Helium inhalation enhances vasodilator effect of inhaled nitric oxide on pulmonary vessels in hypoxic dogs

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1Department of Thoracic and Cardiovascular Surgery and 2Department of Medicine, Kitasato University, Kitasato 1-15-1, Sagamihara, Kanagawa 228-8555; and 3Department of Cardiovascular Sciences, Tokyo Women’s Medical University, Tokyo 162-8666, Japan

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Nie, Masaki, Hirosuke Kobayashi, Motoaki Sugawara, Tomoyuki Tomita, Kuniyoshi Ohara, and Hirokuni Yoshimura. Helium inhalation enhances vasodilator effect of inhaled nitric oxide on pulmonary vessels in hypoxic dogs. Am J Physiol Heart Circ Physiol 280: H1875–H1881, 2001.—There are theoretical and experimental indications that the presence of He as a balance gas markedly increases the diffusion velocity of other gases contained in a gas mixture. We allowed dogs with pulmonary vasoconstriction induced by hypoxia to inhale a mixture of 5 parts per million (ppm) of nitric oxide (NO) and O2 balanced with He (NO in He) instead of N2 (NO in N2). The dilating effect of NO in He and NO in N2 on the pulmonary artery was evaluated by determining conventional pulmonary hemodynamic parameters, mean pulmonary artery (PA) pressure (MPAP), and pulmonary vascular resistance indexed to body surface area (PVRI), pulmonary impedance (Z), and the recently developed hemodynamic index, time-corrected wave intensity (WI). The main findings in this study were as follows: 1) hypoxia increased MPAP, PVRI, Z at 0 Hz (Z0), Z at the first harmonics, characteristic impedance (Zc), the reflection coefficient (Γ), and the first peak of WI; 2) NO in N2 reduced Z0 and Γ; and 3) NO in He reduced the first peak of WI and reduced Z0 and Γ more than NO in N2. The enhanced vasodilatory effect of NO in He might be associated with facilitated diffusion of NO diluted in the gas mixture with He. In conclusion, increased efficacy of NO in He offers the possibility to reduce the inhaled NO concentration.

INHALATION OF OXYGEN BALANCED with He instead of N2 was used to treat asthma patients (2, 13, 16). O2 and He mixtures have lower density than mixtures of O2 and N2 and reduce turbulence in the flow due to their low Reynolds numbers (20). However, He-O2 viscosity is actually greater than that of air. Therefore, inhalation of He-O2 might be disadvantageous in lungs with intact airways, because its greater viscosity compared with air should require more driving pressure (and hence more effort) to achieve streamlined flow through small airways (7).

There are also theoretical and experimental indications that a mixture of two (17) or more (5, 6) gases interferes with their diffusion velocities, and that the presence of He as a balance gas markedly increases the diffusion velocity of other diluted gases in a mixture. Therefore, inhalation of He mixtures might be beneficial in gas transport even in lungs with an intact airway, because diffusional transport is important in peripheral airways and alveoli.

Because inhaled nitric oxide (NO) dilates constricted pulmonary vessels and reduces pulmonary artery (PA) pressure without inducing systemic hypotension (10, 27), NO inhalation is widely used to treat patients with pulmonary hypertension (23, 29). We hypothesized that inhalation of NO in He enhances the diffusional transport of NO in peripheral airways and alveoli and therefore enhances the vasodilator effect of inhaled NO on constricted pulmonary vessels during hypoxia. It has been reported that inhaled NO only partially reverses hypoxic pulmonary vasoconstriction in dogs (30) in contrast to sheep (3, 10, 28, 32) and humans (9). Therefore, to investigate the facilitated diffusion of NO in the presence of He, we used intact dogs and allowed the dogs with pulmonary vasoconstriction induced by hypoxia to inhale a mixture of 5 parts per million (ppm) NO and O2 balanced with He (NO in He).

The specific question we attempted to answer in this study was whether NO in He improves pulmonary hemodynamics more than NO inhalation balanced with N2 (NO in N2) in our intact animal model.

Dilating effects on the pulmonary arteries were evaluated by 1) conventional pulmonary hemodynamic parameters: mean pulmonary artery pressure (MPAP), and pulmonary vascular resistance indexed to body surface area (PVRI); 2) pulmonary impedance analysis; and 3) a recently developed hemodynamic index, time-corrected wave intensity (WI) (24).

MATERIALS AND METHODS

Animals and measurements. Eight Beagle dogs weighing 12.0–22.0 kg were anesthetized with intravenous pentobarbital sodium and intubated with a tracheal tube. Intubated animals were ventilated with a Harvard respirator (Model 650; Harvard Apparatus, South Natick, MA) with room air. The ventilator was set to give a tidal volume of approximately 10 ml/kg and a respiratory frequency of 20 breaths/min. The lungs were ventilated with a tidal volume of approximately 10 ml/kg and a respiratory frequency of 20 breaths/min. The lungs were ventilated with a tidal volume of approximately 10 ml/kg and a respiratory frequency of 20 breaths/min.

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bital sodium (35 mg/kg). Anesthesia was maintained by con-

inuous intravenous infusion of pentobarbital sodium (4 mg/kg \times 1^{-1} \text{h}^{-1}), and muscles were paralyzed with pancuro-
nium (0.1 mg/kg) every 2 h. Animal care was performed in

accordance with the guidelines of the Animal Care Commit-
tee of Kitasato University, and the conduct of this study conformed to the Guide for the Care and Use of Laboratory

Animals published by the National Institutes of Health.

The animals were intubated and mechanically ventilated at 25 breaths/min and tidal volume of 12 ml/kg with positive end-expiratory pressure maintained at 3 cmH2O. Core body temperature was maintained at 37–39°C with an external heater. A 5.5-Fr thermoliation PA catheter (model P575-

EH, Abbott; Chicago, IL) was inserted via the left femoral

vein. Blood gas content was calculated by using a

analyzer (model ABL505, Radiometer; Copenhagen, Den-

mark). A 5.5-Fr thermoliation PA catheter was located at just downstream of the PA valve. Because the thermoliation PA catheter was located and kept in the main PA, we could not measure the pulmo-

nary capillary wedge pressure. Therefore, we used PA end-

diastic pressure (PAWP) as an estimate of left atrial pressure (LAP). In a preliminary study, we confirmed that LAP was stable throughout the study during hypoxia as well as normoxia, and PAWP was identical to LAP at normoxia, but PAWP was significantly higher than LAP at hypoxia. Pres-

ures were measured (in mmHg) at normoxia, PAWP = 5.3 \pm

0.2 (means \pm SE; n = 8), and LAP = 5.2 \pm 0.2; at hypoxia in

N2, PAWP = 13.1 \pm 0.4, and LAP = 5.2 \pm 0.4; at hypoxia in

He, PAWP = 12.4 \pm 0.2, and LAP = 5.2 \pm 0.2; at hypoxia in

5 ppm NO in N2, PAWP = 10.7 \pm 0.5, and LAP = 5.3 \pm 0.4;

at 5 ppm NO during hypoxia with He, PAWP = 9.0 \pm 0.4, and

LAP = 5.3 \pm 0.3. Therefore, we used PAWP at normoxia as an estimate of LAP throughout the study. The pressure and flow velocity signals of the catheter-tipped microsensors were fed via a pressure amplifier (model AP-621G, Nihonkoden; To-

kyo, Japan) and a flow amplifier (700–1,500, Narco Bio-

Systems) into a computer (PowerBook 2400c/180, Macintosh; Cupertino, CA) by using a 16-bit analog-to-digital trans-

former (model MP100A, Biopac Systems) at a sampling in-
terval of 2 ms (500 Hz). The filter characteristics of the 3-dB
cutoff frequency were 20 Hz of the one-pole Batterworth type for the pressure sensor and 30 Hz of the two-pole Batter-

worth type for the flow sensor. Electrocardiogram signals were also fed into the computer. An arterial catheter was placed in the femoral artery to measure mean systemic arterial pressure (MSAP) and to obtain arterial blood samples. Blood samples for mixed venous blood analysis were obtained through the PA catheter. The P02, Pco2, and pH values of blood were measured at 37°C with an automated blood gas analyzer (model ABL505, Radiometer; Copenhagen, Den-

mark) and were corrected for body temperature measured via

the PA catheter. We calculated cardiac output by Fick’s principle by dividing the minute oxygen consumption rate (expired volume per minute times the difference between the oxygen fraction in inspired gas and mixed expired gas) by the difference between the oxygen content of arterial and mixed venous blood. Blood gas content was calculated by using a standard formula (19). In a preliminary study, the cardiac output values obtained by Fick’s principle were found not to
differ from those obtained by the thermoliation method. The cardiac output values from Fick’s principle in means \pm SE by

n = 8 and those from thermoliation were the following (in

l/min): 3.6 \pm 0.5 and 3.1 \pm 0.0 at normoxia, 2.0 \pm 0.3 and 2.1 \pm 0.1 at hypoxia, 2.0 \pm 0.3 and 2.1 \pm 0.1 at hypoxia in

N2, 1.9 \pm 0.2 and 2.2 \pm 0.2 at hypoxia in He, 1.1 \pm 0.1 and 2.8 \pm

0.1 at hypoxia in 5 ppm NO in N2, and 2.5 \pm 0.5 and 2.7 \pm 0.0 at hypoxia in 5 ppm NO in He, respectively. The cardiac output values were indexed to body surface area (CI). The body surface area (in m2) for dogs was calculated by using the standard formula: body wt (kg)0.687 \times 0.1, and PVRI was calculated as (MPAW \times PAWP) \times 79.29 CI.

The inspiratory oxygen fraction, FiO2, was adjusted to 0.21
during normoxia and to 0.08–0.11 during hypoxia to adjust arterial Po2 (PaO2) to \sim 30 mmHg. The gas mixtures were produced by using three mass flow controllers (model 1259C, MKS; Andover, MA), one each for pure O2, N2, and He, respectively. The O2, N2, and He were of research level purity (>99.999%). The O2 fraction in the inspired gas was continuously monitored with an oxygen sensor (model OMD-100, Aika; Tokyo, Japan), and the He fraction was monitored with an He sensor (model XP-314, Shin Cosmos Denki; Osaka, Japan). The NO gas was supplied from a nitrogen-balanced 800 ppm NO gas mixture (model XP-314, Shin Cosmos Denki; Tokyo, Japan) to the inlet arm of the ventilator at a flow rate of \sim 60–140 ml/min via a microflowmeter (RK1150, Kofloc; Tokyo, Japan). The NO2 level was measured with an electrochemical sensor (NOX-BOX II, Bedfont Scientific; Kent, UK), and it was found to be 0.6 ppm in 800 ppm NO in N2 gas. The mean NO concentra-

tion in the inspired gas was continuously monitored with a chemiluminescence NO-NOx analyzer (model ECL-88US, Yanako; Kyoto, Japan) and adjusted to 5 ppm. Because the response of chemiluminescence NO-NOx analyzer is not rapid enough to detect the fluctuation of NO level during inspiration, we introduced CO2 gas instead of 800 ppm NO in

N2 to the inlet arm of the ventilator at a flow rate of 60

ml/min to examine the fluctuation of introduced gas, and we measured the CO2 concentration with the use of a high-

response capnography (Respina 1H26, San-ei; Tochigi, Jap-
n). As a result, the CO2 concentration peaked in early ex-

piration phase and returned to the plateau level during the late expiration and whole inspiration phase, indicating stable NO concentration during inspiration in this study.

The concentration of inhaled NO, 5 ppm, was chosen on the basis of a preliminary dose-response study of inhaled NO level and MPAP during hypoxia. Inhalation of 5 ppm NO in He decreased MPAP to the lowest plateau level, which was lower than the MPAP level that inhalation of 40 ppm NO in

N2 could achieve.

The study protocol sequence consisted of the following eight steps: 1) normoxia, 2) hypoxia, 3) NO during hypoxia, 4) NO during hypoxia with He, 5) NO during hypoxia with

N2, 6) hypoxia with N2, 7) hypoxia with He, and 8) normoxia with N2.

After an interval time for stabilization of MPAP at each step (from 15 to 30 min), MPAP, cardiac output, MSAP, and core body temperature were measured, and arterial and mixed venous blood samples were drawn. Meanwhile, ex-

pired gas was collected in a gastight Tedler bag for 5 min, and pulmo-
nary artery pressure and flow velocity were measured with the catheter-tipped microsensors for 10 s, eliminating the effect of breathing on the measurements by stopping the ventilator in end expiration.

Impedance analysis. The impedance was calculated from

the pressure (P) wave and bulk flow (Q) wave, which was obtained by multiplying instantaneous flow velocity (U wave) by the effective cross-sectional area of the main pulmonary artery. The effective cross-sectional area was obtained by dividing cardiac output per minute by an integral of the U wave for 1 min.
The impedance analysis was carried out according to a standard algorithm (18) with the use of a wave analysis program (Igor Pro version 3.1, WaveMetrics; Lake Oswego, OR). Briefly, a Hamming window was applied to each bin of data files composed of 4,096 points to reduce side-lobe leakage. Fast Fourier transform was then performed, and the PA input impedance \( Z_{\text{in}}(\omega) \) was calculated as a function of frequency \( (\omega) \) by using the formula \( Z_{\text{in}}(\omega) = P(\omega)/Q(\omega) \), and its modulus and phase were obtained at every P wave and Q wave until 15 Hz, which had amplitudes >2% of pressure and flow pulse amplitudes. Whenever ANOVA for repeated measures detected significance, data for specific effects of \( Z_{\text{in}}(\omega) \) spectra calculated were then corrected for the phase responses of the PA pressure and flow transducers and amplifiers.

An impedance at 0 Hz \( (Z_0) \), which represents total PVRI, was calculated from the PA input impedance spectrum. An impedance at the first harmonics \( (Z_1) \) was also derived. The characteristic PA impedance \( (Z_c) \) was calculated from the PA input impedance modulus spectra as the mean of the magnitude of \( Z_{\text{in}}(\omega) \), between 2 and 15 Hz, and the reflection coefficient \( (\Gamma) \) was calculated as:

\[
\Gamma = \frac{(Z_0 - Z_1)}{(Z_0 + Z_1)}
\]

**Time-corrected WI.** Pressure and flow signals on the time domain were measured when ventilation was stopped in end expiration. The flow velocity signals were time shifted 6–12 ms to adjust the time delay of the flow velocity signals compared with the pressure waves. We performed three-point numerical differentiation of the pressure and flow waves and calculated WI as:

\[
WI = \frac{dP}{dt} \times \frac{dU}{dt}
\]

where \( dP/dt \) is the first derivative of pressure development over time and \( dU/dt \) is the change in flow velocity over time.

**Statistical analysis.** Values are expressed as means ± SE unless otherwise stated. To confirm stability throughout the experimental steps, Student’s paired t-test was applied to the following steps: 1) normoxia and normoxia with \( \text{N}_2 \), steps 1 and 2; 2) hypoxia and hypoxia with \( \text{N}_2 \), steps 2 and 6; and 3) NO during hypoxia and NO during hypoxia with \( \text{N}_2 \), steps 3 and 5. Because there were no statistical differences in any of the parameters between the corresponding experimental steps, the corresponding data for normoxia and normoxia with \( \text{N}_2 \), hypoxia and hypoxia with \( \text{N}_2 \), and NO during hypoxia and NO during hypoxia with \( \text{N}_2 \) were averaged and shown as normoxia, \( \text{N}_2 \)-hypoxia, and NO in \( \text{N}_2 \). The data of He-hypoxia were also shown to indicate that no significant effect of He was detected by Student’s paired t-test compared with \( \text{N}_2 \)-hypoxia. Whenever ANOVA for repeated measures detected significant differences among the data during normoxia, \( \text{N}_2 \)-hypoxia, with NO in \( \text{N}_2 \), and with NO in He, the data for the specific effects of 1) hypoxia, comparing normoxia with \( \text{N}_2 \)-hypoxia, 2) NO in \( \text{N}_2 \), comparing \( \text{N}_2 \)-hypoxia with NO in \( \text{N}_2 \), and 3) “NO in He,” comparing NO in \( \text{N}_2 \) with NO in He, were evaluated by using Student’s paired t-test with the Bonferroni correction as a post hoc test.

**RESULTS**

Conventional hemodynamic parameters and blood gas analysis. Heart rate did not change during hypoxia (see Table 1). MPAP significantly increased during hypoxia, and NO inhalation in He significantly reduced MPAP compared with NO inhalation in \( \text{N}_2 \). PVRI increased during hypoxia and remained at a similar level during NO inhalation in \( \text{N}_2 \) and NO inhalation in He. CI significantly decreased during hypoxia, but it did not change both at NO inhalation in \( \text{N}_2 \) and NO inhalation in He. PaO2 decreased during hypoxia but remained at a similar level at all experimental steps during hypoxia. PaCO2 remained constant throughout the experiment. The hemodynamic and blood gas data of \( \text{N}_2 \)-hypoxia did not differ from those of He-hypoxia.

**Impedance analysis.** \( Z_0 \) significantly increased during hypoxia and significantly decreased after NO inhalation (see Table 2). It decreased further after NO inhalation in \( \text{N}_2 \). \( Z_0 \) significantly increased during hypoxia but did not change either after NO inhalation in \( \text{N}_2 \) or after NO inhalation in He. \( \Gamma \) significantly increased during hypoxia, and decreased significantly.

**Table 1. Conventional hemodynamic parameters and blood gas analysis**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>( \text{N}_2 )-Hypoxia</th>
<th>He-Hypoxia</th>
<th>NO in ( \text{N}_2 )</th>
<th>NO in He</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Fl}_{\text{O}_2} )</td>
<td>0.21 ± 0</td>
<td>0.11 ± 0.005(^b)</td>
<td>0.11 ± 0.006</td>
<td>0.11 ± 0.005</td>
<td>0.11 ± 0.005</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>147 ± 9</td>
<td>164 ± 8</td>
<td>166 ± 7</td>
<td>165 ± 9</td>
<td>164 ± 8</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>17.6 ± 1.8</td>
<td>24.9 ± 2.2(^a)</td>
<td>24.8 ± 2.3</td>
<td>21.9 ± 1.9</td>
<td>19.5 ± 1.9(^d)</td>
</tr>
<tr>
<td>MSAP, mmHg</td>
<td>147 ± 3.7</td>
<td>157 ± 4.7</td>
<td>158 ± 4.3</td>
<td>152 ± 7.2</td>
<td>150 ± 8.1</td>
</tr>
<tr>
<td>PVR, dyn·s·cm(^{-5})</td>
<td>207 ± 23</td>
<td>274 ± 150(^b)</td>
<td>738 ± 141</td>
<td>595 ± 116</td>
<td>389 ± 73</td>
</tr>
<tr>
<td>PVRI, dyn·s·cm(^{-5}·m(^{-2})</td>
<td>345 ± 50</td>
<td>1,249 ± 229(^b)</td>
<td>1,232 ± 288</td>
<td>993 ± 229</td>
<td>653 ± 151</td>
</tr>
<tr>
<td>SVRI, dyn·s·cm(^{-5}·m(^{-2})</td>
<td>3,672 ± 429</td>
<td>7,122 ± 823(^b)</td>
<td>6,903 ± 625</td>
<td>6,191 ± 898</td>
<td>5,590 ± 906</td>
</tr>
<tr>
<td>CI, l·min(^{-1}·m(^{-2})</td>
<td>11,777 ± 1,693(^b)</td>
<td>11,351 ± 1,501</td>
<td>10,209 ± 1,796</td>
<td>9,341 ± 1,862</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.42 ± 0.03</td>
<td>7.42 ± 0.02</td>
<td>7.41 ± 0.03</td>
</tr>
<tr>
<td>pCO2, mmHg</td>
<td>106.1 ± 6.4</td>
<td>40.9 ± 3.9(^b)</td>
<td>37.0 ± 4.1</td>
<td>38.0 ± 4.4</td>
<td>37.5 ± 3.8</td>
</tr>
<tr>
<td>pCO2, mmHg</td>
<td>51.2 ± 1.8</td>
<td>32.2 ± 2.8(^a)</td>
<td>31.1 ± 3.6</td>
<td>31.1 ± 3.4</td>
<td>30.8 ± 3.5</td>
</tr>
<tr>
<td>pCO2, mmHg</td>
<td>34.1 ± 2.1</td>
<td>32.5 ± 1.7</td>
<td>32.2 ± 2.8</td>
<td>32.5 ± 2.2</td>
<td>32.5 ± 1.8</td>
</tr>
<tr>
<td>pCO2, mmHg</td>
<td>37.2 ± 2.1</td>
<td>34.4 ± 2.1</td>
<td>34.5 ± 3.0</td>
<td>33.9 ± 3.0</td>
<td>34.7 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), 8 dogs in each group. \( \text{Fl}_{\text{O}_2} \), fraction of inspired oxygen; HR, heart rate; MPAP, mean pulmonary arterial pressure; MSAP, mean systemic arterial pressure; PVR, pulmonary vascular resistance; PVRI, PVRI indexed to body surface area; SVR, systemic vascular resistance; SVRI, SVR indexed to body surface area; CI, cardiac index; pH, arterial pH; pHv, venous pH; PaO2, arterial PO2; PaCO2, venous PO2; PaCO2, arterial PCO2; PaCO2, venous PCO2. Whenever ANOVA for repeated measures detected significance, data for specific effects of the following were evaluated using Student’s paired t-test with the Bonferroni correction as a post hoc test: 1) “hypoxia,” comparing normoxia with \( \text{N}_2 \)-hypoxia, \( *P < 0.05 \) and \( \&P < 0.01 \); 2) “NO in \( \text{N}_2 \),” comparing \( \text{N}_2 \)-hypoxia with NO in \( \text{N}_2 \), \( \&P < 0.05 \); 3) “NO in He,” comparing NO in \( \text{N}_2 \) with NO in He, \( *P < 0.05 \) and \( \&P < 0.01 \).
after NO inhalation in N₂. It decreased further after NO inhalation in He.

**Time-corrected WI.** WI had two positive peaks (see Table 3 and Fig. 1 for examples). During hypoxia, the first peak was increased due to increased dP/dt and dU/dt, and negative waves were occasionally observed between the first and second peaks. NO inhalation in N₂ did not decrease the first peak significantly, but NO inhalation in He reduced the first peak significantly due to decreased dP/dt and dU/dt. The second positive peak did not change during any of the experimental steps.

**DISCUSSION**

**Main findings.** This study had three main findings: 1) hypoxia increased MPAP, PVRI, Z₀, Z₁, γ, and the first peak of WI and decreased CI; 2) NO inhalation in N₂ reduced Z₀ and γ; and 3) NO inhalation in He reduced MPAP and the first peak of WI significantly and further decreased Z₀ and γ.

**Conventional hemodynamic parameters and blood gas analysis.** Hypoxia increased MPAP and PVRI, despite decreased CI, indicating the presence of hypoxic pulmonary vasoconstriction. Because cardiac output, heart rate, and blood gas values did not change significantly during hypoxia, with or without NO inhalation in N₂ or He, we were able to evaluate the effects of NO in N₂ and NO in He on MPAP and PVRI as well as other hemodynamic parameters, impedance parameters, and the time-corrected WI uninfluenced by cardiac output level, heart rate, and blood gases. At the same oxygenation level, a similar cardiac output and heart rate should be sufficient for oxygen delivery to the tissues.

Compared with NO in N₂, MPAP decreased significantly after NO inhalation in He, indicating a greater vasodilator effect of NO in He. It is likely that NO delivery to the periphery of the lungs was facilitated by He more than by N₂. As Frostell et al. (10) reported, NO inhalation had no effect on the systemic circulation, and its effects were confined to the pulmonary circulation.

**Impedance analysis.** A considerable change in Z₀ has been reported (25) during hypoxia, whereas it was reported that hypoxic pulmonary vasoconstriction was associated with insignificant changes in pulmonary vascular impedance (8, 15).

In our study, hypoxia was found to increase Z₀, Z₁, Zₐ, and γ, and NO inhalation decreased Z₀ and γ, indicating improvement in resistance and vascular impedance matching by inhalation of NO. NO in He further decreased Z₀ and γ, indicating a greater improvement in resistance and impedance matching. The vasodilator effect of 40 ppm of NO has been reported (31) to be preserved during exposure of cats to anoxia, and it completely reversed the severe pulmonary vasoconstriction. Maggiorini et al. (15) reported that inhalation of 150 ppm of NO reversed Z₀ and Z₁ at hypoxia.

All of this evidence suggests that the vasodilator effect of NO attenuates hypoxic pulmonary vasoconstriction and that this effect is facilitated in the presence of He.

**Time-corrected WI.** The WI is positive for forward traveling (compression and expansion) waves and negative for backward traveling (reflected compression and expansion) waves (24). In our study, WI had two positive peaks in the pulmonary artery, the same as in the systemic circulation. The initial peak was located in early ejection phase. A compression wave traveling from the right ventricle during early ejection accounts for the acceleration of blood flow and pressure rise in the pulmonary artery. The initial peak, which represents the intensity of a forward traveling compression wave, would be expected to vary with the contractile state of the right ventricle and with its afterload, as shown in the left ventricle (11). During hypoxia, the increased pulse wave speed, as shown by the increase in Z₀, and the increased afterload were expected to decrease the first peak of WI, but hypoxia increased WI by increasing dP/dt and dU/dt, indicating the major contribution by increased contractile performance of the right ventricle. It is possible that sympathetic nerve stimulation (4) and inotropic substances secreted at hypoxia, such as epinephrine, enhance right ventric-
ular contractility, increasing the rates of pressure and flow changes in the pulmonary artery.

NO in He decreased the initial peak significantly. The decrease in the initial peak is attributed to the decreased demand for right ventricular contractility to maintain cardiac output, likely due to decreased afterload, i.e., the vasodilator effect of inhaled NO. However, it may also be attributable to the inhibitory effect of inhaled NO on the right ventricular contractility. This possibility should be further investigated by evaluating right ventricular performance.

Between the first peak and second peak, i.e., during the sustained middle phase of ejection, WI was zero in normoxia and during hypoxia with NO inhalation, indicating that blood flow continues in the absence of significant net wavefront travel and implying that right ventricular shortening matched pulmonary artery outflow. The momentum of the flowing blood dominated right ventricular ejection with little wave energy flux being transmitted from the ejecting heart to the ejected blood.

Negative waves were occasionally observed during hypoxia, indicating backward waves, i.e., reflection waves. Backward waves did not have sharp peaks but consisted of several broad peaks, indicating several reflection sites in pulmonary arterial vessels during hypoxia. Inhaled NO is reported to uniformly dilate vessels, from large pulmonary arterial vessels to peripheral vessels, ensuring uniform blood flow due to reduced reflection waves.

The second peak occurred during the late ejection phase. Analysis of WI has shown that the heart itself stops blood flow in the aorta during late ejection phase before closure of the aortic valve by generating forward expansion waves traveling in the aorta from the left ventricle toward the periphery (12, 21, 22). The same as in the systemic circulation, the second peak in the pulmonary artery in late ejection phase indicates that the right ventricle also stops blood flow in the pulmonary artery by generating forward expansion waves traveling in the pulmonary artery from the right ventricle toward the peripheral vessels. Hypoxia and NO inhalation did not alter the magnitude of the second peak, because NO inhalation decreased the magnitude of the negative dP/dt at the second peak, but it did increase the magnitude of the negative dU/dt.

**NO inhalation with He.** Inhalation of NO in He improved pulmonary hemodynamics more than inhalation of NO in N₂, although this vasodilatory effect was partial (66% decrease in the increase in PVRI by NO in He during hypoxia, in contrast to 28% decrease by NO in N₂) and less than that at normoxia (i.e., 100% recovery). This result was similar to the report by Romand et al. (30) of NO inhalation in a canine model with hypoxic pulmonary vasoconstriction, suggesting that NO and hypoxia act on the vasoconstriction response via different reactions and/or different receptors in dogs. Regional electrophysiological diversity among pulmonary vascular smooth muscle cells is reported (1) to be a major determinant of segmental differences in vascular reactivity to hypoxia and NO.

Therapeutic use of a He-O₂ mixture was first described by Barach (2). To the extent that gas flow through obstructed airways is turbulent, inhalation of lower density gas may preserve laminar flow at high flow rates by reducing the Reynolds number (20), thereby reducing airway resistance. In addition, because the pressure drop during turbulent flow in large airways is proportional to $\rho u^2$, where $\rho$ is the density and $u$ is the flow velocity of a gas mixture in the airway, a gas mixture with lower density decreases the...
pressure drop. An 80:20 He-O₂ mixture, Heliox, has a density one-third that of air.

However, gas velocity rapidly decreases beyond the third-generation central airways in the normal bronchial tree, because airway cross-sectional area increases, and thus the resistive pressure drop for laminar flow in the peripheral airways is independent of gas density. The viscosity of the He-O₂ mixture is actually greater than that of air. Therefore, it has been considered that inhalation of 80% He-20% O₂ should be useful only in those conditions in which a decrease in gas density is beneficial; such a decrease permits greater flow for the same driving pressure when air flow is turbulent. This occurs only where flow is rapid or where irregularities in the lumen of air passages cause eddy currents (7). Therefore, the administration of He-O₂ mixture would not be beneficial for oxygen transport in the lungs without airway narrowing, and inhalation of He-O₂ might be disadvantageous because its greater viscosity compared with air should require more driving pressure to achieve laminar flow through small airways (7). A possible reduction in airway resistance in this study may not have been a major cause of the improved hemodynamics because the lungs are intact and the ventilation rate was kept constant throughout the experiment, irrespective of the level of resistance.

The characteristics of diffusion involving two species of gas, called binary diffusion (17), have been reported to be quite different from diffusion of one component, and the characteristics of diffusion involving more than three species of gas, multicomponent diffusion, have also been reported to be different from diffusion of two (5). This notion has also been verified experimentally (6). On the basis of the blood gas data in this study, it is likely that transport of O₂ and CO₂ was not facilitated by He. In contrast, on the basis of the hemodynamic data, it is suggested that He facilitated the diffusion velocity of NO, possibly due to the diluted amount of NO in the gas mixture. However, it should be noted that there is no direct evidence of facilitated NO diffusion in this study, and the mechanism of the enhanced vasodilatory effect of NO in He needs to be further investigated.

NO inhalation is widely used to treat patients with pulmonary vasoconstriction, including patients with primary pulmonary hypertension (23), persistent pulmonary hypertension of the newborn (29), postoperative pulmonary vasoconstriction complicating congenital heart defects (26), and limited scleroderma with isolated pulmonary hypertension (33). The observed decrease in MPAP during NO inhalation in He may be easily obtained by an increased NO concentration in N₂. However, it is recommended that the concentration of inhaled NO be as low as possible (34), because NO combines with O₂ to produce NO₂, and may damage pulmonary tissue. To comply with the demand to minimize the inhaled dose of NO, breath-by-breath delivery of spikes of concentrated gas has been proposed to lower its concentration (14). NO inhalation balanced with He is another potential tool for enhancing the vasodilator effect of NO on pulmonary vessels or for reducing the NO concentration while maintaining adequate pulmonary vasodilation. Although 90% of He in inspired gas is an unrealistic proportion for clinical applications, NO in He may be applied to patients with pulmonary hypertension without respiratory failure, in whom N₂ can be replaced with He in a sufficient proportion of the inspired air. In conclusion, administration of NO in He improved pulmonary hemodynamics more than NO in N₂.

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