Effects of active vasoconstriction and total flow on perfusion distribution in the rabbit lung

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Effects of active vasoconstriction and total flow on perfusion distribution in the rabbit lung. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1465–R1473, 1997.—We analyzed the effects of hypoxic vasoconstriction and total flow on the distribution of pulmonary perfusion in 38 isolated left rabbit lungs perfused under zone 3 conditions. Lungs were suspended in an upright position, oriented to the apicobasal line. Distributions of regional perfusion rates (RPR) along the vertical and horizontal axes were determined using nonradioactive microspheres labeled with heavy metal elements, which were detectable with X-ray fluorescence spectrometry. Changing the O2 concentration of a respirator and an extracorporeal membrane oxygenator independently, respective influences of active vasoconstriction; isolated perfused lung; nonradioactive microsphere; gravity

regionally pulmonary perfusion; hypoxic pulmonary vasoconstriction; isolated perfused lung; nonradioactive microsphere; gravity

ZONAL CONCEPTS were proposed by West et al. (25) to explain vertical differences in the regional pulmonary perfusion rate (RPR), indicating that gravity is a decisive factor in the distribution of RPR. However, several recent studies (2, 3, 8–14, 16, 23) have demonstrated that there are multiple phenomena that may not be explained solely by a gravity-dependent mechanism. These recent data suggest that a distinct mechanism, unrelated to gravity, may work as one of the determinants of RPR, indicating that distributional changes in RPR induced by certain stimuli should be attributed to both the gravitational and the nongravitational effects.

Hypoxia in alveolar regions (alveolar hypoxia) is a physiologically important factor regulating an appropriate match between ventilation and pulmonary blood flow through active vasoconstriction [i.e., hypoxic pulmonary vasoconstriction (HPV)]. The effects of HPV on the RPR distribution have been observed and analyzed using data from the gravitational aspect (5–7, 22, 24). To the best of our knowledge, however, there has been no systematic study [except in that reported by Kuwahira et al. (19), which focused on nongravitational aspects] about the effects of HPV on the RPR distribution. Although Kuwahira et al. (19) reported that HPV modulates the RPR distribution apart from gravity, their findings might not eliminate the importance of gravity as a possible determinant for RPR, because they used small rat lungs, which are expected to be minimally influenced by gravitational effects. Furthermore, since previous studies analyzing the effects of HPV on the distribution of RPR were performed under in vivo conditions, the effects of active vasoconstriction induced by hypoxia may not have been distinguishable from those caused by enhanced cardiac output, which generally accompanies alveolar hypoxia. In addition, no study has been attempted to clarify the potential roles of hypoxia in the pulmonary arteries (PA hypoxia) in determining the RPR distribution, from either the gravitational or the nongravitational aspect, although PA hypoxia is believed to cause some degree of HPV (20).

This study was designed to analyze regional pulmonary perfusion, influenced by hypoxic vasoconstriction and total flow, not only from the vertical (gravitational) but also from the horizontal (nongravitational) aspect, under zone 3 conditions. The distributions of RPR were determined in the left lungs divided into four isogravitational planes along the apicobasal line. Each plane was further partitioned into several pieces in the ventral-to-dorsal direction. RPR in each piece was measured on the basis of the X-ray fluorescence activity of heavy metal elements.

MATERIALS AND METHODS

Nonradioactive microspheres and X-ray fluorescence spectrometry. The use of nonradioactive microspheres and X-ray fluorescence spectrometry for measurement of blood flow distribution has previously been described in detail (18, 21). Briefly, nonradioactive microspheres (Sekisui Plastic) made of inert plastic were labeled with different types of stable heavy metal elements. The average diameter was 15 μm. The
X-ray fluorescence activity of a stable heavy metal element was examined with a wavelength dispersive spectrometer (PW 1480, Phillips) and was used as a measure of the RPR. Microspheres labeled with barium, iodine, zirconium, or bromine were used in the present study.

Isolated perfused lungs. Detailed description of isolated perfused lung preparation was given elsewhere (26–27). Briefly, male rabbits (weighing from 2.5 to 3.0 kg, n = 38), were anesthetized with an intravenous injection of pentobarbital sodium (25 mg/kg). Heparin (3,000 U) was also injected intravenously. Tracheotomy was performed, and the rabbits were ventilated by means of a respirator (model 665, Harvard Apparatus) with room air. After a median sternotomy, the main pulmonary artery and the left atrium were cannulated using rigid polyethylene tubes with diameters of 4.5 and 2.5 mm, respectively. Blood was collected from the left ventricle prior to the atrial cannulation. Thereafter, the lungs and heart were removed en bloc and suspended in an upright position, oriented to the apicobasal line, by the tracheotomy tube. The right lung was separated by ligating the hilum, and only the left lung was ventilated with a tidal volume of 20 ml and at a respiratory rate of 20 breaths/min. Positive end-expiratory pressure of 2 mmHg was also applied to prevent the lung from collapsing. The left lung height was −4 cm, whereas its width at the base was −5 cm when it was suspended in an upright position. The left lung thus prepared was perfused with a modified Krebs-Henseleit buffer. Three percent bovine serum albumin was added to maintain isosmotic pressure. Allogenic red blood cells washed with cold phosphate-buffered saline, the pH of which had been adjusted to 7.4, were also added to the perfusate, producing a final hematocrit of 4.9 ± 0.2% (mean ± SE, n = 38). We used only the left lung to avoid disparities in the distribution of pulmonary perfusion between the right and left lungs. Because preliminary experiments showed that the flow distribution between the right and left lungs was very sensitive to the position of the catheter inserted into the main pulmonary artery (data not shown), we used only the left lung for determining the regional distribution of blood flow. Perfusion was established under zone 3 conditions, achieved by adjusting the height of the perfusate reservoir. The perfusate was pumped from the reservoir through an extracorporeal membrane oxygenator (ECMO) with a surface area of 0.5 m² and a hematocrit of 4.9 ± 0.2% (mean ± SE, n = 38). The perfusate containing blood was injected into the pulmonary arterial tube through the PE-50 catheter and then washed with cold saline for 30 min. The microspheres were suspended in 1.0 ml of 0.1% sodium dodecyl sulfate, was injected into the pulmonary arterial tube with a hand-held magnet. A PE-50 catheter was inserted into the pulmonary arterial tube from a side port placed 30 cm upstream from the pulmonary artery. The microspheres were injected through the catheter. Just downstream from the tip of the catheter, the pulmonary arterial tube was looped, and a small iron ball with a diameter of 3 mm was placed in the looped tube to achieve a uniform microsphere-perfusate mixture. The temperature of the perfusate was maintained at 37°C with a heat exchanger. The lungs and heart were covered with a thin vinyl film to maintain sufficient humidification and prevent heat loss from the perfused lung surface. The total circulating volume was adjusted to 300 ml. Pulmonary arterial pressure (Ppa) and left atrial pressure (Pla) were continuously monitored with pressure transducers (TP-400T, Nihon Kohden) connected with small cannulas whose tips were located in the larger catheters inserted into the main pulmonary artery and the left atrium and recorded throughout the experiment (RMP-6008M, Nihon Kohden). The zero point of vascular pressures was set at the top of the apex. Airway pressure was continuously measured, in a similar way, at the end of the tracheotomy tube. Lung weight was also recorded continuously with a counterbalanced force-displacement transducer (TB-611T, Nihon-Kohden). Pulmonary vascular resistance (PVR) was calculated by dividing the difference between Ppa and Pla by the total perfusion rate. Perfusate Po2, PcO2, and pH were measured with appropriate electrodes (model 1306, IL).

Experimental conditions. Four different conditions were obtained by changing the fractional concentrations of O2 of the respirator and ECMO and also the perfusion rate. For control (n = 13), both the respirator and ECMO delivered a gas mixture composed of 95% O2-5% CO2. For PA hypoxia (n = 10), ECMO ventilation was carried out with 2% O2-5% CO2, whereas isolated lungs were ventilated with 95% O2-5% CO2 by the respirator. Under all the experimental conditions described above, the perfusion rate was fixed at 0.4 ml·min⁻¹·g wet lung tissue⁻¹, corresponding to 40–50 ml/min, depending on the left lung weight (control-flow groups). For the increased-flow group (n = 6), the perfusion was made at a flow rate of 1.2 ml·min⁻¹·g wet lung tissue⁻¹, yielding the total flow rate of 120–150 ml/min in the left lung, and the gas mixture containing 95% O2-5% CO2 was supplied through both the respirator and ECMO. For the control group, the perfusion rate was adjusted to a level similar to that in the control by changing the height of the reservoir.

Microsphere injection. Just before microsphere injection, the ventilator was switched off at the end-expiratory position. Immediately thereafter, the tracheal tube was shut to maintain the lung volume at functional residual capacity. The reservoir height was finely adjusted so that mean Ppa was held at the level exceeding airway pressure by 1 mmHg, thus ensuring that the zone 3 condition was certainly established during microsphere injection. Under each experimental condition, only one kind of the microsphere was injected after pressures of airway, pulmonary artery, and left atrium had been stabilized. A total of 1.0 × 10⁶ microspheres, suspended in 1.0 ml of 0.1% sodium dodecyl sulfate, was injected into the pulmonary arterial tube through the PE-50 catheter and then flushed with 1.0 ml of normal saline over 2 min. Immediately after microsphere injection, the reservoir was refilled. Before and during the injection, the suspension was mixed mechanically in a syringe to avoid aggregation of the spheres. Injected microspheres were also thoroughly mixed with the perfusate in the looped tube by moving the iron ball within the tube with a hand-held magnet.

Determination of RPR. Perfusion was stopped at 5 min after microsphere injection. Perfusate containing blood was rinsed from the lung. Thereafter, the left lung was fixed at a total lung capacity with 40 ml of 10% formaldehyde injected into the trachea at a pressure of 20 cmH₂O. The fixed lung was then divided into 17 pieces (Fig. 1). First, the left lung was cut into four horizontal planes. Planes 1–4 were defined as isogravitational planes at the apical region, upper-middle lung field, lower-middle lung field, and the base, respectively. Each horizontal plane was subsequently divided into three to five pieces according to the vascular anatomy of the rabbit lung, which had been preliminarily determined by soft X-ray analysis using contrast medium injected through the main pulmonary artery. In the left lung, a large artery ran straight from the hilum to the base. Small arteries branched off from the large artery almost perpendicularly. Another small artery, exiting the hilum and running toward the apex, was also identified. Plane 1 was partitioned into pieces 1–3, all of which were located in the upper lobe. The small artery was located in piece 2. Plane 2 was divided into pieces 4–8. Both
the hilum and the large artery were contained in piece 7. Piece 4 and most of pieces 5 and 6 belonged to the upper lobe, whereas half of piece 7 belonged to the lower lobe. Similarly, plane 3 was cut into pieces 9–13, all of which were located in the lower lobe. Piece 11 contained the large artery. Plane 4 was partitioned into pieces 14–17, all belonging to the lower lobe. Among these 17 tissue samples, pieces 1, 4, 9, and 14 were close to the ventral portion of the thorax and as such were defined as the ventral region in each horizontal plane. Because pieces 3, 8, 13, and 17 were in contact with the dorsal thorax, they were considered to be the dorsal region in the plane. Remaining pieces were defined as medial regions. The lung sectioning was performed with a sharp knife in the same way, by one of the authors, being as careful with the reproducibility as possible. Visible connective tissue, vessels, and airways were all removed. The tissue samples were dried at 60°C and weighed. The samples were placed in hard plastic tubes and completely dissolved by adding 40 ml of 2 N KOH solution at 60°C followed by at least 2 days incubation. The solutions were centrifuged (3,000 revolutions/min for 10 min). After the supernatant had been discarded, the precipitate was aspirated and trapped on filter papers. The X-ray fluorescence activity of the heavy metal containing microspheres was determined with an X-ray spectrophotometer.

Gas analysis and stability of isolated perfused lungs. \( P_{O_2} \) values in the perfusates entering and leaving the lung were, respectively, 540 ± 36 and 535 ± 25 mmHg under control conditions \( (n = 13) \). Reducing the inspired \( O_2 \) concentration to 2%, while maintaining 95% \( O_2 \) with ECMO (alveolar hypoxic condition), decreased \( P_{O_2} \) in the perfusion medium leaving the lung at 49 ± 9 mmHg \( (n = 5) \), a value considerably lower than that obtained for the perfusate flowing into the lung \( (643 ± 14 \text{ mmHg}, n = 9) \). In contrast, \( P_{O_2} \) in the perfusate entering the lung was reduced to 40 ± 4 mmHg, whereas that leaving the lung was maintained at 499 ± 47 mmHg under PA hypoxic conditions \( (n = 10) \). In the increased-flow group, \( P_{O_2} \) values in the perfusion medium entering and leaving the lung were, respectively, adjusted to 567 ± 10 and 537 ± 24 mmHg \( (n = 6) \), values not statistically different from those obtained for the control group. The \( pH \) in the perfusion circuit was adjusted at 7.4 and did not differ significantly among any experimental conditions employed.

Increases in lung weight during perfusion were very subtle. Lung weight during perfusion increased by 0.1 ± 0.1, 0.1 ± 0.0, 0.2 ± 0.1, and 0.3 ± 0.1 g in the control \( (n = 13) \), alveolar hypoxia \( (n = 9) \), PA hypoxia \( (n = 10) \), and increased-flow \( (n = 6) \) groups, respectively, indicating minimal pulmonary edema under these experimental conditions.

Both \( P_{pa} \) and \( P_{pa} \) were maintained at fairly constant levels during the experimental period.

Statistical analysis. \( P_{O_2} \), \( P_{CO_2} \), and \( pH \) in the perfusate entering and leaving the lung under each experimental condition were statistically evaluated by the paired t-test. Significant differences in various indexes among the three different experimental conditions, i.e., control, alveolar hypoxia, and PA hypoxia, with a fixed total perfusion rate were evaluated with one-way analysis of variance (ANOVA) followed by multiple-comparison analysis using the Scheffé method. Statistically significant differences between the control-flow and increased-flow groups were evaluated with the nonpaired t-test. Two-way ANOVA, followed by multiple comparison by the Scheffé method was used to compare RPR in different planes and among different pieces in the same plane under each experimental condition. Reproducibility of microsphere technique was examined by calculating the limits of agreement proposed by Bland and Altman \( (4) \). \( P < 0.05 \) indicated statistically significant differences. Values are means ± SE.

RESULTS

Gas analysis and stability of isolated perfused lungs. \( P_{O_2} \) values in the perfusates entering and leaving the lung were, respectively, 540 ± 36 and 535 ± 25 mmHg under control conditions \( (n = 13) \). Reducing the inspired \( O_2 \) concentration to 2%, while maintaining 95% \( O_2 \) with ECMO (alveolar hypoxic condition), decreased \( P_{O_2} \) in the perfusion medium leaving the lung at 49 ± 9 mmHg \( (n = 9) \), a value considerably lower than that obtained for the perfusate flowing into the lung \( (643 ± 14 \text{ mmHg}, n = 9) \). In contrast, \( P_{O_2} \) in the perfusate entering the lung was reduced to 40 ± 4 mmHg, whereas that leaving the lung was maintained at 499 ± 47 mmHg under PA hypoxic conditions \( (n = 10) \). In the increased-flow group, \( P_{O_2} \) values in the perfusion medium entering and leaving the lung were, respectively, adjusted to 567 ± 10 and 537 ± 24 mmHg \( (n = 6) \), values not statistically different from those obtained for the control group. The \( pH \) in the perfusion circuit was adjusted at 7.4 and did not differ significantly among any experimental conditions employed.

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Reproducibility of microsphere technique. Before investigating the distribution of RPR, we examined the reproducibility of consecutive injections, performed at 10-min intervals under control conditions, of micro-
spheres labeled with different heavy metals. The linear regression equation estimated from 34 data points was
\[ y = 1.0x + 3.3 \quad (r = 0.97, \ P < 0.0001) \], where \( x \) is RPR calculated from the first injection, and \( y \) is the value from the second injection. Mean and standard deviation of differences between \( x \) and \( y \) were \(-1\) and \(0.8\), respectively, resulting in the 95% confidence interval for the differences between two measurements to be
\[-2.6\] and \[0.6 \text{ ml·min}^{-1} \cdot \text{g}^{-1}\]. The values appeared to be fairly small, indicating the acceptable reproducibility of RPR determined by injecting microspheres labeled with various heavy metals into isolated perfused lungs.

The dry weights of pulmonary tissue samples cut into 17 pieces averaged \(35 \pm 1.0\) \(\text{mg} \) (\(n = 646\)). Pieces with the same number did not vary markedly in weight among the four experimental conditions employed.

Vascular pressures and PVR. \(Ppa\) was significantly elevated in the alveolar hypoxia group by \(-4\) mmHg on the average (Table 1). PVR also increased in alveolar hypoxia group. \(Ppa\) in the increased-flow group was augmented to a level comparable to that observed under conditions of alveolar hypoxia, but PVR was decreased. \(Pia\) in the alveolar hypoxia and increased-flow groups were essentially the same as that of the control group. The PA hypoxia group showed \(Ppa\), \(Pia\), and PVR values statistically identical to those obtained in the control.

Distribution of RPR along vertical axis. In control, RPR increased stepwise from the apex to the base (Fig. 2), i.e., RPR in the lower-middle lung field (plane 3) and that at the lung base (plane 4) were about four times larger than that at the apex (plane 1), leading to distinct inhomogeneity in the RPR distribution along the vertical axis.

In alveolar hypoxia, compared with control, higher RPR in the apical (plane 1), as well as upper-middle lung fields (plane 2), but lower RPR at the lung base (plane 4) were observed. In addition, RPR at the lung base (plane 4) was significantly lower than that in the upper-middle lung field (plane 2). Consequently, alveolar hypoxic conditions appeared to evoke an inhomogeneous distribution of RPR along the vertical axis, which was qualitatively different from that obtained in control.

In PA hypoxia, RPR in the upper-middle lung field (plane 2) tended to increase, in contrast to control, whereas increase in RPR at the apex (plane 1), which was evident under alveolar hypoxic conditions, was not observed. The RPR in the apical region (plane 1) was clearly diminished compared with those in other planes. Furthermore, the RPR at the lung base (plane 4) was somewhat lower than that in the lower-middle lung field (plane 3) but well above that at the apex (plane 1), resulting in an RPR distribution that would be qualitatively intermediate between control and alveolar hypoxia.

Increased flow rate produced a higher RPR than that of the control in all planes. The distribution of RPR in increased-flow group was found to be inhomogeneous because RPR at the lung base (plane 4) was significantly lower than those in other planes.

Horizontal distribution of RPR in control. No significant differences in RPR were observed among the pieces at the apex (plane 1) in control (Fig. 3). Qualitatively the same trend was observed in the upper-middle lung field (plane 2), resulting in a fairly homogeneous distribution of RPR in the isogravitational planes mainly located in the upper lobe. Control group showed decreased RPR in the ventral piece (piece 9), whereas RPR was increased in the medial portions (pieces 10–12) in the lower-middle lung field (plane 3). At the lung base (plane 4), RPR in the dorsal region (piece 17) was significantly reduced, leading to an inhomogeneous distribution of RPR along the horizontal axis in the isogravitational planes in the lower lobe.

Horizontal distribution of RPR in alveolar hypoxia. Although alveolar hypoxia showed enhanced total RPR in plane 1 (Fig. 2), compared with those of control, the augmented flow seemed to be fairly homogeneously distributed (Fig. 3), as there was no significant difference in RPR among any piece of plane 1. Total RPR in plane 2 was also higher than that in control (Fig. 2). Because RPR values in pieces 4–6 were much higher than those observed in control, the enhanced flow in plane 2 under alveolar hypoxic conditions seemed to be caused by the increase in RPR in the ventral and

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Table 1. Vascular pressures and pulmonary vascular resistance

<table>
<thead>
<tr>
<th>Condition</th>
<th>(Ppa) (mmHg)</th>
<th>(Pia) (mmHg)</th>
<th>PVR (mmHg·ml·min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td>9.6 ± 0.6</td>
<td>2.9 ± 0.2</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Alveolar hypoxia (n = 9)</td>
<td>13.7 ± 1.2*(\dagger)</td>
<td>2.9 ± 0.2</td>
<td>0.27 ± 0.03*(\dagger)</td>
</tr>
<tr>
<td>PA hypoxia (n = 10)</td>
<td>9.1 ± 0.7</td>
<td>2.9 ± 0.2</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Increased flow (n = 6)</td>
<td>13.9 ± 0.9*(\dagger)</td>
<td>3.2 ± 0.3</td>
<td>0.10 ± 0.01*(\dagger)</td>
</tr>
</tbody>
</table>

* Significantly different vs. control, † significantly different vs. PA hypoxia.

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Values are means ± SE. \(Ppa\), pulmonary arterial pressure; \(Pia\), left atrial pressure; PVR, pulmonary vascular resistance; PA, pulmonary artery. 

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Fig. 2. Regional pulmonary perfusion rate (RPR) of planes. Vertical bars, SE; PA, pulmonary artery. * Significantly different from control, #significantly different from PA hypoxia; a–c, statistical difference vs. planes 1–3, respectively. Flow to each lung plane is given as \(\text{ml·min}^{-1} \cdot \text{g dry weight of plane}^{-1}\).
medial pieces. In plane 3, alveolar hypoxia apparently lowered the RPR in the dorsal piece (piece 13), in which the RPR was markedly reduced compared with those in other pieces, including pieces 9–11. Furthermore, RPR in piece 13 was significantly lower than that obtained in control. However, there was no significant reduction in RPR in the ventral portion (piece 9) compared with that in control. Although total RPR in plane 4 was reduced in alveolar hypoxia (Fig. 2), RPR distribution was qualitatively similar to that in control, i.e., significantly reduced RPR in piece 17 compared with that in piece 14. However, the most remarkable finding was that RPR values in pieces 15 and 16, in the setting of alveolar hypoxia, were diminished, whereas those in pieces 14 and 17 did not differ significantly from those in control.

Horizontal distribution of RPR in PA hypoxia. RPR in any of the pieces in plane 1 was statistically comparable to that in control (Fig. 3). Although total RPR in plane 2 was significantly higher than that in control (Fig. 2), the increased flow appeared to be mainly distributed to the medial region (piece 6), the trend being qualitatively similar to that observed in alveolar hypoxia. In plane 3, RPR in the medial regions (pieces 10 and 11) was significantly higher than that in either the ventral (piece 9) or the dorsal (piece 13) piece. There was considerable reduction in RPR in piece 13, causing a distinct inhomogeneous RPR distribution in plane 3. In plane 4, diminished RPR in the dorsal region (piece 17) was observed, again inducing an inhomogeneous RPR distribution.

Horizontal distribution of RPR at increased flow rate. Although RPR values in most of the pieces in increased-flow group were obviously high compared with those in control-flow group (Fig. 4), the RPR in piece 13 (plane 3) was much lower than that obtained in control. Total RPR in plane 4 was increased by a factor of three when compared with control (Fig. 2). However, only the ventral (piece 14) and medial (piece 15) regions received higher RPR than those in control, whereas the RPR in other pieces (pieces 16 and 17) did not differ signifi-

![Fig. 3. Effects of hypoxic pulmonary vasoconstriction (HPV) on RPR distribution in the isogravitational plane. Vertical bars, SE. *Significantly different from control, #significantly different from PA hypoxia; a–g, statistical difference vs. pieces 9–12 and 14–16, respectively. Flow of each lung piece is presented as ml·min⁻¹·g dry weight of the piece⁻¹.](http://ajpregu.physiology.org/)

![Fig. 4. Effects of increased flow rate on RPR of pieces: □ and △, control flow (0.4 ml·min⁻¹·g wet lung tissue⁻¹) and increased flow (1.2 ml·min⁻¹·g wet lung tissue⁻¹), respectively. Vertical bars, SE. *Significantly different from control; a–i, statistical difference vs. pieces 1, 4, 6, 9, 10–12, 14, and 15, respectively. Flow into each lung piece is given as ml·min⁻¹·g dry weight of the piece⁻¹.](http://ajpregu.physiology.org/)
cantly from those in control. RPR distribution in increased-flow group was remarkably inhomogeneous in any of the isogravitational planes, i.e., RPR values in the ventral portions (pieces 1, 4, and 9) in planes 1–3 were, respectively, diminished compared with those in the medial portions, including pieces 2, 6, 10, and 11. In addition, the RPR in the dorsal regions (pieces 8, 13, and 17) in planes 2–4 were markedly reduced in contrast to RPR in the medial regions (pieces 6, 10–12, and 15).

DISCUSSION

Critique of methods. We examined RPR, subdividing the left lung into 17 pieces, which were not as numerous as those reported by Glenny et al. (8–11), who had analyzed RPR in the canine lung. This was due not only to substantially smaller lung size of the rabbit but also to the detection limit of X-ray dispersive spectrometer applied in the current study. The preliminary examination showed that fluorescence activity of a heavy metal labeling microspheres was unable to be precisely detected when the lung parenchyma was cut into >17 pieces. Although increase in the number of injected microspheres allowed detection of the fluorescence signal emitted from the lung piece with much smaller size, it made the perfused lung system incompletely unstable. As demonstrated by Bassingthwaighte et al. (1) as well as Glenny et al. (8–11), the size of the piece inspected has a significant influence on the estimated magnitude of perfusion inhomogeneities, i.e., the smaller the sample size, the higher the sensitivity for detecting perfusion inhomogeneities, suggesting that the extent of RPR inhomogeneities obtained in the current study may be underestimated because of a relatively large sample size.

The other crucial issue warranting consideration is that we conducted the perfusion of isolated lungs with the buffer containing 3% albumin and a small quantity of erythrocytes (hematocrit, 4.9%), indicating that the perfusate viscosity would appreciably differ from that of the whole blood. Therefore, we cannot preclude the possibility of some inconsistency between the RPR determined in the current study and that under in vivo conditions. However, perfusion with the buffer having a physiological hematocrit made the isolated lung system very unstable at the time of microsphere injection, especially under alveolar hypoxia and increased-flow conditions. Therefore, we preferred the perfusate with a low hematocrit, in which all the necessary experiments had been able to be securely performed.

Increased flow rate distinctly reversed the RPR distribution along the vertical axis compared with that in the control (Fig. 2), such a phenomenon having not been observed in the experiments done under in vivo conditions (5–7, 19, 22, 24). We believe that this may be attributable to our specific experimental conditions. In the current study, the lung is excised from the thorax, i.e., the lung is not supported by the thoracic wall structure, which significantly confines the overall lung configuration during the lung distention. Without the thoracic wall, the lung expansion caused by increased flow may not coincide with that observed under in vivo conditions.

Gravitational-dependent and -independent mechanisms determining RPR. Since West et al. (25) proposed the zonal concept to explain the vertical difference in regional pulmonary blood flow, gravity has been considered to be a principle factor governing the distribution of pulmonary blood flow at a constant cardiac output. They showed that regional pulmonary perfusion rates could increase down the lung, along the gravitational axis, depending on the mechanical interactions of vascular hydrostatic and alveolar surface pressures. However, the zonal concept could not account for the relative lack of a vertical gradient in pulmonary blood flow in prone dogs (14). Several groups of investigators (2, 3, 8–11, 13, 14, 19) proposed that the gravity-independent differences in regional pulmonary blood flow may exist and are attributed to resistances that are firmly determined by the anatomic structure of the pulmonary vasculature. Most of the studies as described above were done under in vivo conditions, in which various zones were expected to exist. To avoid the complicated effects of different zones on determining regional pulmonary blood flow, we used isolated lungs and examined the perfusion distribution under only the zone 3 condition, in which pulmonary capillaries were expected to be perfused more homogeneously than in other zones. Despite these efforts to eliminate gravitational effects causing zonal variation, RPR along the vertical axis determined at flow rate of 0.4 ml·min⁻¹·g wet lung tissue⁻¹ was distinctly inhomogeneous and was augmented down the lung (Fig. 2). As proposed by West et al. (25), the RPR distribution under zone 3 conditions may be affected by gravity to some extent. Although the driving pressure (Ppv − pulmonary venous pressure (Ppv)) remains fixed down the zone 3 lung because gravity causes Ppa and Ppv to increase equally per centimeter of distance, the degree of pulmonary vessel distention may be variable depending on the transmural pressure difference, which is influenced by gravity and increases down the zone. Thus our findings are consistent with the concept of gravity working as one of the important factors affecting the RPR distribution, at least in rabbit lungs. However, we also found distinct RPR differences within isogravitational planes, especially in the lower parts of the lung (Fig. 3). The finding requires the gravity-independent factor as another important mechanism for determining RPR distribution.

Effects of active vasoconstriction on RPR distribution along the vertical axis. Since Fowler and Read (7) reported increasing blood flow to the upper regions of the lung during acute exposure to inspired gas with a low O₂ concentration, alveolar hypoxia has been known to exert an antigravitational effect on the RPR distribution (5–7, 22, 24). The antigravitational redistribution of pulmonary blood flow during alveolar hypoxia has been attributed to capillary recruitment in the upper portion of the lung along with an elevation in pulmo-
nary arterial pressure (5, 22, 24). It has also been suggested that selective vasoconstriction in the lower lung plays a part in this redistribution (6, 7). However, earlier studies were performed under in vivo conditions, suggesting that the results might be influenced by enhanced cardiac output, usually accompanied by acute exposure to alveolar hypoxia, in addition to the active vasoconstriction caused by hypoxia. As described below, enhanced flow may contribute to vascular expansion and/or capillary recruitment in upper lung fields, thereby leading to potential difficulty in separating the effects derived solely from active vasoconstriction from those due to increased flow. To overcome this difficulty, we examined the role of alveolar hypoxia in regulating the distribution of pulmonary perfusion in isolated perfused lungs at a fixed flow rate. The experimental results showed that alveolar hypoxia significantly shifted pulmonary perfusion upward, whereas lung base pulmonary perfusion was diminished compared with other planes (Fig. 2). These findings may indicate that active vasoconstriction induced by exposure to alveolar hypoxia is substantially inhomogeneous down the gravitational axis and occurs mainly at the lung base. Our experimental findings are consistent with those observed under in vivo conditions (5–7, 22, 24).

Low PO2 in pulmonary arterial blood (PA hypoxia) is predicted to operate as a physiological determinant of pulmonary perfusion distribution. To the best of our knowledge, however, no previous study has analyzed the direct effects of PA hypoxia on the regional distribution of pulmonary perfusion. This may reflect the difficulty encountered in observing the effects of PA hypoxia alone in the in vivo condition. To overcome this obstacle, we used an isolated lung preparation with two oxygennators, including an artificial respirator connected to the trachea and ECMO, the latter of which allowed us to separately adjust gas compositions in the pulmonary artery. Hyman et al. (17) reported that PA hypoxia appeared to induce significant HPV in cats, whereas Hauge (15) reported that PA hypoxia alone was not capable of inducing HPV in isolated rat lungs. Our experimental results are compatible with those reported by Hauge (15), i.e., overall PVR was not elevated during PA hypoxia induced by a gas mixture containing 2% O2, through ECMO (PO2 in pulmonary artery, 40 mmHg), while maintaining the inspired O2 concentration in the respirator at 95%. Although overall PVR was not significantly increased, PA hypoxia produced an RPR distribution along the vertical axis that was qualitatively different from that obtained in control (Fig. 2). First, PA hypoxia augmented RPR in the upper-middle lung field (plane 2). Second, PA hypoxia significantly diminished RPR at the lung base (plane 4) compared with that in the lower-middle lung field (plane 3), a finding qualitatively consistent with that in alveolar hypoxia, although the extent was less marked but different from that observed in control. These findings suggest that, although overall pressure change in the pulmonary circulation is not evident, PA hypoxia causes local vasoconstriction that may take place mainly at the lung base just as it does under alveolar hypoxic conditions.

Effects of active vasoconstriction on RPR distribution along the horizontal axis. Analysis of RPR within isogravitational planes demonstrated that hypoxia-induced pulmonary vasoconstriction, which reduces the RPR there, does not occur homogeneously even in the isogravitational plane (Fig. 3). Our experimental results suggest that the basomedial region has relatively high reactivity to, but basodorsal region is insensitive to, hypoxic stimulation. On the other hand, the dorsal area in the lower-middle lung field as well as the dorsal area or nearby area in the upper-middle lung field have a relatively high reactivity responding to hypoxic stimulation. At the apex, alveolar hypoxia showed a fairly homogeneous RPR distribution along the horizontal axis, indicating that vascular reactivity in the apical region would be similar. However, we may not conclude that all vessels in the apical region have the essentially identical reactivity to hypoxia, as the degree of RPR inhomogeneities predicted in the current study may be somewhat distorted due to a relatively large sample size (1, 8–11). These findings may imply that hypoxia-induced active vasoconstriction exerts a significant redistribution of pulmonary perfusion in the isogravitational direction within most of the lung fields excepting the apical regions.

During PA hypoxia, qualitatively the same trend as that obtained at alveolar hypoxia was investigated only in the upper-middle lung field in which the RPR at the medial portion was appreciably augmented (Fig. 3). This may be explained by the extent of vasoconstriction due to PA hypoxia alone not being as strong as that indicated by alveolar hypoxia (Table 1).

To the best of our knowledge, this may be the first report providing a direct evidence for local differences in hypoxia-elicited active vasoconstriction from both gravitational and nongravitational aspects.

Effects of increased flow on RPR along the vertical and horizontal axes. The difference in RPR at the lung base between control-flow and increased-flow groups was only 40 ml·min⁻¹·g tissue dry weight⁻¹, whereas that in other planes ranged from 130 to 240 ml·min⁻¹·g tissue dry weight⁻¹ (Fig. 2), indicating that the vessels at the lung base were already distended or almost opened at control-flow condition and would behave as if they had the low vascular conductance. At the lung base, areas with low vascular conductance are found to be located predominantly in the dorsal and adjacent portions (Fig. 4). Although dorsal portions of the lower- and upper-middle lung fields had also a relatively low vascular conductance, vascular conductance in the apicodorsal region was not reduced. We also found that ventral portions of the apical, upper-, and lower-middle lung fields have somewhat lower vascular conductances, whereas the basoventral portion does not (Fig. 4). Medial portions in all planes appear to possess a higher vascular conductance. Although our experimental results show that most of the dorsal portions have
relatively low vascular conductance, the findings may be unique to the rabbit lung and may not be applied to other animals. Beck and Rehder (3) demonstrated that, in contrast to the findings obtained in the current study, dorsal regions of the canine lung have higher vascular conductances than ventral regions, independent of the vertical orientation of the lung. The interspecies differences in distribution of vascular conductance were reported in the myocardium, as well (1).

In conclusion, the RPR distribution down the vertical axis under zone 3 conditions may be determined by both gravity-dependent and -independent mechanisms. Alveolar hypoxia and PA hypoxia significantly diminished the RPR in the lung base, indicating that HPV may not occur homogeneously throughout the lung but occur primarily at the lung base. RPR distributions along the horizontal axes under alveolar and PA hypoxia conditions demonstrated that strong HPV takes place in medial regions at the lung base. Analyses of the RPR distributions along the horizontal axes at different flow rates revealed that vascular conductance may not be uniform throughout the lung and that regions with low vascular conductance appear to be located mainly in the dorsal area of the lower portion of the lung.

Perspectives

On the basis of the experimental findings, we are convinced that pulmonary microvascular networks have extremely complicated natures, i.e., both reactivity to hypoxia and vascular conductance are perceptibly different among the regions. Some regions have low sensitivity to hypoxic stimulation in addition to low vascular conductance, but others have high sensitivity to hypoxia associated with low vascular conductance. These facts indicate that the vascular distensibility, which is determined by anatomic characteristics of the pulmonary microvasculature in a given area, is not closely correlated with the reactivity to hypoxia, which reflects physiological and/or biological natures of the microvasculature. These findings bring a couple of important issues to be addressed. 1) Why does the local vascular conductance, which is expected to be anatomically fixed, appreciably differ among the regions? Are there any qualitative differences in the anatomy of the pulmonary microvascular networks not only among the planes with different heights in the gravitational direction but also within the certain isogravitational plane? 2) What kinds of sources should be taken into account for explaining the quantitative differences in vascular responsiveness to hypoxia? Are there any differences in the densities of hypoxia-sensitive potassium and/or calcium channels on the vascular smooth muscle cells among the different regions? Does the production of endothelial vasodilators (such as nitric oxide and prostacyclin) by hypoxic vasoconstriction significantly differ among the regions? Detailed approaches to these questions will largely extend our understanding of the pulmonary microcirculatory kinetics with intricate characteristics.

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