Inhaled Nitric Oxide
A Selective Pulmonary Vasodilator Reversing Hypoxic Pulmonary Vasoconstriction

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Background. We examined the effects of inhalation of 5–80 ppm nitric oxide (NO) gas on the normal and acutely constricted pulmonary circulation in awake lambs.

Methods and Results. Spontaneous breathing of nitric oxide (an endothelium-derived relaxing factor) at 40 ppm or more reversed acute pulmonary vasoconstriction within 3 minutes either because of infusion of the stable thromboxane endoperoxide analogue U46619 or because of pulmonary hypertension due to breathing a hypoxic gas mixture. Systemic vasodilation did not occur. Pulmonary vasodilation by NO inhalation was produced during infusion of U46619 for periods of 1 hour without observing evidence of short-term tolerance. Pulmonary hypertension resolved within 3–6 minutes of ceasing NO inhalation. In the normal lamb, the pulmonary vascular resistance, systemic vascular resistance, cardiac output, left atrial and central venous pressures were unaltered by NO inhalation.

Conclusion. Breathing 80 ppm NO for 3 hours did not increase either methemoglobin or extravascular lung water levels or modify lung histology compared with those in control lambs. (Circulation 1991;83:2038–2047)

Nitric Oxide (NO) was reported in 1987 to be an important endothelium-derived relaxing factor. The vascular relaxant effect of NO on arterial strips is antagonized by substances such as hemoglobin that bind NO extracellularly with high affinity, thereby inactivating NO. The common nitrovasodilators, nitroprusside and glyceryl trinitrate, act by releasing NO, probably intracellularly, to mimic the effect of endogenous NO. We examined the effects of inhalation of 5–80 ppm gaseous NO on the normal and acutely constricted pulmonary circulation of awake lambs. We hypothesized that inhalation would allow NO to diffuse directly into the smooth muscle of pulmonary vessels and produce vasodilation while preventing NO from being exposed to hemoglobin. We believed an inhalation strategy would surmount the central problem of intravenous administration of vasodilators to dilate the lung’s vessels. The dosage of vasodilators is limited during intravenous administration because of concomitant dilation of the systemic circulation, which causes peripheral hypotension, right ventricular ischemia, and consequent heart failure. Inhaled NO can potentially dilate pulmonary vessels and yet because of its rapid reaction with hemoglobin, it would prevent systemic vasodilation.

There is evidence that hypoxic pulmonary vasoconstriction of isolated pulmonary artery rings is enhanced by putative inhibitors of NO synthesis, including NG-monomethyl-L-arginine. In addition, intravenous aliquots of dissolved NO dilate the isolated angiotensin II–primed rat lung perfused with an acellular Krebs’ solution containing meclofenamate.

NO is the major oxide of nitrogen formed during a variety of high-temperature combustion processes and is a common air pollutant. NO is produced in the hot reducing atmosphere near the glowing cone of a cigarette and is inhaled in smoke at concentrations of 400–1,000 ppm. Thirty minutes of exposure of rats to 1,000 ppm NO and rabbits to 43 ppm NO for 6 days causes neither pulmonary pathological effects (according to light and electron microscopy) nor pulmonary edema (by gravimetry). Humans have breathed up to 30 ppm NO for 15 minutes with minor effects on gas exchange and respiratory function. The US Occupational Safety and Health Administration has set the time-weighted average NO value as 25 ppm. Because NO has a much greater affinity for...
hemoglobin than does carbon monoxide, NO has been used clinically to measure the lung's diffusion capacity.

Our main goal was to examine the ability of NO inhalation to reverse hypoxic pulmonary vasoconstriction because it is an important adaptation of lung vessels, which can at times contribute to severe acute pulmonary hypertension (e.g., high altitude pulmonary edema). We studied awake unanesthetized lambs to avoid general anesthesia, which can blunt hypoxic vasoconstriction. In addition, we planned to develop a dose–response curve to inhaled NO during intravenous infusion of a potent pulmonary vasoconstrictor, the stable thromboxane analogue U46619.

Methods

All studies were approved by the Animal Studies Committee of our hospital. Eight Suffolk lambs weighing 25–35 kg underwent a sterile thoracotomy to place a left atrial line, a tracheostomy, and a femoral artery line under general endotracheal anesthesia with halothane and oxygen 3 days before the study. After this recovery period, the lambs underwent sterile placement of a 7F thermodilution pulmonary artery catheter (Edwards Laboratories, Santa Ana, Calif.) through a jugular vein under local anesthesia.

During the study, the tracheostomy was connected to a non-rebreathing circuit consisting of a 5-l reservoir bag and a one-way valve to separate inspired from expired gas. Expired gas was scavenged and discarded. The inspired gas was a precise mixture of oxygen and nitrogen immediately diluted with NO to produce the correct inspired concentration. With volumetrically calibrated flowmeters, varying quantities of NO mixed with N₂ were substituted for pure N₂ to obtain the desired inspired NO concentration at an inspired oxygen concentration (FIo₂) of 0.6–0.7. We chose 60–70% oxygen to avoid hypoxic vasoconstriction. The reservoir bag was emptied after each level of NO inhalation. NO was obtained from Air Products and Chemicals, Inc., (Allentown, Penn.), as a mixture of 235 ppm NO in pure N₂. Chemiluminescence analysis demonstrated less than 12 ppm NO₂ in this mixture.

Mean and phasic pulmonary artery pressure (PAP), left atrial pressure (LAP), systemic arterial pressure (SAP), and central venous pressure (CVP) were continuously monitored using calibrated pressure transducers (model 1280C, Hewlett-Packard, Palo Alto, Calif.) zeroed at the left atrial level and a four-channel recorder (model 7754A, Hewlett-Packard). Cardiac output (CO) was measured by thermodilution as the average of two determinations after injection of 5 ml 0°C Ringer's lactate. Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were computed by standard formulas.

Protocol

Eight lambs were studied awake, spontaneously breathing, and eating ad libitum. The series of studies (A–D) were completed, on separate days, on each lamb when the following exclusion criteria did not occur: a peripheral white blood cell count less than 4,000 or more than 12,000/mm³, mean PAP more than 20 mm Hg, and a core temperature more than 40.1°C.

A. Control NO inhalation. After baseline measurements, six of the eight lambs were allowed to breathe 80 ppm NO for 6 minutes at FIO₂ 0.6–0.7. Hemodynamic measurements were obtained at 3 and 6 minutes during NO inhalation and were repeatedly obtained 3 and 6 minutes after ceasing NO inhalation.

B. Dose–response study of intermittent NO inhalation during U46619 infusion. Eight lambs breathing oxygen at FIO₂ 0.6–0.7 were given an infusion of a potent pulmonary vasoconstrictor, the stable endoperoxide analogue of thromboxane (5Z=9α,13E,15S)-11,9-(E-poxymethano) prosta-5, 13-dien-l-0ic acid (U46619, Upjohn, Kalamazoo, Mich.) at a rate of 0.4–0.8 μg/kg/min to increase the mean PAP to 30 mm Hg.

To obtain a pulmonary vasodilator dose–response curve during U46619 infusion, after a stable hemodynamic period, each of the eight lambs breathed a series of NO and O₂ mixtures of 5, 10, 20, 40, and 80 ppm NO for 6 minutes. Each level of NO exposure was followed by 6 minutes of breathing the oxygen mixture without NO. Then, a second exposure to 5, 10, and 20 ppm NO for 6 minutes was performed, and each lamb was examined for the occurrence of acute tolerance. Subsequently, each lamb was studied during a control period of breathing the oxygen mixture 6 minutes after ceasing U46619 infusion. Methemoglobin levels were measured before and after the study.

C. Study of tolerance after 1 hour of NO inhalation during U46619 infusion. Four awake lambs were given a U46619 infusion to raise their mean PAP to 30 mm Hg. Pulmonary and systemic hemodynamic measurements were obtained. Then, the lambs breathed 80 ppm NO at FIO₂ 0.6–0.7 for 1 hour. Repeated measurements were obtained at 3, 6, 15, 30, and 60 minutes of NO breathing and again at 3 and 6 minutes after ceasing NO breathing. Then, U46619 infusion was discontinued, and repeated hemodynamic measurements were obtained after 10 minutes.

D. NO breathing during hypoxic exposure. Five of the awake lambs were studied during a period of breathing a hypoxic gas mixture to induce acute hypoxic pulmonary hypertension. We used a nonbreathing circuit containing a 25-l reservoir bag, and the FIO₂ was reduced to 0.06–0.08 to produce a mean PAP near 25 mm Hg at a PaO₂ near 30 mm Hg. Then, either 40 or 80 ppm NO was added to the inspired gas mixture at the same FIO₂. Total gas flows were maintained at 35 l/min to prevent rebreathing due to hyperventilation. The inspired FIO₂ was monitored with an electrode, and pure CO₂ was added to the inspired gas to maintain the end-tidal CO₂ concentration (model LB-2, Beckman, Schiller Park, Ill.) at 4.5–6%. Measurements of central hemodynamics and gas exchange were obtained at baseline, while
breathing air, during hypoxia, and at 3–6 minutes of NO breathing during hypoxia.

E. Toxicology at 1 and 3 hours of 80 ppm NO inhalation. Thirteen additional lambs were included in this study to examine the effects of longer NO exposure. Twenty-four hours before study, a tracheostomy was performed during a brief halothane and O₂ anesthetia. Then, lambs were allowed to breathe either a gas mixture of room air and O₂ at FIO₂ 0.3–0.4 for 3 hours (control group, n = 5) or air and O₂ at the same FIO₂ containing 80 ppm NO for 1 hour (n = 5) or 3 hours (n = 3). Each animal was then killed with potassium chloride administered intravenously during a brief halothane anesthesia. The lungs were quickly excised and separated. The right lung was dissected to remove bronchi greater than 5 mm in diameter and was used to determine the percentage of extravascular lung water content by the method described by Peterson et al. Twenty-five appropriate tissue blocks were selected from each lobe of the right lung and placed in Trumps' fixative (1% glutaraldehyde and 4% formaldehyde in phosphate buffer, pH 7.2) and then processed for pathological examination. Five-micron resin-embedded sections were stained with hematoxylin and eosin. Methemoglobin levels were measured before and after 1 and 3 hours of NO inhalation.

Statistical Analysis

All data are expressed as mean±SEM. Data were compared by a two-way analysis of variance (ANOVA, version 5.16, SAS Institute, Cary, N.C.) or a paired t test (dose–response slopes). A p value less than 0.05 was considered to indicate statistical significance. For the dose–response study, the dose–response slopes, and the ANOVA were calculated with the values from both the early and the late series for the 5, 10, and 20 ppm NO doses. By averaging these values, the effect of time was minimized. For the hypoxic exposure study, comparisons were performed with paired t tests.

Results

A. Control NO Inhalation

In the six lambs spontaneously breathing 80 ppm NO for 6 minutes, we measured no change of mean PAP, PVR, SAP, CO or SVR (Figure 1).

B. Dose–Response Study of Intermittent NO Inhalation During U46619 Infusion

At all dose levels, NO inhalation produced a prompt reduction of the pulmonary hypertension caused by U46619 infusion. The onset of pulmonary vasodilation occurred within seconds after beginning NO inhalation, and the vasodilator effect was nearly maximal within 3 minutes (Figure 2). Termination of NO inhalation caused a return to the prior level of vasoconstriction within 3–6 minutes. The inhaled NO pulmonary vasodilator dose–response curve of eight lambs is shown in Figures 3 and 4. Note that 5 ppm NO (an inhaled lung dosage of approximately 0.9 µg/kg/min) significantly reduced the PAP, and an almost complete vasodilator response occurred by inhalation of 40 or 80 ppm NO. Mean methemoglo-
bin levels were 1.06±0.13% before and 1.29±0.33% after (not significant) the U46619 infusion during inhalation of the various NO mixtures (n=7). After consideration of the minor reduction over time of baseline PAP during U46619 infusion, the vasodilator response of the second exposure was compared with breathing 5, 10, and 20 ppm NO; no significant reduction from the prior series of exposures occurred. Regression analyses of NO concentration during U46619 infusion with SVR, CO, or SAP showed that the slopes were not significantly different from zero.

C. Study of Tolerance After 1 Hour of NO Inhalation During U46619 Infusion

In four lambs, inhalation of 80 ppm NO for 1 hour during U46619 infusion produced sustained pulmonary vasodilation to a normal PAP and PVR for 1 hour, and pulmonary hypertension promptly recurred after ceasing NO inhalation (Figure 5). There was no short-term tolerance to NO vasodilation. Methemoglobin levels ranged from 0.6% to 2.0% (1.10±0.25%) after 1 hour of 80 ppm NO inhalation and did not differ significantly from the control value (0.68±0.14%).

D. NO Breathing During Hypoxic Exposure

In all five lambs, acute hypoxia produced pulmonary hypertension and a marked increase of cardiac output. In each instance when 40 or 80 ppm NO was added to the inspired hypoxic gas mixture, pulmonary hypertension was relieved and pulmonary artery pressure returned to control levels while the lambs breathed air despite a maintenance of elevated CO (Table 1).

E. Toxicology of 1 and 3 Hours of 80 ppm NO Inhalation

Effects of 1 and 3 hours of 80 ppm NO inhalation are summarized in Table 2. Five lambs were killed after breathing 80 ppm NO through the tracheostomy for 1 hour. The extravascular lung water level of lambs breathing 80 ppm NO for 1 hour was 74.8±0.9% and did not differ from the control value of 74.2±1.4% obtained in five lambs. The extravascular lung water level of three lambs breathing 80 ppm NO for 3 hours was 73.9±1.2%, and these values were not significantly different from control levels. Methemoglobin levels after 1 or 3 hours of breathing 80 ppm NO did not change from control values. All the lungs from NO breathing and control lambs showed microscopic evidence of a scattered low-grade inflammatory response. There were no consistent histological differences between the control and NO breathing groups.

Discussion

Our studies demonstrate that inhaled NO can act as a selective local pulmonary vasodilator without causing systemic vasodilation. NO inhalation reversed the pulmonary hypertension caused by infusing the stable endoperoxide analogue U46619 without decreasing systemic arterial pressure (Figure 2). The pulmonary vasodilator effect of NO inhalation for 1 hour did not exhibit short-term tolerance in lambs (Figure 5). Intravenous infusion of nitroprusside and nitroglycerine can also reduce the pulmonary vasoconstriction produced in dogs by U46619 infusion, but such infusion causes marked concomitant systemic arterial vasodilation and hypotension. NO directly combines with heme to form the paramagnetic species, nitrosyl-heme, which is the active species responsible for the activation of guanylate cyclase by NO and, thereby, for stimulating the accumulation of intracellular cyclic GMP in vascular smooth muscle. We believe rapid combination with hemoglobin in red blood cells inactivates inhaled NO, restricting inhaled NO vasodilation to vessels in the lung and preventing systemic vasodilation. Thus, in normal lambs breathing 80 ppm for 6
minutes, PAP, PVR, SAP, or SVR did not change (Figure 1). The normally low-resistance pulmonary vasculature had no dilatory response during NO breathing, and the SVR was not altered.

Hypoxic pulmonary constriction is an important regulatory mechanism in both the normal and diseased lung. Pulmonary hypertension due to alveolar hypoxia occurs at high altitude and contributes to high-altitude pulmonary edema and right ventricular failure. Pulmonary hypertension due to alveolar hypoxia is common in the Adult Respiratory Distress Syndrome, augmenting pulmonary edema and leading to hypoxia and death. Others have shown that both intravenous infusion of nitroprusside and nitroglycerin can reverse acute pulmonary vasoconstriction and hypertension due to breathing 12.5% O$_2$ in normal humans. However, there was marked concomitant systemic vasodilation and systemic hypotension. The ability of NO inhalation at 40 and 80 ppm to antagonize hypoxic pulmonary vasoconstriction without reducing systemic blood pressure or SVR may allow a new therapeutic strategy for the treatment of these types of acute pulmonary hypertension, provided the toxicity of NO does not preclude prolonged pulmonary exposure. This topic will be discussed considering: 1) oxidation of NO to NO$_2$, 2) pulmonary effects of inhaling NO and NO$_2$ at high and low levels, 3) NO and hemoglobin interaction, and 4) the metabolic fate of NO.

**Oxidation of NO to NO$_2$**

The inhalation toxicology of inhaled NO and NO$_2$ is difficult to ascertain separately because of oxidation of NO to NO$_2$ in the presence of oxygen, the rate
of oxidation being dependent on the initial concentration of NO and the FIO2.28 For example, in air at 20°C, a 10,000 ppm NO mixture undergoes 50% conversion from NO to NO2 in 24 seconds, whereas a 10 ppm NO mixture undergoes 50% conversion to NO2 in about 7 hours.29

The level of NO2 in our gas mixtures was less than 5% of the NO concentration as measured by chemiluminescence.23 We believe there was minimal conversion of NO to NO2 during the brief mean transit time (<30 seconds) from the gas reservoir bag to alveoli. Thus, our lambs were exposed to no more than 4 ppm NO2 at the highest dose (80 ppm NO); we did not believe it was necessary to chemically absorb NO2 from the inspired gas.20

**Airway and Pulmonary Effects of Inhaling NO and NO2 at High Levels**

Inhalation of gas mixtures containing high concentrations of NO and NO2 can be rapidly lethal in humans and can cause severe acute lung damage with pulmonary edema and marked methemoglobinemia.31 Greenbaum et al32 exposed anesthetized dogs to 5,000–20,000 ppm NO, and the exposure produced rapid death due to a critical reduction of arterial oxygen content caused by methemoglobinemia, a right to left shunt due to severe alveolar edema, and acidemia with a rightward shift of the oxyhemoglobin dissociation curve. A rapid reduction of lung compliance was measured during exposure to 20,000 ppm NO. The investigators believed32 that conversion of high levels of inhaled NO to NO2 followed by subsequent transformation to nitric and nitrous acid resulted in an acid pneumonitis.

**Pulmonary Effects of Inhaling NO and NO2 at Low Levels**

More recent studies examined exposure to lower levels of NO. Stavert and Lehnert14 reported that rats exposed to 15 minutes of 1,500 ppm NO showed no increases of extravascular lung water and no evidence of histopathological changes. In their study, the conversion of NO to NO2 was minimized by using high flow rates to reduce gas residency time in the exposure chamber as well as partially filling the gas mixing chamber with soda lime to chemically absorb

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**FIGURE 4.** Pulmonary artery pressure (PAP) dose–response curves (n=8, mean±SEM) for nitric oxide (NO) inhalation in lambs given an infusion of U46619. Top panel: Mean PAP vs. NO concentration. Bottom panel: Percent maximal change of PAP vs. NO concentration. PAP (% max) was computed as: \( PAP (\% \text{ max}) = 100 \times \frac{\text{PAP}_{\text{HT}} - \text{PAP}_{\text{NO}}}{\text{PAP}_{\text{HT}} - \text{PAP}_{\text{C}}} \), where \( \text{PAP}_{\text{HT}} \) is the elevated mean PAP level recorded immediately before NO inhalation, \( \text{PAP}_{\text{NO}} \) is the mean PAP measured after 6 minutes of NO inhalation, and \( \text{PAP}_{\text{C}} \) is the mean control value obtained before infusion of U46619.
NO₂. They reported that NO₂ contamination during NO exposure was less than 15 ppm. In other studies, exposure of rats to 25–100 ppm NO₂ for 30 minutes resulted in histological evidence of lung injury. At more than 50 ppm NO₂, 5 minutes of inhalation resulted in significant lung injury, with increases of extravascular lung water, extravasated erythrocytes, type II pneumocyte hyperplasia and accumulation of fibrin, polymorphonuclear cells, and alveolar macrophages in the alveoli. Little evidence for NO toxicity exists with exposures less than 100 ppm in normal rats and rabbits. Hugod reported on exposure of rabbits for 6 days to air containing a mixture of 3.6 ppm NO₂ and 43 ppm NO. Using two different procedures for tissue fixation from control and exposed animals and a blind evaluation of light and electron microscopy results, Hugod reported no evidence of acute NO toxicity.

Evans et al studied young rats exposed to 2.0 and 17 ppm NO₂ for up to 360 days. Early evidence of cell proliferation in terminal bronchioles and alveoli was found in both groups after 2–3 days but was normalized after 5 days. Epithelial hyperplasia of terminal bronchioles and an increased turnover rate of type II alveolar cells were noted. The same investigative group noted that after exposure to 2 ppm NO₂ for 24–72 hours pulmonary changes included loss of cilia, hypertrophy, and focal epithelial hyperplasia of the terminal bronchioles with an apparent return to normal (adaptation to exposure) after 21 days of continuous exposure to NO₂. Species differences may exist in pulmonary susceptibility to NO₂ toxicity as

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**TABLE 1. Alterations of Hemodynamics and Gas Exchange**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypoxia + 40–80 ppm NO</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIO₂</td>
<td>0.21</td>
<td>0.06–0.08</td>
<td>0.06–0.08</td>
</tr>
<tr>
<td>Pao₂ (mm Hg)</td>
<td>70.8±4.4</td>
<td>28.2±1.4*</td>
<td>31.1±1.7*</td>
</tr>
<tr>
<td>Pvo₂ (mm Hg)</td>
<td>36.8±2.5</td>
<td>16.6±1.8*</td>
<td>19.8±3.2</td>
</tr>
<tr>
<td>Paco₂ (mm Hg)</td>
<td>33.9±1.4</td>
<td>38.6±2.6</td>
<td>40.0±2.7</td>
</tr>
<tr>
<td>pHa</td>
<td>7.47±0.01</td>
<td>7.42±0.03</td>
<td>7.40±0.03</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>16.7±0.6</td>
<td>28.3±2.2*</td>
<td>18.7±1.1†</td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>5.2±0.8</td>
<td>6.4±0.5</td>
<td>4.2±1.0</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>4.55±0.13</td>
<td>7.08±0.22*</td>
<td>7.56±0.79*</td>
</tr>
<tr>
<td>PVR (mm Hg/l/min)</td>
<td>2.51±0.11</td>
<td>3.07±0.25</td>
<td>2.01±0.35†</td>
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<tr>
<td>SAP (mm Hg)</td>
<td>103±6</td>
<td>113±7</td>
<td>106±5†</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>3.0±1.3</td>
<td>3.5±0.8</td>
<td>2.8±1.6</td>
</tr>
<tr>
<td>SVR (mm Hg/l/min)</td>
<td>21.7±1.4</td>
<td>16.2±0.9*</td>
<td>13.7±1.0*</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n=5 lambs.

NO, nitric oxide; FIO₂, inspired oxygen concentration; PAP, pulmonary arterial pressure; LAP, left atrial pressure; CO, cardiac output; PVR, pulmonary vascular resistance; SAP, systemic arterial pressure; CVP, central venous pressure; SVR, systemic vascular resistance.

*p<0.01 value differs from control; †p<0.01 NO + hypoxia value differs from hypoxia.
demonstrated by Erlich, who noted that squirrel monkeys and hamsters tolerated inhaling a dose of NO, 10-fold higher than that tolerated by mice.

von Nieding and coworkers examined pulmonary mechanics and gas exchange in more than 100 healthy humans and in 132 patients with respiratory disease breathing 1–30 ppm NO for up to 15 minutes. At concentrations of 15 ppm NO or greater, they reported a small reversible decrease in arterial oxygen tension and a minor increase of airway resistance. They also measured a decreased arterial oxygen tension and a reduced diffusion capacity for CO after the subjects had been breathing 5 ppm NO2 or more for 15 minutes. An altered diffusion capacity for CO did not occur after breathing 40 ppm NO.

In our lamb studies, the frequent finding of endemic pneumonia with regional bronchopneumonia in control lambs did not allow us to histologically differentiate lambs breathing 80 ppm NO for 3 hours from control lambs. In the future, inhalation toxicology studies should be pursued in an experimental animal species without endemic low-grade pulmonary inflammation. We found no increase of extravascular lung water levels in these animals after breathing 80 ppm NO for 1 and 3 hours (Table 2).

**NO and Hemoglobin Interaction**

The heme structure of hemoglobin has great affinity for NO, and we believe that inhaled NO is inactivated by hemoglobin, thereby restricting its vasodilator activity to the lung. NO combines with hemoglobin forming nitrosyl hemoglobin with an affinity 1,500 times higher than that measured for carbon monoxide. Nitrosyl hemoglobin is oxidized to methemoglobin when oxygen is present, by a poorly understood mechanism. Methemoglobin, in turn, is metabolized into nitrate. In the reaction process of NO with hemoglobin, damage to erythrocyte membranes has been observed. Oda and coworkers studied mice after 6 months of exposure to 10 ppm NO and reported that the mice displayed signs of increased red blood cell turnover with enlarged spleens and increased bilirubin levels. Spleen weights increased significantly after 2 weeks of exposure. Nitrosyl hemoglobin levels remained low (0.13%); methemoglobin levels were 0.2% in both control and exposed mice.

It is uncertain what the critical duration and level of NO exposure in humans will be before red blood cell degenerative processes become significant. In studies by von Nieding et al of humans breathing 20–30 ppm NO for 15 minutes, the methemoglobin levels increased by a mean of 0.52% from an initial mean value of 0.72%. Our lambs had no significant increase of methemoglobin levels when measured before and after 1 and 3 hours of breathing 80 ppm NO (Table 2).

**Metabolic Fate of NO**

Hemoglobin and related hemoproteins bind NO with great affinity and form methemoglobin. NO gas is unstable and undergoes spontaneous oxidation to NO2 and higher oxides of nitrogen as outlined above. As a dilute solution exposed to oxygen, NO has a half-life of less than 10 seconds because of rapid oxidation to inorganic nitrite and nitrate. In the presence of superoxide, the half-life of NO is greatly reduced whereas in the presence of superoxide dismutase, its half-life is significantly increased.

NO is cleared from inhaled air by approximately 80% during quiet breathing and by up to 90% during deep breathing. Goldstein et al examined the distribution in monkeys of an inhaled dose of 0.3–0.9 ppm NO2. They reported that after a few minutes 50–60% of the inhaled NO2 was found in the lung and spread to other organs through the blood stream. Yoshida and Kasama measured a high nitrogen-15 content in blood serum and urine of rats and mice after inhalation of 138 to 880 ppm 15NO. Within 24 hours, about 40% of the inhaled 15N was excreted into urine.

It is possible that inhalation of NO will not dilate chronically constricted pulmonary vessels if pulmonary vascular musculature is too distant from the alveolus to allow diffusion in sufficient concentrations or if downstream venous muscle is protected by intravascular hemoglobin. In a report of seven patients with primary pulmonary hypertension breathing 40 ppm NO, only a minor dilatory effect on PAP and reduction of PVR was noted. A dose–response curve was not reported. These patients were awaiting heart and lung transplantation and suffered from chronic obstructive pulmonary hypertension rendering their vascular cross-sectional area fixed and restricted. Because NO inhalation is readily performed and vasodilator effects commence within minutes after beginning administration, brief periods of NO inhalation may provide a simple and selective test of pulmonary vasodilatory potential. Patients with chronic pulmonary hypertension vasodilating during NO breathing could then be tested with intravenous agents that are suitable for long-term administration.

In this study, we did not measure the effects of NO inhalation on airway mechanics. However, we note with interest that there are experimental data indicating that NO may be one of the recently described epithelium-derived relaxing factors causing local bronchodilation somewhat analogous to what has been demonstrated in the blood vessel wall.

In conclusion, inhalation of low levels of NO for brief periods warrants further study as a selective...
pulmonary vasodilator in acute and chronic lung disease associated with pulmonary hypertension. NO inhalation, thus, represents a practical application of basic vascular research. This mode of direct vasodilator delivery to the lung may allow novel diagnostic and therapeutic applications advancing our understanding and treatment of pulmonary vascular diseases. However, a number of toxicological studies remain to be performed; for example, toxicity to the lungs caused by long-term breathing of NO has not been examined and therefore, NO for long-term inhalation therapy cannot be recommended. Long-term toxicological studies of NO inhalation by animals with preexisting acute or chronic lung disease are also necessary. If long-term inhalation of low levels of NO or NO-releasing compounds proves both safe and effective at reducing pulmonary hypertension, a new era in the selective treatment of pulmonary vascular disease will have begun.

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