Pulmonary arterial dilation by inhaled NO: arterial diameter, NO concentration relationship

JASON BENTLEY,1 DAVID RICKABY,1 STEVEN T. HAWORTH,1 CHRISTOPHER C. HANGER,2 AND CHRISTOPHER A. DAWSON1,3,4

Departments of 1Physiology, and 2Anesthesiology, Medical College of Wisconsin, Milwaukee 53226; 3Department of Biomedical Engineering, Marquette University, Milwaukee 53201; and 4Research Service, Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53293

Received 1 May 2001; accepted in final form 22 June 2001

Pulmonary arterial dilation by inhaled NO: arterial diameter, NO concentration relationship. J Appl Physiol 91: 1948–1954, 2001.—The objective of this study was to determine the nitric oxide (NO) concentration and vessel diameter dependence of the pulmonary arterial dilation induced by inhaled NO. Isolated dog lung lobes were situated between a microfocal X-ray source and X-ray detector and perfused with either blood or plasma. Boluses of radiopaque contrast medium were injected into the lobar artery under control conditions, when the pulmonary arteries were constricted by infusion of serotonin and when the serotonin infusion was accompanied by inhalation of from 30 to 960 parts/million NO. Arterial diameter measurements were obtained from X-ray images of vessels having control diameters in the 300- to 3,400-μm range. Serotonin constricted the vessels throughout the size range studied, with an average decrease in diameter of ~20%. The fractional reversal of the serotonin-induced constriction by inhaled NO was directly proportional to inhaled NO concentration, inversely proportional to vessel size, and greater with plasma than with blood perfusion in vessels as large as 3 mm in diameter. The latter indicates that intravascular hemoglobin affected the bronchoalveolar-to-arterial luminal NO concentration gradient in fairly large pulmonary arteries. The data provide information regarding pulmonary arterial smooth muscle accessibility to intrapulmonary gas that should be useful as part of the database for modeling the communication between intrapulmonary gas and pulmonary arterial smooth muscle cells in future studies.

serotonin; pulmonary vascular resistance; pulmonary X-ray angiography; dog lung; nitric oxide

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org

http://www.jap.org
part of the database for modeling the communication between intrapulmonary gas and pulmonary arterial smooth muscle cells in future studies.

METHODS

X-ray angiographic image data were obtained from perfused dog lung lobes prepared as previously described (1). Each of 15 dogs [13.3 ± 4.6 (SD) kg body wt] was anesthetized with pentobarbital sodium (30 mg/kg iv), heparinized (1,250 IU/kg), and exsanguinated via a carotid artery catheter. During the exsanguination procedure, ~12 ml/kg of saline solution containing 10% dextan (Rheomacrodex, 40 kDa) were infused. After exsanguination, the chest was opened, and cannulas were placed in the left lower lobar artery, vein, and bronchus. The lobe was excised and suspended from the bronchus along an X-ray source (Fein Focus FXE-100.20; 3-μm focal spot) and a detection system (North American Imaging X-ray image intensifier with Sony charge-coupled device camera) as previously described (3). The lobar artery and vein were connected to the temperature-controlled (35°C) perfusion system primed with a volume of ~1 liter of either autologous blood (Hct = 32.7 ± 2.8; n = 7) or a mixture of autologous and homologous plasma (n = 8). A roller pump (Masterflex) pumped the blood or plasma from a reservoir into the lobar artery at a flow rate of 12.2 ± 1.9 (SD) ml·min⁻¹·g⁻¹ lobe wet weight for lobes weighing 31.1 ± 11.5 (SD) g wet weight. The lobar venous effluent drained back into the reservoir, the height of which was adjusted to set the venous pressure at 4.5 ± 0.7 mmHg. Lobar arterial, venous, and bronchial pressures were monitored continuously. The vascular pressures were referenced to the level of the X-ray focal spot, i.e., the center of the field of view of the X-ray image. Under the control conditions, lobar arterial pressure was 14.5 ± 0.7 mmHg for blood-perfused lobes and 12.4 ± 0.7 mmHg for plasma-perfused lobes.

The lobe was ventilated with a gas mixture containing ~15% O₂, 5.6% CO₂, balance N₂. This resulted in a P O₂ of 120 ± 11 Torr, P CO₂ of 41 ± 4 Torr, and pH of 7.31 ± 0.04 in the blood or plasma perfusate. Tidal volume was 136 ± 29 ml, and breathing frequency was 16.4 ± 2.6 breaths/min. End-expiratory pressure was maintained at 4.2 ± 0.5 mmHg by using a water overflow system.

To determine arterial diameters under the various experimental conditions, the inflow tubing included an injection loop that allowed the introduction of a 4-ml bolus of radiopaque contrast medium, 61% isopamidol (Isovue 300), into the lobar arterial inflow without affecting the pressure or flow (1). Before each bolus injection, the ventilation was halted at end expiration, and the bolus was injected within 1 s. The reported vascular pressures were also measured during this expiratory pause.

Once the preparation of the lobe was completed and the perfusion pressure was stabilized, the ventilator was stopped at end expiration, and a bolus was injected under "control" conditions. Then, to study the dilatory effects of NO inhalation, it was necessary to constrict the vessels. This was accomplished by continuous infusion of serotonin (20–200 μg/min) into the arterial cannula. The infusion rate was adjusted to produce an increase in perfusion pressure of ~30–40% with flow constant. With the level of constriction during serotonin infusion established, another bolus was injected. Then, NO was added to the inspired gas mixture by mixing 7,500 parts/million (ppm) NO in N₂ with a bias flow stream of O₂, CO₂, and N₂ to obtain concentrations of 30, 120, 480, or 960 ppm in the gas mixture at the inflow to the piston respirator. It took ~20 s from the time the NO was added to the ventilating gas until the lobar arterial pressure had stabilized at its new lower level, and between 45 and 60 s after the addition of NO, another bolus was injected. The lungs were then ventilated with the NO-free gas mixture until the perfusion pressure returned to a steady level, and another bolus was injected. This procedure was repeated for each of the four NO concentrations in random order of NO concentration. The entire sequence involving 12 bolus injections (including the one under control conditions before the serotonin infusion and two at the end after recovery from the serotonin infusion) took ~36 min. The sequence was carried out one, two, or three times on an individual lung lobe.

We also exposed the lung lobes to the highest NO concentration without infusing serotonin to determine whether there was any NO-reversible baseline tone in the vessels, even without the added constrictor stimulus. Any decrease in perfusion pressure was <1 mmHg, consistent with previous observations (1, 2) that the vessels in this preparation have little tone unless an external stimulus is applied.

For each bolus of contrast medium passing through the pulmonary vasculature, video images were recorded at 30 frames/s using an S-VHS videocassette recorder. The diameter of the field of view at the magnification settings used ranged from ~4.5 cm in diameter at low magnification to 0.32 cm at high magnification. The recorded images were analyzed off-line to determine the internal diameters of 4–20 vessels per field of view, with the number depending on the number of measurable vessels in the field of view, which in turn depended on magnification and vascular architecture in the particular field. For each diameter measurement, a region of interest was placed over the image of the vessel, and the videotape automatically advanced frame by frame until the frame having maximum absorbance during the passage of the contrast medium was identified. The average background image, calculated by averaging image pixel intensities for 10 image frames before the appearance of the contrast bolus, was subtracted from the maximum absorbance image. Line scans orthogonal to the axis of the vessel shadow obtained were obtained, and the diameter was estimated from the measured absorbance data using the previously described cylindrical model-based algorithm (4, 8). A "measurable" vessel was one for which convergence of the optimization routine was obtained when the vessel was at its smallest (i.e., serotonin constricted) diameter. Some vessels were narrowed to such an extent during serotonin infusion that, even if still visible, they were not measurable by this criterion. The two control injections at the end of the sequence were used to calibrate the imaging system for calculating vessel diameters in micrometers. These two injections were carried out with the lung at two different positions a measured distance apart on a line perpendicular to the X-ray beam. This measured distance (μm) was divided by the fraction of the total image diameter moved by the subject vessel across the image to obtain the calibration factor for that vessel.

In all, 2,748 diameter measurements were made on 229 vessels (117 and 112 in the blood and plasma groups, respectively). The range of vessel diameters under the control conditions was from ~300 to 3,400 μm. The control diameter (Dc) of a vessel was taken to be the diameter before serotonin infusion. This was compared with the average of the two diameters bracketing each NO exposure to obtain the serotonin response for comparison with the diameter during NO inhalation, as described below.

To determine the effect of NO inhalation on the pulmonary vascular resistance in the intact dog, each of two dogs (10.4 and 13.0 kg body wt) was anesthetized with pentobarbital...
sodium (30 mg/kg iv). An endotracheal tube was placed and attached to a bias flow system that allowed administration of inspired NO to the spontaneously breathing dog. A balloon catheter was positioned in a pulmonary artery via an external jugular vein such that balloon inflation resulted in a rapid fall in catheter tip pressure to the arterial wedge pressure. Catheters were also placed in each femoral artery and in a femoral vein. The tip of the femoral venous catheter was advanced to the vena cava near the entrance to the right atrium. After placement of the catheters, the dog was heparinized (1,250 IU/kg). One femoral arterial catheter was used to monitor systemic arterial pressure. The other was connected to a Gilford dye densitometer via a roller pump so that the concentration of indocyanine green dye could be monitored in the arterial blood. The pump flow rate was set at 20 ml/min, and the blood was returned via the femoral venous catheter. The femoral venous catheter was also used to inject 0.5-ml boluses containing indocyanine green dye (0.5 mg) for measurement of the cardiac output. For the cardiac output calculation, the area under the indocyanine green dye concentration curve was determined after semilogarithmic extrapolation of the downslope of the concentration vs. time curve in the usual fashion.

The protocol used to determine the pulmonary vascular resistance vs. NO concentration relationship in the intact dogs was similar to that for the isolated lungs. Mean pulmonary arterial pressure, wedge pressure, and cardiac output were measured under control conditions. Then an infusion of serotonin was begun at 41 or 102 µg/min via the femoral venous catheter to increase the pulmonary arterial wedge pressure difference by ~7 mmHg. The pressure and cardiac output measurements were repeated, and NO was introduced into the inspired air at a concentration of 30, 120, 480, or 960 ppm, and the measurements were repeated. This sequence was repeated until all four NO concentrations had been delivered and bracketed by measurements with no NO in the inspired gas. The pulmonary vascular resistance was calculated as the pulmonary arterial-wedge pressure difference divided by the cardiac output.

RESULTS

The patterns of the perfusion pressure (arterial-venous pressure difference) response to serotonin infusion and to NO inhalation during serotonin infusion at each stage of the experimental protocol for both blood- and plasma-perfused lung lobes are shown in Fig. 1. The “control” perfusion pressure and increases in perfusion pressure resulting from serotonin infusion were greater during blood than plasma perfusion as expected because of the higher blood viscosity. The symmetry of the “M” shape of the pressure graphs reflects the extent of reversibility of the NO and serotonin responses. The differences in the pressure responses to NO inhalation are emphasized by the Fig. 2 representation, wherein the fraction of the serotonin-induced increase in perfusion pressure reversed by NO inhalation is graphed. A one-way analysis of variance with repeated measures within blood or plasma groups reveals a significant NO concentration-dependent reversal of the serotonin-induced pressure increase within both blood- (P < 0.004) and plasma-perfused lungs (P < 0.001). A two-way analysis of variance between blood and plasma indicated that mean reversal of the serotonin response by NO inhalation was greater (P < 0.001) in the plasma- than in the blood-perfused group after allowing for the effects of NO concentration.

To provide a sense of the raw image data, example images from three stages of the experimental protocol from one low magnification field of view of a perfused lung lobe are shown in Fig. 3. The narrowing of the vessels resulting from the serotonin infusion and the dilatation resulting from NO inhalation during serotonin infusion are evident.

The goal of the serotonin infusion was to achieve constriction throughout the measurable diameter range so that the NO-induced dilatation could be measured. The mean decreases in diameter were 22.8 ± 14.5 (SD) % for blood- and 21.3 ± 10.0 % for plasma-perfused lung lobe. The changes in diameter in response to serotonin (5-hydroxytryptamine) infusion (\(AD_{5-HT}\)) for the vessels grouped into four bins according to vessel diameter are shown in Fig. 4. Because all
of the vessels did not narrow by the same fraction in response to serotonin infusion, but there was not a significant correlation between $D_{5-HT}$ and the change in diameter in response to inhaled NO ($\Delta D_{NO}$) for any NO concentration, the NO-induced dilation is represented by the ratio of $\Delta D_{NO}$ to $\Delta D_{5-HT}$ ($\Delta D_{NO}/\Delta D_{5-HT}$) in Figs. 5 and 6. The relationships between individual $\Delta D_{NO}/\Delta D_{5-HT}$ and their respective $D_c$ were evaluated by using Eq. 1 for each NO concentration

$$\frac{\Delta D_{NO}}{\Delta D_{5-HT}} = -m_1 + \frac{(1 + m_2) \left( \log \left( \frac{D_c}{m_3} \right) \right)^{m_2}}{1 + \left( \log \left( \frac{D_c}{m_3} \right) \right)^{m_2}}$$

(1)

where $D_c$ is in $\mu$m, and the $m_i$ are the fitting coefficients. The sigmoid form of Eq. 1 was chosen because the range of $\Delta D_{NO}/\Delta D_{5-HT}$ is constrained by the fact that the maximum diameter of a vessel is approximately equal to $D_c$ and the minimum diameter cannot be much smaller than that resulting from the initial serotonin constriction in the absence of NO. In actuality, the latter is not a true minimum because a further decrease in large vessel diameter in response to NO is possible because of the fact that, with constant flow, downstream dilation reduces upstream pressure. Thus the offset, $m_1$, was included.

Equation 1 fits to the individual data points, and the averaged data grouped into the four bins by $D_c$ are

![Graph](image1.png)

**Fig. 5.** Effect of inhaled NO concentration on the diameter dependence of the NO-induced reversal of the serotonin-induced constriction. The vertical scale is the fraction of the serotonin-induced vasoconstriction, $\Delta D_{5-HT}$, that was reversed by NO, $\Delta D_{NO}$. The symbols represent the means ± SE of the data in the same bins as in Fig. 4 for the designated NO concentrations of 30, 120, 480, and 960 ppm. The lines are Eq. 1 fit to the individual vessel data. The NO concentration effect was significant for both blood and plasma perfusion (see text).
plotted to emphasize the NO concentration effects in Fig. 5 and to emphasize the difference between blood and plasma in Fig. 6. The fitting coefficients are given in Table 1. Equation 1 represented the data significantly better than did the mean in each case (F test, \( P < 0.001 \)). The rightward shifts with increasing NO concentration and removal of the blood cells reflect the dilation of larger arteries. The statistical significance of the NO concentration dependence was evaluated by comparing the variances about Eq. 1 fitted to the pooled data (either blood or plasma) with those obtained from the fits to the data for the individual NO concentrations (19). Similarly, for each NO concentration, the plasma vs. blood comparison was made by comparing the variances about the pooled data fits to the blood or plasma fits. The \( F \) test revealed a significant NO concentration effect within both plasma- and blood-perfused groups (\( P < 0.001 \)), and the blood vs. plasma difference was significant at all NO concentrations (30 ppm, \( P < 0.05 \); 120 ppm, \( P < 0.001 \); 480 and 960 ppm, \( P < 0.01 \)). Equation 1 provides a means for making these comparisons and for summarizing the trends in the data, but the \( m_i \) are too highly correlated in the fitting procedure to have specific physical or physiological interpretations. Similarly, extrapolation beyond the diameter range studied would not appear to be justified.

The vascular resistance data for the intact dogs are graphed in Figs. 7 and 8 in a similar fashion to the pressure data for the isolated lungs in Figs. 1 and 2. They suggest that the total vascular resistance response to a given NO concentration was about the same in the intact animals as in the isolated lungs.

**DISCUSSION**

The results reveal a NO concentration and diameter-dependent vasodilatory effect of inhaled NO in dog pulmonary arteries and provide quantification of the relationships in the diameter range studied. This study has some similarities to the elegant study of Shirai et al. (28), wherein the arterial diameter responses to inhaled NO in cat lung pulmonary arteries with basal tone and with additional tone induced by alveolar hypoxia were studied. That study demonstrated that inhaled NO affected pulmonary arteries in cat lungs in the 100- to 1,000-\( \mu \)m-diameter range. Adding 40 ppm NO in the inhaled air eliminated the hypoxic vasoconstriction throughout the measured diameter range, indicating that inhaled NO has access to the vascular smooth muscle of vessels in the entire range of diameters also affected by alveolar PO\(_2\). The present study differs in the species and vasoconstrictor stimulus used. The dog lung extends the diameter range available for study, but, more importantly for the objectives of the present study, its pulmonary arteries are essentially devoid of basal tone in this experimental preparation (1, 2). Basal tone would have a potentially confounding effect because it would be difficult to rule out the possibility that any diameter dependence of the NO response is simply a reflection of the diameter depen-

**Table 1. Coefficients for Eq. 1 fit to the \( \Delta D_{NO}/\Delta D_{5-HT} \) data as in Figs. 4 and 5**

<table>
<thead>
<tr>
<th>Condition</th>
<th>( m_1 )</th>
<th>( m_2 )</th>
<th>( m_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 ppm</td>
<td>0.194</td>
<td>-8.97</td>
<td>2.68</td>
</tr>
<tr>
<td>120 ppm</td>
<td>0.333</td>
<td>-14.25</td>
<td>2.87</td>
</tr>
<tr>
<td>480 ppm</td>
<td>-0.060</td>
<td>-9.53</td>
<td>2.95</td>
</tr>
<tr>
<td>960 ppm</td>
<td>-0.246</td>
<td>-13.97</td>
<td>2.98</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 ppm</td>
<td>0.680</td>
<td>-7.15</td>
<td>3.13</td>
</tr>
<tr>
<td>120 ppm</td>
<td>0.143</td>
<td>-6.79</td>
<td>3.00</td>
</tr>
<tr>
<td>480 ppm</td>
<td>-0.244</td>
<td>-6.49</td>
<td>3.00</td>
</tr>
<tr>
<td>960 ppm</td>
<td>0.329</td>
<td>-7.42</td>
<td>3.75</td>
</tr>
</tbody>
</table>

\( \Delta D_{NO} \), change in diameter in response to inhaled NO; \( \Delta D_{5-HT} \), change in diameter in response to serotonin (5-hydroxytryptamine) infusion; ppm, parts/million; \( m_1, m_2, m_3 \); fitting coefficients. Coefficients have no units but are for control diameters in \( \mu \)m.

**Fig. 7.** Pulmonary vascular resistance (means ± SE; \( n = 2 \)) in the intact dogs plotted in a fashion similar to Fig. 1.
dence of the basal tone. The use of the infused vasoconstrictor stimulus (serotonin), as opposed to ventilation with low oxygen, tends to separate the question of NO access from that of constrictor stimulus access. The asymmetry between the serotonin-induced constriction and NO-induced dilation implies diameter-dependent NO accessibility to the serotonin-activated smooth muscle cells. Although a contribution by some other factor(s), such as a vessel size-dependent gradient in the NO transduction mechanism [e.g., in soluble guanylate cyclase activity (6)], cannot be ruled out, the progressive rightward shift with increasing NO concentration suggests that saturation of the vasodilator mechanism did not play a dominant role in determining the $\Delta D_{\text{NO}}/\Delta D_{5,\text{HT}}$ vs. $D_e$ relationship.

The NO concentrations used were high compared with concentrations commonly used in inhalation therapy (29) or to reverse experimental hypoxic vasoconstriction (24, 28). Although it is not surprising that it would take relatively high concentrations of NO to affect large vessels, we were somewhat surprised by the high NO concentrations required to achieve a maximum effect on total pulmonary vascular resistance in the blood-perfused lungs. In this regard, the NO concentration-response relationship for reversing the serotonin-induced increase in total pulmonary vascular resistance in the dog lungs was similar to that for reversing the angiotensin II-induced resistance increase in rat lungs (21). Because the rat lung study was also carried out in isolated lungs, we considered the possibility that the NO concentration-total vascular resistance response might be somehow shifted in isolated lungs compared with that in the intact animals used in other studies (24, 28, 29). However, we found that the pulmonary vascular resistance concentration-response relationship was not substantially different in the intact dogs. Thus isolation of the lungs does not appear to be the important variable, and a more likely possibility seems to be related to the size of the constricted vessels. We used serotonin specifically to obtain constriction over a wider range in diameters than what is obtained, for example, with alveolar hypoxia (1). Most of the vessels in the size range studied apparently make relatively little contribution to the total vascular resistance under control conditions (1). This can be appreciated by noting that the ~20% average decrease in diameters resulting from serotonin infusion would have increased the resistance of these vessels by >140%, assuming that resistance is inversely proportional to the fourth power of diameter, whereas total lobar vascular resistance increased by only ~40% (see Fig. 1). Thus the upper bound on their possible contribution to the total resistance under control conditions would be <30% [i.e., 40/140 (1)], assuming that the vessels that were not measured did not increase their resistance in response to serotonin. On the other hand, the observation that both narrowing of large vessels and elevated total vascular resistance remained, even when the NO concentration was high, suggests that the vessels in the measured size range made a substantial contribution to the increase caused by serotonin.

Removing blood cells has been observed to augment the decrease in total pulmonary vascular resistance induced by inhaled NO (21) and the transmission of the dilatory effects of inhaled NO downstream from the pulmonary capillaries (21, 22, 25). This is presumed to reflect the NO scavenging effects of the hemoglobin (14, 21, 22, 25). In the present study, the plasma experiments were motivated by consideration of the possibility that, by removing the intravascular sink for NO, the vascular smooth muscle NO concentration would be increased at a given inhaled NO concentration. Thus a flattening of the NO diffusion gradient between the air spaces and the perfusate might allow the NO access to larger, further upstream vessels. The results appear to be consistent with that possibility and suggest that gas exchange through the walls of fairly large arteries can in fact have a significant effect on their smooth muscle response to intrapulmonary gas composition. Thus the implication of the effect of removing the blood cells is that there is sufficient NO transport between pulmonary gas and blood in arteries in the NO responsive size range such that removing the hemoglobin increased the NO concentration within the smooth muscle cells.

In conclusion, we have measured the pulmonary arterial diameter dependence of the dilator effect of inhaled NO. The results have implications with regard to vessel accessibility and the efficacy of inhaled NO in pulmonary vasodilator therapy. However, in addition, the expectation is that the data will provide part of the database for modeling of the influence of exchange of gases between intrapulmonary air and pulmonary arterial blood on pulmonary arterial tone. In that context, the serotonin-activated vascular smooth muscle might be thought of as a NO sensor situated between the gas and blood.

This study was supported by National Institutes of Health Grants R01-HL-19298, T32-HL-07852, and T32-GM-O837706, and by the Department of Veterans Affairs.

Fig. 8. Fraction (means ± SE; n = 2) of the pulmonary vascular resistance increase in response to serotonin infusion that was reversed by NO inhalation for the 4 NO concentrations in the intact dogs plotted in a fashion similar to Fig. 2.
REFERENCES


