Regulation of pulmonary circulation by alveolar oxygen tension via airway nitric oxide

HIROSHI IDE,1 HITOSHI NAKANO,1 TOSHIYUKI OGASA,1 SHINOBU OSANAI,1 KENJIROU KIKUCHI,1 AND JUN IWAMOTO2

1Department of Medicine, 2Division of Applied Physiology, School of Nursing, Asahikawa Medical College, Asahikawa 078-8510, Japan

Regulation of pulmonary circulation by alveolar oxygen tension via airway nitric oxide. J. Appl. Physiol. 87(5): 1629–1636, 1999.—The effects of airway (AH) and vascular hypoxia (VH) on the production of nitric oxide (NO; V\textsubscript{NO}) were tested in isolated buffer-perfused (BFL) and blood-perfused rabbit lungs (BLL). To produce AH and/or VH, the lung was ventilated with 1% \textsubscript{O}2 gas, and/or the perfusate was deoxygenated by a membrane oxygenator located on the inlet limb to the pulmonary artery. We measured exhaled NO (V\textsubscript{NO}), accumulation of perfusate NO\textsubscript{x}, and arterial pressure (P\textsubscript{pa}) during AH (inspired \textsubscript{O}2 fraction = 0.01) and/or VH (venous \textsubscript{P}O\textsubscript{2} = 26 Torr). In BFL, a pure AH without VH caused decreases in V\textsubscript{NO} and NO\textsubscript{x} accumulation with an increase in P\textsubscript{pa}. However, neither V\textsubscript{NO}, NO\textsubscript{x} accumulation, nor P\textsubscript{pa} changed during VH. Similarly, in BLL, only AH reduced V\textsubscript{NO}, although NO\textsubscript{x} accumulation was not measurable because of Hb. When alveolar \textsubscript{P}O\textsubscript{2} was gradually reduced from 152 to 0 Torr for 20 min, AH reduced V\textsubscript{NO} curvilinearly from 73.9 ± 8 to 25.6 ± 8 nl/min in BFL and from 26.0 ± 2 to 5.2 ± 1 nl/min in BLL. This plot was analogous to that of a substrate-velocity curve for an enzyme obeying Michaelis-Menten kinetics. The apparent Michaelis-Menten constant for O\textsubscript{2} was calculated to be 23.2 \textmu{}M for BLL and 24.1 \textmu{}M for BFL. These results indicate that the V\textsubscript{NO} in the airway epithelium is dependent on the level of inspired \textsubscript{O}2 fraction, leading to the tentative conclusion that epithelial NO synthase is \textsubscript{O}2-sensitive over the physiological range of alveolar \textsubscript{P}O\textsubscript{2} and controls pulmonary circulation.

AN IMPORTANT FUNCTION of the lung is to match local perfusion with ventilation to preserve arterial oxygenation. Blood perfusion in the lung is redistributed according to alveolar ventilation; the \textsubscript{P}O\textsubscript{2} of the alveolus is, therefore, a major determinant of local blood flow. Such a mechanism has been described as hypoxic pulmonary vasoconstriction (HPV) (37). The main site of vasoconstriction in response to hypoxia has been demonstrated to be precapillary pulmonary arteries, and the primary stimulus for HPV is low alveolar \textsubscript{P}O\textsubscript{2} (P\textsubscript{A\textsubscript{O}2}), whereas low \textsubscript{P}O\textsubscript{2} in mixed venous blood is only a weak stimulus (6, 35). A comparison of airway (AH) and vascular hypoxia (VH) was attempted in isolated animal lungs, and it demonstrated that AH had a greater effect on HPV (20, 39). HPV can be produced by direct hypoxia onto vascular smooth muscle (21) or mediated by an endothelial mechanism (1, 9, 15, 16, 30, 36). Nitric oxide (NO), a potent vasodilator, is a highly diffusible and volatile gas and is synthesized enzymatically by NO synthase (NOS) from L-arginine and molecular \textsubscript{O}2 (23). In the respiratory system, it has been demonstrated that NOS immunoactivity is localized in the nasal epithelium (31), airway epithelium, and pulmonary vascular endothelium (2, 19, 33). Thus NO may play an important role in the regulation of airway function and pulmonary circulation. NO has been detected in the exhaled air of humans and animals (9). It has been demonstrated that hypoxic ventilation decreased the concentration of exhaled NO in isolated animal lungs (4, 8, 24). Several studies demonstrated that the NO production (V\textsubscript{NO}) in the pulmonary endothelium was either attenuated (38) or potentiated (11) by hypoxia. Controversy remains over the issue of the role of NO in pulmonary hypoxic vasoconstriction. Rengasamy and Jones (28) found very low values of the Michaelis-Menten constant (K\textsubscript{m}) for \textsubscript{O}2 in three NOS isoforms from isolated cell preparations, suggesting that the substrate (\textsubscript{O}2) is not saturated for these NOS isoforms and hypoxia per se may be able to change the production rate of NO quickly. To clarify the responsiveness of V\textsubscript{NO} in the lung tissues to low \textsubscript{O}2, and the relationship between local V\textsubscript{NO} and pulmonary vasoconstriction, we attempted to evaluate the production of NO in the airway epithelium and the vascular endothelium during AH and/or VH in buffer-perfused (BFL) or blood-perfused rabbit lungs (BLL).

MATERIALS AND METHODS

Preparation of the isolated Lung Model

Male \textsuperscript{1} Japanese albino rabbits (3.5–4.0 kg body wt) anesthetized with pentobarbital sodium (30 mg/kg iv) were intubated and ventilated by a respirator (model 683, Harvard Apparatus) with NO-free room air. The right common carotid artery was cannulated and used for heparinization (1,000 U/kg) and phlebotomy (100–150 ml of blood letting). After thoracotomy, the main pulmonary artery and left atrium (pulmonary vein) were cannulated via the apex of the right and left ventricle, respectively. The lungs with heart and trachea were excised en bloc and placed in a housing chamber kept at 37°C. The cannulas from each pulmonary vessel were connected to a closed-circuit perfusion system containing 250 ml of a circulating solution of Krebs-Henseleit buffer that contained (in mM) 119.2 NaCl, 4.7 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 1.2 MgSO\textsubscript{4}, 25 NaHCO\textsubscript{3}, 3.2 CaCl\textsubscript{2}, and 15 glucose. The perfusion system consisted of a membrane oxygenator (SILOX-S 0.3 MERA), a roller pump (Master Flex, Cole-Parmer Instrument), and a reservoir (Fig. 1). After the lungs were rinsed thoroughly with buffer solution, the perfusion system was closed (BFL), or, after 100

http://www.jap.org

8750-7587/99 $5.00 Copyright © 1999 the American Physiological Society
ml of buffer solution were taken away, autologous blood was added into a reservoir and hematocrit was adjusted to ~14% (BLL). Finally, the pump rate (flow rate) was adjusted to 100 ml/min for stabilization. The pH and PCO2 of the buffer were adjusted to 7.4 and 40 Torr, respectively, by ventilating the isolated lungs at 0.9 l/min (30 cycles × 30 ml) of minute ventilation (Ve) with the use of a gas mixture of 20% O2-5% CO2-balance N2 (standard gas). The perfusate returning from the reservoir to the lung was passed through a membrane oxygenator, where the PO2 (venous PO2 (PvO2) of the perfusate was manipulated before it entered the pulmonary artery. The O2 level of ventilatory gas was also changed, whereas the CO2 level was kept at 5%. The system could be used to manipulate the O2 levels in the pulmonary arteries, pulmonary veins, and the airway lumen separately. For instance, when a membrane oxygenator gave enough O2 to the perfusate while the lungs were being ventilated with a gas mixture containing zero O2, the perfusate in the artery had much higher O2 than that in the pulmonary vein, and the airway epithelia were exposed to extreme anoxia. Thus a combination of AH and VH may yield a great opportunity to explore the effects of low O2 on the perfusate in the artery had much higher O2 than that in the pulmonary vein, and the airway epithelia were exposed to extreme anoxia. Thus a combination of AH and VH may yield a great opportunity to explore the effects of low O2 on the gas-exchange apparatuses could elicit localized hypoxia in various portions in the lung. See text for details. LA, left atrium; PA, pulmonary artery.

Fig. 1. Diagram of circuit arrangement for perfusion of isolated rabbit lung and the measurement system for exhaled nitric oxide (NO) and perfusate NO2/NO3. Ventilatory gas was continuously monitored, and perfusate was sampled every 1 min. Perfusate circulated through isolated lung and a membrane oxygenator. Combination of these gas-exchange apparatuses could elicit localized hypoxia in various portions in the circuit. See text for details. LA, left atrium; PA, pulmonary artery.

where [NO] is the mean concentration of exhaled NO expressed in parts/billion and Ve is expressed in l/min.

Measurements of NOx (NO2/NO3) in the Perfusion

The measurement of NOx consists of two parts. First, we measured NO2 by reducing each sample (20 µl) in the purge vessel in which 5 ml of 1 N acetoacetate and 50 mg of potassium iodine were already ventilated with 100% N2 gas. In this purge vessel, NO2 in the sample was instantly converted to NO and transferred to an NO analyzer

\[ \text{NO}_2 + I^- + 2H^+ \rightarrow \text{NO} + \frac{1}{2} I_2 + H_2O \]

The calibration with the use of 1 µM NaNO2 solution was perfectly linear for the wide range of NO2 (10^{-11}–10^{-9} mol). However, reduction by this method is only valid for nitrite, not for nitrate. Hence NO2 was reduced to NO3 by using Aspergillus nitrate reductase (ANR) and NADPH. In brief, a 100-µl aliquot of the sample from the reservoir was mixed with 120 µl of distilled water, 40 µl of 50 µM NADPH, and 40 µl of 1 U ANR and then incubated at 36°C for 1 h. Thereafter, a 20-µl aliquot from this mixture was injected into the purge vessel for further reduction and measurement in the form of NO. We also performed the calibration of NO3 using NaN3 and found a similar linearity in a wide range (10^{-11}–10^{-9} mol). The value obtained from the two-step reduction with ANR and potassium iodine was the total of values for NO2 and NO3; hence, to obtain the real value for NO2, we subtracted NO3 from the total value. Accumulation of NOx in the perfusate was calculated from the slopes of plots under each condition.

Experimental Protocols

Protocol 1: combination of hypoxia: AH, VH, and AH + VH.

After a stabilization period, the isolated lungs (n = 12 each for BLL and BFL) were used to measure exhaled NO and NOx in the perfusate. During the control period, the postoxygenator (prelung) perfusate PvO2 was kept at 100 Torr by standard gas ventilation and normoxic oxygenation in the membrane oxygenator. After 20 min of the control period, PvO2 was reduced to <30 Torr for 20 min by decreasing O2 in the membrane oxygenator (VH). Thereafter, the hypoxic challenge (ventilation with 1% O2) was either immediately performed for 20
min (AH + VH) or started after 10 min of the recovery period, allowing PvO2 to return to the control level (AH). During the course of the experiments, aliquots of the perfusate were sampled from the pulmonary vein every 2 min to measure accumulation of perfusate NOx.

Protocol 2: gradual AH in BLL. A 95% N2-5% CO2 gas mixture was slowly added to the standard gas to obtain a gradual decrease in inspired O2 fraction (FiO2) to 0 in the ventilatory gas. The O2 level was later restored to 20%.

Protocol 3: gradual AH in BFL. The same procedure in protocol 2 was repeated in BFL.

Statistical Analysis

In protocol 1, comparisons were made between control values and values obtained at VH, AH, and VH + AH by use of an ANOVA. In protocols 2 and 3, the values of exhaled NO and Ppa gradually changed as the O2 concentration was lowered. We averaged these parameters every 500 ms for following values of the O2 level. As shown in RESULTS, the relationship between VNO and the O2 level was found to follow a Michaelis-Menten kinetics (quasi-Michaelis-Menten kinetics); hence, we applied a Lineweaver-Burk plotting technique to test the linearity between 1/V and 1/S, the reciprocals of reaction velocity and substrate concentration, respectively. For this analysis, regression analysis was used. All data are expressed as means ± SE. Differences were considered significant when P values were <0.05.

RESULTS

Effects of Combinations of VH and AH

In the present study, oxygenation of the perfusate was manipulated by using two oxygenating systems, i.e., a membrane oxygenator and the isolated rabbit lung. Two oxygenators were serially connected, and the perfusate in the limbs between oxygenators contained different levels of O2, depending on the performance of each oxygenator. For the incoming perfusate to the lung, the membrane oxygenator was used to decrease the O2 level (PvO2) to produce a VH condition. With the use of 1% O2 gas, PvO2 was lowered from 145 ± 4 to 27 ± 2 Torr (Table 1). This low-O2 perfusate mainly stimulated the pulmonary artery portion when the lung was ventilated with normoxic gas to raise the O2 level [arterial PO2 (PaO2)] up to 118 ± 4 Torr (Table 1). In such conditions, exhaled NO did not change. There was no rise in Ppa in this condition.

When the isolated lung was temporarily ventilated with 1% O2 to produce an AH condition, the O2 levels of PvO2 and PaO2 became 138 ± 7 and 48 ± 3 Torr,

Table 1. Exhaled NO output and perfusate gas analysis in blood-perfused lung

<table>
<thead>
<tr>
<th>Condition</th>
<th>Exhaled NO Output, n/min</th>
<th>Prelung PpO2, Torr</th>
<th>Postlung PaO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>28.9 ± 2</td>
<td>145 ± 4</td>
</tr>
<tr>
<td>VH</td>
<td>12</td>
<td>28.4 ± 2</td>
<td>27 ± 2*</td>
</tr>
<tr>
<td>AH</td>
<td>6</td>
<td>12.2 ± 1*</td>
<td>138 ± 7</td>
</tr>
<tr>
<td>VH + AH</td>
<td>6</td>
<td>11.6 ± 1*</td>
<td>17 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of lungs. NO, nitric oxide; VH, vascular hypoxia; AH, airway hypoxia; PVo2, venous PO2; PAo2, arterial PO2. *P < 0.05 compared with corresponding control value.

respectively. Thus AH mainly stimulated the airway area and/or the pulmonary vein portion. There was a prompt decrease in exhaled NO with a gradual increase in Ppa (mean rise: 7.5 ± 1.6 mmHg from the baseline), followed by a rapid recovery of exhaled NO when ventilation was returned to normoxia. The mean exhaled NO decreased to 12.2 ± 1 n/min (P < 0.05), which was ~42% of the control value.

When both an oxygenator and the lung were used to deoxygenate the perfusate in all limbs to achieve AH + VH, the O2 levels of PvO2 and PaO2 were 17 ± 2 and 15 ± 2 Torr, respectively. This was a massive stimulation to the entire portion of the circuit, including the airway and the pulmonary circulation. In such a severe hypoxic condition, the exhaled NO level was as low as 11.6 ± 1 n/min (P < 0.05 vs. control), which was, however, only slightly lower than that in the AH condition. The mean Ppa increased by 7.7 ± 1.5 mmHg during AH + VH, which was also slightly higher than that in the AH condition. Thus the responses of exhaled NO to AH + VH were comparable to those during AH. Hence, the hypoxic response of epithelial NO was seen as exhaled NO in BLL, but the hypoxic response of endothelial NO was unclear. To clarify the hypoxic response of endothelial NO, we measured exhaled NO and the accumulation of perfusate NOx in BFL. Although, in BFL, the basal value of exhaled NO was approximately twofold higher than that in BLL (Table 2), the responses of exhaled NO to hypoxic stimuli tended to be similar to those in BLL. There was a prompt decrease in exhaled NO with a gradual increase in Ppa (mean rise: 4.7 ± 0.8 mmHg from the baseline), followed by a rapid recovery in exhaled NO when FiO2 was returned to normoxia (Fig. 2). The accumulation of perfusate NOx did not change in the VH condition but significantly decreased in the AH condition (3.4 ± 1 vs. 1.5 ± 1 nmmol/min, P < 0.05). Additional VH to AH did not differ from that in AH alone (Table 2).

Effects of Gradual Hypoxia on Ppa

Lowering the ventilatory O2 level induced a gradual decrease in exhaled NO as well as a gradual increase in Ppa (Fig. 3). Thus there was a linear relationship between changes in Ppa and PO2 in BFL as well as BLL (Fig. 4). This clearly demonstrated a dose dependency of HPV. The range of PO2 for changing Ppa was 0–100 Torr. The addition of blood to the perfusate increased

Table 2. Exhaled NO output, perfusate NOx, and perfusate gas analysis in buffer-perfused lung

<table>
<thead>
<tr>
<th>Condition</th>
<th>Exhaled NO Output, n/min</th>
<th>Perfusion NOx, nmol/min</th>
<th>Prelung PpO2, Torr</th>
<th>Postlung PaO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>62.9 ± 6</td>
<td>3.4 ± 1</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>VH</td>
<td>12</td>
<td>60.5 ± 6</td>
<td>3.3 ± 1</td>
<td>27 ± 1*</td>
</tr>
<tr>
<td>AH</td>
<td>6</td>
<td>41.1 ± 5*</td>
<td>1.5 ± 1*</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>VH + AH</td>
<td>6</td>
<td>36.3 ± 6*</td>
<td>1.5 ± 2*</td>
<td>26 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of lungs. *P < 0.05 compared with corresponding control value.

Downloaded from http://jap.physiology.org/ on April 8, 2017
the gain of the responses to hypoxia in the pulmonary circulation (Fig. 4).

Effects of Hypoxia on Exhaled NO

In Fig. 5, the mean values of $V_{\text{NO}}$ from six isolated lungs were plotted for varying $P_{\text{O}_2}$ values from protocols 2 and 3. There were curvilinear relationships between $P_{\text{O}_2}$ and the $V_{\text{NO}}$ rate in both BFL and BLL, whereas the relationship in BFL exhibited a higher amount of NO at the same $O_2$ level. In addition, $V_{\text{NO}}$ in BFL did not fall to the zero level at $F_{\text{IO}_2} = 0.5$. However, both curves were almost identical to each other except at the basal level of $V_{\text{NO}}$. Moreover, the shape of the curves was also analogous to the plots for enzymes obeying Michaelis-Menten kinetics. To examine whether these relationships together express a uniform enzymatic reaction, we attempted to analyze the curve using a Lineweaver-Burk plot, which demonstrated a beautiful linearity between $1/P_{\text{O}_2}$ and $1/V_{\text{NO}}$ (Fig. 6). This suggests that the epithelial NOS in the airway wall has Michaelis-Menten kinetics. An apparent $K_m$ value for $O_2$ in BLL was estimated as 23.2 $\mu$M, which corresponded to $\sim$19 Torr of partial pressure, and the maximal rate of reaction velocity ($V_{\text{max}}$) was 23.3 nl/min. An apparent $K_m$ value for $O_2$ in BFL was 24.1 $\mu$M, which corresponded to $\sim$19 Torr of partial pressure, and $V_{\text{max}}$ was 52.6 nl/min.

DISCUSSION

Involvement of Epithelial NO in the Perfusate NOx

As well as being found in the alveolar macrophage, it has been histologically demonstrated that NOS is present in the bronchoalveolar epithelial cells and vascular endothelium (2, 19, 32). In isolated animal lung models, two fractions of the lung NO have been detected in the form of exhaled NO and NOx in the perfusate (8, 24, 34). Usually the exhaled NO is attributed mainly to the NO produced in the airway epithelium (15, 26, 27), and the perfusate NOx is attributed mainly to the NO produced in the vascular endothelium (18, 34). For the perfusate NOx, it has been established that high levels of NO in the ventilatory gas may diffuse to the vascular smooth muscle during NO inhalation therapy (29). In the present study, decreased exhaled NO (NO in the airway) by hypoxia was coupled with a reduced accumulation of perfusate NOx (Table 1). This suggests that the production of NO in the epithelium was decreased by hypoxia and the diffusion of NO toward the vessels was decreased. Indeed, inhalation of different concentrations of NO gas caused a linear increase in the perfusate NOx accumulations (34). According to the study by Spriestersbach et al. (34), 800 parts/billion of NO in the ventilatory gas caused an accumulation of 2 nmol/min of the perfusate NOx in the

![Fig. 2. Example of real-time recording of exhaled NO, O2 concentration, and pulmonary arterial pressure (Ppa) at slower- (A) and faster-sweep speed (B) in isolated buffer-perfused lung (BFL; protocol 1). Vascular hypoxia (VH) induced by a membrane oxygenator affected neither exhaled NO nor Ppa, whereas airway hypoxia (AH) challenge via airway exhibited stepwise decrease in exhaled NO with gradual increase in Ppa. Note that changes in exhaled NO occurred within several breaths. ppb, Parts/billion.](https://example.com/fig2)

![Fig. 3. Examples of real-time recording of exhaled NO, O2 concentration, and Ppa in isolated blood-perfused lung (BLL; protocol 2; A) and in isolated BFL (protocol 3; B). For both protocols, 95% N2-5% CO2 gas mixture was gradually added to the inhaled standard gas for 20 min to achieve a slow and linear fall of ventilatory O2. NO concentration was gradually decreased with gradual rise in Ppa along with a slow reduction of alveolar O2 concentration from 20 to 0%.](https://example.com/fig3)
isolated rabbit lung. Hence, a reduction of the NOx accumulation by 7 nmol/min during hypoxia (Table 1) may be attributed to a remarkable drop (\(\sim 3\) parts/million) of the tissue NO concentration. During control, the perfusate NOx was 3.3 nmol/min. This value was not changed by VH, whereas the AH diminished the perfusate NOx down to 1.5 nmol/min. Therefore, this unchanged portion of the basal value (1.5 nmol/min) may be attributed to the endothelial production of NO. However, the behavior of epithelial NO in BLL is different from that in BFL, because Hb acts as a huge sink for NO (7, 13). Hence, the pressure gradient of NO between airway and vascular lumen in BLL is considered to be greater than that in BFL. Thus diffusion of epithelial NO toward the vasculature (backward NO diffusion) should be greater in BLL. This view is supported by the fact that exhaled NO in BLL is significantly less than in BFL (Tables 1 and 2). Indeed, the amount of exhaled NO is dependent on the amount of NO cleared in the alveolus (15) after its production in the epithelium. In this regard, the values of exhaled NO (\(V_{\text{NO}}\)) in BLL did not express the whole production of NO from the airway epithelium, because a fraction of NO diffusing backward is cleared by Hb during the inspiratory period, and the remainder of epithelial NO was exhaled during the expiratory period. As indicated in Tables 1 and 2, the remainder of NO, i.e., exhaled NO in BLL, would be approximately one-half of the total production of epithelial NO. Therefore, the behavior of exhaled NO in BLL solely reflects the NO produced within the epithelium.

Characteristics of Epithelial NO in Relation to Alveolar \(O_2\) Level

Gustafsson et al. (9) demonstrated that hypoxia reduced exhaled NO in the rabbit. Although they made the interpretation that endothelial NO was reduced, it is widely accepted that hypoxia causes a reduction of exhaled NO in various animals (4, 8, 24). In the present study, we observed that exhaled NO was reduced along with a decrease in \(P_{\text{AO}_2}\) (Fig. 5). The curvilinear relationship between \(P_{\text{AO}_2}\) and \(V_{\text{NO}}\) recalls a plot of an enzyme that obeys quasi-Michaelis-Menten kinetics. Indeed, Rengasamy and Jones (28) reported that three isoforms of NOS exhibited Michaelis-Menten kinetics. Hence, we attempted to use a double-reciprocal plotting technique (Lineweaver-Burk plot) to analyze the relationships between \(P_{\text{O}_2}\) and \(V_{\text{NO}}\). However, the \(V_{\text{NO}}\) value for zero \(P_{\text{O}_2}\) was not zero. This background portion of \(V_{\text{NO}}\) was omitted before plotting. We simply subtracted 4.4 nl/min in BLL and 25.6 nl/min in BFL from all the data and replotted (Fig. 6). The apparent \(K_m\) value for \(P_{\text{O}_2}\) was estimated to be 23.2 \(\mu M\) in BLL and 24.1 \(\mu M\) in BFL (19 Torr). In cultured bovine aortic endothelial cells, the \(K_m\) value for \(P_{\text{O}_2}\) has been reported to be 7.7 \(\pm\) 1.6 \(\mu M\) (28). The \(K_m\) values of enzymes such as cytochrome P-450 and cytochrome oxidase that utilize \(O_2\) as a substrate have been shown to range from 1 to 9 \(\mu M\) (3, 17).

Different \(V_{\text{max}}\) Values in BFL and BLL

The apparent \(K_m\) values in BLL and BFL were 23.2 and 24.1 \(\mu M\), respectively. On the other hand, \(V_{\text{max}}\) in BLL and BFL were 23.3 and 52.6 nl/min, respectively. The difference in \(V_{\text{max}}\) was probably attributed to the
presence of blood in the perfusate, because blood may reduce the expiration of NO produced in the airway (15). The V\textsubscript{NO} was assumed to reflect the production rate of NO in the airway by the following hypothesis. There is an exhaled fraction and a cleared fraction of NO in the airway. Basically, NO produced in the airway may be cleared mostly by Hb in the pulmonary circulation during the inspiratory period (cleared fraction) and/or expired and measured as V\textsubscript{NO} during the expiratory period (exhaled fraction) (14). Thus it is obvious that V\textsubscript{NO} is not equivalent to the real production rate of NO in the airway but is a balance of cleared and exhaled fractions. As long as the ventilation is kept constant, the amount of NO cleared in the alveolus is simply determined by the total period of expiration, i.e., expiration-to-inspiration ratio. Hence, V\textsubscript{NO} can be close to one-half of the real production rate of NO in the airway when the expiration-to-inspiration ratio = 1. This probably explains a lower V\textsubscript{NO} in BLL. Thus it is speculated that higher V\textsubscript{max} results from the greater amount of V\textsubscript{NO} in BFL. Indeed, V\textsubscript{max} in BFL was close to twice that in BLL.

Contribution of eNOS and Epithelial NOS to HPV

We supposed that NO brought by epithelial NOS and eNOS was in the exhaled NO, but the exhaled NO might reflect the NO produced via epithelial NOS because of the clearance of NO by Hb in the pulmonary circulation. These NOS are probably constitutive NOS and might be brain type NOS (40). NOS on the vascular bed seems to behave as an adaptive change in upregulation with vascular remodeling (40). However, the acute response of exhaled NO with hypoxia merely shows biochemical change, and epithelial NOS works on so-called HPV, and the mechanism of ventilation-perfusion (V\textsubscript{A}/Q\textsubscript{O}) matching.

Airway NO and Hypoxic Vasoconstriction

As mentioned in Involvement of Epithelial NO in the Perfusate NOx, the AH solely reduced the airway NO without any changes in the basal NO level from the endothelia (Tables 1 and 2), because a decreased portion of perfusate NOx during hypoxia was attributed to the decreased airway NO. Such decreases of exhaled...
NO and PO2 were accompanied by a proportional rise in \( P_{pa} \) (Fig. 4). From these results, it may be interpreted that the reduction of the vascular tone is regulated by NO diffused from the adjacent vascular endothelium, the relationship between PO2 and NO synthesis is still controversial (12). AH may either inhibit or increase production of NO in the lung (9, 11). We have demonstrated in the present study that the endothelial \( \text{V} \text{NO} \) was not suppressed by moderate VH, despite the fact that HPV occurred (Table 2). Therefore, the findings in the present study that the airway epithelial NO can control the pulmonary vascular tone in the physiological range of PO2 may reconcile these conflicting facts. In this regard, it is conceivable that the epithelial NOS per se might act as an "O2 sensor" in the airway. The K\( m \) value obtained in the present study was merely an approximation obtained from the ex vivo experiment. Hence, no direct comparison can be made between the present value and precise values obtained from in vitro settings (28), although it is still worth indicating that our ex vivo K\( m \) values are close to the K\( m \) values in vitro. We believe that such characteristics of NOS may be essential to the O2 sensitivity of the pulmonary circulation.

O2 Sensing Mechanism Involves the Sensing of PaO2

Generally, the Hb-containing perfusate should have a higher O2 content than the buffer perfusate. Hence, in BFL, the PO2 of the local tissue receiving O2 from perfusate/blood was expected to be higher than that in BFL. A higher O2 level of perfusate/blood might well have counteracted HPV in BLL, which, however, was not observed in the present study. Rather, an enhancement of HPV was demonstrated in our results (Fig. 4) as well as in other studies (10, 22, 39). Such an effect of blood has been attributed to unknown chemical mediators (5) or deformity of red blood cells (10). Unfortunately, the available data regarding the effect of Hb on HPV are scanty, and, in particular, the magnifying effect of Hb on various levels of HPV induced by varying F\( I_\text{O2} \), has not been studied. In the present study, we have demonstrated an increased sensitivity (gain) in HPV for various PO2 (Fig. 4). The responsiveness of the pulmonary pressure to hypoxia was greatly augmented with the addition of blood. This fact leads us to consider the role of Hb in the O2 sensitivity in HPV. The location of the O2-sensing mechanism may be crucial to the effect of blood in the pulmonary circulation onto the O2 sensitivity. If an O2 sensor is located in the vicinity of vascular smooth muscles, the higher tissue O2 level in BLL could not enhance HPV. It has been proposed that the O2 sensor or O2-sensing mechanism may exist in the bronchoalveolar compartment (39). If this is the case, the blood in the pulmonary circulation could have increased the diffusion of alveolar O2 toward the perfusate, which, in turn, could lower the PAO2, giving a more severe hypoxia in the vicinity of the bronchoalveolar compartment (Fig. 7A). This is a state of AH that eventually diminished the V\( \text{NO} \) on the epithelial NOS that follows Michaelis-Menten kinetics (Figs. 5 and 6). Altogether, the O2 level in the bronchoalveolar compartment seemingly modulates the production rate of NO, which diffuses toward the pulmonary arteriole to change vascular caliber (Fig. 7B); in other words, the NOS in the bronchoalveolar compartment can sense PAO2. It has been proposed that NO is a strong candidate for the mechanism that matches alveolar ventilation and pulmonary perfusion (V\( \text{A/Q} \)) (25), although the blood O2 level has been believed to modulate V\( \text{NO} \) in the vascular endothelium. Our findings indicate that the epithelial NO has a pivotal role in controlling the pulmonary circulation by sensing the O2 level in the bronchoalveolar compartment. Further study is needed to clarify the physiological significance of NOS in the mechanism of V\( \text{A/Q} \) matching.

Address for reprint requests and other correspondence: J. Iwamoto, Division of Applied Physiology, School of Nursing, Asahikawa Medical College, 4–5 Nishikagura, Asahikawa 078–8510, Japan (E-mail: j1103@asahikawa-med.ac.jp).

Received 25 April 1998; accepted in final form 30 June 1999.

REFERENCES


