Hypoxia recruits intrapulmonary arteriovenous pathways in intact rats but not isolated rat lungs

Melissa L. Bates,1 Brendan R. Fulmer,1 Emily T. Farrell,1 Alyssa Drezdon,1 David F. Pegelow,1 Robert L. Conhaim,3 and Marlowe W. Eldridge1,2

1Department of Pediatrics and the John Rankin Laboratory of Pulmonary Medicine, 2Departments of Biomedical Engineering and Kinesiology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin; and 3Department of Surgery, University of Wisconsin School of Medicine and Public Health, and William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin

Submitted 2 August 2011; accepted in final form 13 March 2012

Bates ML, Fulmer BR, Farrell ET, Drezdon A, Pegelow DF, Conhaim RL, Eldridge MW. Hypoxia recruits intrapulmonary arteriovenous pathways in intact rats but not isolated rat lungs. J Appl Physiol 112: 1915–1920, 2012. First published March 15, 2012; doi:10.1152/japplphysiol.00985.2011.—Intrapulmonary arteriovenous anastomoses (IPAVS) directly connect the arterial and venous circulations in the lung, bypassing the capillary network. Here, we used solid, latex microspheres and isolated rat lung and intact, spontaneously breathing rat models to test the hypothesis that IPAVS are recruited by alveolar hypoxia. We found that hypoxia recruits IPAVS in the intact rat, but not the isolated lung. IPAVS are at least 70 μm in the rat and, interestingly, appear to be recruited when the mixed venous PO2 falls below 22 mmHg. These data provide evidence that large-diameter, direct arteriovenous connections exist in the lung and are recruitable by hypoxia in the intact animal.

arteriovenous anastomoses; hypoxia; intrapulmonary shunts; lung; microspheres

INDUCIBLE INTRAPULMONARY ARTERIOVENOUS anastomoses (IPAVS) are large-diameter pathways that connect the pulmonary arterial and venous circulations, bypassing the lung’s capillaries. Studies using saline contrast echocardiography demonstrate that IPAVS are closed in healthy, adult humans, breathing room air [fractional inspired O2 (FIO2) = 0.21] at rest (7, 8, 15, 18, 19). However, they are recruited with exercise at ≥60% of maximal exercise capacity in most individuals (7). Indeed, transpulmonary bubble passage has been observed in ~90% of participants tested while performing maximal exercise in our laboratory. Exercise with hypoxia (FIO2 = 0.12) causes the pathways to be recruited at lower workloads (18) than with normoxia and IPAVS can be recruited at rest in many individuals breathing an FIO2 < 0.10 (15). Hypoxia apparently closes these pathways, preventing the passage of saline bubble contrast, even with high-intensity exercise (19).

However, saline bubble echocardiography is limited in that it provides only qualitative measurements of IPAVS recruitment (12). Furthermore, saline contrast yields no information about IPAVS diameter. Thus, although saline contrast is a useful, minimally invasive tool for making repeated measures of IPAVS recruitment in humans, there is an additional need for a model using solid particles of known diameter to investigate the mechanism of IPAVS recruitment and their impact on gas exchange. We have previously described 25-μm solid microspheres in dogs to verify the recruitment of IPAVS during normoxic exercise. Here, we aimed to establish a rat model of IPAVS recruitment in hypoxia and to determine the diameter of IPAVS in this species.

We conducted four experiments using our previously described fluorescent microsphere method to test the hypothesis that IPAVS > 50 μm are recruited by alveolar hypoxia in the rat. In the first experiment, we ventilated isolated rat lungs with normoxic (FIO2 = 0.21) or hypoxic gas (FIO2 = 0.08) and assessed whether large-diameter microspheres traverse the lung under each condition. When we found that hypoxia did not recruit IPAVS in the isolated rat lung, we conducted a second experiment in which we exposed intact, anesthetized, spontaneously breathing rats to normoxic or hypoxic gas (FIO2 = 0.12) and found evidence of transpulmonary microsphere passage in hypoxia. In the third and fourth experiments, we varied the microsphere size to determine IPAVS diameter and investigated the effect of varying the FIO2 on IPAVS recruitment.

METHODS

Male Sprague-Dawley (250–350 g) rats (Harlan Laboratories) were maintained in standard animal housing on a 12:12-h light-dark cycle and offered water and chow ad libitum. All protocols were approved by the institutional animal care and use committee at the University of Wisconsin-Madison. Each of the experiments described in detail in subsequent sections used the same general approach. Isolated lungs or intact rats were exposed to normoxic or hypoxic gas for 5 min with IPAVS recruitment assessed by quantifying the transpulmonary passage of microspheres. Blood gases were always obtained before the injection of microspheres. Additionally, we dissected, examined, and probed the atrial and ventricular septa of all rats reported in this manuscript. Two rats with evidence of an atrial-level shunt were excluded from our data set. Careful postmortem analysis of the animals included in our data set revealed no intracardiac conduit, including a patent foramen ovale, that could allow for transcardiac microsphere passage.

Experiment 1: effect of alveolar hypoxia on IPAVS recruitment in isolated lungs. Lungs were isolated and prepared using previously well-described methods (5, 6, 20, 32). Briefly, rats were anesthetized with ketamine and xylazine (50–100 mg/kg and 5 mg/kg ip) and secured supine. A polyethylene catheter was placed in the femoral vein and infused with heparin (6,000 U/kg) to prevent lung thrombus formation during isolation. The femoral artery was then cut and blood was flushed from the animal by perfusing 10 ml isotonic phosphate-buffered saline (PBS, pH = 7.4) into the femoral vein. The trachea was isolated, cannulated with polyethylene tubing, and the lungs inflated with 5 ml of air. A midline sternotomy was performed, the chest wall removed, and fluid-filled polyethylene catheters were...

http://www.jappl.org

Address for reprint requests and other correspondence: M. L. Bates, The Univ. of Wisconsin, H6/551 Clinical Sciences Center, 600 Highland Ave., Madison, WI 53792 (e-mail: mlbates@pediatrics.wisc.edu).

placed in the pulmonary artery and left atrium. Lungs were left in situ in the thorax and 3 ml PBS was added to the chest cavity to prevent dehydration and to minimize friction between the lung and chest wall. The lungs were mechanically ventilated with FiO₂ = 0.08 (n = 5) or 0.21 (n = 4), balance N₂, 30–45 times per minute (3–4 ml/inflation) to a peak inflation pressure of 15 cmH₂O with 5 cmH₂O expiratory pressure.

The pulmonary arterial catheter was connected to a continuous-flow reservoir system containing 5% albumin in heparinized PBS (1,000 U/l, pH = 7.4). This reservoir was supplied by a pump and had an overflow outlet so that the height of the fluid column could be maintained at a constant level. The perfusion pressure was thus determined by the height of the fluid column above the pulmonary artery, which we set to 20 cmH₂O. The left atrial catheter was maintained at the level of the atrium so that the outlet pressure equaled 0 cmH₂O and the entire left atrial effluent was collected. Lungs were ventilated and perfused for 5 min before beginning our study protocol.

One million 15 ± 12% μm green fluorescent microspheres (Duke Scientific) were suspended in 4 ml PBS as previously described (20) and injected in four 1-ml boluses (1/4 of 4 ml with 250,000 microspheres per bolus) via an injection port in the pulmonary arterial catheter. Microspheres were injected in multiple boluses over 20 min, instead of as a single injection, to minimize the risk of microsphere clumping in large-diameter vessels. The entire left atrial effluent was vortexed and vacuum filtered through a filter with 8-μm pores (Millipore, Billerica, MA). Microspheres trapped in the filters were imaged using a fluorescent microscope and counted.

**Experiment 2:** effect of alveolar hypoxia on IPAVS recruitment in intact rats. Rats were anesthetized with ketamine and xylazine (50–100 mg/kg and 5 mg/kg ip) and placed on an electric homeothermic blanket. Body temperature was measured with a rectal probe and the blanket was adjusted to maintain a body temperature of 36–38°C. Once a surgical plane of anesthesia was verified, the neck was exposed and the carotid artery and jugular vein were isolated. A 0.5-mm microtip pressure-volume catheter (Millisar Instruments, MPVS Ultra and SPR-839, Houston, TX) was introduced via the carotid artery into the left ventricle to measure left ventricular pressure and volumes. Pressure-volume data were acquired with a PowerLab data acquisition device and analyzed using the LabChart software package (AD Instruments, Colorado Springs, CO). Before each use, the catheter was calibrated according to the manufacturer’s instructions using a sample of each animal’s own blood. The electronic pressure calibration was verified before use (Veri-Cal, Utah Medical Products, Midvale, UT).

To measure right ventricular pressure, a Tygon catheter (0.010 in. ID, 0.030 in. OD) was connected to a pressure transducer (Hospira, Lake Forest, IL) and introduced into the right ventricle via the jugular vein. The final location of the catheter was assessed by monitoring the pressure waveform during placement and was verified postmortem.

A second polyethylene catheter was introduced into the superior vena cava via the same jugular vein for microsphere injection. A third polyethylene catheter was placed in the femoral artery for blood gas analysis. Rats were then sealed into a custom-built Plexiglas chamber and exposed to hypoxia ventilated lungs (5.1 ± 1.3 ml/min) than hypoxia ventilated lungs (1.3 ± 0.8 ml/min) and 0.12. After 5 min, blood gases were drawn to verify that the rat was hypoxic. Each rat received an injection of one million microspheres of a single size (10 μm ± 18%, 25 μm ± 12%, 50 μm ± 12%, or 70 μm ± 7%, n = 4 each group, total n = 16) via the femoral catheter. Each rat received an injection of a single microsphere size. The rat was then euthanized by exsanguination and the kidneys were collected and analyzed as described.

**Results**

Experiment 1: effect of alveolar hypoxia on IPAVS recruitment (isolated lungs). The number of microspheres found in the venous effluent of isolated rat lungs with normoxic and hypoxic ventilation was equal and trivial (78 ± 10, P = 0.0, 43) and then transferred to mean transpulmonary microsphere passage <0.01% (Table 1). Perfusion flow was higher in normoxia ventilated isolated lungs (8.9 ± 1.3 ml/min) than hypoxia ventilated lungs (5.1 ± 1.5 ml/min, P = 0.005) and tended to decline, although not significantly, as a result of the injection of 1 × 10⁶ microspheres (7.5 ± 1.5 ml/min in normoxia, P = 0.16 vs. 2.8 ± 1.8 ml/min in hypoxia, P = 0.08).

**Experiment 2:** effect of alveolar hypoxia on IPAVS recruitment (intact rats). As expected, hypoxia (FiO₂ = 0.12) depressed arterial Po₂ relative to normoxia (28.8 ± 6.9 vs. 65.4 ±
Table 1. The effect of fractional inspired oxygen on the transpulmonary passage of 15-μm microspheres in isolated rat lungs

<table>
<thead>
<tr>
<th>Group 1: Normoxia (FIO₂ = 0.21)</th>
<th>Group 2: Hypoxia (FIO₂ = 0.08)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung 1</td>
<td>Lung 1</td>
</tr>
<tr>
<td>Lung 2</td>
<td>Lung 2</td>
</tr>
<tr>
<td>Lung 3</td>
<td>Lung 3</td>
</tr>
<tr>
<td>Lung 4</td>
<td>Lung 4</td>
</tr>
<tr>
<td>Lung 5</td>
<td>95</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>78 ± 62</td>
</tr>
</tbody>
</table>

Hypoxia did not enhance the transpulmonary passage of microspheres. FIO₂, fractional inspired oxygen.

4.4 mmHg, P < 0.001), although rats exposed to normoxia still demonstrated arterial hypoxemia that may have been caused by regional lung atelectasis in these spontaneously breathing animals. There was no difference in arterial Pco₂ (23.8 ± 7.7 vs. 28.1 ± 7.7 mmHg, P = 0.43) or pH (7.41 ± 0.19 vs. 7.38 ± 0.15, P = 0.79). Hypoxia increased the number of 15-μm microspheres that bypassed the pulmonary circulation and lodged in the kidney (0 ± 1 vs. 1.19 ± 1.925, P = 0.008) (see Table 2). More than 100 microspheres were observed in the single kidneys of five of six rats exposed to hypoxia. Two microspheres were observed in the kidney of only a single rat exposed to normoxia. No microspheres were observed in the kidneys of the remaining four rats. Again, we note the passage of 100 microspheres as important because, assuming the kidney receives 10% of the cardiac output, this translates to 0.1% of the microspheres having bypassed the lung.

The effects of hypoxia and microsphere injection on cardio-pulmonary hemodynamics are given in Table 3. As expected, hypoxia elevated the right ventricular systolic pressure and, consequently, the total pulmonary vascular resistance relative to normoxia. Rats exposed to hypoxia demonstrated no hemodynamic changes in response to microsphere injection. Microsphere injection in normoxia caused a transient 10-mmHg increase in right ventricular systolic pressure (P = 0.005) that resolved at the termination of the injection, but no other hemodynamic changes were noted.

Experiment 3: assessment of IPAVS size. More than 100 microspheres were found in the single kidney of two of four hypoxic rats injected with 10-μm microspheres (79 ± 93), five of six rats injected with 15-μm microspheres (1,119 ± 1,925, data from experiment 2), three of four rats injected with 25-μm microspheres (4,979 ± 5,815), three of four rats injected with 50-μm microspheres (4,641 ± 6,913), and four of four rats injected with 70-μm microspheres (637 ± 801) (Fig. 1A). The injection of 70-μm microspheres within the range of microsphere numbers that could be injected reproducibly (2.5 × 10⁵ to 2 × 10⁶) resulted in death within 5 min of injection. For that reason, larger sizes were not attempted in intact animals. Although the mean number of microspheres lodged in the kidney appears higher for the 25- and 50-μm spheres, the SD is also quite high. The higher mean is the result of one rat in each group with a very large number of microspheres (>10,000) and one rat in each group with <100 microspheres. An ANOVA revealed no overall effect of sphere size on the number of microspheres lodged in the kidney (P = 0.27). Therefore, data were pooled and are shown in Fig. 1B.

Assuming that the each kidney receives 10% of the cardiac output, and that this is unchanged by hypoxia, this translates to 2.2 ± 4.1% of the microspheres having bypassed the pulmonary circulation in the hypoxic condition. This is certainly an underestimation given that exposure to hypoxia decreases the renal blood flow ~40% in the anesthetized rat (22).

Experiment 4: relation between FIO₂ and IPAVS recruitment. As expected, PaO₂ (R² = 0.88, P < 0.001), PVO₂ (R² = 0.52, P < 0.001), and Paco₂ (R² = 0.54, P < 0.001) decreased with decreasing FIO₂. However, decreasing FIO₂ had no effect on PVco₂ (R² = 0.07, P = 0.20). Very few 25-μm microspheres were found in the kidneys of rats with PaO₂ > 30 mmHg and PVO₂ > 22 mmHg (5 ± 3 microspheres, n = 16). However, more than 100 microspheres were found in the kidneys of six of eight rats with PaO₂ < 30 mmHg and PVO₂ < 22 mmHg (966 ± 529, P < 0.001) (see Fig. 2). There was no linear correlation between the number of microspheres found in the kidneys of hypoxic rats and FIO₂, PVO₂, or PaO₂ (R² < 0.10 and P > 0.05 for each comparison).

DISCUSSION

We postulated that alveolar hypoxia would be sufficient to recruit IPAVS in isolated rat lungs and intact rats. Surprisingly, even a severe hypoxic stress that routinely recruits IPAVS in humans failed to recruit IPAVS in isolated lungs. This apparent negative finding, however, may provide insight into the anatomic location or regulation of IPAVS in the rat. In the intact rat, we found that hypoxia recruits IPAVS when PaO₂ was <30 mmHg and PVO₂ was <22 mmHg. Further, we show that IPAVS are at least 70 μm in diameter.

Why does hypoxia fail to recruit IPAVS in the isolated rat lung? As we have done previously (32), we used both isolated lungs and intact animals to investigate IPAVS recruitment. We found transpulmonary microsphere passage in isolated lungs ventilated with normoxia, although the number of transversing microspheres was very low (0.008%). This is similar to our findings in baboon (0.01%), human (0.06%), and dog lungs (0.001%) (20, 32). Microsphere passage was not enhanced by hypoxia. There are several key differences between the intact animal and isolated lung that may explain our findings.

The lack of IPAVS recruitment in the isolated lung may provide clues about the anatomic location of these pathways. The isolated lung is perfused exclusively between the pulmonary artery and left atrium and lacks an intact bronchial circulation. Although we have generally referred to these

Table 2. Number of 15-μm microspheres found in the kidneys of intact, spontaneously breathing rats exposed to normoxia or hypoxia

<table>
<thead>
<tr>
<th>Group 1: Normoxia (FIO₂ = 0.21)</th>
<th>Group 2: Hypoxia (FIO₂ = 0.08)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 1</td>
<td>11</td>
</tr>
<tr>
<td>Rat 2</td>
<td>204</td>
</tr>
<tr>
<td>Rat 3</td>
<td>5,004</td>
</tr>
<tr>
<td>Rat 4</td>
<td>380</td>
</tr>
<tr>
<td>Rat 5</td>
<td>859</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>254</td>
</tr>
</tbody>
</table>

Exposure to FIO₂ = 0.08 increased the number of microspheres found in the kidney compared with exposure to FIO₂ = 0.21 (*P = 0.008).
Resistance is approximated as the quotient of the right ventricular systolic pressure and cardiac output.†

Group 2: FIO2 required for IPAVS recruitment. Also, the fact that the cardiac output was not elevated by hypoxia in our experiments suggests that increased pulmonary blood flow is also not required for IPAVS recruitment.

As expected, we did observe elevations in pulmonary vascular pressure with hypoxia exposure and cannot rule out the possibility that IPAVS recruitment is mediated by elevations in pulmonary artery pressure. Evidence in humans in support of pressure-mediated recruitment of these pathways is conflicting. For example, Stickland et al. (33) found that elevating pulmonary artery pressure had minimal impact on IPAVS recruitment.

What recruits intrapulmonary arteriovenous anastomoses with hypoxia and exercise? Although the original purpose of these experiments was to test the question of whether exposure to a hypoxia recruits IPAVS, our findings in the intact rat lend additional insight into the mechanism by which IPAVS are recruited. During exercise, especially at workloads where we have observed IPAVS recruitment, P_{CO2} is elevated. However, P_{CO2} did not change with hypoxia exposure in our experiments, providing evidence that elevated P_{CO2} is not required for IPAVS recruitment. Also, the fact that the cardiac output was not elevated by hypoxia in our experiments suggests that increased pulmonary blood flow is also not required for IPAVS recruitment.

Table 3. Right heart pressure and cardiac output in intact, spontaneously breathing rats exposed to normoxia (n = 5) or hypoxia n = 6

<table>
<thead>
<tr>
<th>Group 1: FIO2 = 0.21</th>
<th>Heart Rate, beats/min</th>
<th>Stroke Volume, μl</th>
<th>Cardiac Output, ml/min</th>
<th>RV Systolic Pressure, mmHg</th>
<th>RV Diastolic Pressure, mmHg</th>
<th>Resistance, mmHg·ml⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinjection</td>
<td>319 ± 123</td>
<td>90.8 ± 28.7</td>
<td>29 ± 9</td>
<td>19 ± 3</td>
<td>3 ± 5</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>Injection</td>
<td>289 ± 16</td>
<td>126.9 ± 34.6</td>
<td>36 ± 8</td>
<td>29 ± 1†</td>
<td>5 ± 4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Postinjection</td>
<td>323 ± 30</td>
<td>98.9 ± 30.0</td>
<td>32 ± 10</td>
<td>22 ± 1</td>
<td>4 ± 5</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: FIO2 = 0.12</th>
<th>Heart Rate, beats/min</th>
<th>Stroke Volume, μl</th>
<th>Cardiac Output, ml/min</th>
<th>RV Systolic Pressure, mmHg</th>
<th>RV Diastolic Pressure, mmHg</th>
<th>Resistance, mmHg·ml⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinjection</td>
<td>283 ± 47</td>
<td>73 ± 18</td>
<td>21 ± 7</td>
<td>44 ± 11†</td>
<td>3 ± 7</td>
<td>2.3 ± 0.9†</td>
</tr>
<tr>
<td>Injection</td>
<td>268 ± 32</td>
<td>94 ± 30</td>
<td>25 ± 7</td>
<td>48 ± 11</td>
<td>6 ± 7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Postinjection</td>
<td>265 ± 27</td>
<td>78 ± 29</td>
<td>21 ± 9</td>
<td>45 ± 14</td>
<td>6 ± 7</td>
<td>2.4 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Right ventricular systolic pressure is used as a surrogate for pulmonary artery systolic pressure. Thus total pulmonary vascular resistance is approximated as the quotient of the right ventricular systolic pressure and cardiac output. †P < 0.05 compared with preinjection in normoxia, adjusted for multiple comparisons.

pathways as “intrapulmonary” arteriovenous pathways, they may contain an intrabronchial component. The bronchial circulation contains both pre- and postcapillary anastomoses to the pulmonary circulation (30, 34) and the short, muscular Sperr arteries that anastomose the pulmonary arteries to the bronchial arteries may provide a site of active control. These connections are 35–100 μm in diameter in the neonatal lung and appear to functionally close after birth. They remain present in the adult lung and it is hypothesized that there may be physiological or pathological conditions that could lead to their reopening (10).

It is also possible that there are factors missing from the isolated lung that are necessary for IPAVS recruitment. For example, the sympathetic nervous system is activated by both exercise and hypoxia, and catecholamine-mediated vasodilation could explain IPAVS recruitment in both of these conditions. In dogs and humans, epinephrine infusion causes intrapulmonary shunting. This hypoxemia is reduced by 100% O2 breathing or propranolol infusion (2, 3). Furthermore, epinephrine infusion causes the transpulmonary passage of albumin microspheres in the dog (23). We have noted the passage of albumin macroaggregates in exercising humans and the cessation of transpulmonary bubble passage with 100% O2 breathing (17, 19). If a functional sympathetic nervous system is required, hypoxia would not recruit IPAVS in our isolated lung model that lacks innervation and circulating humoral factors.

As in our previous studies, the isolated lungs in these experiments were positive-pressure ventilated and perfused in situ with a room temperature 5% albumin solution that was exposed to the ambient environment. This albumin solution has a high P_{O2}, low P_{CO2}, and nonphysiological temperature. It is possible that these nonphysiological conditions prevented IPAVS recruitment in the isolated lung. Each of these variables may have an independent effect on IPAVS recruitment.

What recruits intrapulmonary arteriovenous anastomoses with hypoxia and exercise? Although the original purpose of these experiments was to test the question of whether exposure to a hypoxia recruits IPAVS, our findings in the intact rat lend additional insight into the mechanism by which IPAVS are recruited. During exercise, especially at workloads where we have observed IPAVS recruitment, P_{CO2} is elevated. However, P_{CO2} did not change with hypoxia exposure in our experiments, providing evidence that elevated P_{CO2} is not required for IPAVS recruitment. Also, the fact that the cardiac output was not elevated by hypoxia in our experiments suggests that increased pulmonary blood flow is also not required for IPAVS recruitment.

As expected, we did observe elevations in pulmonary vascular pressure with hypoxia exposure and cannot rule out the possibility that IPAVS recruitment is mediated by elevations in pulmonary artery pressure. Evidence in humans in support of pressure-mediated recruitment of these pathways is conflicting. For example, Stickland et al. (33) found that elevating pulmonary artery pressure had minimal impact on IPAVS recruitment.

Fig. 1. Number of microspheres observed in the kidney as a function of microsphere size (A) and distribution of transpulmonary microsphere passage (B) (n = 22). Transpulmonary microsphere passage was observed in 17/22 rats and microsphere size was not related to the number of microspheres found in the kidney (P = 0.27). In the total population, 2,170 ± 4,118 microspheres were found in the kidney.
Rosenweig et al. (29) demonstrated the presence of corner capillaries through distended corner capillaries. Microspheres with a diameter of 15 \( \mu \text{m} \) were used to study transpulmonary passage of microspheres. Still, we appreciate that one possible interpretation of the greyhound lungs perfused at 37–74 mmHg is that the greatest mean capillary diameter observed in exercising humans. Laurie et al. (15) also found no causal relationship between the onset of IPAVS recruitment and ultrasound-assessed pulmonary artery pressure in human volunteers exposed to hypoxia. However, lower pulmonary artery pressures have been observed in exercising humans with evidence of IPAVS recruitment compared with those without (14).

We examined the effect of altering FIO\(_2\) on IPAVS recruitment and, as expected, arterial PO\(_2\) declined as a function of decreasing FIO\(_2\) and, interestingly, IPAVS recruitment occurred only with PaO\(_2\) < 30 mmHg and PV\(_{O2}\) < 22 mmHg. This PaO\(_2\) is much lower than those observed in humans with transpulmonary bubble passage (18) and, although we cannot rule out the potential confounding effect of anesthesia, we speculate that the signal for IPAVS recruitment may be on the venous side of the circulation. It is interesting that the PV\(_{O2}\) observed in our study when IPAVS were recruited is similar to the PV\(_{O2}\) during exercise and simulated altitude (27), although direct measurements of PV\(_{O2}\) and IPAVS recruitment have not been made simultaneously in humans or animals. Future studies in large-animal models, where the venous and arterial oxygen tensions can be isolated and controlled individually, would be valuable in testing this mechanism further.

**How large are intrapulmonary arteriovenous anastomoses?** We used 15-\( \mu \text{m} \) microspheres to assess the fraction of blood flow bypassing the pulmonary capillary bed and traveling through direct intrapulmonary arteriovenous anastomoses. The rationale for choosing this size microsphere was based on data suggesting that the greatest mean capillary diameter observed in greyhound lungs perfused at 37–74 mmHg is 6.5 \( \mu \text{m} \) and the largest capillaries observed were no larger than 13 \( \mu \text{m} \) (9). Still, we appreciate that one possible interpretation of the transpulmonary passage of 15-\( \mu \text{m} \) microspheres is passage through distended corner capillaries \( \approx 20 \mu \text{m} \) (9, 28, 29). Rosenweig et al. (29) demonstrated the presence of these corner capillaries in exercising humans. Laurie et al. (15) also found no causal relationship between the onset of IPAVS recruitment and ultrasound-assessed pulmonary artery pressure in human volunteers exposed to hypoxia. However, lower pulmonary artery pressures have been observed in exercising humans with evidence of IPAVS recruitment compared with those without (14).

We examined the effect of altering FIO\(_2\) on IPAVS recruitment and, as expected, arterial PO\(_2\) declined as a function of decreasing FIO\(_2\) and, interestingly, IPAVS recruitment occurred only with PaO\(_2\) < 30 mmHg and PV\(_{O2}\) < 22 mmHg. This PaO\(_2\) is much lower than those observed in humans with transpulmonary bubble passage (18) and, although we cannot rule out the potential confounding effect of anesthesia, we speculate that the signal for IPAVS recruitment may be on the venous side of the circulation. It is interesting that the PV\(_{O2}\) observed in our study when IPAVS were recruited is similar to the PV\(_{O2}\) during exercise and simulated altitude (27), although direct measurements of PV\(_{O2}\) and IPAVS recruitment have not been made simultaneously in humans or animals. Future studies in large-animal models, where the venous and arterial oxygen tensions can be isolated and controlled individually, would be valuable in testing this mechanism further.

**Summary.** We demonstrate that IPAVS at least 70 \( \mu \text{m} \) in diameter are recruited by hypoxia in the intact rat. These are extensively recruited in zone 1, allowing arterially perfused dye to enter the pulmonary veins. To verify that we were observing the recruitment of true arteriovenous anastomoses and not the passage of microspheres through corner capillaries, we tested microspheres up to 70-\( \mu \text{m} \) diameter in intact rats exposed to hypoxia. The Strahler model of the rat vascular tree places 70-\( \mu \text{m} \) vessels at the level of fifth-generation pulmonary arteries (13). Assuming a corner capillary diameter of 20 \( \mu \text{m} \), a 2% change in diameter per mmHg increase in capillary transmural pressure (11, 16, 26), and an initial transmural pressure equal to half the pulmonary artery (20 mmHg) and left atrial pressures (5 mmHg), 140 mmHg transmural pressure would be required to distend a corner capillary sufficiently to allow the passage of a 70-\( \mu \text{m} \) particle. Distension of a 6.5-\( \mu \text{m} \) alveolar capillary to 70 \( \mu \text{m} \) would require 488 mmHg transmural pressure. Thus it seems impossible that these large-diameter microspheres are passing through distended capillaries.

**What are the clinical and pathological consequences of recruiting intrapulmonary arteriovenous anastomoses?** The ability to modulate IPAVS may have important clinical consequences. In addition to its role in gas exchange, the lung is an important biological filter and IPAVS provide a route by which large-diameter particles can bypass this filter. Fifty percent of pediatric strokes are cryptogenic, occurring in the absence of hypercoagulative state, trauma, infection, or apparent cardiovascular disease (4, 21). Paradoxical embolism (an arterial embolism caused by a thrombus with a venous origin) has been considered as a possible mechanism, but only 45% of stroke patients have a patent foramen ovale to allow these thrombi to bypass the lung filter (1). Direct arteriovenous connections may serve as an alternative pathway to allow potentially dangerous blood clots, air bubbles, fat particles, or parasites to bypass the lung filter and reach the brain. It is particularly relevant that emboli similar in size to our 70-\( \mu \text{m} \) microspheres cause function performance deficits when injected into the cerebral circulation in rats (25). We examined the brains of a small subset of rats and found cerebral embolization by our microspheres (Fig. 3).

**Summary.** We demonstrate that IPAVS at least 70 \( \mu \text{m} \) in diameter are recruited by hypoxia in the intact rat. These
findings support previous observations made in human research participants. Microspheres do not traverse the isolated hypoxic lung, but are found in the systemic circulation of intact, hypoxicemiac rats. Although the mechanism of recruitment remains unknown, microsphere passage is observed at \( P_{\text{O}_2} < 22 \text{ mmHg} \), suggesting a role for the mixed venous \( P_{\text{O}_2} \) in IPAVS recruitment.

**REFERENCES**