Altered reactivity of pulmonary vessels in postobstructive pulmonary vasculopathy

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Chronic ligation of one pulmonary artery causes structural remodeling of the pulmonary vascular bed and angiogenesis of the bronchial vessels (14, 29, 32). These characteristic alterations are termed postobstructive pulmonary vasculopathy (POPV). Clinically, POPV resembles large pulmonary arterial embolism, some forms of pulmonary hypertension, and conditions with elevated bronchial blood flow (14). The morphological changes of the pulmonary vascular bed in POPV consist of medial thickening, a reduction in the diameter of pulmonary arteries, muscularization of nonmuscular arteries, patchy intimal thickening, and an increased density of myoendothelial junctions (14, 16). These alterations could profoundly influence the reactivity of pulmonary vessels to vasoactive stimuli. Indeed, in both canine and rodent models of POPV, we found a markedly augmented responsiveness of pulmonary arteries to serotonin (5-HT) and endothelin and of veins to histamine (15, 24).

The mechanisms for the exaggerated responsiveness of pulmonary vessels in POPV to substances such as histamine and 5-HT are unclear. Putative explanations include structural alterations such as a decreased baseline diameter and increased medial thickness (16), endothelial dysfunction (8, 10), and alterations in the smooth muscle proper (4). In the canine model of POPV, the hyperreactivity of the pulmonary vessels, assessed by a disproportionate increase in segmental pulmonary vascular resistance (15), could be caused by their narrower internal diameter and thicker media without necessarily enhanced smooth muscle contractility (17). Thus an in vitro system, in which we could measure smooth muscle contraction directly rather than indirectly with a parameter such as vascular resistance, is preferable to determine whether smooth muscle constriction is truly enhanced in POPV. Recently, we successfully adapted a lung explant technique, used to study airway constriction in the rat (5), to pulmonary vessels of guinea pigs and rats (24, 25). In this preparation, vascular smooth muscle contraction or relaxation can be directly measured; moreover, the explants can be fixed and morphometric measurements made on the same vessels studied with the pharmacological agents.

Endothelial cells modulate the responses of the underlying smooth muscle to vasoactive agonists by releasing endothelium-derived relaxing and contracting factors (6). Nitric oxide (NO) is a major endothelium-derived relaxing factor, and ACh, histamine, bradykinin, and several other agents stimulate the endothelium to release NO (6, 21). Recent in vitro studies with isolated arteries have demonstrated that endothelium-dependent NO-mediated relaxation is reduced in several vascular diseases, leading to exaggerated contractile responses to vasoactive substances (8, 10). Thus, in POPV, a similar mechanism could account for the hyperreactivity to histamine and 5-HT because, in addition to directly constricting vascular smooth muscle, these agents stimulate the endothelium to release NO and thereby reduce the constriction (6).

Thus the principal aims of the present study were to 1) directly compare the contractile responses of individual intrapulmonary arteries and veins to histamine and to 5-HT in POPV lungs with those in contralateral control lungs by using the lung explant technique; and 2) determine whether structural alterations and/or
impaired endothelial NO-mediated relaxation contributed to alterations in the contractile responses.

METHODS

Surgical Procedure for Pulmonary Artery Ligation

Animals were used according to a protocol approved by the McGill University Animal Care Committee. The procedure previously described for dogs was adapted to guinea pigs (15). Briefly, 24 male Hartley guinea pigs (Charles River, St. Constant, PQ) weighing 556 ± 30 g were anesthetized with pentobarbital sodium (35 mg/kg ip) and placed in the prone position for intubation. A 14-gauge polyethylene tubing with a needle stylet angled at −15° was passed through the mouth into the trachea, and animals were ventilated with 30% O2–70% N2 at 60 breaths/min with a tidal volume of 5–7 ml by means of a Harvard Apparatus rodent ventilator (model 680, Millis, MA).

For the ligation, the animals were placed in the supine position, and, by using sterile technique, a left thoracotomy was performed via the third or fourth intercostal space. The left pulmonary artery was ligated with a 4-0 silk suture ~2 mm beyond its bifurcation from the main pulmonary artery. The chest was closed, and the lung was reexpanded with negative suction and positive-pressure ventilation. Postoperatively and daily for 3 days, 5 mg/kg trimethoprim and 25 mg/kg sodium sulfadiazine were injected subcutaneously.

Preparation of the Lung Explants

The procedure was performed as previously described (24, 25). Briefly, the animals were anesthetized with pentobarbital sodium (40 mg/kg ip), heparinized through the dorsal vein of the penis (3,000 U/kg), and intubated through a tracheotomy with sterile polyethylene tubing 1.9 mm in diameter. The abdomen was opened, and animals were exsanguinated by cutting the abdominal aorta. After removal of the anterior chest wall, the pulmonary vessels were washed in situ with 10 ml Ringer lactate containing 20 U/ml heparin through a catheter inserted into the main pulmonary artery for the right control lungs or into the left atrium for the left ligated lungs. The heart and lungs were excised en bloc, and the lungs were inflated to near total lung capacity with 1% agarose in bicarbonate-buffered culture medium (BCM) at 37°C. The preparation was left to cool for 20 min at 4°C. Then the lungs were separated from the heart, and in some animals (those used for the study of the dilator responses; see Vasodilator Responses to ACh and SNP), the volumes of the left and right lungs were measured by fluid displacement (Ringer lactate) in a sterile 50-ml syringe, before being embedded in 4% agarose in bicarbonate-buffered minimum essential medium. After 30 min at 4°C, the lung-agarose block was sectioned with a hand-held microtome blade into 0.5- to 1.0-mm-thick transverse slices. These were examined with an inverted microscope (model 1MT-2, Olympus, Tokyo, Japan), and those that contained at least one cross section of a vessel were placed in a 30-mm culture well insert within a six-well plate containing 2 ml of BCM and incubated overnight at 37°C in 5% CO2–95% air.

Image Acquisition

The culture dish inserts containing the lung explants were transferred to six-well plates containing 2 ml of HEPES-buffered culture medium (HCM) (5) and placed on the stage of an inverted microscope (model LH50A, Olympus). Arteries and veins were identified and imaged with a video camera (model CDS, Sony, Nagano, Japan), and images were recorded with a videodisc recorder (model TQ2026F, Panasonic, Osaka, Japan). To distinguish arteries from veins, we used the following criteria: 1) the arteries usually accompanied airways, whereas veins were at a distance from them, and 2) arterial walls had a thick media and their inner lining was slightly wrinkled, whereas veins were thinner and wrinkles were inconspicuous. The identities of the vessels were confirmed by histological examination (see Histology and Morphometry).

Experimental Protocol

Vasoconstrictor responses to histamine and 5-HT. In a first set of experiments, we tested vasoconstrictor responses to histamine and 5-HT in 10 guinea pigs, 3 mo after ligation. After baseline images of the vessels were generated, 10⁻¹¹ M histamine or 5-HT was added to the vessels. Twenty seconds later (which corresponded to the time at which the peak contractile response occurred), images of the vessels were taken. This procedure was repeated with increasing concentrations up to 10⁻³ M for histamine and 10⁻⁴ M for 5-HT.

Vasoconstrictor responses to ACh and SNP. In the second set of experiments, we tested vasodilator responses to ACh and SNP in two groups of guinea pigs; group 1 consisted of six animals ligated for 1 mo, and group 2 consisted of eight animals ligated for 10 mo. The rationale for using these two time points was that after prolonged increased vasoactivity to histamine and 5-HT, we also wanted to examine the effect of the duration of ligation on the vasodilatory responses; thus we chose a duration shorter than the 3 mo and a much longer time, i.e., 10 mo. All vessels were first precontracted with 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F₂α (U-46619) at 3 × 10⁻⁶ M in group 1 and at 10⁻⁵ M in group 2; preliminary experiments showed that at these concentrations, U-46619 produced contraction ~80% of maximum and that the contraction was stable for at least 20 min. Cumulative concentration responses to ACh or SNP were generated in one-log-unit intervals from 10⁻¹¹ to 10⁻⁴ M by using the same procedure as for histamine and 5-HT as described in Vasodilator Responses to histamine and 5-HT.

In each animal, we usually used 12 explants from each lung and in each explant observed one artery and/or one vein, and in a few instances two veins; each vessel was only studied once. In the experiments of the constrictor responses to histamine and 5-HT, we studied 62 arteries and 66 veins from 82 explants of the control lungs and 36 arteries and 64 veins from 68 explants of the lungs with POPV; in group 1 of the experiments of the dilator responses, we studied 32 arteries and 41 veins from 43 explants of the control lungs and 34 arteries and 32 veins from 47 explants of the POPV lungs; and in group 2 we studied, 38 arteries and 63 veins from 67 explants of the control lungs and 36 arteries and 64 veins from 68 explants of the POPV lungs.

Image and Data Analysis

The stored images were digitized by using a 80386 Intel-based microcomputer equipped with a frame-grabber board (PIP1024B, Matrox, Montreal, PQ). The digitized images were then transferred to a scientific workstation (model RS6000, IBM, Armonk, NY), and measurements of luminal area were made with Galileo image processing software (Inspiraplex, Montreal, PQ). The contractile responses of arteries or veins to histamine, 5-HT, and U-46619 were expressed as percent change in luminal area over baseline, and the responses to ACh and SNP were expressed as percent reversal of vessel precontraction induced by U-46619, as carried out in previous studies from our own or others’ laboratories (3, 5, 24–27, 30, 33).
Arteries and veins in the explants were readily identifiable by the criteria described in Image Acquisition. Representative video and corresponding light microscopic images of arteries from right and left lungs are in shown in Fig. 1, A–F. Light microscopy revealed that the pulmonary arteries were muscular in type and had a thick and complete inner elastic lamina, a media composed of compact smooth muscle cells, and a thin attenuated external elastic lamina often seen only with Van Gieson’s elastic stain. The veins were also muscular in type, but their media, relative to arteries, was thinner, and their internal elastic lamina was also much thinner or frequently absent. The architecture and morphology of the control right lungs were normal with few bronchial vessels around the larger pulmonary vessels and airways and with inconspicuous lymphatic vessels. In contrast, the left lungs with the ligated pulmonary artery showed a marked increase in bronchial blood vessels and lymphatics in the adventitia of pulmonary arteries and veins and in the walls of airways. The parenchyma was normal in the right lungs but focally fibrotic in the left lungs. The results of the morphometric measurements are given in Table 1. The internal diameters were smaller in both arteries and veins of lungs with POPV compared with controls (P < 0.05). The external diameters were also reduced in the POPV lungs compared with the controls (P < 0.05). There was no significant effect of either POPV or the duration of ligation on medial muscle thickness of arteries and veins (P > 0.05).

Vasoconstrictor responses to histamine and 5-HT. At 3 mo after ligation of the pulmonary artery, histamine produced concentration-dependent constriction of pulmonary arteries and veins in both control and POPV lungs (Fig. 2). The constriction of the veins to histamine was significantly enhanced in POPV. In addition, the maximal responses of pulmonary veins were significantly greater in POPV than in the controls (P < 0.05; Table 2), although the pD2 values did not differ significantly from those of the controls. In contrast to the veins, with histamine, the arteries had significantly reduced pD2 values in POPV (Table 2), and, at concentrations of 10^-8 and 10^-7 M (Fig. 2), histamine produced significantly less contraction of arteries in POPV than of control arteries (P < 0.05); their maximal responses, however, were similar to those of controls.

Like histamine, 5-HT produced a concentration-dependent contraction of arteries and veins (Fig. 3). After reaching a peak, the contraction of the arteries in the control lungs waned in a concentration-dependent fashion; in distinction, the contractile responses remained stable in the lungs with POPV. In contrast to histamine, the maximal responses of the arteries in POPV to 5-HT were significantly greater than those of control arteries (Table 2), although the pD2 values in the control and POPV lungs did not differ. The effect of 5-HT on veins was not modified by POPV.

### Results

#### Effects of Pulmonary Artery Ligation

Between the left pulmonary artery ligation and the final experiment, the guinea pigs gained weight in proportion to the duration of ligation: 156 ± 41 g after 1 mo ligation, 380 ± 34 g after 3 mo ligation, and 536 ± 21 g after 10 mo ligation. The volumes of the left POPV lungs (10.8 ± 0.3 ml at 1 mo and 10.8 ± 0.8 ml at 10 mo) were significantly lower (P < 0.05) than those of the right control lungs (21.3 ± 0.7 and 28.9 ± 1.8 ml at 1 and 10 mo, respectively).

#### Statistical Analysis

Values are means ± SE, with n indicating the number of animals from which the vessels were obtained; this was the same n used for all the analyses, and the contralateral right lungs were the controls. Statistical analyses were performed by using proprietary software (Systat, Evanston, IL). For the comparisons of concentration-response curves between POPV and control lungs, two-way ANOVA was used. When the F value was significant (P < 0.05), the Tukey’s test or Student’s paired t-test was used to examine differences at each concentration. For comparisons of maximal responses, pD2 values, we used Student’s paired or unpaired t-test. Differences were considered statistically significant at P < 0.05.

### RESULTS

#### Effects of Pulmonary Artery Ligation

From these responses, concentration-response curves of arteries and veins were constructed by plotting the mean values against concentrations. The potency and maximum responses were determined for each individual vessel, and potency was expressed as the negative log of the molar concentrations of EC50 (pD2).

#### Histology and Morphometry

At the end of each experiment, the explants were fixed by immersion in 10% buffered formalin, processed by using standard histological technique, and embedded in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin and, in selected instances, with Van Gieson’s elastic stain. The arteries and veins that were used for pharmacological study were identified on the basis of maps drawn at the time of image acquisition. Morphometric measurements were then made on those vessels that had an intact wall, by using previously described methods (13, 14), with an ocular micrometer on a optical microscope (Leitz, Wetzlar, Germany). We measured the inner diameter at a magnification of ×100 or ×250 and medial smooth muscle thickness at a magnification of ×250 or ×400 at the same position; the sum of inner diameter and 2× medial smooth muscle thickness was the external diameter. We made measurements on 21 arteries and 23 veins from the control lungs and on 19 arteries and 17 veins from POPV lungs ligated for 1 mo, 46 arteries and 36 veins from the control lungs, and 34 arteries and 46 veins from the POPV lungs ligated for 3 mo, and 27 arteries and 36 veins from the control lungs and 29 veins from the control lungs ligated for 10 mo.

#### Drugs

All drugs were purchased from Sigma Chemical (St. Louis, MO). Histamine (dihydrochloride), 5-HT (hydrochloride), ACh (chloride), and SNP were prepared as stock solutions in HCM, from which working dilutions were prepared fresh daily. For U-46619 the stock solution was used directly.

#### RESULTS

### Vasoconstrictor Responses to ACh and SNP

The maximal response values for precontraction (combined data for ACh and SNP) obtained with U-46619...
before the relaxants were applied were, at 1 mo,
31.59 \pm 3.83\% for control arteries, 46.83 \pm 2.31\% for
control veins, 34.76 \pm 2.59\% for POPV arteries, and
52.46 \pm 4.3\% for POPV veins. The values at 10 mo were
27.65 \pm 2.85\% for control arteries, 46.78 \pm 2.71\% for
control veins, 29.47 \pm 2.22\% for POPV arteries, and
48.99 \pm 2.76\% for POPV veins. Analysis by time period
and vessel type showed no significant differences be-
tween control and POPV groups (P > 0.05).

After precontraction with U-46619, ACh produced
concentration-dependent relaxation of pulmonary arter-
ies; the relaxant effect of ACh in POPV lungs did not
differ from controls at 1 mo (Fig. 4) but was signifi-
cantly enhanced at 10 mo after ligation (Fig. 5). ACh
did not relax veins of either control or POPV lungs;
indeed, in both, it caused contraction. Unlike ACh, SNP
induced a concentration-dependent relaxation of both
arteries and veins; the relaxant effect on veins in POPV
lungs was greater than in controls at 1 mo (P < 0.05,
Fig. 6) but not at 10 mo after ligation (Fig. 7). The pD2
values of arteries and veins for ACh and SNP, however,
were not significantly altered by POPV (Table 2).

**DISCUSSION**

The data in this study reveal three principal effects of
POPV. First, there was an increased constriction of
pulmonary arteries to 5-HT and of veins to histamine, a
Table 1. Effects of POPV on morphometric measurements of pulmonary arteries and veins in guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>Arteries, µm</th>
<th></th>
<th>Veins, µm</th>
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<tbody>
<tr>
<td></td>
<td>VID ID MT ED</td>
<td>VID ID MT ED</td>
<td></td>
<td></td>
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<tr>
<td>1 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>500 ± 54</td>
<td>419 ± 45</td>
<td>53 ± 3</td>
<td>524 ± 49</td>
</tr>
<tr>
<td>POPV</td>
<td>404 ± 44*</td>
<td>302 ± 19*</td>
<td>56 ± 5</td>
<td>414 ± 19*</td>
</tr>
<tr>
<td>3 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>585 ± 37</td>
<td>571 ± 40</td>
<td>48 ± 2</td>
<td>666 ± 40</td>
</tr>
<tr>
<td>POPV</td>
<td>366 ± 33*</td>
<td>343 ± 32*</td>
<td>51 ± 3</td>
<td>444 ± 32*</td>
</tr>
<tr>
<td>10 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>581 ± 31</td>
<td>453 ± 38</td>
<td>47 ± 3</td>
<td>546 ± 38</td>
</tr>
<tr>
<td>POPV</td>
<td>375 ± 28*</td>
<td>301 ± 36*</td>
<td>56 ± 5</td>
<td>414 ± 33*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6–10 animals. 1, 3, and 10 mo refer to duration of pulmonary artery ligation; contralateral lungs were the controls. **P < 0.05 vs. controls.

Table 2. Effects of POPV on responses of pulmonary arteries and veins of guinea pigs to histamine, 5-HT, ACh, and SNP

<table>
<thead>
<tr>
<th></th>
<th>Arteries</th>
<th>Veins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximal response, %</td>
<td>pD2</td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35.3 ± 4.7</td>
<td>6.71 ± 0.12</td>
</tr>
<tr>
<td>POPV</td>
<td>38.2 ± 5.9</td>
<td>6.16 ± 0.12*</td>
</tr>
<tr>
<td>5-HT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.8 ± 1.5</td>
<td>8.61 ± 0.12</td>
</tr>
<tr>
<td>POPV</td>
<td>24.4 ± 2.6*</td>
<td>8.98 ± 0.31</td>
</tr>
<tr>
<td>ACh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-31.8 ± 8.4</td>
<td>7.88 ± 0.27</td>
</tr>
<tr>
<td>POPV</td>
<td>-29.5 ± 5.1</td>
<td>7.62 ± 0.29</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-75.7 ± 3.9</td>
<td>7.48 ± 0.21</td>
</tr>
<tr>
<td>POPV</td>
<td>-74.3 ± 7.0</td>
<td>7.43 ± 0.18</td>
</tr>
<tr>
<td>10 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>-41.8 ± 8.5</td>
<td>7.81 ± 0.15</td>
</tr>
<tr>
<td>POPV</td>
<td>-69.8 ± 9.2*</td>
<td>8.02 ± 0.26</td>
</tr>
<tr>
<td>SNP</td>
<td>-56.7 ± 8.3</td>
<td>7.91 ± 0.12</td>
</tr>
<tr>
<td>POPV</td>
<td>-67.1 ± 5.4</td>
<td>7.97 ± 0.36</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6-10 animals. One and 10 mo refer to duration of pulmonary artery ligation; contralateral lungs were the controls. Negative values denote relaxation. pD2, negative log of molar concentrations of EC50; 5-HT, serotonin; SNP, sodium nitroprusside. *P < 0.05 vs. controls.

Reduced sensitivity of arteries to histamine, and a failure of the constriction in the arteries to wane at higher concentrations of 5-HT, as they did in controls. Second, not only was there no evidence of endothelial dysfunction but also the relaxation of the arteries to ACh was enhanced at 10 mo after ligation; moreover, relaxation of the veins to SNP was increased at 1 mo after ligation. Third, the diameters of the arteries and veins were significantly reduced at all times after ligation, with no significant medial thickening.

POPV has been previously produced primarily in dogs (14, 15, 29), although it was also produced in the rat (24, 32). Qualitatively, the morphological changes in guinea pigs are the same as in canine lungs: the numbers of bronchial vessels and lymphatics around airways and pulmonary vessels are increased in lungs with POPV compared with those of controls (11, 14).
chial vessels proliferated; the structural changes of pulmonary vessels, however, were not presented in detail.

We found previously in in situ perfused canine lungs with POPV a markedly increased response of arteries to 5-HT and of veins to histamine, with no significant effect of 5-HT on veins or of histamine on arteries (15).

In the present in vitro study of POPV in guinea pigs, maximal responses of arteries to 5-HT and of veins to histamine were also increased. In contrast, histamine was less potent in the POPV arteries than in controls, and 5-HT constricted the veins of control and POPV lungs to the same degree. These results point to drug-
specific and vessel-specific alterations of the responses to these two pharmacological agents.

Before considering the mechanisms of altered vasoreactivity in POPV, let us briefly examine the question of whether the right lung, perfused with a greater flow than one in a nonligated animal, is indeed normal. Although it is theoretically possible that increased shear stress in the contralateral lung could alter, particularly increase, reactivity, our own studies in dogs, rats, and guinea pigs have failed to reveal significant differences in morphology or in physiological and pharmacological responses that could produce this result (11–16, 24, 26). To further address this point, we compared the vasoreactivity in the contralateral right lungs of animals with POPV with those of our previous controls (25, 27) and found that, when there was difference, it was in the direction of a small reduction in reactivity in the contralateral lungs of POPV compared with “true controls,” best explainable by differences in age of the guinea pigs (older in the POPV experiments).

What then are the mechanisms of the increased reactivity to vasoactive agents such as histamine and 5-HT in POPV? One obvious potential explanation could be the structural alterations either in the pulmonary vessels themselves or in the surrounding parenchyma. In our previous studies in the in situ-perfused canine lobes, medial thickening, peripheral muscularization, and a reduced luminal diameter could be invoked to explain part of the hyperresponsiveness of the arteries to 5-HT (15). Indeed, according to the law of Laplace, the transmural pressure against which vessels contract is proportional to tension and inversely proportional to radius, and thus the pressor response will be greater with a smaller lumen even though the tension generated in the smooth muscle is unchanged. Similarly, for a given degree of contraction, a narrower vessel will increase its resistance more than a wider vessel, because resistance, according to Poiseuille’s equation, is inversely proportional to radius to the fourth power (17). The above reasoning, however, does not apply to lung explants in which we measured smooth muscle contraction directly, not pressure or resistance.

In the present study, although the reduction in vascular luminal diameter was significant in POPV, the pharmacological specificity of the hyperreactivity that we observed argues against the possibility that it was due to this structural alteration: indeed, despite their reduced diameters, the maximal responses of the arteries were greater only to 5-HT and not to histamine, whereas the veins showed an increased maximal response only to histamine and not to 5-HT. Our data on the maximal response values for U-46619 (used to precontract the vessels before application of the relaxants) also support the notion of a specific hyperreactivity to 5-HT and histamine because, with U-46619, there was only a small, nonsignificant increase in the maximal response of arteries and veins of POPV lungs compared with controls. Furthermore, although medial muscle thickening is certainly capable of enhancing contractility (18), this was not observed in our study (Table 1), and thus the specifically altered constrictor responses occurred despite an unchanged muscle thickness. In fact, the findings that vascular diameters were reduced and medial muscle thickness remained unchanged suggests that, if anything, muscle mass was reduced overall, further supporting the concept that the increased reactivity cannot be explained by these structural alterations. In a related study (24), we found that contractile responses to endothelin-1 and endothelin-3 of only pulmonary arteries, not veins, were significantly increased in POPV.

With regard to parenchymal alterations, we have described in POPV an elevated lung elastance and increased reactivity (11) that, if anything, should increase the resistance against which the vascular smooth muscle must contract. Thus the fact that we observed an increased contractility to the pharmacological agents argues against the alterations in the surrounding parenchyma explaining our results. To summarize, we believe that structural alterations play a minor role, if any, in explaining the augmented vasoreactivity of POPV in the present study.

A second potential explanation for the increased vasoconstriction could be defective endothelium-derived NO-mediated relaxation because it is known that denudation of the endothelium or inhibition of NO production increases constrictive responses to histamine and 5-HT (7,19). In the present study, we used ACh, which acts indirectly through release of NO (23), to test endothelium-derived NO production and SNP, which directly releases NO (22), to test the responsiveness of vascular smooth muscle to NO, and found that ACh dilated pulmonary arteries but not veins and that SNP dilated both. The finding that the responses of
pulmonary arteries to ACh and SNP were not altered 1 mo after pulmonary artery ligation suggests that endothelium-derived NO-mediated relaxation was intact and thus that endothelial dysfunction was not present. Ten months after ligation, however, dilator responses to ACh were augmented, because of either increased endothelial NO production or increased responsiveness of vascular smooth muscle to NO. The fact that the responses to SNP were not altered in POPV suggests that an increased release of NO rather than an increased response of the smooth muscle to it explains the enhanced response to ACh. In the pulmonary veins, ACh produced contraction but not relaxation. This further contraction in pulmonary veins of guinea pigs was probably the result of the release of vasoconstrictor prostanoids because, in a separate study, our laboratory (25) found that it could be abolished by indomethacin.

SNP caused greater relaxation of the veins after 1 mo of POPV compared with controls. Other investigators have also found increased responsiveness to vasodilators of small but not large pulmonary arteries in hypertensive animals. For example, Orton et al. (20), in neonatal calves with severe pulmonary hypertension, found that maximal responses to ACh were increased in structurally altered resistance vessels in vivo but reduced in isolated lobar pulmonary arteries. Wanstaff et al. (31) reported that in rats with monocrotaline-induced pulmonary hypertension, maximal relaxation to SNP was increased in arteries with an internal diameter of 200–500 μm but reduced in main pulmonary arteries; the existence of an inherent tone in the small arteries was proposed to explain the hyperresponsiveness. The arteries in POPV share some structural alterations with those in pulmonary hypertension, including a reduced internal diameter and an increased wall-to-radius ratio (14). The internal diameters of the arteries that we studied were under 400 μm in diameter, within the range of small vessels in the monocrotaline-treated pulmonary hypertensive rats (31).

The increment in endothelial NO-mediated relaxation of the pulmonary arteries in POPV of 10-mo duration was unexpected and obviously cannot explain the hyperreactivity of the pulmonary vessels in POPV. This increased relaxation, however, is conducive to the perfusion of pulmonary vessels and thus could perhaps represent an adaptive response to the reduced blood flow, as observed in other conditions (9). Moreover, it complements and provides an explanation for the results, in the arteries of lungs with POPV, of the reduced sensitivity to the contractile effects of histamine (Table 2), and of the reduced responses at the lower concentrations of this agent (10−4 and 10−7 M; Fig. 2). Indeed, it is known that histamine simultaneously stimulates the endothelium to release NO while contracting the pulmonary vessels of guinea pigs (1, 23); thus, if endothelial NO-mediated relaxation is increased, sensitivity to histamine would be expected to decrease. This histamine-induced endothelium-dependent relaxation is most prominent at lower concentrations (as observed in the present study) because, at higher concentrations, it is masked by its predominant contractile effect (1, 25). In further support of this, even though in the present study we did not test responses to histamine in the presence of an inhibitor of NO synthase, our laboratory had done so in a prior study and found that differences between the maximal responses of arteries and veins to histamine were attributable to endothelium-derived NO-mediated effects (27).

Although there was variation in the duration of ligation between the constrictor and the dilator experiments, we believe that the results from the effects of the latter can be used to interpret the findings in the experiments with the former. Indeed, in our previous experiments in dogs, the physiological results were consistent over a wide range of duration of ligation (11, 14). Thus, in the present study, we opted for a ligation period of 3 mo to investigate constriction, as this was similar to the canine lungs. To examine potential mechanisms, however, we decided on two time periods, 1 and 10 mo, similar to the extremes of the experiments in canine lungs, to ascertain whether there was a time-dependent effect. The postligation duration of 3 mo falls between the time points of 1 and 10 mo, at which time there were increases in the endothelial NO-mediated relaxation in pulmonary arteries.

With 5-HT, we found that in POPV, there were both an increased maximal contraction and a failure of the arteries to relax at high concentrations (Fig. 3). The increased contraction of the arteries to 5-HT in POPV may be partly explained by their failure to relax at the high concentrations because the control arteries showed pronounced relaxation at higher concentrations of 5-HT (Fig. 3). Cushing and Cohen (4) showed that high concentrations of 5-HT (above 10−7 M) produced concentration-dependent relaxation of canine coronary arteries devoid of endothelium, although the receptor responsible for this effect had not been characterized. Similar findings were reported in guinea pig airways in vitro by Baumgartner et al. (2), who found that 5-HT contracted smooth muscle at low concentrations and relaxed it at higher concentrations directly via activation of 5-HT2 receptors, independent of the epithelium. Thus, in POPV, receptors on smooth muscle mediating relaxation at high concentrations of 5-HT or downstream signal transduction pathways may be impaired, leading to failure of relaxation and persistence of contraction.

In summary, we found in the present study that chronic ligation of one pulmonary artery results in alterations in pulmonary vascular structure and in vasoreactivity. Although alterations in vasoreactivity are a common phenomenon of vascular diseases, the mechanisms and pathological significance for these remain elusive. Structural alterations, including decreased vascular diameter and increased medial thickness, are frequently invoked to explain an increased vasoreactivity (17). The present study suggests that the exaggerated vessel-specific vasoconstriction to 5-HT and to histamine in POPV, however, are not attributable to the structural alterations. This study also suggests that endothelial NO-mediated relaxation of
pulmonary vessels in POPV is intact and even is augmented. Because neither altered structure nor endothelial dysfunction appears to explain the increased vasoreactivity in POPV, we speculate that it could be caused by altered receptors on the smooth muscle or by abnormal signal transduction pathways, fruitful avenues for further exploration.

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