Single-breath diffusing capacity of NO independent of inspiratory NO concentration in rabbits

HARTMUT HELLER AND KLAUS-DIETER SCHUSTER
Department of Physiology, University of Bonn, D-53115 Bonn, Germany

Heller, Hartmut, and Klaus-Dieter Schuster. Single-breath diffusing capacity of NO independent of inspiratory NO concentration in rabbits. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2055–R2058, 1997.—Pulmonary diffusing capacity of NO (DLNO) was determined by performing single-breath experiments on six anesthetized paralyzed supine rabbits, applying inspiratory concentrations of NO (FINO) within a range of 10 parts per million (ppm) ≤ FINO ≤ 800 ppm. Starting from residual volume, the rabbit lungs were inflated by 50 ml of a NO-nitrogen-containing indicator gas mixture. Breath-holding time was set at 0.1, 1, 3.5, and 7 s. Alveolar partial pressure of NO was determined by analyzing the end-tidal portion from expirates, with the use of the respiratory mass spectrometry. In the six animals, pulmonary diffusing capacity of NO averaged DLNO = 1.92 ± 0.21 ml·mmHg⁻¹·min⁻¹ (mean ± SD value). Despite extreme variations in FINO, we found very similar DLNO values, and in three rabbits we found identical values even at such different FINO levels of 80 ppm or 500, 20, or 200 ppm as well as 10 or 80 ppm. There was also no dependence of DLNO on the respective duration of the single-breath maneuvers. In addition, the time course of NO removal from alveolar space was independent of applied FINO levels. These results suggest that DLNO determinations are neither affected by chemical reactions of NO in alveolar gas phase as well as in lung tissue nor biased by endogenous release of NO from pulmonary tissue. It is our conclusion that the single-breath diffusing capacity of NO is able to provide a measure of alveolar-capillary gas conductance that is not influenced by the biochemical reactions of NO.

pulmonary diffusing capacity of nitric oxide

NITRIC OXIDE (NO) has been successfully introduced as a new test gas for studying alveolar-capillary diffusion. Because of the extremely high affinity of NO for hemoglobin and its very fast rate of association with hemoglobin, it has been suggested that NO uptake in pulmonary capillary blood is not limited by chemical reaction with hemoglobin (2, 5, 8, 9). Thus pulmonary diffusing capacity of NO (DLNO) has been thought to represent a close estimate of the membrane component in alveolar-capillary gas transfer. However, chemical reactions of NO in alveolar gas phase and lung tissue could affect DLNO determinations by contributing to the removal of NO from alveolar space in addition to alveolar-capillary diffusion (9). Furthermore, endogenous NO generated in lung tissues (6, 14) should also be taken into account. If significant, pulmonary NO output would increase alveolar partial pressure of NO, creating an underestimation of the true diffusive removal rate.

The aim of our work was to check the potency of chemical reactions and the endogenous production of NO as factors capable of distorting measurements of pulmonary diffusing capacity. For this purpose, we performed two series of DLNO determinations on each of six mechanically ventilated rabbits, by applying single-breath maneuvers at two different levels of inspiratory concentrations of NO (FINO), which ranged between 10 and 800 parts per million (ppm). In each series, breath-holding times of 0.1, 1, 3, 5, and 7 s were executed. It was hypothesized that 1) if there were a chemical transformation of NO to NO₂ in alveolar gas phase or a reversible binding of NO at lung tissue, DLNO should increase with increasing breath-holding time; 2) an irreversible binding of NO at lung tissue should distort the time course of NO removal from alveolar space; 3) an endogenous NO output of significant magnitude (compared with FINO) should produce detectable traces of NO in alveolar gas.

METHODS

Single-breath maneuvers were performed in six anesthetized paralyzed supine rabbits (Chinchilla cross-breed, mean body weight 4.2 kg, range 3.4–5.6 kg). The experiments were approved by the local animal ethics committee. The animals were anesthetized with pentobarbital sodium (11 mg·kg⁻¹·h⁻¹) and paralyzed by alcuronium (0.08 mg·kg⁻¹·h⁻¹). The endotracheal tube was connected to a computerized ventilatory servo-system that we had designed for steady mechanical ventilation of small laboratory animals as well as to perform lung function testing. The animals were ventilated with room air. Sufficient conditions of normoxic ventilation were checked by repeatedly analyzing the partial pressure of oxygen in arterial blood samples (ABL1, Radiometer, Copenhagen, Denmark). We replaced fluid loss by intravenously infusing Ringer solution (5 ml·kg⁻¹·h⁻¹).

Preparation of indicator gas mixture. To avoid a spontaneous transformation of NO to NO₂, we prepared oxygen-free gas mixtures containing 10–800 ppm NO in nitrogen (N₂). NO (NO 2.5, Messer Griesheim, Cologne, Germany) was led through diluted KOH, subsequently collected with a KOH-containing syringe, and finally injected into gas-tight flexible aluminum bags (Plastigas-Beutel, Linde Gase, Cologne, Germany), which had repeatedly been washed out with N₂.

Experimental protocol. After induction of anaesthesia, we recorded pressure-volume curves in each animal by inflating and deflating the lungs in predefined volume steps. The airway pressure was measured with a differential pressure transducer (Dr. Fenyves and Gut, Basel, Switzerland) stopping the computerized procedure at airway pressures smaller than −20 cmH₂O or greater than +20 cmH₂O (related to atmospheric pressure). We defined the lung volume attained at −20 cmH₂O of airway pressure as residual volume. In a separate set of single-breath maneuvers, the rabbit lungs were inflated with a NO/N₂ gas mixture and were subsequently deflated to determine anatomic and apparatus dead space by applying Fowler’s graphical method (3) to fraction-volume curves for NO. The results were used to gauge the effective time available for NO disappearance from alveolar space, which was necessary for calculating DLNO.

Starting from the residual volume, we inflated the rabbit lungs using 50 ml of the NO-N₂ mixture. We set the time
intervals for both inspiration and expiration at 0.6 s and those of breath-holding at 0.1, 1, 3, 5, and 7 s. After executing the breath-holding periods, alveolar gas was sampled by deflating the lungs via a spiral stainless steel tube (3.5 mm ID, length 5 m), thus storing the entire expire (≈50 ml) within the tube. Then mechanical ventilation of the animals was continued with room air. In each rabbit, the two different levels of $P_{ANO}$ as well as the various breath-holding times were applied in random order. The gas stored within the tube was dried by freezing and was continuously sucked into the inlet system of a respiratory mass spectrometer. To record NO backpressure, the same experimental procedure was repeatedly applied by inflating the rabbit lungs with pure nitrogen.

Mass spectrometry. We used a variable-collector magnetic sector respiratory mass spectrometer (modified M3, Varian MAT, Bremen, Germany) to continuously record partial pressures of oxygen ($O_2$) and carbon dioxide ($CO_2$) during continued mechanical ventilation as well as to analyze the alveolar partial pressures of NO and Ar. The relevant gases NO, $O_2$, Ar, and $CO_2$ were detected, setting ion collectors at the following mass-to-charge ratios ($m/z$): 30 ($NO$), 32 ($O_2$), and 44 ($CO_2$). We determined Ar ($m/z = 40$) at the $CO_2$-44-ion collector by repeatedly changing the accelerating voltage (peak jump). To gain an optimal resolving power for NO analyses, we put the ion collectors at a maximal distance to the ion source. As previously introduced (12), we reduced drift errors and cross-talk effects by repeatedly comparing the dry sample gas with a reference gas, which only differed in the NO content. We determined the alveolar partial pressure of NO ($P_{ANO}$) within the alveolar gas sample by starting at the end-tidal volume (which was ≈10 times greater than dead space) and continuing analysis as long as $P_{ANO}$ values remained constant (alveolar plateau). Using a 2-m heated steel inlet capillary, the gas sampling rate of the mass spectrometer was reduced to 5 ml/min. The detection limit for NO was 0.07 ppm at $FINO = 10$ ppm and 0.5 ppm at $FINO = 800$ ppm.

Calculations for $DL_{NO}$. We processed the $P_{ANO}$ values by performing calculations of the $DL_{NO}$ on the basis of differential equations for inspiration, breath-holding, and expiration, as has been previously introduced (4, 13).

**Inspiration:**
\[
\frac{d}{dt} (P_{ANO} \cdot \beta_g \cdot V_A) = P_{INO} \cdot \beta_g \cdot V_I - DL_{NO} \cdot P_{ANO}
\]

**Breath-holding:**
\[
\frac{d}{dt} (P_{ANO} \cdot \beta_g \cdot V_A) = -DL_{NO} \cdot P_{ANO}
\]

**Expiration:**
\[
\frac{d}{dt} (P_{ANO} \cdot \beta_g \cdot V_A) = -DL_{NO} \cdot P_{ANO} - P_{ANO} \cdot \beta_g \cdot V_E
\]

where $P_{INO}$ is the inspiratory partial pressure of NO; $V_I$ and $V_E$ are the inspiratory and expiratory flows (ml BTPS/s), respectively; and $\beta_g$ is the capacitance coefficient for the gas phase at 37°C (0.00116 ml STPD · mmHg⁻¹ · ml⁻¹ BTPS) according to Piiper et al. (11). $V_A$ (ml BTPS) is the effective alveolar distribution volume of NO. We determined $V_A$ (as well as residual volume) from the Ar washout, induced by inflating the rabbit lungs with the Ar-free NO-N$_2$ indicator gas mixtures.

We calculated $DL_{NO}$ by performing a trial- and- error approximation method (Newton’s iteration procedure) on the coupled system of equations that we obtained from integrating Eqs. 1–3. First, the starting value of $DL_{NO}$ was set at $DL_{NO} = 2$ ml · mmHg⁻¹ · min⁻¹, and $P_{ANO}$ was calculated by using Eqs. 1–3. If the calculated value of $P_{ANO}$ differed from the measured one, $DL_{NO}$ was appropriately adjusted and the procedure was repeated. There was simple convergence to within a tolerance of 0.01%, usually within five iterations. The three-equation methodology presented has already been reported to improve the accuracy and precision in measurements of pulmonary diffusing capacity significantly when either carbon monoxide (4) or $CO_2$ (13) was used.

As previously described (13), we considered the variable alveolar volume to be one compartment. We neglected a backpressure for NO since we did not detect significant NO traces in alveolar gas samples when using pure nitrogen. Finally, we also ignored the capability of lung tissues for taking up NO as it would constitute a further path of NO removal from alveolar space, which is indistinguishable from $DL_{NO}$ when calculated by applying the three-equation methodology.

Data analysis. Values are expressed as means ± SD. To assess a dependence of the single-breath diffusing capacity of NO on the inspiratory concentration of NO used, we performed regression analysis on the $DL_{NO}$-to-$FINO$ relationship. In addition, the $DL_{NO}$ values obtained in each animal at the two different inspiratory concentrations of NO were compared using Student’s paired $t$-test. At each $FINO$ level, regression analysis was applied to the change in $DL_{NO}$ values with increasing duration of the single-breath maneuvers (hypothesis 1). Furthermore, nonlinear regression analyses were used to assess the time course of NO removal from alveolar space (hypothesis 2). The significance level was set at $P < 0.05$.

**RESULTS**

Continuous recordings of alveolar partial pressures of $O_2$ and $CO_2$ revealed values around 95 mmHg ($O_2$) as well as 38 mmHg ($CO_2$), reflecting normoxic ventilatory conditions. During the single-breath maneuvers, the end-tidal partial pressure of $O_2$ ranged between 20 and 25 mmHg. The results of determinations made in measuring the single-breath diffusing capacity of NO ($DL_{NO}$) are presented in Table 1. The overall mean value was $DL_{NO} = 1.92 ± 0.21$ ml · mmHg⁻¹ · min⁻¹. Despite extreme variations in inspiratory concentration of NO ($FINO$), we found very similar $DL_{NO}$ values (and in 3 rabbits identical values), e.g., even at $FINO = 10$ ppm as well as $FINO = 800$ ppm (rabbit F). There was no significant deviation between $DL_{NO}$ mean values obtained in the same animal and no dependence of $DL_{NO}$ on the $FINO$.}

Table 1. $DL_{NO}$ at various levels of $FINO$

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Residual Volume, ml</th>
<th>$D_{NO}$, ml · mmHg⁻¹ · min⁻¹</th>
<th>$FINO$, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.7 ± 0.2 (5)</td>
<td>1.70 ± 0.05 (5)</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>16.3 ± 0.3 (5)</td>
<td>1.84 ± 0.18 (5)</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>14.6 ± 0.4 (7)</td>
<td>2.20 ± 0.27 (5)</td>
<td>20</td>
</tr>
<tr>
<td>D</td>
<td>13.7 ± 1.2 (6)</td>
<td>2.10 ± 0.17 (5)</td>
<td>80</td>
</tr>
<tr>
<td>E</td>
<td>13.1 ± 0.2 (6)</td>
<td>1.68 ± 0.20 (5)</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>13.1 ± 1.8 (9)</td>
<td>2.00 ± 0.30 (5)</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are means ± SD of no. of measurements (in parentheses). $DL_{NO}$, pulmonary diffusing capacity of NO; $FINO$, inspiratory concentration of NO; ppm, parts per million.
values on $F_{\text{INO}}$ levels ($D_{\text{LNO}} = 1.94 - 8 \cdot 10^{-5} \cdot F_{\text{INO}}, r = -0.12, n = 12, P > 0.72, \text{power} = 0.8$).

Table 2 shows the results of regression analyses. At each $F_{\text{INO}}$ level, the single-breath diffusing capacity was independent of entire duration of maneuvers performed, which also applied to the entirety of the 60 $D_{\text{LNO}}$ determinations (see Fig. 1). The time course in disappearance of NO from alveolar space was also independent of $F_{\text{INO}}$. At each level, the ratio of alveolar partial pressures of NO obtained at the end of gas sampling ($P_{\text{ANO}}$) to initial values ($P_{\text{AONO}}$, as calculated from $F_{\text{INO}}$ and Ar washout) decreased monoeXponentially with increasing duration of the single-breath maneuvers, an aspect that is also valid for the total of 60 $P_{\text{ANO}}/P_{\text{AONO}}$ determinations (see Fig. 2). Linear transformation yielded that none of the intercept values of $P_{\text{ANO}}/P_{\text{AONO}}$ were different from unity at time zero.

DISCUSSION

The main finding of the present study is that determinations of the single-breath diffusing capacity of NO are independent of $F_{\text{INO}}$ within the studied range of 10 ppm $\leq F_{\text{INO}} \leq 800$ ppm. There was also no dependence of $D_{\text{LNO}}$ values on the respective duration of single-breath maneuvers ($t$) over a range of $1.3 \text{s} \leq t \leq 8.2 \text{s}$.

Chemical reaction of NO with alveolar gas. If there had been a significant decay of NO apart from diffusive removal, based on a chemical transformation to $NO_2$, $D_{\text{LNO}}$ should have been more and more overstated with increasing duration of single-breath maneuvers as well as increasing values of $F_{\text{INO}}$, an aspect that our findings were unable to corroborate. Furthermore, Meyer et al. (9) showed that a chemical reaction of NO in gas phase (300 ppm NO in helium by addition of 12% $O_2$) was much too slow (drop to 100 ppm NO within 1 h) to bias $D_{\text{LNO}}$ data obtained from their rebreathing experiments on dogs. During the breath-holding periods of our single-breath study, the $O_2$ concentration within alveolar space was $\approx 3\%$ as we added 50 ml of $O_2$-free NO-$N_2$ gas mixture to residual volume (14.4 $\pm$ 1.4 ml), leading to even lower reaction rates. All in all, we conclude that our experiments have not been biased by a chemical transformation of NO in the presence of oxygen.

Chemical reaction of NO in lung tissue. NO is a highly reactive chemical species. Therefore, an interaction between NO and lung tissue is also to be taken into account: if NO is reversibly absorbed by lung tissue, its effective alveolar distribution volume would exceed that of Ar [the solubilities of both gases in water are close at $0.04 \text{ ml STPD} \cdot \text{ml}^{-1} \cdot \text{atm}^{-1}$ (7)]. As alveolar volume is implicit in the calculation of the single-breath diffusing capacity, we evaluated the role of such an interaction as might possibly bias our results. We recalculated $D_{\text{LNO}}$ in rabbit E on the basis of our experimental data ($F_{\text{INO}} = 10$ ppm), but set alveolar values on $F_{\text{INO}}$ levels ($D_{\text{LNO}} = 1.94 - 8 \cdot 10^{-5} \cdot F_{\text{INO}}, r = -0.12, n = 12, P > 0.72, \text{power} = 0.8$).

Table 2. Results of linear regression analysis on $\Delta D_{\text{LNO}}$ vs. $t$ as well as nonlinear regression analysis on $P_{\text{ANO}}/P_{\text{AONO}}$ vs. exp($t$) of no. of measurements at various levels of $F_{\text{INO}}$

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>$F_{\text{INO}}$, ppm</th>
<th>$\Delta D_{\text{LNO}}$ vs. t</th>
<th>$P_{\text{ANO}}/P_{\text{AONO}}$ vs. exp($t$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>slope/SDslope</td>
<td>$</td>
<td>r</td>
</tr>
<tr>
<td>A</td>
<td>100 (5)</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>B</td>
<td>200 (5)</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>300 (5)</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>D</td>
<td>40 (5)</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>E</td>
<td>200 (5)</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>F</td>
<td>300 (5)</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>G</td>
<td>40 (5)</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>H</td>
<td>10 (5)</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>I</td>
<td>800 (5)</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>J</td>
<td>10 (5)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>K</td>
<td>800 (5)</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

$\Delta D_{\text{LNO}}$, deviation between single $D_{\text{LNO}}$ values and $D_{\text{LNO}}$ mean values obtained at each $F_{\text{INO}}$ level; t, entire duration of single-breath maneuvers; $P_{\text{ANO}}/P_{\text{AONO}}$, ratio of alveolar partial pressures of NO obtained at the end of maneuvers to initial values; $|r|$, absolute value of correlation coefficient. For slope $\neq 0$, intercept $\neq 1$ as well as $P < 0.05$: slope/SDslope, $(\text{intercept} - 1)/\text{SD}_{\text{intercept}} > 3.182$; for $P < 0.05$: $|r| > 0.9$. 

Fig. 2. Ratio of alveolar partial pressures of NO obtained at the end of maneuvers ($P_{\text{ANO}}$) to initial values ($P_{\text{AONO}}$) related to various duration of single-breath maneuvers ($t$). Nonlinear regression analysis: correlation coefficient $= -0.98 (n = 60, P < 0.001); \text{intercept value at time zero: } P_{\text{ANO}}/P_{\text{AONO}} = 1.0 \pm 0.04$. 

Fig. 1. Deviation between single pulmonary diffusing capacity of NO ($D_{\text{LNO}}$) values and $D_{\text{LNO}}$ mean values ($\Delta D_{\text{LNO}}$) obtained at respective levels of inspiratory concentration of NO against duration of single-breath maneuvers ($t$). Linear regression analysis: correlation coefficient $= -0.21 (n = 60, P > 0.2)$. 

The table above shows the results of linear regression analysis on $\Delta D_{\text{LNO}}$ vs. $t$, as well as nonlinear regression analysis on $P_{\text{ANO}}/P_{\text{AONO}}$ vs. exp($t$) for various levels of $F_{\text{INO}}$.
volume at respective values that were 30, 60, and 90% higher. We obtained an underestimation of DLNO by 2, 37, and 95% at a breath-holding time of 0.1 s, but found an increasing overestimated result with increasing duration of maneuvers, attaining values of 22, 41, and 56% at a breath-holding time of 7 s. In the same animal, we calculated even more distinct discrepancies when using experiments performed on FINO = 800 ppm. In the face of the time independence of the DLNO values, a reversibly binding of NO at lung tissue hence appears to be insignificant, at least when using 10 ppm ≤ FINO ≤ 800 ppm.

If NO is irreversibly bound to tissue throughout the single-breath maneuvers, the time course of NO removal from alveolar space should have been affected in as far as the zero-time intercept of the PANO/PAONO-time relationship is different than unity. In this connection, it should be emphasized that we calculated PANO/PAONO = 1 at time zero (see Fig. 2), although we included values obtained from 10 ppm as well as 800 ppm experiments. From equality in the zero-time intercept with unity, we inferred that NO could not have been subjected to an irreversible reaction with lung tissue unless it developed proportionally to the partial pressure of NO in the gas phase.

Endogenous production of NO. Recently, it was shown that NO is also endogenously generated by rabbit lung tissue (6, 14). Assuming endogenous NO outputs were of significant magnitude compared with values of FINO, we should have obtained significant traces of NO in alveolar gas after having the animals inhale pure nitrogen or room air. However, we were unable to detect such traces of NO (detection limit: 0.07 ppm NO).

There is, incidentally, no reason to expect an influence of the endogenous NO output on the single-breath diffusing capacity as we determined a lower limit of NO at a removal rate of 30 nmol/min in rabbit E (DLNO = 1.68 ml·mmHg⁻¹·min⁻¹ at FINO = 10 ppm), although this constitutes a 15-fold higher value compared with exhalation rates of endogenously produced NO in isolated perfused rabbit lungs [2 nmol/min (14)]. Evidently, even at 10 ppm NO its alveolar concentration was much too high to detect an influence of endogenous NO on measurements of the single-breath diffusing capacity of NO. However, this observation may be qualified by the findings of Muramatsu et al. (10), who showed an increased endothelial NO production in chronic hypoxia-induced hypertensive rat lungs (13 nmol/min), indicating that DLNO measurements are possibly limited during conditions of altered physiology at least when using 10 ppm NO.

Perspectives

The relative roles of chemical reaction and endogenous production of NO in their ability to influence determinations of pulmonary diffusing capacity of NO were assessed by performing single-breath experiments on six mechanically ventilated rabbits. Using very different inspiratory concentrations of NO as well as a large range of breath-holding times, we nevertheless obtained unchanged values of DLNO. This finding suggests that the single-breath diffusing capacity of NO is able to provide a measure of alveolar-capillary gas conductance that is unbiased by biochemical reactions of the indicator gas at least within the range of 10 ppm ≤ FINO ≤ 800 ppm. Another aspect of our findings would also seem worthy of discussion, since NO is known to mediate vasodilatation in the pulmonary circulation (1). In this respect, our finding that DLNO values were almost identical at FINO = 10 ppm as well as at FINO = 800 ppm may be taken as an indication that within this range of inspiratory concentrations NO did not influence pulmonary diffusion conditions by changing the effective pulmonary capillary surface area (which, among other things, depends on pulmonary capillary blood volume). In any event, to what extent the interpretation of DLNO data is limited by functional inhomogeneities of pulmonary gas exchange remains a question requiring further research and investigation.

We gratefully acknowledge the expert technical assistance of Christa Pusch, Barbara Schreiber, and Bernd Eixmann.

Address for reprint requests: H. Keller, Dept. of Physiology, Univ. of Bonn, Nussallee 11, D-53115 Bonn, Germany.

Received 22 May 1997; accepted in final form 28 August 1997.

REFERENCES