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

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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˙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% O2 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˙NO), accumulation of perfusate NOx, and pulmonary arterial pressure (Ppa) during AH (inspired O2 fraction = 0.01) and/or VH (venous PO2 = 26 Torr). In BFL, a pure AH without VH caused decreases in V˙NO and NOx accumulation with an increase in Ppa. However, neither V˙NO, NOx accumulation, nor Ppa changed during VH. Similarly, in BLL, only AH reduced V˙NO, although NOx accumulation was not measurable because of Hb. When alveolar PO2 was gradually reduced from 152 to 0 Torr for 20 min, AH reduced V˙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 O2 was calculated to be 23.2 µM for BLL and 24.1 µM for BFL. These results indicate that the V˙NO in the airway epithelium is dependent on the level of inspired O2 fraction, leading to the tentative conclusion that epithelial NO synthase is O2 sensitive over the physiological range of alveolar PO2 and controls pulmonary circulation.

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 O2 (23). In the respiratory system, it has been demonstrated that NOS immunoreactivity 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˙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 J ones (28) found very low values of the Michaelis-Menten constant (Km) for O2 in three NOS isoforms from isolated cell preparations, suggesting that the substrate (O2) 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˙NO in the lung tissues to low O2, and the relationship between local V˙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 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 volume of Krebs-Henseleit buffer that contained (in mM) 119.2 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, 3.2 CaCl2, 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

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ml of buffer solution were taken away, autologous blood was added into a reservoir and hemocrit 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 controlled. NO2/NO3. Ventilatory gas was continuously monitored, and perfusate was sampled every 1 min. Perfusate circulates 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.

Measurements of Physiological Parameters and Exhaled NO

Pulmonary arterial pressure (Ppa) and pulmonary venous pressure were measured with pressure transducers (AP-601G, Nihon Koden). Airway pressure was also measured with a low-pressure transducer (TP-603T, Nihon Koden) placed at the site proximal to the pulmonary artery. The exhaled NO was continuously measured by a chemiluminescence analyzer (NOA 270B, Sievers) from the outlet limb of a tracheal tube. The concentrations of O2 and CO2 were also monitored via this outlet by a gas analyzer (Respina IH26, Nihon Koden). Airway pressure was also measured with a low-pressure transducer (TP-603T, Nihon Koden). An electromagnetic flowmeter (MFV-1100, Nihon Koden) was placed at the site proximal to the pulmonary artery. The exhaled NO was continuously measured by a chemiluminescence analyzer (NOA 270B, Sievers) from the outlet limb of a tracheal tube. The concentrations of O2 and CO2 were also monitored via this outlet by a gas analyzer (Respina IH26, Nihon Koden). Airway pressure was also measured with a low-pressure transducer (TP-603T, Nihon Koden).

A quantitative measurement of exhaled NO (VNO) can be obtained by measuring both NO and Ve:

\[ \dot{V}_{NO} (\text{nl/min}) = [\text{NO}] \times \dot{V}_e \]

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 Perfusate

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.

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

The calibration with the use of 1 µM NaNO3 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. NO2 was also performed the calibration of NO3 using NaNO3 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 NO3, we subtracted NO2 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 PV02 was kept at 100 Torr by standard gas ventilation and normoxic oxygenation in the membrane oxygenator. After 20 min of the control period, PV02 was reduced to <30 Torr (anoxia) 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 corresponding values of the O2 level. As shown in RESULTS, the relationship between PVC 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 arterial 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, 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 nl/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 Pao2 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 nl/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 nmol/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>
<thead>
<tr>
<th>Condition</th>
<th>Exhaled NO Output, nl/min</th>
<th>Prelung Pao2, Torr</th>
<th>Postlung Pao2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.9 ± 2</td>
<td>145 ± 4</td>
<td>157 ± 4</td>
</tr>
<tr>
<td>AH</td>
<td>12</td>
<td>28.4 ± 2</td>
<td>27 ± 2*</td>
</tr>
<tr>
<td>VH</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. *P < 0.05 compared with corresponding control value.
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.

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˙NO from six isolated lungs were plotted for varying PO2 values from protocols 2 and 3. There were curvilinear relationships between PO2 and the V˙NO rate in both BFL and BLL, whereas the relationship in BFL exhibited a higher amount of NO at the same O2 level. In addition, V˙NO in BFL did not fall to the zero level at FIO2 = 0. However, both curves were almost identical to each other except at the basal level of V˙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/PO2 and 1/V˙NO (Fig. 6). This suggests that the epithelial NOS in the airway wall has Michaelis-Menten kinetics. An apparent Km value for O2 in BLL was estimated as 23.2 µM, which corresponded to ~19 Torr of partial pressure, and the maximal rate of reaction velocity (Vmax) was 23.3 nl/min. An apparent Km value for O2 in BFL was 24.1 µM, which corresponded to ~19 Torr of partial pressure, and Vmax 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
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 (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\({\dot{\text{N}}}_\text{O}\)) 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 PAO\(_2\) (Fig. 5). The curvilinear relationship between PAO\(_2\) and V\({\dot{\text{N}}}_\text{O}\) 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 PO\(_2\) and V\({\dot{\text{N}}}_\text{O}\). However, the V\({\dot{\text{N}}}_\text{O}\) value for zero PO\(_2\) was not zero. This background portion of V\({\dot{\text{N}}}_\text{O}\) 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 Km value for O\(_2\) was estimated to be 23.2 \(\mu\text{M}\) in BLL and 24.1 \(\mu\text{M}\) in BFL (~19 Torr). The Km 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 \(\mu\text{M}\) (3, 17).

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

The apparent Km values in BLL and BFL were 23.2 and 24.1 \(\mu\text{M}\), 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

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**Fig. 4.** Changes (\(\Delta\)) in Ppa from baseline in response to alveolar O\(_2\) tension. ○, BFL, n = 6 lungs; ●, BLL, n = 6 lungs. Ppa was linearly increased as O\(_2\) tension decreased from 100 to 0 Torr in both BFL and BLL. Hypoxic vasoconstriction in BLL was significantly larger than that in BFL. Data are means ± SE.

**Fig. 5.** NO production (V\({\dot{\text{N}}}_\text{O}\)) response to alveolar O\(_2\) tension. ○, BFL, n = 6 lungs; ●, BLL, n = 6 lungs. V\({\dot{\text{N}}}_\text{O}\) decreased curvilinearly when O\(_2\) tension was changed from 152 to 0 Torr. Relationship between alveolar O\(_2\) tension and V\({\dot{\text{N}}}_\text{O}\) was analogous to plot of substrate-velocity curve for an enzyme that obeys Michaelis-Menten kinetics. Mean V\({\dot{\text{N}}}_\text{O}\) values at 0 Torr of O\(_2\) tension are 25.6 nl/min in BFL and 4.4 nl/min in BLL. Data are means ± SE.
presence of blood in the perfusate, because blood may reduce the expiration of NO produced in the airway (15). The VNO 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 VNO during the expiratory period (exhaled fraction) (14). Thus it is obvious that VNO 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, VNO 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 VNO in BLL. Thus it is speculated that higher Vmax results from the greater amount of VNO in BFL. Indeed, Vmax in BFL was close to twice that in BLL.

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/Q) 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 P O 2 were accompanied by a proportional rise in Ppa (Fig. 4). From these results, it may be interpreted that the reduction of airway NO is responsible for the hypoxic vasoconstriction. Although it seems to be accepted that the vascular tone is regulated by NO diffused from the adjacent vascular endothelium, the relationship between P O 2 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 V 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 P O 2 may reconcile these conflicting facts. In this regard, it is conceivable that the epithelial NOS per se might act as an "O 2 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 O 2 sensitivity of the pulmonary circulation.

O 2 Sensing Mechanism Involves the Sensing of P A O 2

Generally, the Hb-containing perfusate should have a higher O 2 content than the buffer perfusate. Hence, in BLL, the P O 2 of the local tissue receiving O 2 from perfusate/blood was expected to be higher than that in BFL. A higher O 2 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 O 2 has not been studied. In the present study, we have demonstrated an increased sensitivity (gain) in HPV for various P O 2 (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 O 2 sensitivity in HPV. The location of the O 2-sensing mechanism may be crucial to the effect of blood in the pulmonary circulation onto the O 2 sensitivity. If an O 2 sensor is located in the vicinity of vascular smooth muscles, the higher tissue O 2 level in BLL could not enhance HPV. It has been proposed that the O 2 sensor or O 2-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 O 2 toward the perfusate, which, in turn, could lower the P A O 2 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 NO on the epithelial NOS that follows Michaelis-Menten kinetics (Figs. 5 and 6). Altogether, the O 2 level in the bronchoalveolar compartment seemingly modulates the production rate of NO, which diffuses toward the pulmonary arterial to change vascular caliber (Fig. 7B); in other words, the NOS in the bronchoalveolar compartment can sense P A O 2. It has been proposed that NO is a strong candidate for the mechanism that matches alveolar ventilation and pulmonary perfusion (V A/Q ˙) (25), although the blood O 2 level has been believed to modulate V NO in the vascular endothelium. Our findings indicate that the epithelial NO has a pivotal role in controlling the pulmonary circulation by sensing the O 2 level in the bronchoalveolar compartment. Further study is needed to clarify the physiological significance of NOS in the mechanism of V A/Q ˙ matching.

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