The lung diffusing capacity for nitric oxide in rats is increased during endotoxemia

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Stitt, John T., and Arthur B. DuBois. The lung diffusing capacity for nitric oxide in rats is increased during endotoxemia. *J Appl Physiol* 90: 1049–1056, 2001.—Rats, when injected with endotoxin, begin to exhale nitric oxide (NO) within 1 h. This study measured the diffusing capacity for NO in the lungs of rats (DL\textsubscript{NO}) under both control and endotoxemic conditions, and it also estimated the rate at which endogenous NO (V\textsubscript{PNO}) enters the distal compartment of the lung, both in control rats and during endotoxemia. DL\textsubscript{NO} increased from 0.68 ± 0.12 (SE) ml·min\textsuperscript{-1}·mmHg\textsuperscript{-1} in control rats to 1.17 ± 0.25 ml·min\textsuperscript{-1}·mmHg\textsuperscript{-1} in endotoxemic rats. V\textsubscript{PNO} was 2.6 ± 0.5 nl/min in control rats and attained a value of 218.6 ± 50.1 nl/min at the height of NO exhalation 3 h after the endotoxin. We suggest that increased DL\textsubscript{NO} reflects an increase in pulmonary membrane diffusing capacity, caused by a pulmonary hypertension that is due to neutrophil aggregation in the lung capillaries. DL\textsubscript{NO} may also be increased by an enlarged pulmonary capillary volume because of the vasodilatory effects of the endogenous NO that is produced by the lung in response to the endotoxin. NO production by the lungs in response to endotoxin is unique in that it is the only situation reported to date in which pathologically induced increases in NO exhalation originate from the alveolar compartment of the lung, as opposed to the small conducting airways.

adult respiratory distress syndrome; lipopolysaccharides; lung injury; pulmonary diffusing capacity

RECENTLY, THERE HAS BEEN CONSIDERABLE interest in the phenomenon of exhaled nitric oxide (NO) gas in both humans and animals (1, 6, 7, 8, 12). It has been proposed that a number of lung disorders, including exacerbated asthma, bronchiectasis, and inflammatory responses, can lead to increases in the concentrations of NO measured in expired air (14, 17, 18). There is also good evidence that NO is both produced and absorbed at different rates along the human airway. The lowest NO concentrations are found in the alveolar expirate of the lung, the conducting airways contain somewhat higher concentrations, but by far the highest concentrations are measured in the nasopharyngeal airway (6, 7, 19, 20). Obviously, the concentration of NO that is measured in air at any particular level in the airway is determined by the rate of production, the rate of its absorption, and the rate of ventilation. Little can be concluded about the nature of any increase in NO concentration in expired air from any level of the respiratory tract unless both the rates of NO production and NO absorption at that particular site are known. Furthermore, unless proper precautions are taken during expiratory sampling, contamination between regions can lead to erroneous results (6, 19, 20, 25).

Because of its pulmonary vasodilator properties, exogenously administered NO, at concentrations of 20–50 parts per million (ppm), is now frequently used in the treatment of newborn pulmonary hypertension and during adult respiratory distress syndrome, or ARDS (22, 23). However, the manner in which NO diffuses across the lung into the pulmonary circulation and the ultimate fate of this inhaled NO have not been thoroughly studied nor are they well understood (2–4). NO is thought to be avidly scavenged by the red blood cells and irreversibly bound to hemoglobin as methemoglobin, although recently Stamler’s group (Jia et al., Ref. 16) has postulated that the NO molecule can be bound by thiol bonds to cysteine residues on the amino termini of the hemoglobin rather than by the heme moiety. Furthermore, the blood oxygen tension (PO\textsubscript{2}) is postulated to affect the nature of this form of NO binding and thereby regulate allosterically the loading and unloading of NO by the hemoglobin molecule at different sites within the circulation, depending on the prevailing PO\textsubscript{2} levels of the blood. However, the importance of this concept has recently been challenged (9) and therefore must still be regarded as unsubstantiated.

The recent literature in this area has mainly been concerned with increases in NO concentrations that are detected within the smaller conducting airways, related to the exacerbation of asthma and other inflammatory conditions (8, 17, 18, 25). Little attention has been paid to the alveolar compartment per se, because it is thought that all NO in this compartment is removed by the pulmonary circulation (5). However, although it is realized that the lung alveoli play a major role in the uptake of NO (e.g., absorbing the NO that is picked up during the passage of inhaled air through the nasopharyngeal region in humans), they may also produce NO. There have been a few reports in the literature documenting very low levels of NO [<5.0 parts per billion (ppb)] in end-tidal expired air. Hyde et

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al. (14) measured both the rate of production and the rate of uptake of NO in the distal portion of the healthy human lung and speculated on how they might be affected during conditions of lung injury. However, to our knowledge, there has been no systematic study of the production and absorption of NO by the alveolar portion of the lung during lung injury. We are particularly interested in this problem, because we have observed high concentrations of NO in the mixed-expired air of rats in which acute lung injury was initiated by endotoxemia (28, 30). We postulated that this exhaled NO is produced in response to an indirect action of endotoxin on the pulmonary capillary endothelium and that this endogenous NO crosses the pulmonary blood-to-air membrane, enters the alveolar compartment of the lung, and a portion of it is then exhaled in the end-tidal gas.

Rats that have been injected intravenously with a lipopolysaccharide (LPS) endotoxin soon begin to exhibit high concentrations of NO gas in their exhaled air (26, 28, 30). This increase in NO concentration first appears within 60 min after the LPS injection, reaches a plateau at ~3 h, and then gradually declines during the next 6-h period. We have shown that the NO is produced from within the lungs and is not carried to the lungs and unloaded there by the systemic circulation. We have evidence that circulating neutrophils are essential for this NO response to occur and that an interaction between the LPS-activated neutrophils, aggregating within the lung vasculature, and the lung capillary endothelium appears to be the cause of the production of NO by the lung (13, 26, 29, 30).

Our previous study demonstrated that, despite the fact that they were producing endogenous NO from within their lungs, endotoxemic rats were capable of absorbing more NO when it was inhaled from an exogenous source than were control rats (29). The purpose of this study was to compare the NO diffusion capacity of the rat lung (DlNO) under control and endotoxemic conditions and also to determine the rate of production of endogenous NO (VpNO) by the lungs in both the control and endotoxemic, LPS-treated states. In this study the measurement of the rate of NO exchange was confined to the distal portion of the respiratory tract in rats for the following three reasons. First, the rats were tracheotomized to the level of the carina; second, the frequency of ventilation chosen (60 breaths/min) virtually eliminated the conducting airways as significant contributors to the NO concentration in the exhaled air; and, finally, when end-tidal samples of exhaled NO were compared with samples of the mixed-expired gas, physiological dead space was calculated to be, as expected, ~25% of tidal volume. End-tidal vs. mixed-expired CO₂ samples yielded a similar physiological dead space fraction.

The steady-state lung diffusion capacity for any suitable inhaled gas is determined by measuring the rate of uptake of that gas across the alveolar membrane into the circulation at a known partial pressure of the gas within the alveolar compartment minus the back pressure from the blood. The most commonly used gas in such studies is carbon monoxide (CO) because of its low solubility and its ability to bind preferentially to hemoglobin (2–4). Recently, there has been a renewed interest in the lung diffusion of NO because of the widespread therapeutic use of inhaled NO in the treatment of pulmonary hypertension or lung injury, such as ARDS. NO has properties similar to CO that make it suitable for the determination of lung diffusion capacity, although it is more than twice as soluble as CO in aqueous solution (4, 11), which in turn increases its lung membrane diffusion coefficient. It binds very tightly with blood hemoglobin to form methemoglobin, thus reducing the back pressure from the blood. The derivations of the equations used to determine steady-state DlNO and the determination of VpNO in control and endotoxemic rats are contained in the APPENDIX.

**MATERIALS AND METHODS**

The animals used in this study were five male Sprague-Dawley rats weighing between 250 and 350 g. They were first anesthetized with pentobarbital sodium (50 mg/kg ip) and then tracheotomized, and a catheter was inserted via the left femoral vein and advanced to the inferior vena cava. The animals were placed supine and paralyzed with gallamine triiodate (15 mg/kg iv). The endotracheal tube was attached to a Harvard small animal ventilator and set for a minute ventilation rate of 180 ml/min, a tidal volume (VT) of 3.0 ml, and a respiratory frequency rate of 60/min. Air supplied to the intake port of the ventilator was cleansed of ambient NO by drawing it through a permanganate/charcoal absorber attached to the intake. Anesthesia was maintained during the experiments by intravenously infusing a solution of pentobarbital (6.0 mg/ml) and gallamine (4 mg/ml) contained in 0.9% NaCl at a rate of 1.0 ml/h. Systemic arterial blood pressure was measured by a Statham transducer connected to a PE-60 catheter inserted into the left common carotid artery. The preparation is illustrated in Fig. 1.

Experiments were conducted at an ambient temperature of 22°C between 0800 and 1600; rectal temperature was monitored continually throughout each experiment and was kept above 37°C by the intermittent application of infrared heat. Mixed-expired air was continuously withdrawn from the ventilator outflow via a 10-ml exhaust plenum at a rate of

![Fig. 1. Schema of the preparation used in this study. See text for a description.](http://jap.physiology.org/)
18 ml/min and passed through a Sievers 270B chemiluminescence NO detector that had both a detection threshold and a sensitivity of ~1.0 ppb for gaseous NO, allowing us to determine continuously the fraction of NO in mixed-expired gas ($F_{ENO}$). The fraction of NO in the end-tidal gas ($F_{ANO}$) was measured by using a solenoid-actuated valve, driven by the ventilator to repeatedly sample, via a sidearm on the endotrachial tube, the final 0.3-ml fraction of each $V_T$ exhaled. Because the ventilation rate was 60/min, this meant that a quasi-continuous flow of end-tidal air (~18 ml/min) passed through the NO detector as demanded by the vacuum pump. Both the mixed-expired and end-tidal circuits were independently tested for leaks by replacing the rat with an “artificial” lung (the airtight finger of a vinyl glove, having a volume of ~6 ml), which was attached to the endotrachial tube. A 5-liter Tedlar bag containing a known concentration of NO (previously determined directly by the NO meter) was attached to the intake port of the ventilator, and the “vinyl lung” was ventilated with this gas, just as in a normal experiment. We could divert sampling from the “mixed-expired” to the “end-tidal” circuit by turning a three-way stopcock and thus compare the two NO concentrations being measured. Because this lung did not absorb any NO, the end-tidal values exactly matched the mixed-expired values, and both were identical to the NO concentration of the gas mixture in the bag that was supplying the vinyl lung via the intake port of the ventilator. In this manner, we assured ourselves that there were no leaks in either the mixed-expired or the end-tidal circuits and that there was no sample dilution.

The results using this technique are illustrated by Fig. 2, which shows an experiment in which an LPS-treated rat is breathing NO-free air. It can be seen that the appearance of NO in mixed-expired air starts at ~60 min and reaches a plateau at +3 h. End-tidal values were measured where indicated, by use of the endotracheal sidearm, and it can be seen that $F_{ANO}$ exceeds $F_{ENO}$ by the ratio of the ventilatory dead space. Mixed-expired and end-tidal values of CO$_2$ were also measured by passing the mixed-expired or the end-tidal samples through a Beckman infrared CO$_2$ meter, placed in series before the NO analyzer. Mixed-expired CO$_2$ was lower than end-tidal CO$_2$ by ~25%, and end-tidal gas concentrations were used as an estimate of alveolar gas concentrations. Calculation of the physiological dead space ($V_D$) by using $V_T$, $F_{ENO}$, and $F_{ANO}$ for inhalation of NO, exhaled NO, and exhaled CO$_2$, respectively, yielded identical values for $V_D$. This permitted us to calculate ($V_T - V_D$) and, hence, alveolar ventilation ($V_A$). Deeper sighs (twice normal, i.e., $V_T$ ~6 ml) were induced once every 10 min to minimize lung atelectasis.

The LPS endotoxin (prepared from *Escherichia coli*, batch no. 82F-4012) was purchased from Sigma Chemical (St. Louis, MO). It was dissolved in sterile 0.9% saline, and all injections were made at a dose of 1.0 mg/kg via the femoral/vena caval catheter.

The ability of the lungs to extract inhaled NO was measured by connecting three different concentrations of NO (approximately at 600, 1200, and 1,800 ppb), carried in air in a Tedlar bag attached to the intake port of the ventilator for 4 min, and by measuring the respective concentrations of NO in the resulting mixed-expired and end-tidal samples. This was first performed in a randomized fashion on each rat to obtain control NO uptake values and was then repeated 3 h after the induction of endotoxemia. The plots of the mixed-expired concentrations of NO ($[NO]_E$) and the end-tidal concentrations of NO ($[NO]_A$) vs. the inspired concentrations of NO ($[NO]_I$) yielded regression lines whose slopes (m) described the fraction of NO that was not absorbed by the whole lung ($m_{ENO}$) or by the alveoli ($m_{ANO}$), first under control and then during endotoxemic conditions. These values, determined by data taken from the five rats, were used to calculate values for $D_{LNO}$ under control and then under endotoxemic conditions. We also calculated the rate at which endogenously produced NO entered the lungs during control and endotoxemic conditions, using the equations whose derivations are described in the Appendix. Time control experiments had been conducted previously to ensure that ventilation of the animals for a period of ~3 h, per se, did not alter the ability of their lungs to extract inhaled NO (29).

Statistical analyses of the data were carried out using standard unpaired t-tests, regression analysis, and, where appropriate, ANOVAs. When data were averaged, means ± SE are given. Statistical significance was assumed at the level of $P < 0.05$.

RESULTS

Figure 3 illustrates the uptake of inhaled NO in control rats, in which both $[NO]_I$ and $[NO]_A$ are plotted for three different levels of $[NO]_I$ over a range of 500–2,000 ppb. The slopes of the resulting two regression lines intersect close to the axis at $[NO]_I = [NO]_E = [NO]_A = 5.3$ ppb, indicating that little endogenous NO is produced by control rats.

Measurement of $[NO]_E$ and $[NO]_A$, taken when $[NO]_I = 0$, show that they are 3.2 ± 0.2 and 4.2 ± 0.2 ppb, respectively. The slopes of the two lines represent the NO fraction that remains unabsorbed in the entire lung, including $V_D$ ($m_{ENO} = 0.41 ± 0.02$), and in the end-tidal compartment ($m_{ANO} = 0.22 ± 0.02$). These two values, along with $V_T$, permitted us to calculate $V_D$ and thus ($V_T - V_D$) to evaluate $D_{LNO}$ by use of Eq. 1

$$D_{LNO} = V_A \times (1 - m_{ANO})/(P_B - P_{H_2O}) \times m_{ANO}$$

where ($V_T - V_D$) = 2.27 ± 0.14 ml; respiratory frequency (RF) = 60 breaths/min; $P_B - P_{H_2O} = 713$ Torr; $V_A = (V_T - V_D) \times RF = 136.2 ± 8.1$ ml/min ATP; $m_{ANO} =$
0.22 ± 0.02; DLNO = 136.2 ml/min · (1 − 0.22)/(713 Torr · 0.22); and DLNO = 0.68 ± 0.12 ml · min⁻¹ · Torr⁻¹. Pb represents barometric pressure.

Additionally, the rate of entry of endogenously produced NO into the alveolar compartment of the lungs of the control rats was calculated using Eq. 2

\[ V_\text{PNO} = F_\text{ANO} \times (V_A + (P_B - P_{H_2}O) \times DLNO) \]  

(2)

where \( F_\text{ANO} = 4.2 \pm 0.2 \times 10^{-9} \), when \( F_{INO} = 0; V_A = 136.2 \pm 8.1 \) ml/min at ATP; \( P_B - P_{H_2}O = 713 \) Torr; \( DLNO = 0.68 \pm 0.12 \) ml · min⁻¹ · Torr⁻¹. \( V_\text{PNO} = 4.2 \times 10^{-9} \cdot (136.2 \text{ ml/min} + (713 \text{ Torr} \cdot 0.68 \text{ ml/min} \cdot \text{Torr}^{-1})); \)
\[ V_\text{PNO} = 2.6 \pm 0.5 \times 10^{-6} \text{ ml/min}. \]

The amount of endogenous NO that is produced by control rats is very small, such that, when rats were breathing NO-free air, \( F_{ENO} \) and \( F_{ANO} \) were 3.2 ± 0.2 and 4.2 ± 0.2 ppb, respectively, and were therefore just measurable by the Sievers NO analyzer, which had a detection threshold of ~1 ppb.

The rats were then made endotoxemic by injecting them with LPS, and we waited 3 h until exhalation of endogenous NO reached its plateau level (namely, Fig. 2). The NO uptake experiments were repeated, and the results obtained are summarized in Fig. 4.

It is noted that slopes of the two lines are reduced compared with the control animals (\( m_{E_{NO}} = 0.35 \pm 0.02; m_{ANO} = 0.14 \pm 0.02 \)) and that they intersect on the line of identity, in which \( [NO]_E = [NO]_A = [NO]_I = 261.5 \) ppb. This attests to the accuracy of the measurements that we performed. Furthermore, when \( [NO]_I = 0 \), it can be seen that \( [NO]_A = 224.7 \) ppb and \( [NO]_E = 169.9 \) ppb. Because \( [NO]_A > [NO]_E \), this confirms that endogenously produced NO comes from the alveolar compartment rather than from the conducting airways, which are dead space. DLNO was again calculated using Eq. 1, where \( (V_T - V_D) = 2.27 \pm 0.12 \) ml; \( RF = 60 \) breaths/min; \( P_B - P_{H_2}O = 713 \) Torr; \( V_A = (V_T - V_D) \times RF = 136.2 \pm 7.2 \) ml/min ATP; \( m_{ANO} = 0.14 \pm 0.02; DLNO = 136.2 \text{ ml/min} \cdot (1 - 0.14)/(713 \text{ Torr} \cdot 0.14) \);

and \( DLNO = 1.17 \pm 0.25 \text{ ml/min} \cdot \text{Torr}^{-1} \).

The rate of entry of the endogenously produced NO into the alveolar compartment of the endotoxemic rats was calculated using Eq. 2, where \( F_{ANO} = 224.7 \pm 7.6 \times 10^{-9} \) when \( F_{INO} = 0; V_A = 136.2 \pm 7.2 \) ml/min at ATP; \( P_B - P_{H_2}O = 713 \) Torr; and \( DLNO = 1.17 \pm 0.25 \text{ ml/min} \cdot \text{Torr}^{-1} \). \( V_\text{PNO} = 224.7 \times 10^{-9} \cdot (136.2 \text{ ml/min} + (713 \text{ Torr} \cdot 1.17 \text{ ml/min} \cdot \text{Torr}^{-1})); \)
\[ V_\text{PNO} = 218.6 \pm 50.1 \times 10^{-6} \text{ ml/min}. \]

Of the 218.6 ml of endogenous NO entering the lungs each minute, 0.86 (188 ml) leaves by diffusion out into the pulmonary circulation, whereas 0.14 (30.6 nl) remains within the alveolar compartment and is exhaled at an end-tidal concentration of 30.6 nl/136.2 ml = 224.7 ppb = [NO]_A when \( [NO]_I = 0 \).

To illustrate both between-animal variability and the reproducibility of our technique, Table 1 contains estimates of both DLNO and V_\text{PNO}, calculated using individual data from the five rats in the study. It can be seen that the range of DLNO in normal rats was 0.53 to 0.86, with a mean of 0.68 ± 0.08 ml · min⁻¹ · Torr⁻¹, and V_\text{PNO} varied from 2.0 to 3.0 with a mean of 2.6 ± 0.21 ml/min. When the rats were made endotoxemic, DLNO had a range of 0.87 to 1.39 with a mean of 1.18 ± 0.11 ml · min⁻¹ · Torr⁻¹, and V_\text{PNO} varied from 158.1 to 280.7 with a mean value of 220.9 ± 20.7 ml/min. These mean values are not very different from those calculated with the pooled data, and, indeed, their overall variability is actually somewhat less.
DISCUSSION

A “steady-state” determination of lung diffusion capacity, as opposed to other methods such as “single breath” or “rebreathing” techniques, is usually regarded as a more physiologically useful method of determining gas uptake by the lung. This is because it is determined under conditions of normal, uninterrupted respiration and at normal tidal volumes, although such a determination might be criticized on the grounds that it is also more subject to inhomogenieties of both regional ventilation and perfusion within the lung. The method we used relies on the determination of the fraction of inspired NO that remains in end-tidal air at several different values of FINO. It permitted us to demonstrate that NO uptake was linear over a range of different gas concentrations and then to average this NO uptake by determining the slope of the line obtained by plotting FANO as a function of F INO. Furthermore, by determining both F ENO and F ANO simultaneously for each level of F INO, we were able to determine the Bohr dead space for NO and use it to calculate alveolar ventilation and to show that the preponderance of NO uptake occurred within the end-tidal region of the lung. The respiratory rate of 60 breaths/min that we used (normal for rats) also ensured that any contamination of the end-tidal air by “conducting airway NO,” picked up during its passage through the lower conducting airways, was negligible. This meant that the end-tidal samples were more representative of true alveolar gas. Alveolar tidal volume (VT – VD) was calculated using F ENO and FANO according to the equation

\[
(V_T) \times F_{E_{NO}} = (V_T - V_D) \times F_{A_{NO}}
\]

and more generally by

\[
(V_T) \times (1 - m_{E_{NO}}) = (V_T - V_D) \times (1 - m_{A_{NO}})
\]

The fact that the calculated dead spaces for CO2 and NO are identical might at first sight seem a bit surprising. However, we are sure that this is not due to sample dilution in the measurement of either the mixed-expired or the end-tidal gas concentrations. Our reasons are as follows. First, we independently verified the accuracy of both measurements. Second, when the CO2 dead space is

Table 1. Individually calculated values of V̇A, ḊNO and V̇PNO for each of the 5 rats that comprised this study

<table>
<thead>
<tr>
<th>Rat No</th>
<th>mENO</th>
<th>mANO</th>
<th>V̇A, ml/min</th>
<th>ḊENO</th>
<th>V̇PNO, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>0.46</td>
<td>0.33</td>
<td>0.26</td>
<td>0.13</td>
<td>133.2</td>
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<td>2</td>
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<td>0.34</td>
<td>0.18</td>
<td>0.12</td>
<td>133.8</td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td>0.38</td>
<td>0.19</td>
<td>0.18</td>
<td>133.2</td>
</tr>
<tr>
<td>4</td>
<td>0.38</td>
<td>0.37</td>
<td>0.26</td>
<td>0.16</td>
<td>150.6</td>
</tr>
<tr>
<td>5</td>
<td>0.45</td>
<td>0.35</td>
<td>0.22</td>
<td>0.12</td>
<td>126.9</td>
</tr>
</tbody>
</table>

mENO, fraction of nitric oxide (NO) not absorbed by the whole lung; mANO, fraction of NO not absorbed by the alveoli; V̇A, alveolar ventilation; ḊNO, lung NO diffusing capacity; V̇PNO, rate NO enters distal compartment of lung; C, control; E, endotoxemic.
calculated, it is based on the assumption that the dead space contains no CO₂ and that all CO₂ in the mixed-expired sample comes from the end-tidal compartment. Thus [CO₂]A is greater than [CO₂]E, and the difference between [CO₂]A and [CO₂]E, along with the tidal volume, is used to calculate the dead space. However, in the case of calculating the NO dead space, we used two different methods. First, as can be seen in Fig. 3, when normal animals are ventilated with an NO gas mixture, [NO]A is less than [NO]E, because the lungs absorb more NO than they produce. Thus, in this case, NO dead space is calculated with the assumption that the NO in the dead space remains unabsorbed. However, in the case of endotoxemic rats (Fig. 2), when the animals breathe NO-free air, [NO]A is greater than [NO]E, and, as in the case of CO₂, the assumption is that all NO in the mixed-expired sample comes from the end-tidal compartment. Both calculations yield identical NO dead spaces, which also agree with the calculated CO₂ dead space. When endotoxemic rats are ventilated with NO gas mixtures (Fig. 4), once the concentration of NO in the inspired gas mixture exceeds that of the endogenous end-tidal NO concentration, [NO]A becomes less than [NO]E, because there is now a net absorption of NO; however, the calculation of NO dead space yields a similar result. Finally, the fact that in Fig. 4 the two lines representing mixed-expired and end-tidal concentrations intersect exactly on the line of identity also strongly militates against any errors in sample measurement.

We suggest that the reason that the CO₂ and NO dead spaces are similar has more to do with the nature of the dead spaces themselves. In the first place, the physiological dead space is small (~0.73 ml, which is ~25% of the tidal volume employed in the study). Furthermore, because the trachea is cannulated to the level of the carina, a significant part of this dead space is inert tubing, thus reducing the portion of total dead space that is even available for gas exchange. The frequency of respiration (60 breaths/min) reduces residence time of the gas within the dead space, thereby further reducing the opportunity for gas exchange. For these reasons, we think that the dead space gas exchange of NO is negligible in tracheotomized rats and therefore that the CO₂ and NO dead spaces are identical.

In the case of the control rats, because VT was always set at 3.0 ml and mENO and mEₘₙ were 0.41 ± 0.02 and 0.22 ± 0.02, respectively, as determined by the slopes of the two lines in Fig. 3, this yielded a value for (VT − VD) = 2.27 ± 0.14 ml. The (VT − VD) value accorded well with the value for (VT − VD) as determined by using FECO₂ and FACO₂, this demonstrates that virtually all of the NO uptake occurred in the alveolar compartment of the lung, as might be expected from the argument we have just made.

The extremely low concentrations of endogenous NO that were detected in both mixed-expired and end-tidal air when the control rats were breathing NO-free air (3.2 and 4.2 ppb, respectively) indicate that very little NO is produced within the normal rat lung. When we calculated a value for V˙PNO during the control period, according to Eq. 2, the result was 2.6 nl/min. It is interesting to note that Hyde et al. (14), using the "breathholding" technique in normal humans, calculated that the distal lung produced NO at a rate of 540 nl/min (14). When we consider that the mass of humans is ~200 times that of rats (70 vs. 0.30 kg), there is a surprising scalar congruity between Hyde's results and our own. Indeed, this scalar congruity also extends to the value for DLNO that we determined for normal rats. To our knowledge, there are no published data on the diffusing capacity for NO in rats. However, our value of 0.68 ml·min⁻¹·Torr⁻¹ is fairly well with the published values for humans (2.1 ml·min⁻¹·Torr⁻¹·kg⁻¹) (10, 13) and that for dogs at 54.0 ml·min⁻¹·Torr⁻¹ (2.6 ml·min⁻¹·Torr⁻¹·kg⁻¹) (20). This seems to accord well with the proposal of Weibel (32) that lung diffusing capacity is directly proportional to perfused lung surface area and, hence, to body mass (32).

When rats were made endotoxemic, the concentrations of NO in the mixed-expired and end-tidal samples rose to an average of 170 and 225 ppb, respectively, by the end of 3 h. Several conclusions may be drawn. First, because the end-tidal concentration exceeded the mixed-expired concentration, it is clear that this NO was coming from the alveolar compartment. Furthermore, calculated (VT − VD) remained unchanged at 2.27 ± 0.12 ml, and the ratio of the NO concentrations in the mixed-expired and end-tidal gases (170/225 = 0.76) is exactly equal to the ratio of (VT − VD) to VT (2.27/3.00 = 0.76), indicating that endogenously produced NO came entirely from the end-tidal compartment of the lung and not from the dead space-conducting airways. When uptake of exogenous NO was measured in the endotoxemic rats after their endogenous NO production had reached its plateau, the residual fraction of NO in both the mixed-expired (mENO = 0.35 ± 0.02) and end-tidal (mAₘₙ = 0.14 ± 0.02) fractions was less (P < 0.01) than it was for the normal rats (mENO = 0.41 ± 0.02 and mAₘₙ = 0.22 ± 0.02, respectively). This indicated that lung uptake of NO had increased, and, by using Eq. 1, we determined that DLNO had increased by 73% from 0.68 to 1.17 ml·min⁻¹·Torr⁻¹. It also confirmed our earlier observation that the uptake of inhaled exogenous NO was greater in LPS-treated rats than in normal rats, despite the fact that the endotoxemic rats were also producing significant amounts of endogenous NO within the alveolar compartment (29). Using Eq. 2, we calculated that, on average, V˙PNO increased almost 85-fold, from 2.6 nl/min in the control state to 218.6 nl/min during endotoxia.

Given this demonstrated increase in the avidity of their lungs for NO, it may seem surprising that endotoxemic rats actually exhale any NO from the alveolar compartment. However, at the alveolar ventilation employed, although the majority (~86%) of inhaled NO entering the alveolar compartment is taken up by the pulmonary circulation, the remaining portion (~14%) is exhaled. We assume that this uptake is equally as true for endogenously produced NO as it was for inspired exogenous NO. We propose that the endogenous
NO is produced by the action of adherent neutrophils on the capillary endothelial cells in the pulmonary circulation and that a measurable proportion of this NO finds its way into the alveolar compartment, whence it is exhaled in the end-tidal air. This represents more of a lung vascular response to LPS than a lung parenchymal response, and it is different from the asthmatic condition, in which small airway inflammatory responses are thought to be responsible for the increased NO concentrations in the exhaled air (14, 17, 18, 25).

At this point, we can only speculate about the mechanisms that produce the large increase in the diffusing capacity for NO in the endotoxemic rat lung. According to the widely accepted Roughten and Forster (24) model, overall diffusing capacity of the lung for a gas results from a combination of the diffusing conductance of the lung gas-blood membrane barrier, the gas uptake conductance of the erythrocytes per unit blood volume ($\theta$), and the lung capillary blood volume ($V_c$). Thus, for NO diffusion, these factors are related according to the equation

$$\frac{1}{DL_{NO}} = \frac{1}{Dm_{NO}} + \frac{1}{(\theta_{NO} \times V_c)}$$

where $Dm_{NO}$ is NO membrane diffusing capacity. To increase $DL_{NO}$ from 0.68 to 1.17 ml $\cdot$ min$^{-1}$ $\cdot$ Torr$^{-1}$ would require a substantial increase in one or more of the three components that comprise $Dm_{NO}$, namely, $Dm_{NO}$, $\theta_{NO}$, and $V_c$. It seems unlikely that the erythrocyte NO uptake conductance ($\theta_{NO}$) would be altered by endotoxin because it is governed by the hemoglobin content of the blood cells and pulmonary emacrat. This then leaves the membrane conductance and/or pulmonary $V_c$ as possible sources of increased uptake of NO. Although one might readily conceive that an increase in lung capillary volume could occur in response to locally produced NO, because NO is a vasodilator (15, 31), it seems unlikely that this alone could bring about an increase in $DL_{NO}$ of the magnitude that we observed. The major resistance to diffusion of NO across the lung is believed to reside in the alveolar membrane component, rather than in the blood component; indeed, several studies have proposed that the ($\theta_{NO} \times V_c$) component has an almost infinite conductance (11).

Thus we are led to the conclusion that endotoxin also acts to increase $Dm_{NO}$. This could be caused by the increased capillary pressure and vascular congestion within the pulmonary circulation that is caused by LPS (10, 13, 29). The pulmonary hypertension that occurs during the early phase of endotoxin-induced lung injury appears to be due to an aggregation of neutrophils within the lung capillaries, which eventually leads to pulmonary edema (13, 26, 29). This hypertension would increase both the lung $V_c$ and the portion of the total alveolar/capillary network within the lung that is perfused or blood filled, thereby increasing the area of lung vasculature that is available for NO diffusion and uptake. An additional possible cause for the increase in

$DL_{NO}$ is the increase in cardiac output and $\dot{V}O_2$ that accompanies both the febrile and early “cardiodynamic” stages of endotoxemia. It is well established that both factors increase lung diffusion capacity, in the same manner that exercise increases the oxygen diffusing capacity of the lung (32). For example, Meyer et al. (21) demonstrated that, by using dinitrophenol to increase the cardiac output and $\dot{V}O_2$ in anesthetized dogs, they could increase $DL_{NO}$ by >50%.

In conclusion, we have demonstrated that the intravenous action of LPS on rats causes an increase in the production of NO within the alveolar compartment of the lungs, while it concurrently increases the diffusing capacity of the lungs for NO uptake. This phenomenon is different from previously described pathological conditions in which exhaled NO concentrations are increased, because the increased NO arises from the alveolar compartment of the lungs, and its source appears to be the vascular component of the lungs, rather than the airway smooth muscle. We believe that this early manifestation of NO in the expired air of endotoxin-treated rats is a very prompt and reliable marker for the onset of the acute lung injury that is caused by septicemia.

**APPENDIX**

**Determining the Diffusing Capacity of Rat Lungs**

$V_{Dl_{NO}} = V_{INO} + V_{PNO} - V_{FINO}$ (A1)

$P_{ANO} \cdot DL_{NO} = V_A \cdot [(P_{ANO}/V_A) - F_{ANO}]$ (A3)

However, $F_{ANO}$ may also be defined as $F_{ANO} = m_{ANO} \cdot ([P_{INO} + (V_{INO}/V_A)] or F_{ANO}/m_{ANO} = F_{INO} + (V_{INO}/V_A)$, where $m_{ANO}$ is the fraction of the theoretical initial concentration of NO that remains in the alveoli after diffusion occurs. Substituting into Eq. A3, we get

$P_{ANO} \cdot DL_{NO} = V_A \cdot [(F_{ANO}/m_{ANO}) - F_{ANO}]$ (A4)

$P_{ANO} \cdot DL_{NO} = V_A \cdot F_{ANO} \cdot [1/m_{ANO} - 1]$ (A5)

However, because $F_{ANO} = P_{ANO}/(P_B - P_{H_2O})$, substituting this into Eq. A5 we get

$DL_{NO} = V_A \cdot (1 - m_{ANO})/[(P_B - P_{H_2O}) \cdot m_{ANO}]$ (1)

Equation 1 is from the text; and $m_{ANO}$ is evaluated by plotting $F_{ANO}$ against $P_{INO}$ for several different values of $P_{INO}$ and determining the slope of the resulting regression line.

**Determining the Rate of Endogenous NO Production in Rat Lungs**

$$\frac{NO}{produced} = \frac{NO}{exhaled} + \frac{NO}{diffused} - \frac{NO}{inhaled}$$

$$V_{PNO} = (V_A \cdot F_{ANO}) + (P_{ANO} \cdot DL_{NO}) - (V_A \cdot F_{INO})$$
However, when rats are breathing NO-free air, then $F_{\text{NO}} = 0$.

$$V_{\text{PNO}} = (V_A F_{\text{ANO}} + (P_{\text{ANO}} \times DL_{\text{NO}}))$$

$$V_{\text{PNO}} = F_{\text{ANO}} \times [V_A + (P_B - P_{\text{H}_2\text{O}}) \times DL_{\text{NO}}]$$

where $F_{\text{ANO}}$ is the alveolar concentration when rats are breathing NO-free room air (i.e., when $F_{\text{NO}} = 0$).

REFERENCES


