Role of airway nitric oxide on the regulation of pulmonary circulation by carbon dioxide

Yasushi Yamamoto, Hitoshi Nakano, Hiroshi Ide, Toshiyuki Ogasa, Toru Takahashi, Shinobu Osanai, Kenjiro Kikuchi, and Jun Iwamoto. Role of airway nitric oxide on the regulation of pulmonary circulation by carbon dioxide. *J Appl Physiol* 91: 1121–1130, 2001.—The effects of hypercapnia (CO₂) confined to either the alveolar space or the intravascular perfusate on exhaled nitric oxide (NO), perfusate NO metabolites (NOₓ), and pulmonary arterial pressure (Ppa) were examined during normoxia and progressive 20-min hypoxia in isolated blood- and buffer-perfused rabbit lungs. In blood-perfused lungs, when alveolar CO₂ concentration was increased from 0 to 12%, exhaled NO decreased, whereas Ppa increased. Increments of intravascular CO₂ levels increased Ppa without changes in exhaled NO. In buffer-perfused lungs, alveolar CO₂ increased Ppa with reductions in both exhaled NO from 93.8 to 61.7 (SE) nl/min (P < 0.01) and perfusate NOₓ from 4.8 to 1.8 nmol/min (P < 0.01). In contrast, intravascular CO₂ did not affect either exhaled NO or Ppa despite a tendency for perfusate NOₓ to decline. Progressive hypoxia elevated Ppa by 28% from baseline with a reduction in exhaled NO during normocapnia. Alveolar hypercapnia enhanced hypoxic Ppa response up to 50% with a further decline in exhaled NO. Hypercapnia did not alter the apparent Kₘ for O₂, whereas it significantly decreased the Vₘₐₓ from 66.7 to 55.6 nl/min. These results suggest that alveolar CO₂ inhibits epithelial NO synthase activity noncompetitively and that the suppressed NO production by hypercapnia augments hypoxic pulmonary vasoconstriction, resulting in improved ventilation-perfusion matching.

Hypercapnia; epithelium; hypoxic pulmonary vasoconstriction

Ventilation-perfusion (V̅A/Q̅) matching in the lung is vital for improving gas exchange and arterial oxygenation. It is well known that hypoxic pulmonary vasoconstriction (HPV) plays an important role in this mechanism and that alveolar hypoxia is a major determining factor for local blood flow (12). In addition to hypoxia, hypercapnia has been demonstrated to evoke pulmonary vasoconstriction (5, 16, 21). Furthermore, the pressor response to hypoxia is augmented by CO₂ inhalation (2, 23). Several studies, however, reported conflicting results that CO₂ elicits pulmonary vasodilation during both normoxia (6, 34) and hypoxia (4). Although controversy persists over the role of CO₂ in the regulation of pulmonary circulation, it has been demonstrated that alveolar hypercapnia evoked pulmonary vasoconstriction, whereas intravascular hypercapnia elicited vasodilation, suggesting that the stimulus of alveolar CO₂ may be different from that of intravascular CO₂ in the regulation of pulmonary circulation (16).

Nitric oxide (NO), a highly diffusible gas with a potent vasodilator action, is synthesized enzymatically by NO synthase (NOS) from L-arginine and molecular O₂ as a substrate (24). In the lung, NOS immunoactivity is localized in airway epithelium and pulmonary vascular endothelium (20). Recently, it has been demonstrated that airway epithelial NOS produces NO from ambient O₂ through the Michaelis-Menten kinetic mechanism in humans (11) and in isolated rabbit lungs (17). It is widely accepted that endogenous NO regulates pulmonary vascular tone (3) and modulates the pressor response to hypoxia (9). We have previously demonstrated that alveolar hypoxia reduces exhaled NO production in isolated rabbit lungs, suggesting that NO released from the epithelium can control the pulmonary circulation (17). On the other hand, it has been reported that CO₂ inhalation also causes a reduction in exhaled NO in rabbits (1, 29) and dogs (7). However, the precise mechanism responsible for inhibition of exhaled NO by CO₂ and its physiological significance in the regulation of pulmonary circulation are unknown. Furthermore, it has not been determined whether either isolated alveolar or intravascular CO₂ contributes to the NO suppression and pulmonary pressor response.

With regard to the action of either NO or CO₂, we hypothesized that NO production in the epithelium would be regulated by alveolar CO₂ tension, but not by intravascular CO₂, and thus the effect of CO₂ on the pulmonary pressor response could be partially mediated by an alteration of airway NO. In addition, we hypothesized that enhancement of HPV by hypercap-
nia would be mediated also via airway NO. In the present study, we measured exhaled NO, perfusate NO metabolites (NOx), and pulmonary arterial pressure (Ppa) during inhalation of various concentrations of CO2 in an isolated blood- and buffer-perfused lung model while separately controlling alveolar and intravascular CO2 levels using a membrane oxygenator. We also examined the effect of CO2 on the lung NO production response to lowering inspired O2 fraction (FiO2) and the combined effects of hypoxia and hypercapnia on pulmonary presor response.

MATERIALS AND METHODS

Preparation of the Isolated Lung

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Asahikawa Medical College. Male Japanese albino rabbits weighing between 3.0 and 3.5 kg were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated by a respirator (model 683, Harvard Apparatus) with NO-free room air. The animal underwent cannulation of the right common carotid artery, which was immediately used for heparinization (1,000 U/kg) and phlebotomy (100–150 ml of blood letting). Subsequently, the chest of the animal was opened, and the heart and great vessels were exposed. After the main pulmonary artery was cannulated via the apex of the right ventricle and the left atrium via the left ventricle, the lungs with the heart and the trachea were excised en bloc and placed in a humidified chamber kept at 37°C. The isolated lungs were then connected to the circuit consisting of a reservoir, a roller pump (Master Flex, Cole-Parmer Instrument), and an extracorporeal membrane oxygenator (ECMO) (SILOX-S 0.3, MERA) located before the pulmonary artery, in which circulatory volume was ~200 ml. After the lungs were rinsed thoroughly with buffer solution of Krebs-Henseleit buffer containing (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 was closed (buffer-perfused lungs), or, after 150 ml of buffer solution were taken away, autologous blood was added into the reservoir and hemotocrit was adjusted to ~15% (blood-perfused lungs). The perfusate returning from the reservoir to the lung passed through the ECMO. The pump rate (flow rate) was adjusted to 100 ml/min during the entire experimental period. The isolated lungs were ventilated at 0.9 l/min (30 ml × 30 cycles) of minute ventilation (V̇E) with the use of a gas mixture of 20% O2-6% CO2-balance N2 (standard gas). The ECMO was also supplied with the standard gas to adjust the perfusate pH to 7.4 and PO2 to 40 Torr, respectively.

Measurements of Physiological Parameters and Exhaled NO

Ppa and pulmonary venous pressure (Ppv) were measured with pressure transducers (model AP-601G, Nihon Koden). Airway pressure (Paw) was also measured with a low-pressure transducer (model TP-603T, Nihon Koden). An electromagnetic flowmeter (model MFV-1100, Nihon Koden) was placed in line proximal to the pulmonary artery to measure pulmonary perfusion rate. Exhaled NO was continuously measured by a chemiluminescence analyzer (model NOA 270B, Sievers) from the outlet limb of the tracheal tube. End-tidal O2 fraction and CO2 fraction (FEtCO2) were also monitored via this outlet by a gas analyzer (Respina IH26, Sanei). Signals from these probes were continuously recorded via a data acquisition system (MacLab, AD Instrument) for real-time recording and later analysis.

A quantitative measurement of exhaled NO production (vNO) can be obtained by measuring both NO and vE

\[
v_{NO} (nl/min) = [NO] \times v_E
\]

where [NO] is the mean concentration of exhaled NO expressed in parts per billion (ppb), and vE is expressed in liters per minute.

Quenching Effect of CO2 on NO Measurements

CO2 is known to cause a decrease in NO measurements because of a quenching effect on the chemiluminescence process (33). Therefore, we examined the influence of CO2 on our NO measurements. A small amount of 100% CO2 gas was put into a plastic bag filled with a gas mixture of NO (100 ppb) and balance N2 while NO and CO2 concentrations were measured (n = 4). The value of NO concentration was calculated from adding the volume of CO2 gas and subtracting the volume drawn by the chemiluminescence NO analyzer and the CO2 gas analyzer.

Measurements of NOx in the Perfusate

The same protocol as protocol IIA was performed in blood-perfused lungs (n = 5). Before this protocol was started, normoxic gas mixtures (20% O2), which contained various concentrations of CO2 (FeCO2 = 0, 0.03, 0.06, 0.09, and 0.12) were prepared. After stabilization with standard gas inhalation, the isolated lung was ventilated with a gas mixture of FeCO2 = 0 for 15 min followed by the standard gas for 10 min. Thereafter, this maneuver was repeated with stepwise decreases in FeCO2 from 0.03 to 0.12. During experimental period, the ECMO was supplied with standard gas. The pre- and postlung perfusate were sampled for gas analysis at the last minute of each alveolar CO2 exposure.

Protocol II B: Intravascular CO2 exposure in buffer-perfused lungs (n = 6). The same protocol as protocol I A was performed in buffer-perfused lungs. In addition, during experimental period, aliquots of the postlung perfusate were sampled at 5-min intervals to measure perfusate NOx accumulation rate, which was calculated from the slope of plots between perfusate NOx concentrations and time.

Protocol II B: Intravascular CO2 exposure in buffer-perfused lungs (n = 6). The same protocol as protocol IB was performed in buffer-perfused lungs. The procedure of perfusate sampling was the same as that of protocol IIA.
followed by an increase to 0.2 without changes in FiCO₂. Next, inspired gas was switched to a gas mixture of 12% CO₂-20% O₂-N₂ balance. Then, a second gradually progressive hypoxic challenge was carried out by adding a 12% CO₂-N₂ balance-hypercapnia gas mixture. During the experimental period, the ECMO was supplied with standard gas to maintain prelung pH at 7.4 and PVO₂ at 40 Torr.

Statistics

In protocols I and II, the effects of each CO₂ exposure on VNO, perfusate NOx accumulation, and Ppa were analyzed by an analysis of ANOVA for repeated measures. When significance was indicated, a post hoc t-test with Bonferroni’s correction for multiple comparisons was used. The relationships among Ppa, CO₂ concentration, and VNO were examined by linear least squares regression analysis. In protocol III, the relationship between VNO and O₂ level, which obeys the Michaelis-Menten kinetics, was analyzed by double-reciprocal plotting (Lineweaver-Burke plots) with linear least squares regression. In all cases, a P value < 0.05 was considered statistically significant. All data presented in the text, tables, and figures represent means ± SE.

RESULTS

Quenching Effect of CO₂ on NO Measurements

As shown in Fig. 1A, each NO reading at various CO₂ concentrations was reduced compared with the actual value of the NO concentration. When the CO₂ concentration was 12%, the NO reading decreased by 4.5% from 85.1 to 81.2 ppb. The average reduction rate of NO measurement as a function of CO₂ was estimated as 0.38% per 1% CO₂ (Fig. 1B).

Blood-perfused Lungs

In protocol I A, stepwise increments of FiCO₂ caused significant increases in postlung Paco₂ and corresponding decreases in pH. In protocol I B, changes in CO₂ levels supplying to the ECMO caused significant alterations in prelung PVO₂ and pH (Table 1). Although we attempted to achieve the same intensity of intravascular hypercapnic acidosis between alveolar and intravascular CO₂ exposure, the intensity of protocol I B

Table 1. VNO, pulmonary arterial pressure, and perfusate gas analysis in blood-perfused lungs

<table>
<thead>
<tr>
<th>Condition</th>
<th>FCO₂</th>
<th>CO₂ fraction of ECMO</th>
<th>VNO, nl/min</th>
<th>Ppa, mmHg</th>
<th>PVO₂, Torr</th>
<th>pH</th>
<th>Paco₂, Torr</th>
<th>pH</th>
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<td>Prelung</td>
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<td>Alveolar CO₂ exposure (n = 5)</td>
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<tr>
<td>0</td>
<td>0.03</td>
<td>0.03</td>
<td>13.3 ± 0.71</td>
<td>25.9 ± 1.91</td>
<td>7.52 ± 0.03</td>
<td>16.3 ± 1.21</td>
<td>7.69 ± 0.03</td>
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<td>0.06*</td>
<td>0.06</td>
<td>0.06</td>
<td>15.5 ± 0.51</td>
<td>34.4 ± 1.91</td>
<td>7.40 ± 0.01</td>
<td>31.7 ± 1.51</td>
<td>7.44 ± 0.02</td>
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<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>21.6 ± 1.11</td>
<td>41.6 ± 1.31</td>
<td>7.36 ± 0.02</td>
<td>40.1 ± 1.61</td>
<td>7.37 ± 0.02</td>
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<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>26.9 ± 1.31</td>
<td>46.9 ± 1.11</td>
<td>7.29 ± 0.01</td>
<td>53.2 ± 1.41</td>
<td>7.24 ± 0.01</td>
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<tr>
<td>In intravascular CO₂ exposure (n = 5)</td>
<td>0.06</td>
<td>0.06</td>
<td>19.9 ± 3.31</td>
<td>29.1 ± 2.31</td>
<td>7.47 ± 0.03</td>
<td>34.5 ± 1.81</td>
<td>7.40 ± 0.02</td>
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<tr>
<td>0.06*</td>
<td>0.06</td>
<td>0.06</td>
<td>19.5 ± 3.21</td>
<td>41.5 ± 0.11</td>
<td>7.35 ± 0.01</td>
<td>44.4 ± 1.11</td>
<td>7.34 ± 0.01</td>
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<tr>
<td>0.06</td>
<td>0.12</td>
<td>0.12</td>
<td>18.5 ± 0.81</td>
<td>76.1 ± 4.71</td>
<td>7.14 ± 0.02</td>
<td>56.7 ± 2.91</td>
<td>7.24 ± 0.01</td>
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Values are means ± SE; n, no. of experiments. VNO, nitric oxide production; Ppa, pulmonary arterial pressure; PVO₂, venous Pco₂; Pao₂, arterial Pao₂; Fco₂, inspired O₂ fraction; ECMO, extracorporeal membrane oxygenator, *Control condition. Significantly different from corresponding control value: †P < 0.05; ‡P < 0.01.
was slightly greater than that of protocol IA, as judged by the end-point measurements (protocol IB, prelung: pH = 7.13, P_{CO2} = 76.1 Torr vs. protocol IA, postlung: pH = 7.19, P_{CO2} = 61.7 Torr at 0.12 of FICO2). Pre- and postlung perfusate P_{O2} were maintained constant to be ~150 Torr. Throughout the entire experimental period, P_{PV} and Paw did not change.

Figure 2 illustrates representative recordings of protocols IA (left) and IB (right). The stepwise increases in FICO2 increased Ppa with reductions in exhaled NO. Increases in intravascular CO2 also caused slight increments of Ppa, whereas exhaled NO did not change. As summarized in Table 1, when FICO2 was increased in a stepwise manner from 0.06 to 0.12, V_{NO} significantly decreased from 20.9 ± 1.1 to 14.2 ± 1.2 nl/min (P < 0.05), whereas Ppa significantly increased from 17.8 ± 1.0 to 23.5 ± 1.9 mmHg (P < 0.05). In contrast, when FICO2 was decreased from 0.06 to 0, V_{NO} increased with a reduction in Ppa. The changes in CO2 level supplying to the ECMO from 0.06 to 0.12 significantly increased Ppa from 17 ± 0.7 to 18.5 ± 0.8 mmHg (P < 0.05) without changes in V_{NO}.

Buffer-perfused Lungs

As shown in Table 2, in protocol IA with buffer perfusion, we were able to maintain prelung perfusate in the isohydric range between ~7.38 to ~7.41 of pH. Similar to protocol I with blood perfusion, the intensity of the hypercapnic acidosis in protocol IB was slightly greater than that of protocol IA as judged by the end-point measurements (protocol IB, prelung: pH ~7.12, P_{CO2} ~75.5 Torr vs. protocol IA, postlung: pH ~7.19, P_{CO2} ~61.8 Torr at 0.12 of FICO2). During the experimental period, pre- and postlung perfusate P_{O2} were maintained constant to be ~150 Torr.

Figure 3 illustrates representative recordings of protocol IB (A) and II B (B). The stepwise rises in FICO2 increased Ppa with significant reductions in exhaled NO. However, increases in intravascular CO2 did not change Ppa and exhaled NO. As summarized in Table 2, when FICO2 was stepwise increased from 0.06 to 0.12, V_{NO} significantly decreased from 77.3 ± 5.1 to 61.7 ± 6.2 nl/min (P < 0.05) and perfusate NOx accumulation also decreased from 2.9 ± 0.3 to 1.8 ± 0.3 nmol/min.

Table 2. V_{NO}, perfusate NOx accumulation, pulmonary arterial pressure, and perfusate gas analysis in buffer-perfused lungs

<table>
<thead>
<tr>
<th>Condition</th>
<th>FICO2</th>
<th>CO2 fraction of ECMO</th>
<th>V_{NO}, nl/min</th>
<th>Perfusate NOx Accumulation, nmol/min</th>
<th>Ppa, mmHg</th>
<th>P_{CO2}, Torr</th>
<th>pH</th>
<th>P_{O2}, Torr</th>
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<tr>
<td>Alveolar CO2 exposure (n = 6)</td>
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<tr>
<td>0</td>
<td>0.06</td>
<td>93.8 ± 7.3†</td>
<td>4.8 ± 0.7†</td>
<td>12.7 ± 2.3</td>
<td>36.6 ± 1.0</td>
<td>7.40 ± 0.01</td>
<td>15.5 ± 1.9‡</td>
<td>7.76 ± 0.05‡</td>
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<tr>
<td>0.03</td>
<td>0.06</td>
<td>87.0 ± 6.6†</td>
<td>3.9 ± 0.5</td>
<td>12.7 ± 2.2</td>
<td>36.9 ± 1.1</td>
<td>7.41 ± 0.01</td>
<td>23.5 ± 1.7‡</td>
<td>7.61 ± 0.04‡</td>
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<td>0.06*</td>
<td>0.06</td>
<td>77.3 ± 5.1</td>
<td>2.9 ± 0.3</td>
<td>13.1 ± 2.3</td>
<td>38.7 ± 0.9</td>
<td>7.39 ± 0.01</td>
<td>38.5 ± 2.2</td>
<td>7.40 ± 0.02</td>
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<tr>
<td>0.09</td>
<td>0.06</td>
<td>70.9 ± 4.5†</td>
<td>2.5 ± 0.6</td>
<td>13.6 ± 2.4</td>
<td>38.0 ± 0.9</td>
<td>7.39 ± 0.01</td>
<td>50.8 ± 1.5‡</td>
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<td>0.12</td>
<td>0.06</td>
<td>61.7 ± 6.2†</td>
<td>1.8 ± 0.3†</td>
<td>14.2 ± 2.2†</td>
<td>39.2 ± 1.4</td>
<td>7.38 ± 0.02</td>
<td>61.8 ± 3.4‡</td>
<td>7.19 ± 0.02‡</td>
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<td>Intravascular CO2 exposure (n = 6)</td>
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<tr>
<td>0.06</td>
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<td>74.5 ± 6.9</td>
<td>2.8 ± 0.5</td>
<td>13.1 ± 1.0</td>
<td>13.3 ± 3.2‡</td>
<td>7.85 ± 0.07‡</td>
<td>29.1 ± 1.8‡</td>
<td>7.52 ± 0.03‡</td>
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<tr>
<td>0.06*</td>
<td>0</td>
<td>74.1 ± 7.0</td>
<td>3.2 ± 0.4</td>
<td>13.2 ± 1.1</td>
<td>37.6 ± 0.6</td>
<td>7.41 ± 0.01</td>
<td>35.2 ± 1.7</td>
<td>7.43 ± 0.02</td>
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<tr>
<td>0.06</td>
<td>0.12</td>
<td>72.6 ± 6.8</td>
<td>2.3 ± 0.4</td>
<td>13.1 ± 1.1</td>
<td>75.5 ± 1.6‡</td>
<td>7.12 ± 0.01‡</td>
<td>48.5 ± 1.9‡</td>
<td>7.31 ± 0.02‡</td>
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Values are means ± SE; n, no. of experiments. NOx, nitric oxide metabolites. *Control condition. Significantly different from corresponding control value: † P < 0.05; ‡ P < 0.01.
whereas Ppa increased from 13.1 ± 2.3 to 14.2 ± 2.2 mmHg (P < 0.05). In contrast, when FICO2 was decreased from 0.06 to 0, VNO increased from 77.3 ± 5.1 to 93.8 ± 7.3 nl/min (P < 0.05) and perfusate NOx accumulation also increased from 2.9 ± 0.3 to 4.8 ± 0.7 nmol/min (P < 0.05) with a slight reduction in Ppa. Increasing the CO2 level supplying to the ECMO from 0.06 to 0.12 did not change either VNO or Ppa despite a decline tendency for NOx accumulation in the perfusate (3.2 ± 0.4 to 2.3 ± 0.4 nmol/min, not significant).

As shown in Fig. 4A, there was significant correlation between FETCO2 and decreases in VNO from its control value (ΔVNO) (r = −0.827, P < 0.01). FETCO2 was significantly correlated also with increases in Ppa from its baseline value (ΔPpa) (r = 0.650, P < 0.01) as shown in Fig. 4B.

**Combination of Hypoxia and Hypercapnia in Buffer-perfused Lungs**

A representative recording of protocol III is shown in Fig. 5. Exhaled NO was reduced along with a gradually progressive decrease in FIO2 with an increase in Ppa in normocapnia (FICO2 = 0.06) (left). When FICO2 was switched to 0.12 (hypercapnia), the progressive hypoxia induced a further decrease in exhaled NO and a more marked rise in Ppa compared with those during normocapnia (right). The results obtained during normoxia and at the end point of progressive hypoxia are summarized in Table 3. During the entire experimental period, Ppv and Paw did not change, and isohydrict conditions of prelung perfusate were maintained. During normocapnia, hypoxia evoked an elevation in Ppa from 9.6 ± 0.5 to 12.3 ± 0.5 mmHg (28% from the baseline) with a reduction in VNO from 94.8 ± 4.2 to 26.2 ± 3.1 nl/min. On the other hand, during hypercapnia, hypoxia increased Ppa from 10.0 ± 0.3 to 15.0 ± 1.5 mmHg (50% from the baseline) with a decrease in VNO from 67.0 ± 7.0 to 18.9 ± 1.7 nl/min.

![Fig. 3. Representative recordings of exhaled NO, FETCO2, and Ppa during alveolar CO2 exposure (A) and intravascular CO2 exposure (B) in a buffer-perfused lung (protocol II). The stepwise increases in FICO2 decreased exhaled NO with a rise in Ppa (A). On the other hand, changes in FvCO2 did not affect either exhaled NO or Ppa (B). Values are means ± SE.](image-url)
Figure 6 displays the effect of hypercapnia on the pressor response (ΔPpa) to various degrees of hypoxia. When FIO2 decreased below 0.06, hypercapnia significantly enhanced the pressor response to hypoxia. Figure 7 illustrates the V˙NO response to FIO2 during normocapnia and hypercapnia. V˙NO decreased curvilinearly along with gradual decreases in FIO2 from 0.2 to 0 under normocapnic condition. Hypercapnia caused a downward shift of this curve. Mean V˙NO values at FIO2 of 0 were 26.2 nl/min during normocapnia and 18.9 nl/min during hypercapnia. To analyze the effect of hypercapnia on the kinetics of the relationships between FIO2 and V˙NO, we subtracted 26.2 and 18.9 nl/min from all the data and replotted the data using a double-reciprocal method (Fig. 8). The apparent value of K_m for O2 during normocapnia was not different from that during hypercapnia (14.4 vs. 14.9 μM), whereas V_max was significantly decreased from 66.7 to 55.6 nl/min by alveolar hypercapnia.

DISCUSSION

Quenching Effect of CO2 on NO Measurements

Inhalation of CO2 gas may lower the reading of the chemiluminescence signal for NO because CO2 and water vapor have some quenching effects on the chemiluminescence process (33). Therefore, before we discuss our results, the influence of CO2 on our NO measurements should be addressed. We found a reduction in the NO reading that was in proportion to an increase in CO2 concentration. The maximum reduction ratio of NO reading was 4.5% at 12% of CO2 concentration (Fig. 1). In terms of the actual measurement in buffer-perfused rabbit lungs, inhalation of 12% CO2 caused a marked decrease in exhaled NO by 35% from the baseline (Table 2). Although we have to take into account the quenching effect of CO2 on the measured values of exhaled NO, the magnitude of this effect seems to be too small to have a major influence on our estimation of the effects of hypercapnia on exhaled NO. In the present study, the average reduction rate of NO reading by quenching was 0.38% per 1% CO2. This reduction rate is less than that of previous report (29), and the American Thoracic Society Board of Directors recommend that the allowable tolerance for quenching should be <1% of NO reading per 1% CO2 (10). Thus we concluded that accuracy of our NO measurements during CO2 inhalation was within the tolerable range for further analysis in the present experimental set-up.

Table 3. Effect of alveolar hypercapnia on V˙NO, Ppa, and blood-gas analysis during normoxia and at the end point of gradual hypoxia

<table>
<thead>
<tr>
<th></th>
<th>V˙NO, nl/min</th>
<th>Ppa, mmHg</th>
<th>PvCO2, Torr</th>
<th>PvO2, Torr</th>
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<th>PaCO2, Torr</th>
<th>PaO2, Torr</th>
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<td>Normoxia</td>
<td>94.8 ± 4.2</td>
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<td>7.37 ± 0.01</td>
<td>38.1 ± 0.3</td>
<td>157.9 ± 5.9</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>26.2 ± 3.1*</td>
<td>12.3 ± 0.5*</td>
<td>38.4 ± 1.0</td>
<td>150.6 ± 5.3</td>
<td>7.35 ± 0.02</td>
<td>38.2 ± 3.4</td>
<td>88.8 ± 5.5*</td>
<td>7.36 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Postlung</td>
<td></td>
<td></td>
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<tr>
<td>Normocapnia</td>
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<tr>
<td>Normoxia</td>
<td>67.0 ± 7.0</td>
<td>10.0 ± 0.3</td>
<td>41.2 ± 2.0</td>
<td>145.4 ± 7.7</td>
<td>7.37 ± 0.01</td>
<td>58.2 ± 4.6</td>
<td>155.8 ± 5.7</td>
<td>7.21 ± 0.04</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>18.9 ± 1.7*</td>
<td>15.0 ± 1.5*</td>
<td>41.7 ± 0.5</td>
<td>148.0 ± 7.5</td>
<td>7.35 ± 0.01</td>
<td>63.7 ± 0.4</td>
<td>86.2 ± 4.7*</td>
<td>7.18 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pao2, arterial PO2. *Significantly different from the value during normoxia, P < 0.01.
NO Inhibition by CO₂

It was demonstrated that inhalation of hypercapnic gas caused a reduction in exhaled NO in rabbits (1, 29) and dogs (7). In contrast, ventilation with hypocapnic gas increased exhaled NO in isolated blood-perfused lungs (8). In the present study, we have further explored the effects of hypercapnia confined to either the alveolar space or intravascular perfusate on the lung NO. Our data indicate that alveolar hypercapnia suppressed both exhaled NO and perfusate NOx, whereas intravascular hypercapnia did not change exhaled NO despite a tendency of perfusate NOx to decline (Table 2). In this experimental setting, exhaled NO is derived mainly from the airway epithelium (18, 27) and perfusate NOx originates mainly from the vascular endothelium (19, 28). Thus we suggest that CO₂ could potentially suppress NO production both in the epithelium and the endothelium, but the inhibitory effect on NO synthesis secondary to alveolar CO₂ exposure is likely much greater than that due to intravascular CO₂ exposure.

NO is enzymatically synthesized by NOS from L-arginine and molecular oxygen by transferring electrons from NADPH (24). Indeed, it has been demonstrated that NO production in the airway is regulated by ambient O₂ tension through a mechanism that obeys Michaelis-Menten kinetics (11, 17). In the present study, hypercapnia caused a downward shift of the curve relating VNO and FIO₂ (Fig. 7), and double-reciprocal plotting (Fig. 8) indicated that the apparent Kₘ was unaltered by hypercapnia, whereas Vₘₚₐₓ was decreased. This kinetic behavior is compatible with noncompetitive inhibition of enzyme (NOS)-substrate (O₂) binding. However, to the best of our knowledge, the precise mechanism responsible for NOS inhibition by CO₂ remains unknown. The most likely explanation for this phenomenon is an influence of pH change on NOS activity because CO₂ can freely cross cell membranes and is promptly catalyzed to H⁺ and HCO₃⁻ by carbonic anhydrase (CA), leading to pH changes. Indeed, it has been demonstrated that NO activity is markedly decreased when intracellular pH (pHᵢ) changes from 7.2 to 6.8 in cultured human endothelial cells (13) and that neuronal NOS activity of rat brain decreases with low pH because of uncoupling of NADPH oxidation (15). Thus rapid changes in pH induced by CO₂ might be a major determinant for the NOS activity both in the epithelium and the endothelium.
The pulmonary capillary bed has been shown to have different catalytic activities and pharmacological sensitivities (30). Thus these different distributions of CA subtypes might, in part, account for the differential regulation of NOS activity by CO₂. Another possible explanation is that the CO₂ molecule itself might directly bind to a different site than substrate O₂ in the enzyme, inducing a conformational change in NOS. However, this cannot fully explain the differential regulation of NOS activity in the present experiment. Further study will be necessary to clarify this mechanism.

Regulation of Pulmonary Circulation by CO₂ via NO

The effect of CO₂ on the pulmonary circulation remains controversial. Several investigators addressed the pulmonary vasoconstrictor action of hypercapnia via increased H⁺ concentration in a wide variety of species and preparations (5, 16, 21), whereas others found conflicting evidence that the CO₂ molecule per se directly dilates smooth muscles of pulmonary vessels with an action similar to that reported in the systemic circulation (6, 34). Thus it has been suggested that the pulmonary pressor response to hypercapnia may be the net result of vasoconstriction due to increased H⁺ concentration and vasodilation due to increased CO₂ tension (32). In the present study with blood perfusion, we found that intravascular hypercapnia increased resting Ppa significantly, indicating that vasoconstrictor action of CO₂ contributed much more to the pressor response than vasodilator action of CO₂ in this species and preparation. In support of this, Baudouin and Evans (4) showed dual vasomotor actions of CO₂, in which, at low resting Ppa, CO₂ is a mild vasoconstrictor, whereas, at higher vascular tone, it acts as a dilator. Furthermore, Yamaguchi et al. (36) demonstrated recently in isolated perfused rat lungs that intravascular hypercapnia elicited a small increment of Ppa despite a significant dilatation of pulmonary venules by using a precise confocal microscopic observation system.

With regard to the endothelial NO production in response to CO₂, it was demonstrated that a NOS inhibitor attenuated hypercapnia-induced tension development in the isolated pulmonary arterial ring preparation, suggesting that vasoconstrictor effects induced by hypercapnia were caused by a reduction in endothelial NO release (22). The present results demonstrated that, in buffer-perfused lungs, alveolar hypercapnia markedly reduced perfusate NOx accumulation with a rise in Ppa, whereas intravascular hypercapnia did not elicit these effects. Endothelial NOS seems to function normally in the present experiment because our laboratory has previously demonstrated in the same preparation that endothelial NO production increases in response to acetylcholine or increments of perfusate flow (26). During buffer perfusion, the pulmonary vascular tone is lower than that during blood perfusion and vascular responsiveness may be blunted because of the low viscosity and/or hematocrit (35). Thus it may be interpreted that the less resting shear stress accounts for the small changes in Ppa and perfusate NOx induced by hypercapnia, although CO₂ can potentially inhibit NO production in the endothelium.

Concerning the site of action of CO₂, Hyman and Kadowitz (16) demonstrated using a crossover lung perfusion system in the intact lamb that alveolar hypercapnia evoked marked pulmonary vasoconstriction, whereas intravascular hypercapnia elicited modest vasodilation, suggesting that alveolar CO₂ might stimulate sensory sites in the alveolar-capillary-venous region, leading to pulmonary vasoconstriction. The novel findings from the present study are that, in both blood- and buffer-perfused lungs, alveolar hypercapnia elicited a pronounced elevation in Ppa compared with intravascular hypercapnia and that this vasoconstrictor is associated with corresponding decreases in exhaled NO. In contrast to the hypercapnia, alveolar hypocapnia decreased Ppa accompanied by increments of exhaled NO. It has been demonstrated that intrinsic NO released from the airway epithelium can control pulmonary vascular tone (17). Thus it is conceivable that alveolar CO₂ might modulate the pulmonary vascular tone secondarily via alterations in airway NO release in addition to its direct action on pulmonary vessels. Moreover, as described previously, the biological actions of CO₂ are mediated by rapid changes in pH due to catalytic activity of lung CA. Swenson et al. (31) demonstrated that local ventilation and perfusion are regulated by the local H⁺ concentration induced by the local CO₂ tension via the CA action, suggesting that lung CA plays a significant role in maintaining V̇A/Q̇ matching in the pulmonary circulation. In this regard, we speculate that alterations in CO₂ tension might change pH, primarily by lung CA followed by changes in NOS activity. Consequently, we postulate that high concentrations of CO₂ in a hypoventilated area would decrease airway NO, leading to pulmonary vasoconstriction, whereas low CO₂ concentrations in hyperventilated area would increase NO, leading to
pulmonary vasodilation, resulting in an improvement of Vo2/Qm matching.

Furthermore, we found that alveolar hypercapnia enhanced HPV under isohydric conditions in the pre-lung perfusate, which was accompanied by further suppression of exhaled NO (Figs. 4 and 5). In the literature, the effect of hypercapnia on HPV has been reported to be controversial, with evidence for vasoconstriction (2, 23) or vasodilation (4, 6). The present results confirm those of Malik and Kidd (23), who showed that, in intact anesthetized dogs, pulmonary vascular resistance increased when hypercapnia with a normal extracellular fluid pH was imposed during hypoxia. Isohydric hypercapnia is frequently observed in chronic lung disease as a result of renal compensation, and this condition plays a significant role in mediating pulmonary hypertension. Regarding the vasodilator action of NO, it is likely that a further reduction in airway NO by alveolar hypercapnia partly accounts for the enhancement of HPV. In chronic lung disease, narrowed airways and poorly ventilated alveoli distribute unevenly. In such areas, CO2 accumulates and O2 fraction decreases in accord with the severity of local hypoventilation. These local conditions would lead to a much greater deficiency of NO production, resulting in a further enhancement of HPV, diverting blood flow away from poorly ventilated areas, thereby bringing about an improvement of Vo2/Qm matching. Thus we conclude that the combination of alveolar hypercapnia and hypoxia may play a significant role in the regulation of pulmonary circulation via alterations in airway NO production.

In conclusion, we found that CO2 inhalation decreased exhaled NO and simultaneously induced pulmonary vasoconstriction, suggesting that CO2 regulates pulmonary circulation via NO released from the epithelium. Furthermore, alveolar CO2 enhanced the hypoxic pressor response with a further reduction in exhaled NO. From these results, we speculate that the combination of hypercapnia and hypoxia in poorly ventilated alveoli would lead to a deficiency of NO production, resulting in an enhancement of local hypoxic vasoconstriction and thereby an improvement of Vo2/Qm matching. Although the precise mechanism is unknown, it is likely that alveolar CO2 inhibits airway epithelial NOS activity via changes in pH. However, further study is necessary to answer these questions.

REFERENCES


