite structural changes induced by rapid ventricular pacing and heart failure are slow to occur in the pulmonary vasculature would help explain the rapid recovery of patients with clinical signs of heart failure secondary to short-term supraventricular tachycardia (2) and in dogs with pacing-induced heart failure (39). The absence of significant medial hypertrophy in small pulmonary resistance microvessels after 1 mo of pacing in the dog suggests that alterations in vascular smooth muscle function at the level of the receptors and/or second-messenger signaling must explain the heightened pulmonary vasoconstrictor responses we observe in paced...
EVLW relationship implies that interstitial compliance of Pt in the septal region where fluid exchange occurs is lost. Consistent with our earlier findings (30, 34, 35), baseline EVLW was significantly elevated in the paced group. In contrast to the predicted effect, Pt in this group remained subatmospheric, with an average value of 1.8 cmH₂O. The similarity between the slopes of the Pt vs. group remained subatmospheric, with an average value of 1.8 cmH₂O, were similar to those reported here (36), when we observed a significant increase in interstitial fibrosis, collagen deposition, and an increment in pulmonary vascular pressure, such as those imposed by exercise (13, 31).

In conclusion, we can find no evidence for vascular structural remodeling in <400-µm pulmonary arteries or veins after 1 mo of pacing-induced heart failure. In contrast, interstitial remodeling does occur, conferring a measure of protection against further edema formation in these hypertensive lungs. This latter adaptation thus adds to the protection resulting from the increased resistance of the capillary endothelial barrier to injury in this model (30, 34). Together, these findings suggest that early adaptations to chronic pulmonary venous hypertension are primarily directed at limiting fluid accumulation in the lung parenchyma. These are important adaptations, allowing a measure of protection against further increments in lung water.

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