Catecholamine-induced opening of intrapulmonary arteriovenous anastomoses in healthy humans at rest

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Laurie SS, Elliott JE, Goodman RD, Lovering AT. Catecholamine-induced opening of intrapulmonary arteriovenous anastomoses in healthy humans at rest. J Appl Physiol 113: 1213–1222, 2012.—The mechanism or mechanisms that cause intrapulmonary arteriovenous anastomoses (IPAVA) to either open during exercise in subjects breathing room air and at rest when breathing hypoxic gas mixtures, or to close during exercise while breathing 100% oxygen, remain unknown. During conditions when IPAVA are open, plasma epinephrine (EPI) and dopamine (DA) concentrations both increase, potentially representing a common mechanism. The purpose of this study was to determine whether EPI or DA infusions open IPAVA in resting subjects breathing room air and, subsequently, 100% oxygen. We hypothesized that these catecholamine infusions would open IPAVA. We performed saline-contrast echocardiography in nine subjects without a patent foramen ovale before and during serial EPI and DA infusions while breathing room air and then while breathing 100% oxygen. Bubble scores (0–5) were assigned based on the number and spatial distribution of bubbles in the left ventricle. Pulmonary artery systolic pressure (PASP) was estimated using Doppler ultrasound, while cardiac output (Qc) was measured using echocardiography. Bubble scores were significantly greater during EPI infusions of 80–320 ng·kg⁻¹·min⁻¹ compared with baseline when subjects breathed room air; however, bubble scores did not increase when they breathed 100% oxygen. At comparable Qc and PASP, intravenous DA (16 μg·kg⁻¹·min⁻¹) and EPI (40 ng·kg⁻¹·min⁻¹) resulted in identical bubble scores. Subsequent studies revealed that β-blockade did not prevent hypoxia-induced opening of IPAVA. We suggest that increases in Qc or PASP (or both) secondary to EPI or DA infusions open IPAVA in normoxia. The closing mechanism associated with breathing 100% oxygen is independent from the opening mechanisms.

INTRAPULMONARY ARTERIOVENOUS ANASTOMOSES (IPAVA) are closed at rest in healthy humans. These vessels can open during exercise (8, 11, 54) or at rest when hypoxic gas mixtures (29, 30) are breathed and can be detected using a technique called saline-contrast echocardiography. Additionally, IPAVA are not open during exercise in subjects breathing 100% oxygen (11, 31). However, the one or more mechanisms that regulate blood flow through IPAVA under these conditions remain unknown.

During exercise, plasma epinephrine (EPI) and dopamine (DA) concentrations both increase (21). In subjects who breathe hypoxic gas mixtures for brief periods of time the change in plasma EPI concentration is reportedly varied depending on the duration and level of hypoxia (49), with most subjects demonstrating an increase (26, 35, 41, 50). Therefore, an increase in plasma EPI or DA concentrations during exercise or when breathing hypoxic gas mixtures may represent a common link, causing IPAVA to open during both of these conditions in healthy humans. Indeed, using 15- to 30-μm radioactive albumin microspheres, Nomoto et al. (40) demonstrated that IPAVA open in dogs infused with EPI. Additionally, Berk and colleagues also suggested a direct effect of EPI increasing venous admixture in anesthetized dogs based on an immediate fall in arterial partial pressure of oxygen (P02) during EPI infusion (2–4), while Hucks et al. (22) suggested a similar effect in patients with left heart failure receiving DA infusion. Thus these catecholamines could potentially be opening IPAVA; however, their role and mechanism of action in opening IPAVA in healthy humans is unknown.

When EPI binds to β₁-adrenergic receptors on pulmonary vascular smooth muscle, it activates a receptor-linked pathway to increase the intracellular concentration of cAMP, leading to pulmonary vascular smooth muscle relaxation (14, 45). Alternately, the binding of EPI to α-receptors on pulmonary vascular smooth muscle leads to vasoconstriction. Under conditions of normal pulmonary vascular tone, EPI infusion appears to favor an increased pulmonary vascular resistance, while EPI infusion during conditions of increased basal tone causes dilation and a decrease in resistance (23, 24, 43, 44, 60). Thus the net change in pulmonary vascular resistance due to EPI infusion is due to a balance between its α- and β-adrenergic effects.

Dopamine stimulates dopaminergic receptors when administered at low doses (0.5–3 μg·kg⁻¹·min⁻¹), weakly stimulates β₁-adrenergic receptors at intermediate doses (3–10 μg·kg⁻¹·min⁻¹), and stimulates α₁-adrenergic receptors at higher infusion rates (42). Binding to dopaminergic receptors on renal vascular smooth muscle induces vasodilation (36), as do higher doses of 5 and 10 μg·kg⁻¹·min⁻¹ (9). In the pulmonary circulation DA has been shown either to have no effect (20), or to increase (15) or decrease (47) vascular resistance.

Thus the purpose of this study was to investigate whether the intravenous infusion of the vasoactive substances EPI or DA opens IPAVA in healthy human subjects at rest while breathing room air and, additionally, to determine whether breathing 100% oxygen prevents IPAVA from opening during a repeated EPI or DA infusion, respectively. We hypothesized that the intravenous infusion of EPI and DA would open IPAVA in healthy human subjects at rest while breathing room air. We also hypothesized that if EPI or DA had a direct effect on IPAVA by binding to a receptor-mediated vasodilatory pathway, then a repeated infusion of EPI or DA would also open IPAVA in the same healthy human subjects who breathe 100% oxygen at rest. We subsequently investigated the contribution of a β-receptor-mediated pathway in the opening of IPAVA in...
METHODS

The University of Oregon Office for Protection of Human Subjects approved this project and all subjects provided verbal and written informed consent prior to participation. All studies were performed in accordance with the Declaration of Helsinki.

Echocardiographic screening and lung function testing. Upon initial screening, 10/21 (48%) subjects demonstrated bubbles in the left heart within three cardiac cycles and were excluded from further participation due to the presence of a patent foramen ovale. Two additional subjects demonstrated one to three bubbles in the left ventricle not due to a patent foramen ovale and were also excluded. The remaining nine subjects (one female) participated in the study protocol. Spirometry, including forced vital capacity, slow vital capacity, and whole-body plethysmography were performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) standards to determine lung function indices, lung volumes, and capacities (38, 59). The single-breath, breath-hold technique was used for determination of lung diffusion capacity for carbon monoxide according to ATS/ERS standards (33) using the Jones and Meade method for timing (25).

Resting epinephrine and dopamine infusions. During a subsequent visit, a 20-gauge intravenous catheter was inserted in each arm and subjects reclined in the left lateral decubitus position at rest before and during all catecholamine infusions. EPI was diluted in sterile saline to 4,000 mg/ml and DA was diluted to 160 μg/ml and delivered at a constant rate using a Harvard Apparatus syringe infusion pump (Pump 22). Bubble injections used for transthoracic saline-contrast echocardiography (TTSCE) were injected through an intravenous catheter placed in the opposite arm. TTSCE was performed at rest, and before and during the infusions of EPI at 20, 40, 80, 160, and 320 ng·kg⁻¹·min⁻¹ for 3–4 min each with minimal breaks between each infusion rate. At the conclusion of the highest EPI infusion rate, subjects took a 30-min break before TTSCE was again performed at rest breathing room air, and then every 10 min throughout a 30-min period breathing an FIO₂ of 0.10. We have previously shown that IPAVA open in subjects at rest breathing this FIO₂ (29). Subjects were then given a 45-min break and breathed room air before the infusion of 10 mg propranolol at an infusion rate of 1 mg/min. After performing TTSCE in these subjects while still breathing room air, subjects again breathed an FIO₂ of 0.10 for 30 min with TTSCE performed at 10-min intervals.

Statistics. All statistical calculations were made using GraphPad Prism statistical software (v.5.0d) and significance was set to P < 0.05. Bubble scores for all EPI infusions in subjects breathing room air and 100% oxygen were analyzed using a Friedman’s test with Dunn’s multiple comparison posttest. Bubble scores obtained before and during all DA infusion rates, and those obtained in subjects breathing hypoxic gas mixtures were analyzed in the same manner. Mean cardiac output and pulmonary artery systolic pressure (PASP) measured during each infusion rate were analyzed using a one-way ANOVA with a Tukey posttest.

RESULTS

Subject characterization and lung function. Anthropometric, pulmonary function, and diffusion capacity data were within normal limits for all subjects and are presented in Table 1.

Bubble scores. Bubble scores for every subject breathing room air and 100% oxygen before and during the infusion of EPI and DA are presented in Fig. 1 and representative echocardiograms are presented in Fig. 2. In subjects who breathed room air during EPI infusion of 80, 160, and 320 ng·kg⁻¹·min⁻¹, bubble scores were significantly greater compared with both the 0 and 20 ng·kg⁻¹·min⁻¹ infusions. However, during EPI infusion when subjects breathed 100% oxygen, the bubble scores never increased compared with

Table 1. Subject characterization and lung function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Height, cm</td>
<td>184.9 ± 6.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.0 ± 6.9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27.8 ± 7.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.7 ± 1.3</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.52 ± 1.06 (95.1 ± 7.4)</td>
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<tr>
<td>FEV₁, liters</td>
<td>4.52 ± 0.74 (95.4 ± 6.5)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>0.82 ± 0.06 (93.9 ± 5.9)</td>
</tr>
<tr>
<td>IC, liters</td>
<td>4.51 ± 0.89 (95.6 ± 16.6)</td>
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<tr>
<td>SVC, liters</td>
<td>5.57 ± 1.06 (96.7 ± 6.6)</td>
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<tr>
<td>ERV, liters</td>
<td>3.45 ± 0.90 (94.4 ± 16.0)</td>
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<td>RV, liters</td>
<td>2.11 ± 0.56 (101.1 ± 25.2)</td>
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<tr>
<td>TLC, liters</td>
<td>3.83 ± 0.78 (104.7 ± 13.9)</td>
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<tr>
<td>FEF₂⁵–₇⁵, l/s</td>
<td>1.68 ± 0.64 (98.9 ± 10.8)</td>
</tr>
<tr>
<td>DLCO₃₁, ml·min⁻¹·mmHg⁻¹</td>
<td>7.34 ± 1.51 (100.0 ± 10.8)</td>
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<tr>
<td>DLCO₃₁, VA, ml·min⁻¹·mmHg⁻¹·1⁻¹</td>
<td>37.4 ± 6.8 (107.8 ± 11.5)</td>
</tr>
<tr>
<td>DLCO₃₁, VA, ml·min⁻¹·mmHg⁻¹·1⁻¹</td>
<td>5.4 ± 1.5 (111.9 ± 12.3)</td>
</tr>
</tbody>
</table>

Values are means ± SD (percent predicted ± SD). BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF₂⁵–₇⁵, forced expiratory flow, midexpiratory phase; SVC, slow vital capacity; IC, inspiratory capacity; ERV, expiratory reserve volume; FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; DLCO₃₁, lung CO-diffusing capacity; DLCO₃₁,VA, DLCO per alveolar volume.
the 0 ng·kg⁻¹·min⁻¹ infusion. Thus, the bubble scores during EPI infusions from 80 to 320 ng·kg⁻¹·min⁻¹ were significantly lower in subjects when breathing 100% oxygen compared to when breathing room air. In subjects breathing air, IPAVA were open in five of seven subjects at the highest infusion rate of DA; however, this set of scores was not significantly greater than the set of bubble scores observed at 0 ng·kg⁻¹·min⁻¹. When 100% oxygen was breathed, the bubble scores during 8 and 16 ng·kg⁻¹·min⁻¹ infusions were significantly lower than scores when room air was breathed.

Breathing an FIO₂ of 0.10 led to increased bubble scores, as expected, and these were not reduced after the infusion of propranolol (Fig. 3).

Cardiac output and PASP. Mean cardiac output and PASP before and during each infusion rate of EPI and DA in subjects who breathed room air and 100% oxygen are presented in Fig. 4. There were no differences in either cardiac output or PASP between room air and 100% oxygen conditions for any infusion rate. The infusions of EPI at 40 ng·kg⁻¹·min⁻¹ through 320 ng·kg⁻¹·min⁻¹ resulted in significant increases in cardiac output compared with the 0 ng·kg⁻¹·min⁻¹ infusion. DA infusion resulted in a significant increase in cardiac output only at the highest infusion rate of 16 μg·kg⁻¹·min⁻¹. Of note, cardiac output measured during the EPI infusion of 40 ng·kg⁻¹·min⁻¹ was the same as cardiac output measured during the DA infusion of 16 μg·kg⁻¹·min⁻¹ (6.17 ± 0.65 l/min vs. 6.16 ± 0.91 l/min, respectively) and the bubble scores were the same (Fig. 1).

There was a significant increase in PASP during the infusion of EPI at 80 through 320 ng·kg⁻¹·min⁻¹, while DA did not result in a significant increase in PASP for any infusion rate. Similar to cardiac output measurements, PASP measurements were the same during the infusion of EPI at 40 ng·kg⁻¹·min⁻¹ and during the infusion of DA at 16 μg·kg⁻¹·min⁻¹ (32.0 ± 6.1 mmHg vs. 31.8 ± 4.5 mmHg, respectively) and bubble scores were essentially identical. Figure 5 demonstrates bubble scores as either a function of cardiac output or as a function of PASP for all bubble injections prior to and during all EPI and DA infusions in subjects who breathed room air. These data revealed variability between individuals in either cardiac output or as a function of PASP for all bubble injections prior to and during all EPI and DA infusions in subjects who breathed room air. These data revealed variability between individuals in either cardiac output or as a function of PASP for both EPI and DA infusions.

Figure 6 demonstrates PASP as a function of cardiac output for all subjects breathing room air before and during all
infusion rates of EPI and DA and identified bubble scores <2 vs. scores ≥2, revealing that increasing cardiac output, or PASP, or both can result in open IPAVA.

DISCUSSION

The main finding of this study was that the intravenous infusion of EPI caused IPAVA to open in healthy humans at rest breathing room air, while the infusion of DA did not result in a significant increase in bubble score for any infusion rate. Additionally, β-blockade did not prevent hypoxia-induced opening of IPAVA. Cardiac output during EPI infusions ≥40 ng·kg⁻¹·min⁻¹ was significantly greater than preinfusion, while DA increased cardiac output only during a DA infusion of 16 μg·kg⁻¹·min⁻¹. PASP also increased significantly during EPI infusions ≥80 ng·kg⁻¹·min⁻¹, but did not increase for any DA infusion rate. However, both the cardiac output and PASP measured during the DA infusion of 16 μg·kg⁻¹·min⁻¹ were the same as those achieved during the infusion of EPI at 40 ng·kg⁻¹·min⁻¹ (Fig. 2) and the bubble scores for these infusion rates were the same (Fig. 1). Conversely, when subjects breathed 100% oxygen while receiving the same infusions of EPI or DA as they did breathing room air, IPAVA were prevented from opening despite similar cardiac output and PASP measurements.

Saline-contrast echocardiography. The limitations of the use of saline contrast echocardiography have been extensively discussed in previous manuscripts (8, 11, 29–31, 54). While neither the size of the bubbles nor the quantification of blood flow through IPAVA can be determined using this technique, the minimum size of bubbles entering the pulmonary microcirculation has been estimated to be 60 to 90 μm in diameter in order for the bubbles to be stable enough to survive and be visualized in the left ventricle (8). We have also shown that neither the internal nor the external partial pressure gas composition of the bubbles affect the ability of TTSCE to detect patent IPAVA (11). Furthermore, extensive anatomical evidence using solid microspheres supports the existence of large-diameter pathways existing in the pulmonary circulation (39, 46, 53, 56, 57). More recently, 50-μm microspheres have been shown to pass through the pulmonary circulation of isolated human and baboon lungs ventilated and perfused under physiologic conditions (32) and 70-μm microspheres pass through the pulmonary circulation of rats breathing hypoxic gas mixtures (1). Finally, the data obtained in the current study using
EPI injections led to vasoconstriction of conventional pulmonary arterioles when the vessels were not already under tension (23). Conversely, if pulmonary vascular tone was elevated, EPI infusion led to dilation via β-receptors, suggesting the possibility of an interplay between α- and β-receptors depending on their location within the pulmonary vasculature and the tone of the vessels. Because the infusion of EPI in our experiments led to an increase in PASP it is unlikely that vasodilation of the conventional pulmonary vasculature occurred.

At the highest DA infusion rate, IPAVA opened in five of seven subjects; however, as a group the bubble scores were not significantly increased for any DA infusion rate. This suggests that the dopaminergic receptors stimulated at low doses of DA infusion probably do not induce vasodilation of IPAVA, while the higher concentrations that stimulate α-adrenergic receptors could theoretically be preventing IPAVA from fully opening. This seems unlikely, however, because the DA concentration used in the current study has not been shown to cause pulmonary vasoconstriction (20) and is not expected to significantly increase flow or pressure (17), which is supported by our data.

**Hypoxia and β-blockade.** We chose to investigate the role of β-receptors in the hypoxia-induced opening of IPAVA during a subsequent visit in five subjects. If the hypoxia-induced opening of IPAVA occurred via a β-receptor-mediated pathway, then IPAVA would have remained closed during the second period of hypoxia. However, all five subjects demonstrated increased bubble scores while breathing an FiO₂ of 0.10 after β-receptor blockade (Fig. 3). This suggested to us that the mechanism that opens IPAVA during EPI or DA infusion was not via a β-receptor-mediated vasodilatory pathway, but rather could be due to the secondary effects of these catecholamine infusions on cardiopulmonary hemodynamics. Thus, EPI and DA infusions increased heart rate and cardiac contractility and increased vasoconstriction of the conventional pulmonary vasculature, which likely led to the increased pulmonary artery pressure and flows we observed. We suggest that it were these changes in pulmonary artery pressure and cardiac output that led to increases in blood flow through IPAVA.

Because IPAVA are open in human subjects and rats breathing hypoxic gas mixtures at rest (1, 29) and during exercise (30), this suggests that blood flow entry into these vessels is occurring upstream of the small pulmonary resistance arteries, which constrict in hypoxic environments and thus may branch from the large conducting vessels, and which demonstrate a lesser degree of hypoxic pulmonary vasoconstriction (34). This permissive or passive opening could allow for a reduction in total pulmonary vascular resistance in the face of increased pulmonary blood flow and help attenuate increases in pressure at the pulmonary capillary or right ventricular afterload as originally suggested by Stickland et al. (54). Indeed, La Gerche et al. (27) demonstrated that individuals demonstrating a greater degree of agitated saline-contrast bubbles traversing the pulmonary circulation have a lower pulmonary resistance compared with those who demonstrate a lesser degree of left-sided contrast. Future work is needed to determine the role of changes in blood flow, pressure, and hypoxic pulmonary vasoconstriction in the recruitment of IPAVA.

**Epinephrine and dopamine effects on pulmonary vasculature.** EPI and DA each have the ability to mediate pulmonary vascular dilation or constriction through their respective α, β, or dopaminergic receptor-mediated pathways. Thus, if β-adrenergic receptors are located on IPAVA, EPI could have theoretically directly induced vasodilation. Conversely, if α-receptors are located on IPAVA, EPI infusion could have induced vasoconstriction of these vessels, potentially limiting even greater flows through IPAVA. Interestingly, using a feline model, EPI injections led to vasoconstriction of conventional pulmonary arterioles when the vessels were not already under tension (23). Conversely, if pulmonary vascular tone was elevated, EPI infusion led to dilation via β-receptors, suggesting the possibility of an interplay between α- and β-receptors depending on their location within the pulmonary vasculature and the tone of the vessels. Because the infusion of EPI in our experiments led to an increase in PASP it is unlikely that vasodilation of the conventional pulmonary vasculature occurred.

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cardiac output, or PASP, or both. We demonstrated an increase in cardiac output during the infusion of 40, 80, 160, and 320 ng·kg\(^{-1}\)·min\(^{-1}\) of EPI and the three highest EPI infusion rates produced bubble scores that were significantly greater than scores at 0 ng·kg\(^{-1}\)·min\(^{-1}\) or 0 µg·kg\(^{-1}\)·min\(^{-1}\), respectively. During DA infusions, cardiac output was significantly increased from rest only at the highest infusion concentration of 16 µg·kg\(^{-1}\)·min\(^{-1}\) and resulted in the same cardiac output as that achieved during the infusion of EPI at 40 ng·kg\(^{-1}\)·min\(^{-1}\) (Fig. 4, A and B). Thus, when bubble scores achieved during the infusion of EPI at 40 ng·kg\(^{-1}\)·min\(^{-1}\) were compared with those achieved during the infusion of DA at 16 µg·kg\(^{-1}\)·min\(^{-1}\) (Fig. 1), the bubble scores were the same. We demonstrated an increase in PASP from baseline in subjects breathing room air when EPI

![Graphs showing cardiac output and PASP with different infusion rates of EPI and DA.](image)

Fig. 4. Mean cardiac output before and during each infusion concentration of EPI (A) and DA (B) in subjects breathing room air and 100% oxygen. Mean PASP before and during each infusion concentration of EPI (C) and DA (D) in subjects breathing room air and 100% oxygen. Circles and squares indicate subjects breathing room air and 100% oxygen during EPI infusions, respectively. Triangles and diamonds indicate subjects breathing room air and 100% oxygen during DA infusions, respectively. *P < 0.05 vs. 0 ng·kg\(^{-1}\)·min\(^{-1}\) or 0 µg·kg\(^{-1}\)·min\(^{-1}\), respectively. NS, no significant difference between room air and 100% oxygen.

![Bubble scores vs. cardiac output and PASP for EPI and DA.](image)

Fig. 5. A: cardiac output vs. bubble score during EPI (circles) and DA (triangles) infusions in subjects breathing room air. B: PASP vs. bubble score during EPI (circles) and DA (triangles) infusions in subjects breathing room air.
was infused at 80, 160, and 320 ng·kg\(^{-1}\)·min\(^{-1}\), while DA infusions did not result in significant increases in PASP. The minimal response by IPAVA to DA infusion may be the result of the minimal increases in cardiac output or PASP compared with those increases that occurred during EPI infusions, because the slopes of the cardiac output vs. PASP relationship were not significantly different between the EPI and DA infusions. In Fig. 5, bubble scores are plotted as a function of either (A) cardiac output or (B) PASP for all EPI and DA infusions. These graphs demonstrate further that during DA infusions the cardiac output and PASP responses were less than they were during the EPI infusions, while the bubble scores track similarly for both drugs.

If a bubble score of 1, which represents only 1–3 bubbles appearing in the left ventricle over the 20 cardiac cycles after the opacification in the right ventricle is considered to be insignificant, then a bubble score of 2 or greater can define when blood begins to flow through open IPAVA. In Fig. 6, PASP is plotted as a function of cardiac output for every bubble injection throughout the EPI and DA infusions in subjects breathing room air and indicates the combinations of flow and pressure that induced IPAVA to open. This demonstrates that there may be some combination of pressure or flow that both opens IPAVA in healthy human subjects at rest, but also that this can be accomplished by individually increasing cardiac output, increasing PASP, or by increasing both cardiac output and PASP. If large-diameter IPAVA branch from pulmonary arteries proximal to the resistance arterioles, catecholamine-induced increases in pulmonary vasoconstriction of conventional pulmonary arterioles may have increased the back pressure at the entrance to IPAVA, directing flow through these vessels even at the relatively low cardiac outputs measured in this study. As such, increasing blood flow through IPAVA may have limited changes in pulmonary artery pressure that would have otherwise occurred due to increases in conventional pulmonary arteriole tone. If \(\beta\)-blockade had reduced the bubble scores and PASP was consequently higher, this would have been evidence that blood flow through IPAVA helps limit even larger increases in pressure. However, because this did not occur, we have no direct evidence for a role of IPAVA in helping to limit increases in pulmonary artery pressure that could otherwise occur.

**IPAVA recruitment and pulmonary gas exchange efficiency.** Previous work investigating the recruitment of IPAVA in healthy humans has suggested a correlation between IPAVA recruitment and impairment of pulmonary gas exchange efficiency (30, 54). While the current study was not designed to investigate the role of IPAVA in impairing pulmonary gas exchange efficiency, recent work by Bryan et al. (5a) showed that the intravenous infusion of either dopamine or dobutamine led to an increase in physiologic shunt fraction (\(Q_s/Q_t\)), which they attributed to the opening of IPAVA. In both the study by Bryan et al. (5a) and in the current study, catecholamine infusions led to increases in cardiac output, yet because the subjects were at rest, the \(\text{VO}_2\) remained constant. Based on the Fick principle, increasing cardiac output with a constant would consequently result in an increase in mixed venous oxygen content. Thus, if blood flow through IPAVA resulted in an increase in venous admixture, we would expect minimal if any changes in \(\text{PaO}_2\) in subjects at rest because of the increase in mixed venous \(\text{PO}_2\), compared with the reduction in mixed venous \(\text{PO}_2\) that occurs during exercise. While changes in \(\text{SpO}_2\) alone can never be considered indicative of changes in pulmonary gas exchange efficiency, we would therefore expect minimal, if any, changes in our measures of \(\text{SpO}_2\) because of the minimal changes in \(\text{PaO}_2\) at the higher catecholamine infusions, combined with the flat portion of the oxyhemoglobin dissociation curve in subjects breathing room air at rest. Accordingly, we detected no change in \(\text{SpO}_2\) throughout each catecholamine infusion. Although our data do not support or refute the possibility that blood flow through IPAVA represents venous admixture, work by Bryan et al. (5a) using a separate pharmacological increase in cardiac output in which IPAVA were opened, showed that \(Q_s/Q_t\) did in fact increase. Taken together, these data suggest that blood flow through IPAVA may act as a shunt.

**Effect of breathing 100% oxygen during EPI and DA infusions on blood flow through IPAVA.** In subjects breathing 100% oxygen in the current study, we demonstrated no increase in bubble scores during the infusion of either EPI or DA. This resulted in bubble scores that were significantly less than those occurring in subjects breathing room air for EPI infusions of 40 and 320 ng·kg\(^{-1}\)·min\(^{-1}\) and for DA infusions of 8 and 16 μg·kg\(^{-1}\)·min\(^{-1}\) despite no differences in cardiac output or PASP between the room air and 100% oxygen conditions. Of note, we have previously demonstrated that neither breathing 100% oxygen nor the duration of breathing 100% oxygen affects the detection of saline contrast microbubbles in vivo (11). In subjects breathing 100% oxygen during exercise, the increased arterial oxygen content can allow cardiac output to be slightly reduced (≈10%) while maintaining a relatively constant oxygen delivery. However, the ≈10% fall in cardiac output still results in higher flows than occur during low to moderate exercise when IPAVA are known to be patent (8, 30). These data suggest that the effect of hyperoxia on IPAVA is not simply due to a reduction in pulmonary artery blood flow. This is supported by data from the current study in which the increases in cardiac output and PASP were the same in subjects breathing room air or 100% oxygen. Furthermore, the reduction in bubble scores during hyperoxic exercise is probably not
due to a reduction in the plasma concentration of EPI or DA because in the current study we infused EPI and DA at the same rate in subjects breathing room air and 100% oxygen.

These data suggest that the effect of breathing 100% oxygen on preventing IPAVA from opening is not simply due to reductions in cardiac output, PASP, or reduced plasma catecholamine concentration; rather, there may be a separate mechanism that actively closes IPAVA or prevents them from opening. The active closure of IPAVA by hyperoxia is an attractive hypothesis because oxygen has been shown to induce constriction in systemic vascular beds based on increases in systemic vascular resistance (16) and coronary vascular resistance (12), and has been implicated in hyperoxia-induced retinal vasoconstriction (7, 18, 55, 62). In the fetal circulation, the ductus arteriosus closes in response to increased oxygen tension (13), which may be mediated by oxygen-sensitive K+ channels (58). Microsphere data indicate that pulmonary blood flow is redistributed in sheep ventilated with 100% oxygen to a similar degree as occurs during hypoxic ventilation (37), which may further suggest active vasomotor activity in response to 100% oxygen. Additional microsphere data demonstrate that ventilating lungs with 100% oxygen reduces the number of large-diameter microspheres collected from the pulmonary venous effluent (39), suggesting a closure of large-diameter pathways within the pulmonary circulation. A potential mechanism could be the increase in the generation of reactive oxygen species (ROS) within 30 min of breathing hyperoxia, leading to increases in intracellular calcium concentration in pulmonary capillary endothelial cells and vasoconstriction of pulmonary vascular smooth muscle cells (5). However, 100% oxygen reduces bubble scores during exercise in healthy humans within 2 min (31), suggesting the response by IPAVA either may occur too quickly to be explained by changes in endothelial function, or that ROS signaling may be acting to close these pathways within that brief time frame when breathing 100% oxygen. Together, these data lend further support to indicate that oxygen could be directly and actively preventing blood flow through IPAVA as we have previously suggested (29). However, the mechanism or mechanisms regulating the hyperoxic closure of IPAVA remain elusive. Despite our suggestion that 100% oxygen prevents blood flow through IPAVA, our measures of cardiac output and PASP at each infusion rate do not suggest a change in peak pulmonary vascular resistance. We believe this is possible because these experiments spanned relatively low cardiac outputs, and that recruitment and distension of the conventional pulmonary vasculature are able to accommodate the blood flow that would normally be perfusing IPAVA, preventing an increase in resistance.

**Similarities between IPAVA and supernumerary arteries.** At rest, IPAVA are not perfused, but we have shown that increases in cardiac output, PASP, or both may recruit these vessels. This is supported by previous work in which Stickland et al. (54) suggested such passive recruitment. Interestingly, the supernumerary arteries first described by Elliott and Reid (10) were also described as being closed under resting conditions, but could be recruited during increases in flow. Because supernumerary arteries branch at right angles from the conventional arteries, blood flow may preferentially follow the more direct conventional artery branching pattern unless increases in pressure, flow, or both, such as during exercise or when breathing hypoxic gas mixtures, direct blood flow through them. Recaverran (48) also described the recruitment of “pre-terminal arterioles” as branching from pulmonary arteries at a 90-degree angle, being closed at rest, and recruited with increases in pulmonary artery pressure (Fig. 7). Shaw et al. (52) further described the dynamic opening of supernumerary arteries as occurring when the conventional artery from which

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**Fig. 7.** Modified schematic of a “preterminal arteriole” branching at a right angle from a pulmonary artery that is closed under resting conditions, but opens due to increased PASP, or cardiac output, or both and delivers a portion of the blood flow to the distal end of the pulmonary capillary bed, effectively acting as an arteriovenous anastomoses. B.P.A., medium sized branch of the pulmonary artery; a, pulmonary arteriole; P.A., preterminal arteriole; P.V., pulmonary venule; Art.Cp., arterial capillary; V.Cp., venous capillary; Cp.Bed, capillary bed. [Reproduced with permission from Recaverran (48)].
they branch is physically stretched. Increases in cardiac output, PASP, or both would accomplish this physical distension of conventional arteries and thus open the baffle valve at the entrance of supernumerary arteries. Additional anatomical evidence for the opening of large-diameter IPAVA is provided in a study by Berk et al. (4) in which the opening of large “angiodi structures” were visualized in lung biopsies taken during EPI infusion, but appeared closed in biopsies taken prior to and after the end of EPI infusion. Furthermore, they suggested that EPI caused a change in pulmonary resistance, which redistributed pulmonary blood flow and would fit with our data suggesting a role for changes in cardiac output, PASP, or both.

Summary. We have demonstrated for the first time that EPI opens IPAVA in healthy humans at rest, which supports previous anatomic work in dogs in which EPI infusions caused an increase in the transpulmonary passage of radioactive microspheres (40). While a direct effect of EPI or DA on IPAVA smooth muscle is a possibility, β-receptor-mediated dilatation of IPAVA appears unlikely. Rather, it appears more likely that the resulting catecholamine-induced increases in either cardiac output, or PASP, or both due to EPI or DA infusions may be passively opening IPAVA, as was first proposed by Stickland et al. (54). IPAVA are prevented from opening in subjects breathing 100% oxygen during either EPI or DA infusions, which suggests that the hypoxic closure mechanism is independent from the opening mechanism and requires further investigation.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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