Endothelin reactivity and receptor profile of pulmonary vessels in postobstructive pulmonary vasculopathy

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Shi, Weibin, Peter Cernacek, Fu Hu, and René P. Michel. Endothelin reactivity and receptor profile of pulmonary vessels in postobstructive pulmonary vasculopathy. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2558–H2564, 1997.—Chronic ligation of one pulmonary artery results in pulmonary vascular remodeling and bronchial angiogenesis, collectively known as postobstructive pulmonary vasculopathy (POPV). To determine whether the reactivity of pulmonary vessels to endothelins (ET) was altered in POPV and to explore potential mechanisms, we ligated the left main pulmonary artery of 18 rats. Four weeks later, using a lung explant technique, we compared POPV lungs with controls for contractile responses of intrapulmonary vessels to ET-1 and ET-3 and for relaxant responses to ET-1 and sodium nitroprusside (SNP) after precontraction with U-46619. Morphometric measurements were made on vessels studied pharmacologically. Competition receptor binding studies with [125I]-labeled ET-1 and unlabeled ET-1 and BQ-123 were performed using membrane proteins of pulmonary vessels. We found, in arteries, that contractile responses to ET-1 and ET-3 were significantly increased and that relaxant responses to ET-1 but not to SNP were reduced; in veins, only relaxation to SNP was increased. Morphometry showed that arteries and veins in POPV had reduced diameters without altered muscle thickness. Receptor binding studies showed that the proportion of ETA receptors in arteries was significantly increased in POPV (66%) vs. controls (54%). We conclude that, in POPV, the increase in reactivity to ET-1 and ET-3 is primarily related to an augmented proportion of ETA receptors.

Keywords: pulmonary arteries; pulmonary veins; receptor binding; lung explant; vascular remodeling

CHRONIC LIGATION of one pulmonary artery causes structural remodeling of the pulmonary vascular bed and angiogenesis of the bronchial vessels (13, 17, 18, 31). These characteristic alterations are termed postobstructive pulmonary vasculopathy (POPV). The pathological 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 increase in myoendothelial junctions (17, 19). These alterations could profoundly influence the reactivity of pulmonary vessels to vasoconstrictor stimuli. Indeed, in the canine model of POPV, we had previously found that the responsiveness of pulmonary arteries to 5-hydroxytryptamine and of veins to histamine was augmented (18). Like these two biogenic amines, endothelins (ET) are synthesized and inactivated in the lung. ET are potent vasoconstrictors and are thought to play a role in pulmonary vascular diseases (7, 16, 20). We therefore postulated that pulmonary vascular responses to ET could also be altered in POPV.

ET-1 and its isoforms ET-2 and ET-3 are 21-amino acid peptides with a characteristic ring structure formed by two disulfide bridges (16). ET-1 and ET-3 are abundantly expressed in endothelial and alveolar epithelial cells of the lung (16). They constrict pulmonary vessels mainly via ETA receptors in rats (30), guinea pigs (3), and humans (2). In contrast, when vascular tone is increased with hypoxia or U-46619, ET-1 and ET-3 act as endothelium-dependent dilators, mediated by ETB receptors on the endothelium, probably acting through production of nitric oxide or activation of K⁺ channels (4, 8, 23). Because ETA and ETB receptors have opposite effects on vascular tone, alterations in the proportion of these two receptors in POPV could lead to abnormal vascular responses to ET.

Thus the principal aims of the present study were 1) to compare the contractile responses to ET-1 and ET-3 in lungs with POPV with those in the contralateral control lungs, 2) to compare in POPV and control lungs dilator responses, after precontraction with U-46619, to ET-1 and sodium nitroprusside (SNP), the latter being an endothelium- and receptor-independent vasodilator (26), and 3) to explore the mechanisms underlying the differences in reactivity, specifically vascular structure and receptor density.

MATERIALS AND METHODS

Surgical Procedure for Pulmonary Artery Ligation

The procedure previously described for dogs was adapted to rats (18). Briefly, 18 male Sprague-Dawley rats (300–400 g) were anesthetized with pentobarbital sodium (35 mg/kg ip), intubated, and ventilated with 30% O₂ at 60 breaths/min with a tidal volume of 5–7 ml. Under sterile conditions, the left pulmonary artery was ligated with a 4-0 silk suture —2 mm beyond the bifurcation from the main pulmonary artery through a left thoracotomy performed in the third or fourth intercostal space. The chest was closed in layers with 4-0 Dexon sutures. The lung was reexpanded with negative suction and positive-pressure ventilation. Postoperative care was provided for the animals for 4 wk by the McIntyre Animal Resources Center, McGill University, before the final experiments were performed.

Contractile and Relaxant Responses of Pulmonary Vessels in Lung Explants

Preparation of lung explants. The responses of pulmonary arteries and veins to ET-1, ET-3, and SNP were examined in lung explants from six rats (483 ± 9 g body wt) 4 wk after ligation. The lung explants from the left lungs with the ligated pulmonary artery and from the contralateral control right lungs were prepared as previously described for airways (5). 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. Their anterior chest wall and upper abdomen were sterilized with 70% ethanol, the abdomen was opened, and animals were exsanguinated by

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cutting the abdominal aorta. After removal of the anterior chest wall, the pulmonary vessels were washed in situ with 10 ml of Ringer lactate containing 20 U/ml heparin. 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) (5). The preparation was allowed to cool for 20 min at 4°C. Then the lungs were separated from the heart, placed in a sterile 50-ml syringe, the needle end of which had been removed, and embedded in 4% agarose in bicarbonate-buffered minimal essential medium at 37°C. 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 under an inverted microscope (IMT-2, Olympus, Tokyo, Japan), and those containing 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 culture medium at 37°C. After 4% agarose in bicarbonate-buffered minimal essential medium at 37°C. 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 under an inverted microscope (IMT-2, Olympus, Tokyo, Japan), and those containing 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 culture medium and incubated overnight at 37°C in 5% CO₂-95% air.

The next morning, the culture dish inserts containing the lung explants were transferred to six-well plates containing 2 ml of N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered culture medium (5) and placed on the stage of an inverted microscope (LH50A, Olympus). Arteries and veins were identified and imaged with a videocamera (CDS, Sony, Nagano, Japan), and images were recorded with a videodisk recorder (TQ2026F, Panasonic, Osaka, Japan). To distinguish arteries from veins, we used the following criteria: 1) the arteries 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. In addition, we confirmed the identities of the vessels by histological examination (see below).

Experimental protocol. First, to examine contractile responses, cumulative dose responses to ET-1 and ET-3 of the pulmonary vessels in the explants of control right lungs and POPV left lungs were studied. After generation of baseline images of the vessels, 10⁻¹⁰ M ET-1 or ET-3 was added to the surface of the explants. Five minutes later (which, according to preliminary experiments, corresponded to time at which peak contractile responses occurred), images of the vessels were taken. Then 3 × 10⁻¹⁰ M ET-1 or ET-3 was added, and images were again taken. This procedure was repeated by half-log intervals until the final concentration of 3 × 10⁻⁶ M was reached.

Second, to study relaxation responses to ET-1 and SNP, arteries and veins were precontracted with 3 × 10⁻⁶ M U-46619, a thromboxane A₂ analogue. Thirty minutes later, cumulative dose-response curves were constructed by adding ET-1 solution in half-log unit intervals from 3 × 10⁻³ M or by adding SNP solution in one-log unit intervals from 10⁻¹¹ M to 10⁻⁸ M. In each explant, we usually observed one artery and/or one vein and, in a few instances, two veins. We examined 57 arteries and 54 veins from 79 explants in the control lungs and 55 arteries and 49 veins from 87 explants in the POPV lungs.

Image and data analysis from explants. The stored images were digitized using a 80386 Intel-based microcomputer equipped with a frame-grabber board (PIPO24B, Matrox, Montreal, QC, Canada). The digitized images were then transferred to a scientific work station (RS6000, IBM, Armonk, NY), and measurements of luminal areas were made with Galileo Image Processing Software (Inspiraplex, Montreal, QC, Canada). The contractile responses of arteries or veins to ET-1, ET-3, or U-46619 were calculated as a percentage of complete vessel closure using the equation:

\[
\text{response} = \left[1 - \left(\frac{\text{area after drug/baseline area}}{\text{area before dilator}}\right)\right] \times 100
\]

Thus a 100% response indicated complete vascular luminal closure, and a 0% response indicated no effect.

The relaxant responses to ET-1 and SNP were expressed as a percentage of precontraction induced by U-46619 using the equation:

\[
\text{response} = \left(\frac{\text{baseline area} - \text{area after dilator}}{\text{baseline area} - \text{area before dilator}}\right) \times 100
\]

Here, −100% indicated a return to baseline state (i.e., before precontraction) and 0% full persistence of the precontracted state.

From these responses, time course and dose-response curves of arteries and veins were constructed by plotting the mean values against time and concentrations, respectively. The half-maximal effective concentration values were determined from each individual vessel and expressed as negative log molar (pD₂) values.

Light Microscopy and Morphometry of Lung Explants

All explants used for experiments were fixed by immersion in 10% buffered formaldehyde solution, processed using standard histological technique, and embedded in paraffin. Sections (5 µm thick) were cut and stained with hematoxylin-eosin. The arteries and veins used for pharmacological study were identified based on maps drawn at the time of image acquisition. Morphometric measurements were then made on those vessels that had an intact wall, using previously described methods (17). With an ocular micrometer on an optical microscope (Leitz, Wetzlar, Germany), we measured the internal luminal diameter at magnifications of ×100 or ×250 and, at the same position, the medial smooth muscle thickness at a magnification of ×400 (for greater precision); the sum of the internal diameter and twice the medial thickness equaled the external diameter.

ET Receptor Binding

Pulmonary arteries and veins from 12 rats (499 ± 13 g body wt) 4 wk after ligation were used to prepare cell membranes as previously described (21). Briefly, arteries and veins were dissected out separately from the hilum down to ~100 µm diameter under a dissecting microscope (Zeiss, Oberkochen, Germany) and snap frozen in liquid nitrogen. The tissues from each animal were homogenized separately on ice in 2 ml of cold tris(hydroxymethyl)aminomethane (Tris) buffer (in mM: 25 Tris-HCl, 2 MgCl₂, 250 sucrose, and 5 HEPES, pH 7.4) with a Polytron (Brinkman, Rexdale, ON, Canada) at 13,000 revolutions/min in six bursts of 15 s each. The homogenate was centrifuged at 1,000 g for 10 min at 4°C, and the supernatant was collected and centrifuged at 35,000 g for 30 min at 4°C. The resulting pellet was resuspended in Tris buffer, aliquoted, and stored at –80°C. Amounts of protein were determined by the dye-binding method with bovine serum albumin as the standard.

The type, density, and affinity of ET receptors in pulmonary vessels were assessed by competitive binding experiments, performed in duplicate. Because of the small sample size, we pooled the membrane proteins from the 12 rats two by two for a total of five or six experiments. ¹²⁵I-labeled ET-1 (50 µl; 14–25 pM) was added to each tube containing either 50 µl of Tris buffer or increasing concentrations of unlabeled
ET-1 or BQ-123. The binding reaction was initiated by adding 100 µl of membrane protein (0.7–2.5 µg/tube for arteries and 3–4 µg/tube for veins) to a final incubation volume of 200 µl. After 3 h at room temperature, the reaction was stopped by addition of 1 ml of cold phosphate-buffered saline containing 0.5% bovine serum albumin and rapid centrifugation at 12,000 g for 5 min. Radioactivity of the resulting pellet was determined in a gamma counter (Packard Minaxi model 5530, Mississauga, ON, Canada) with an efficiency of 80%. Nonspecific binding was measured in the presence of 2 x 10^{-7} M unlabeled ET-1. The dissociation constant (K_d) and maximal binding (B_max) for ET-1 were obtained using the Receptor-Fit Competition Program (London, Chagrin Falls, OH).

Statistical Analysis

Data are presented as means ± SE, with the n indicating the number of animals from which the vessels were obtained; this n was the one used for the analyses. Contralateral right lungs were the controls (17, 18). Statistical analyses were performed using proprietary software (Systat, Evanston, IL). For the comparisons of dose-response curves and maximal responses and pD2 between control right lungs and POPV left lungs or between arteries and veins in the same lungs, two-way analysis of variance (ANOVA) was used. When the F value was significant (P < 0.05), the Tukey test or Student's paired t-test was used to examine differences at each concentration. For comparisons of K_d, B_max, and morphometric measurements, we used the Student's paired t-test. Differences were considered statistically significant at P < 0.05.

RESULTS

Contractile Responses to ET-1 and ET-3

The principal finding was that in POPV the contractile responses to ET-1 and ET-3 of pulmonary arteries were significantly increased compared with controls (P < 0.05); the increase in the responses was significant at ET-1 concentrations from 10^{-8} to 3 x 10^{-6} M (Fig. 1) and at ET-3 concentrations from 3 x 10^{-9} to 3 x 10^{-6} M (Fig. 2). Moreover, in POPV lungs, maximal responses of pulmonary arteries to ET-1 and ET-3 were also significantly increased, although the pD2 values were not altered (Table 1). In contrast, pulmonary veins in POPV did not show altered responses to either ET-1 or ET-3 (Figs. 1 and 2, Table 1).

When we compared responses of pulmonary arteries with those of pulmonary veins in control lungs, the latter had significantly increased maximal responses to both ET-1 and ET-3 (Table 1). In POPV lungs, however, maximal responses of pulmonary arteries and veins were not significantly different. In addition, the maximal responses and pD2 values of both pulmonary arteries and veins were greater for ET-1 than for ET-3 in control lungs; in POPV lungs, however, only pD2 values for ET-1 of pulmonary arteries were greater than those for ET-3 (Table 1).

Table 1. R_max and pD2 values for ET-1, ET-3, and SNP of pulmonary arteries and veins in POPV

<table>
<thead>
<tr>
<th></th>
<th>Arteries</th>
<th>Veins</th>
<th>Arteries</th>
<th>Veins</th>
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<tbody>
<tr>
<td></td>
<td>R_max, %</td>
<td>pD2</td>
<td>R_max, %</td>
<td>pD2</td>
</tr>
<tr>
<td><strong>Contraction</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ET-1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>31.0 ± 3.8*</td>
<td>7.67 ± 0.07*</td>
<td>51.0 ± 6.6†</td>
<td>7.64 ± 0.07*</td>
</tr>
<tr>
<td>POPV</td>
<td>41.0 ± 4.2‡</td>
<td>7.80 ± 0.09*</td>
<td>47.7 ± 4.0</td>
<td>7.50 ± 0.09</td>
</tr>
<tr>
<td>ET-3</td>
<td></td>
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<tr>
<td>Control</td>
<td>23.5 ± 4.1</td>
<td>7.34 ± 0.07</td>
<td>34.1 ± 5.4†</td>
<td>7.24 ± 0.05</td>
</tr>
<tr>
<td>POPV</td>
<td>39.8 ± 3.6‡</td>
<td>7.45 ± 0.07</td>
<td>46.0 ± 7.1</td>
<td>7.35 ± 0.08</td>
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<tr>
<td><strong>Relaxation</strong></td>
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<tr>
<td>ET-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35.5 ± 9.4</td>
<td>9.62 ± 0.15</td>
<td>12.5 ± 3.1</td>
<td>9.96 ± 0.15</td>
</tr>
<tr>
<td>POPV</td>
<td>10.0 ± 1.7†</td>
<td>9.91 ± 0.12</td>
<td>12.9 ± 5.7</td>
<td>9.37 ± 0.35</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
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<tr>
<td>Control</td>
<td>49.9 ± 7.5</td>
<td>7.15 ± 0.41</td>
<td>44.8 ± 7.9</td>
<td>7.39 ± 0.34</td>
</tr>
<tr>
<td>POPV</td>
<td>57.7 ± 6.4</td>
<td>7.22 ± 0.44</td>
<td>60.8 ± 6.1*</td>
<td>7.85 ± 0.41</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 or 6 animals. R_max, maximal responses; pD2, negative log molar values; ET, endothelin; POPV, postobstructive pulmonary vasculopathy; SNP, sodium nitroprusside. *P < 0.05 vs. controls. Responses to ET-1 of arteries but not of veins were significantly enhanced in postobstructive pulmonary vasculopathy (POPV).
Relaxant Responses to ET-1 and SNP

Pulmonary arteries and veins in control and POPV lungs were submaximally contracted with U-46619: the contractile responses were 19.2 ± 4.4 and 29.5 ± 3.8% for control and POPV arteries, respectively, and 18.7 ± 3.3 and 29.1 ± 3.7% for control and POPV veins, respectively; the degree of constriction to U-46619 was significantly greater in POPV arteries and veins than in controls (P < 0.05). In arteries after U-46619, ET-1 caused dose-dependent relaxation at concentrations up to 10^-9 M for control and up to 3 × 10^-10 M for POPV lungs that was significantly smaller in POPV than in the control (Fig. 3, Table 1). In veins, ET-1 also caused dose-dependent relaxation up to 3 × 10^-10 M for the control and 10^-9 M for POPV, but maximal relaxation was not significantly different between them (Fig. 3, Table 1). Above 10^-9 or 3 × 10^-9 M, ET-1 started to contract the arteries and veins. In contrast, SNP produced a pronounced, dose-dependent relaxation of both arteries and veins that was not altered by POPV in arteries but was significantly enhanced in veins (Fig. 4).

Histology and Morphometry

The identity of arteries and veins in the explants was confirmed by histology. The architecture and morphology of the control right lungs were normal with few bronchial vessels around the larger pulmonary vessels and airways. In contrast, the left lungs with the ligated pulmonary artery showed a marked increase in the number of bronchial blood vessels in the adventitia of pulmonary arteries and veins and in the walls of airways. These findings are similar to those previously reported in dogs (17). The parenchyma was normal in the right lungs, but there was mild focal fibrosis in the left lungs. The results of the morphometric measurements of the pulmonary vessels are given in Table 2. 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 POPV lungs compared with controls (P < 0.05). Medial muscle thickness was not altered in either arteries or veins.

ET Receptor Binding Experiments

125I-ET-1 binding was completely displaced by unlabelled ET-1 and partially displaced by BQ-123 in both arteries and veins (Fig. 5). The displacement curves of 125I-ET-1 by ET-1 and BQ-123 were monophasic and best fitted a one-site model. In arteries, the inhibition of 125I-ET-1 binding with BQ-123 was significantly greater (P < 0.05) at concentrations of 10^-7 and 10^-6 M in POPV lungs than in controls (Fig. 5). The inhibition of 125I-ET-1 binding with ET-1 in arteries and veins and with BQ-123 in veins, however, was not significantly different for POPV and control lungs (Fig. 5). B\textsubscript{max} and K\textsubscript{d} for ET-1, and ET\textsubscript{A} as a percentage of total ET receptors are shown in Table 3. The percentage of ET\textsubscript{A} receptors was significantly increased in POPV arteries but not in veins, although the B\textsubscript{max} and K\textsubscript{d} of arteries and veins for ET-1 did not differ significantly between control and POPV lungs.

Table 2. Baseline video image diameter and histological measurements of pulmonary arteries and veins in POPV

<table>
<thead>
<tr>
<th>Video Image ID</th>
<th>ID (µm)</th>
<th>ED (µm)</th>
<th>MT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>439±29</td>
<td>468±17</td>
<td>557±17</td>
</tr>
<tr>
<td>POPV</td>
<td>353±21*</td>
<td>349±23*</td>
<td>432±24*</td>
</tr>
<tr>
<td>Veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>384±17</td>
<td>319±27</td>
<td>352±26</td>
</tr>
<tr>
<td>POPV</td>
<td>316±17*</td>
<td>278±20*</td>
<td>312±20*</td>
</tr>
</tbody>
</table>

Values are means ± SE (in µm) for 6 animals. ID, internal diameter; ED, external diameter; MT, medial thickness. Note that ED = ID + 2MT. *P < 0.05 POPV vs. control.
or BQ-123 in POPV. Alterations in medial thickness, and diameters of the arteries and veins were reduced, without remaining unchanged, whereas their responses to SNP were reduced, whereas their responses to ET-1 in precontracted pulmonary arteries were increased, whereas in rats it remained normal (Table 2). Weibel (31) reported that 2–5 days after pulmonary artery ligation, the bronchial arteries enlarge in rats and that between 5 and 40 days new bronchial vessels grow; in this report, however, the structural changes in pulmonary vessels were not described in detail.

In the contralateral control lungs of rats, we found that contractions to ET-1 and ET-3 were greater in pulmonary veins than in arteries, as previously observed by others (1). We also found that ET-1 was a more potent constrictor of control pulmonary arteries and veins than ET-3. Perreault and Baribeau (25) reported similar findings in normal piglet lungs. Because ET_A receptors are known to mediate constriction in the pulmonary vessels of rats (30), the greater affinity of ET-1 over ET-3 for ET_A receptors may explain their different constrictor effects in pulmonary vessels (16). In addition to inducing constriction, ET-1, in isolated perfused lung preparations of rats precontracted with U-46619 or hypoxia, has been shown to relax pulmonary vessels through ET_B receptors on endothelial cells (8, 9). The present study confirms these observations in in vitro preparations of pulmonary arteries and veins. The right contralateral lungs, although perfused with an increased blood flow in POPV, are believed to be normal both physiologically and morphologically, as indicated by our previous studies (12, 17–19). Indeed, in dogs, we compared lungs from normal dogs with contralateral control lungs from dogs with POPV and found minimal differences in hemodynamics and lung mechanics (12, 18). The increased flow to the right lungs is probably handled primarily by the recruitment of pulmonary alveolar vessels, and the pulmonary arterial pressure remains normal (18).

In lungs with POPV, our first important finding was that the pulmonary arteries but not the veins showed significantly increased contractile responses to ET-1 and ET-3. Increased vasoreactivity to ET-1 has also been reported in pulmonary arteries of pulmonary hypertensive rats (14), portal veins of spontaneously hypertensive rats (11), and aortas of hypercholesteremic rabbits (15). The second principal finding in POPV was that relaxation to ET-1 of the pulmonary arteries was reduced, whereas relaxation to SNP, which acts directly on smooth muscle (26), was unchanged. Decreased relaxant responses to ET have also been found in pulmonary vessels of chronic hypoxic rats (8).

How do we explain the increased contractile responses to ET-1 and ET-3 and the decreased relaxation to ET-1 in POPV? One putative explanation for our findings could be an altered pulmonary vascular structure. Indeed, in pulmonary vessels of the canine POPV model (17) and vessels from rats with systemic hyper-

### Table 3. K_d and B_max for ET-1 and percent ET_A receptors in arteries and veins from competitive binding between 125I-ET-1 and unlabeled ET-1 or BQ-123 in POPV

<table>
<thead>
<tr>
<th></th>
<th>K_d, pM</th>
<th>B_max, fmol/mg protein</th>
<th>ET_A, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>248 ± 79</td>
<td>1,712 ± 533</td>
<td>54.5 ± 2.9</td>
</tr>
<tr>
<td>POPV</td>
<td>247 ± 63</td>
<td>1,767 ± 571</td>
<td>66.1 ± 3.8*</td>
</tr>
<tr>
<td>Veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>172 ± 25</td>
<td>1,421 ± 281</td>
<td>59.2 ± 8.9</td>
</tr>
<tr>
<td>POPV</td>
<td>218 ± 25</td>
<td>1,138 ± 162</td>
<td>61.4 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 (veins) or 6 (arteries) experiments. K_d, dissociation constant; B_max, maximal binding capacity; ET_A, ET_A receptor. *P < 0.05 POPV vs. control.

**DISCUSSION**

In the present study, we compared the in vitro responses to ET, morphometric measurements, and ET receptor binding of pulmonary arteries and veins in lungs with POPV with those of the contralateral control lungs. Our principal findings were that, in POPV, 1) contractile responses of pulmonary arteries but not of veins to ET-1 and ET-3 were increased, whereas their responses to SNP were unchanged, 3) by morphometric analysis, the diameters of the arteries and veins were reduced, without alterations in medial thickness, and 4) receptor binding experiments showed an increased proportion of ET_A receptors in the arteries but not in the veins.

Previously, POPV has been produced primarily in dogs (13, 17, 18), although Weibel (31) produced it in the rat. Qualitatively, the morphological changes in rats resemble those in canine lungs: the numbers of bronchial vessels around airways and pulmonary vessels are increased in lungs with POPV compared with controls (17). Morphometric measurements of pulmonary vessels in rats, however, differed from those in dogs. First, in the latter, only arterial internal diameters were reduced, whereas in the present study both arterial and venous diameters were lower. The likely reason for the prominent reduction in vascular diameters in rats is the reduction in lung volume that followed ligation (27). Second, the medial thickness of the pulmonary arteries in the dogs with POPV was increased, whereas in rats it remained normal (Table 2). Weibel (31) reported that 2–5 days after pulmonary artery ligation, the bronchial arteries enlarge in rats and that between 5 and 40 days new bronchial vessels grow; in this report, however, the structural changes in pulmonary vessels were not described in detail.

In the contralateral control lungs of rats, we found that contractions to ET-1 and ET-3 were greater in pulmonary veins than in arteries, as previously observed by others (1). We also found that ET-1 was a more potent constrictor of control pulmonary arteries and veins than ET-3. Perreault and Baribeau (25) reported similar findings in normal piglet lungs. Because ET_A receptors are known to mediate constriction in the pulmonary vessels of rats (30), the greater affinity of ET-1 over ET-3 for ET_A receptors may explain their different constrictor effects in pulmonary vessels (16). In addition to inducing constriction, ET-1, in isolated perfused lung preparations of rats precontracted with U-46619 or hypoxia, has been shown to relax pulmonary vessels through ET_B receptors on endothelial cells (8, 9). The present study confirms these observations in in vitro preparations of pulmonary arteries and veins. The right contralateral lungs, although perfused with an increased blood flow in POPV, are believed to be normal both physiologically and morphologically, as indicated by our previous studies (12, 17–19). Indeed, in dogs, we compared lungs from normal dogs with contralateral control lungs from dogs with POPV and found minimal differences in hemodynamics and lung mechanics (12, 18). The increased flow to the right lungs is probably handled primarily by the recruitment of pulmonary alveolar vessels, and the pulmonary arterial pressure remains normal (18).

In lungs with POPV, our first important finding was that the pulmonary arteries but not the veins showed significantly increased contractile responses to ET-1 and ET-3. Increased vasoreactivity to ET-1 has also been reported in pulmonary arteries of pulmonary hypertensive rats (14), portal veins of spontaneously hypertensive rats (11), and aortas of hypercholesteremic rabbits (15). The second principal finding in POPV was that relaxation to ET-1 of the pulmonary arteries was reduced, whereas relaxation to SNP, which acts directly on smooth muscle (26), was unchanged. Decreased relaxant responses to ET have also been found in pulmonary vessels of chronic hypoxic rats (8).

How do we explain the increased contractile responses to ET-1 and ET-3 and the decreased relaxation to ET-1 in POPV? One putative explanation for our findings could be an altered pulmonary vascular structure. Indeed, in pulmonary vessels of the canine POPV model (17) and vessels from rats with systemic hyper-

**Fig. 5. Competitive inhibition of 125I-ET-1 binding by unlabeled ET-1 and BQ-123 in membrane proteins from pulmonary arteries (A) and veins (B).** Data were expressed as means ± SE for 5 or 6 experiments. *P < 0.05 vs. control.
tension (22), medial thickening and peripheral muscularization were invoked to explain part of the altered vascular response. However, our present morphometric measurements revealed that the medial thickness of arteries and veins in the rodent model of POPV was not significantly altered, suggesting that the augmented reactivity to ET in POPV is unrelated to vascular medial thickening. The reasons for these morphological differences between the canine and rodent models of POPV are unclear but could be related to species differences or to the duration of ligation, which is much longer in the dog (17). The fact that medial thickening was not altered, however, does not preclude that vascular remodeling may still have occurred (22). This aspect, not specifically the aim of the present study, is being pursued separately. Although the luminal diameters of both pulmonary arteries and veins were significantly reduced in POPV, we do not believe that this played an important role in the altered responsiveness of the arteries. The principal reason, as illustrated in Figs. 1–3, is that despite the fact that both arteries and veins had a reduced luminal diameter, the altered pharmacological responses to both constrictor and dilator agents were observed only in the arteries in POPV, not in the veins. The impaired relaxation in the arteries was also specific to ET-1 and not to SNP and correlated with the increased ET<sub>A</sub>-to-ET<sub>B</sub> receptor ratio that was observed in POPV. To further address the issue of reduced diameter, we performed regression analysis of maximal responses to ET-1 or ET-3 against internal diameter of the corresponding video images in each category of vessel in control and POPV lungs and found no correlation between them (data not shown), supporting the notion that an altered diameter per se does not explain our results.

A second potential mechanism to explain our findings in POPV is the increase in the proportion of ET<sub>A</sub> receptors. Indeed, we found that the percentage inhibition of <sup>125</sup>I-ET-1 binding sites with BQ-123 was significantly increased in POPV arteries compared with controls, indicating that the ET<sub>A</sub>-to-ET<sub>B</sub> receptor ratio was increased (10), although there were no significant differences in total binding sites for ET-1 between control and POPV lungs. This increased ratio can be invoked, on one hand, to explain the increased pulmonary arterial constrictor responses to ET-1 and ET-3 in POPV, since ET<sub>A</sub> receptors on vascular smooth muscle mediate vasoconstriction (6, 24, 30). The fact that the ET<sub>A</sub>-to-ET<sub>B</sub> receptor ratio was similar in veins of control and POPV lungs explains the absence of a difference in their ability to constrict to ET-1 and ET-3. The altered receptor ratio in the arteries, on the other hand, could explain their reduced relaxation responses to ET-1, since these are mediated by ET<sub>B</sub> receptors on endothelial cells (24, 30). It could be argued that our finding of a reduced relaxation response to ET-1 in POPV is secondary to a generalized endothelial dysfunction that may accompany POPV. In a separate study in guinea pigs (28), however, we found that the endothelium-dependent relaxation of pulmonary arteries to acetylcholine (which acts through different receptors) was not impaired, indicating that there is no generalized endothelial dysfunction in POPV.

In our study, one could envisage the possibility that the reduced relaxant responses of POPV arteries to ET-1 were due to their greater degree of precontraction to U-46619 (29). We think that this is unlikely because 1) these arteries did not also show reduced relaxation to SNP and 2) the pulmonary veins in POPV were also more precontracted to U-46619, and yet they did not show a reduced maximal relaxation. The reduced relaxation of pulmonary arteries in POPV probably occurred through mechanisms other than their increased contractility to ET-1, since concentrations required for relaxation were much lower than those for contraction. The reasons for the greater venous relaxation to SNP in POPV are unclear, although this finding was also observed in guinea pigs (28).

In summary, we found, in the present study, increased constriction to ET-1 and ET-3 and decreased relaxation to ET-1 of pulmonary arteries in POPV and attribute this to the increased ET<sub>A</sub>-to-ET<sub>B</sub> receptor ratio rather than to structural changes. Although alterations in vasoreactivity are a common phenomenon in vascular diseases, their mechanisms and pathological significance remain elusive. Structural alterations of vessels, including decreased vascular diameter and increased medial thickness, are frequently invoked to explain the increased vasoreactivity (22). Our findings point to a role for ET in POPV and may provide insights permitting the investigation of mechanisms of abnormal vasoreactivity in other diseases.

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