Role of endogenous nitric oxide in endotoxin-induced alteration of hypoxic pulmonary vasconstriction in mice

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Regional vasconstriction in response to low tissue oxygen tension, i.e., hypoxic pulmonary vasconstriction (HPV), is unique to the pulmonary vasculature and represents the main physiological mechanism to match ventilation and perfusion in the lung. HPV diverts regional pulmonary blood flow away from poorly ventilated toward better ventilated lung regions and thus preserves systemic oxygenation. Despite great efforts, the mechanism of HPV has not yet been fully understood. In recent years there has been growing evidence that the effector mechanism of pulmonary vasconstriction in response to hypoxia is mediated by inhibition of voltage-gated potassium channels, causing membrane depolarization that in turn activates voltage-gated calcium channels in vascular smooth muscle cells (46). The resulting influx of calcium leads to pulmonary smooth muscle contraction. The sensing mechanism seems to be inherent to the pulmonary vascular smooth muscle cells (3), but the exact mechanism is still under debate (1, 52).

HPV has been shown to be impaired in patients with pneumonia, sepsis, or the acute respiratory distress syndrome (29) and in several animal models of endotoxemia (12, 13, 15, 36, 39, 49, 50). This attenuation of HPV increases intrapulmonary shunting and thereby leads to systemic hypoxemia (12, 29, 36). A vast variety of vasoactive mediators have been identified to alter pulmonary vascular tone during lung inflammation, including nitric oxide (NO) [for review, see Barnes and Liu (4)]. Endogenous NO is produced by nitric oxide synthase (NOS) (33). Three different NOS isoforms have been characterized. Neuronal (NOS1) and endothelial (NOS3) NOS are expressed constitutively and produce NO in response to increased intracellular calcium concentration (33). Transcription of the inducible NOS isoform (NOS2) is increased in response to endotoxin and cytokines, such as TNF-α, IL-1β, and IL-6, and leads to increased production of NO (33). NO, either endogenously produced or exogenously administered via infusion or inhalation, stimulates soluble guanylate cyclase to produce the second messenger cGMP from GTP. cGMP activates cGMP-dependent protein kinase, which phosphorylates several intracellular targets, resulting in smooth muscle relaxation (for review, see Ref. 27). Excessive NO synthesis has been suggested as an important mechanism causing systemic hypotension during septic shock (38). NOS2-derived NO production reduces the pulmonary responsiveness to exogenous inhaled NO, alters pulmonary blood flow distribution, and impairs arterial oxygenation in endotoxin (lipopolysaccharide; LPS)-challenged mice (50, 53). We hypothesized that overproduction of NO does not simply counterbalance HPV by NO-mediated vasodilatation but, rather, impairs the HPV mechanism itself during experimental sepsis in analogy to the LPS-induced alteration of the NO-cGMP pathway (53). Therefore, in this study we wanted to clarify the role of endogenous NO production for LPS-induced hyporesponsiveness to HPV in an iso-
lated, perfused mouse lung model. Inhibition of endogenous NO production at 18 h after LPS injection (lung perfusion with nonspecific and NOS2-specific inhibitors) was compared with the absence of NOS2-derived NO production over the entire time period from LPS injection until lung perfusion experiments (NOS2-deficient mice).

**METHODS**

These investigations were approved by the Governmental Animal Care Committee of Baden-Württemberg, Germany. A total of 70 adult male mice weighing 20–35 g, including 28 NOS2-deficient mice (C57BL/6-NOS2tm1Lau; Jackson Laboratory, Bar Harbor, ME) and 42 wild-type mice of the same background (C57BL/6; Jackson Laboratory), were studied.

**Isolated, Perfused, and Ventilated Mouse Lung Model**

Mice were euthanized with an intraperitoneal injection of pentobarbital sodium (200 mg/kg body wt) and placed in a 37°C water-jacketed chamber (Isolated Perfused Lung Size 1 type 839; Hugo-Sachs Elektronik, March-Hugstetten, Germany). The trachea was isolated and tubuted, and the lungs were ventilated with 21% O2-5% CO2-74% N2 (Messer Griesheim, Krefeld, Germany) with the use of a volume-controlled ventilator (MiniVent type 845; Hugo-Sachs Elektronik) at a ventilatory rate of 90 breaths/min, a tidal volume of 10 ml/kg body wt, and an end-expiratory pressure of 2 cmH2O. The lungs were exposed via a midline sternotomy, and a ligature was placed around the aortopulmonary outflow tract. After injection of 10 IU heparin into the right ventricle, the pulmonary artery was cannulated with a stainless steel cannula (internal diameter 1 mm) via the right ventricle. The pulmonary venous effluent was drained via a stainless steel cannula (internal diameter 1 mm) placed through the apex of the left ventricle across the mitral valve and into the left atrium. Left atrial pressure (LAP) was maintained at 2 mmHg throughout the entire experiment. Lungs were perfused with a roller pump (Ismatec Reglo) and dextran (5%; Sigma-Aldrich Chemie, Deisenhofen, Germany) and KH2PO4, 0.50 MgCl2, 0.41 MgSO4, 138.0 NaCl, 4.0 NaHCO3, 0.3 Na2HPO4, and 5.6 glucose. Bovine serum albumin (5%; Serva, Heidelberg, Germany) and dextran (5%; Sigma-Aldrich Chemie, Deisenhofen, Germany) were added to adjust the perfusate pH (7.34 –7.43). Lungs were included in baseline perfusion, the lungs were ventilated with the hypoxic gas mixture.

Pulmonary Vascular Response to Hypoxia After LPS Challenge

Wild-type mice (n = 7) were injected intraperitoneally with 20 mg/kg body wt *Escherichia coli* 0111:B4 LPS (Difco Laboratories, Detroit, MI) dissolved in saline 18 h before isolated lung perfusion. Untreated wild-type mice served as controls (n = 7). Mice lungs of all groups were studied according to the following experimental protocol (a typical original recording is depicted in Fig. 1).

After an initial 10-min baseline perfusion period, pulmonary vascular pressure-flow (P-Q) curves were generated by perfusing the lungs with a flow of 25, 50, 75, and 100 ml·kg⁻¹·min⁻¹ in randomized order for 30 s each. At each flow step, LAP was readjusted to maintain a value of 2 mmHg, and PAP was measured at the end of each period. Pulmonary vascular P-Q curves were analyzed as outlined below.

Flow was then set to 50 ml·kg⁻¹·min⁻¹, and after another 3 min of baseline perfusion, the lungs were ventilated with the hypoxic gas mixture (containing 1% O2). Pilot experiments (n = 5) revealed that the maximal hypoxic pressure response was reached between 5 and 7 min, followed by a slow decline in PAP (data not shown). Therefore, the hypoxic pulmonary vasoconstrictor response (ΔPAP) was defined as the increase in PAP measured at 6 min after initiation of hypoxic ventilation as a percentage of baseline PAP. A second P-Q curve was then generated during hypoxia, as described above. Finally, perfusate flow was reset to 50 ml·kg⁻¹·min⁻¹, normoxic ventilation was reestablished, and PAP was allowed to return to baseline value. In all experiments, perfusion pressure at the end of the experiment did not differ from baseline pressure by >20%.

**Effect of Acute NOS Inhibition on HPV After LPS Challenge**

In a second set of experiments, lungs of LPS-treated and untreated wild-type mice (n = 7 per group) were isolated and perfused, and their hypoxic vasoconstrictor responses, including P-Q curves, were studied according to the experimental protocol outlined above. To study the effect of endogenous NO production on HPV 18 h after LPS treatment, we perfused lungs with either 1 mM Nω-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich Chemie), a nonspecific inhibitor, or 10 μM L-N^6-(1-iminoethyl)lysine (L-NIL; Sigma-Aldrich Chemie), a NOS2-selective NOS inhibitor (34) added to the perfusate.
We choose these doses of l-NAME and l-NIL because similar doses have been shown to effectively inhibit total NOS and NOS2 activity, respectively, in rodent isolated, perfused lung models (19, 21, 40, 53). Additional pilot experiments in untreated and LPS-pretreated mice showed that \( \Delta P \) was similar in LPS-pretreated animals perfused with 10 or 100 \( \mu \)M l-NIL but that 100 \( \mu \)M l-NIL augmented the HP response in untreated control animals, suggesting that this dose of l-NIL may not be completely selective for NOS2 but may also, at least in part, inhibit NOS3 (data not shown).

**Effect of NOS2 Deficiency on HPV After LPS Challenge**

In contrast to just acutely inhibiting NOS2-derived NO production with l-NIL during lung perfusion 18 h after LPS treatment, we also studied NOS2-deficient mice in which LPS-induced NO overproduction by NOS2 was precluded over the entire 18-h period preceding lung perfusion experiments. Thus, to test the hypothesis that NOS2 induction by LPS is necessary for the LPS-induced alteration of HPV, we studied the hypoxic vasconstrictor response (\( \Delta P \)) and the pulmonary vascular P-Q relationship under normoxic and hypoxic ventilation in lungs of LPS-pretreated and untreated NOS2-deficient mice (\( n = 7 \) per group) as described above. In additional experiments, we studied lungs of NOS2-deficient mice with l-NAM added to the perfusate for acute nonspecific NOS-activity inhibition.

**Wet-to-Dry Lung Weight Ratio**

At the end of each experiment, both lungs, excluding hilar structures, were excised and weighed (wet weight). Thereafter, the lungs were dried in an oven overnight at 100°C and then reweighed (dry weight). Lung wet-to-dry weight ratios were calculated by dividing the wet weight by the dry weight (53).

**Analysis of P-Q Curves**

To gain more insight into the respective properties of the pulmonary vasculature during normoxic and hypoxic ventilation, we analyzed the resulting four-point pulmonary vascular P-Q curves on the basis of two different mathematical models.

First, we employed the collapsible vessel model (ohmic-Starling resistor model) of Permutt and Riley (37). This model allows quantification of changes in the shape of P-Q curves via changes in the slope (\( R_{\text{LIN}} \)) and extrapolated pressure axis intercept at zero flow (\( P_{ZF} \)) of a linear regression line (32) of the form

\[
PAP = R_{\text{LIN}}Q + P_{ZF}
\]

where PAP is pulmonary artery pressure (perfusion pressure in our model) and \( Q \) is flow. \( R_{\text{LIN}} \) is interpreted as the mean of parallel ohmic resistances and is assumed to represent the resistance of extra-alveolar, noncollapsible pulmonary vessels (48). Accordingly, \( P_{ZF} \) has been suggested to represent the mean pressure value below which a given pressure would not result in flow through the vessels (also termed “mean critical closing pressure”). Changes in \( P_{ZF} \) in turn were assumed to result from changes in resistance of alveolar, collapsible vessels (16, 48).

However, several limitations regarding the interpretation of \( R_{\text{LIN}} \) and \( P_{ZF} \) have been raised (23, 42), and several authors have proposed alternative mathematical models based on vessel distensibility that may especially account for nonlinearity at low flows (5–7, 43, 57). Alternative mathematical models based on vessel distensibility that may especially account for nonlinearity at low flows (5–7, 43, 57).

**Statistical Analysis**

Linear and nonlinear regression analysis was performed (Statistica for Windows; StatSoft, Tulsa, OK) for P-Q data obtained under normoxic and hypoxic conditions to give \( R_{\text{LIN}} \) and \( P_{ZF} \) values and \( R_0 \) values for each single experiment, respectively. These data as well as \( \Delta P \) and lung wet-to-dry weight ratio data are expressed as means ± SD.

Two-way ANOVA was performed to compare groups. When significant differences were detected using ANOVA, a post hoc least significant difference test for planned comparisons was used (Statistica for Windows). Statistical significance was assumed at a \( P \) value of <0.05.

**RESULTS**

Both wild-type and NOS2-deficient mice injected intraperitoneally with LPS experienced piloerection, diarrhea, and lethargy to a similar degree. The mortality rate 18 h after LPS injection was ~10% and did not differ between wild-type and NOS2-deficient mouse strains.
Pulmonary Vascular Response to Hypoxic Ventilation After LPS Challenge

There was no significant difference in baseline perfusion pressure (data not shown) or pressure-flow relationship characteristics (Table 1) under normoxic conditions between LPS-treated and untreated wild-type mice.

After isolated, perfused mouse lungs were switched from normoxic (inspired fraction of oxygen $F_{IO2}$ of 0.21) to hypoxic ventilation ($F_{IO2}$ of 0.01), PAP started to rise within 2 min and reached its maximum within 6 min regardless of the group studied (Fig. 1). Hypoxic ventilation caused an HPV response ($\Delta$PAP) of 69 ± 17% in isolated, perfused lungs obtained from untreated wild-type mice (Fig. 3). Accordingly, the pulmonary vascular P-Q relationship was shifted to higher pressures at respective flows, resulting in a mean increase in $R_{LIN}$ of 105 ± 19%, in $PZF$ of 43 ± 29%, and in $R_0$ of 156 ± 86%, whereas vessel distensibility $\alpha$ decreased by 57 ± 22% ($P < 0.05$ compared with baseline; Figs. 4 and 5).

In contrast, lungs of wild-type mice that were challenged with LPS did not show any significant vasoconstriction in response to hypoxic ventilation ($\Delta$PAP = 3 ± 7%; $P < 0.001$ vs. untreated controls; Fig. 3). This LPS-induced reduction in $\Delta$PAP was associated with a reduction in the hypoxia-induced increase in $R_{LIN}$, $PZF$, and $R_0$ (Figs. 4 and 5B). Of interest, there was no difference in the hypoxia-induced decrease in $\alpha$ between LPS-pretreated and untreated mice (Fig. 5A).

Effects of Acute NO Synthesis Inhibition on HPV Response After LPS Challenge

Nonspecific NOS inhibition by $L$-NAME. To study the role of endogenous NO production for the reduced pulmonary vasoconstrictor response to hypoxic ventilation 18 h after LPS-injection, in a first set of experiments, we perfused lungs of untreated and LPS-treated wild-type mice with 1 mM $L$-NAME added to the perfusate for nonspecific NO synthesis inhibition (21, 53). Perfusion with $L$-NAME did not affect baseline perfusion pressure or the pulmonary vascular P-Q curve during normoxic ventilation in lungs of both LPS-treated and untreated mice according to analysis with the linear regression model. However, applying the nonlinear regression model, we noted an increase in vessel distensibility factor $\alpha$ as well as in $R_0$ in response to $L$-NAME in both untreated and LPS-challenged mice (Table 1).

Non-specific NOS inhibition by $L$-NAME markedly augmented the HPV response ($\Delta$PAP) in both untreated and LPS-treated mice (Fig. 3). However, $L$-NAME did not fully restore the HPV response in septic mice ($\Delta$PAP = 134 ± 37% in control vs. 26 ± 27% in LPS-pretreated mice; $P < 0.001$).

Table 1. Values of $R_{LIN}$ and $PZF$ and values of $\alpha$ and $R_0$ calculated by linear and nonlinear regression analysis, respectively, for pulmonary vascular pressure-flow curves in isolated, perfused mouse lungs under normoxic conditions

<table>
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<tr>
<th></th>
<th>$R_{LIN}$, mmHg/ml·kg$^{-1}$·min$^{-1}$</th>
<th>$PZF$, mmHg</th>
<th>$\alpha$, %/mmHg</th>
<th>$R_0$, mmHg/ml·kg$^{-1}$·min$^{-1}$</th>
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<td>Wild type</td>
<td>NOS2-ko</td>
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<td>Control</td>
<td>LPS</td>
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<td></td>
<td>72.4±7.0</td>
<td>63.4±9.3</td>
<td>3.3±0.6</td>
<td>1.9±0.07</td>
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<td>71.9±10.0</td>
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<td></td>
<td>61.4±11.2</td>
<td>57.1±14.2</td>
<td>4.0±0.8</td>
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<td>65.6±10.9</td>
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<td>0.16±0.06</td>
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<td>67.1±13.9</td>
<td>3.4±0.8*§</td>
<td>5.2±4.7</td>
<td>0.27±0.17</td>
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Values are means ± SD, $n = 7$ per group. See METHODS for calculation of $R_{LIN}$ and $PZF$ by linear and nonlinear regression analysis.

* $P < 0.05$ vs. no inhibitor. † $P < 0.05$ vs. wild type. ‡ $P < 0.05$ vs. control.
Analysis of hypoxia-induced changes of the pulmonary vascular P-Q relationship revealed that these effects of L-NAME were reflected by an augmentation in $P_{Z\text{F}}$ and $R_0$ in untreated mice that was absent in lungs of LPS-challenged mice (Figs. 4B and 5B). In contrast, nonspecific NOS inhibition did not affect $R_{\text{LIN}}$ and $P_{Z\text{F}}$ in untreated mice but augmented the hypoxia-induced changes in $R_{\text{LIN}}$ and $P_{Z\text{F}}$ in LPS-pretreated mice. The extent of this augmentation reflected the observed partial restoration of $P_{\text{AP}}$ in septic mice by L-NAME perfusion (Figs. 4A and 5A).

**NOS2 Deficiency and HPV Response After LPS Challenge**

In a previous study (53), we showed that NOS2 deficiency protects against LPS-induced alteration of NO-cGMP signal-
ing. Therefore, we studied the HPV response and pulmonary vascular P-Q curves during normoxic and hypoxic ventilation in untreated and LPS-pretreated NOS2-deficient mice.

In contrast to findings in lungs of LPS-challenged wild-type mice perfused with l-NAME or l-NIL, we did not detect any difference in $\Delta P_{\text{AP}}$ as well as in $\Delta R_{\text{LIN}}$, $\Delta P_{\text{ZF}}$, $\Delta \alpha$, and $\Delta R_0$ between untreated and LPS-pretreated NOS2-deficient mice (Figs. 3–5). Although $\Delta P_{\text{AP}}$, $\Delta P_{\text{ZF}}$, $\Delta \alpha$, and $\Delta R_0$ were similar in lungs obtained from untreated wild-type and NOS2-deficient mice, we detected a smaller hypoxia-induced increase in $\Delta R_{\text{LIN}}$ in NOS2-deficient than in wild-type mice. This difference was absent when lungs were perfused with l-NAME added to the perfusate.

**Lung Wet-to-Dry Weight Ratios**

The absence of pulmonary edema was confirmed by the unchanged wet-to-dry lung weight ratios after perfusion experiments. We did not detect any significant difference in wet-to-dry lung weight ratios between any of the studied groups (data not shown). Lung wet-to-dry weight ratios did not correlate with the vasoconstrictor response to hypoxia ventilation.

**DISCUSSION**

The main finding of this study is that the absence of the gene encoding NOS2 completely protects mice from impaired HPV during endotoxemia. Because acute nonspecific and NOS2-specific NOS inhibitions do not completely restore the HPV response in isolated, perfused lungs obtained from mice 18 h after LPS treatment, this impaired HPV may be attributable only in part to pulmonary vasodilation by endogenous NOS2-derived NO production counterbalancing HPV.

**Impaired HPV During Endotoxemia**

In contrast to vessels of the systemic circulation, pulmonary vessels constrict in response to hypoxia (30). This so-called HPV allows the body to redistribute pulmonary blood flow away from poorly ventilated toward better ventilated lung regions (55). However, during inflammatory processes such as pneumonia or sepsis with acute lung injury, HPV may be impaired, causing venous blood to shunt through the lungs without being oxygenated and thereby leading to hypoxemia in humans (29) and in several animal models (13, 15, 36, 39, 49, 50).

Consistent with reports of other authors (2, 11, 54), we found a robust HPV in buffer-perfused isolated mouse lungs with a 75% increase in pulmonary vascular resistance when lungs were ventilated with a gas mixture containing 1% $O_2$ (Fig. 3). Furthermore, we showed that pulmonary vasoconstriction in response to hypoxia was completely abolished in isolated, perfused lungs obtained from mice that were challenged with LPS 18 h before isolated lung perfusion (Fig. 3).

Performing linear regression analysis (37), we demonstrated that hypoxic ventilation caused an increase in $R_{\text{LIN}}$ and $P_{\text{ZF}}$ (Fig. 4) consistent with data obtained in isolated pig lungs (17, 47, 48). However, other authors found an increase in $P_{\text{ZF}}$ by hypoxic ventilation without any change in P-Q slope in isolated, perfused lungs from pigs and dogs (8, 31). When P-Q data were analyzed according to the nondistensible vessel model, we found that $R_0$ doubled and $\alpha$ decreased by 50% when isolated lungs of untreated mice were ventilated with a hypoxic gas mixture (Fig. 5). These findings partially reflect the results of Nelini et al. (35), who found an increase in $R_0$ but no decrease in $\alpha$ following hypoxia in isolated, blood-perfused pig lungs. These differences may be attributable to species and perfusion media differences.

In our study, the hypoxia-induced increases in $R_{\text{LIN}}$, $P_{\text{ZF}}$, and $R_0$ were abolished in LPS-pretreated compared with untreated mice (Figs. 4 and 5B). Of interest, the decrease in $\alpha$ observed during hypoxic ventilation was not affected by endotoxemia (Fig. 5A).

**Endogenous NO Production and HPV**

Three isoforms of NOS have been described to produce NO in the lungs, with only (endothelial) NOS3-derived NO production modulating basal pulmonary vascular tone under physiological conditions (11, 45). Transcription of (inducible) NOS2 is increased in response to endotoxin and cytokines, such as TNF-$\alpha$, IL-1$\beta$, and IL-6, and leads to accelerated production of NO (27). Excessive NO synthesis has been suggested as an important mechanism causing systemic hypertension during septic shock (38).

Nonspecific NOS inhibition by l-NAME did not affect baseline perfusion pressure in our study as has been shown by others (2, 44, 51, 54). In addition, P-Q slope ($R_{\text{LIN}}$) and intercept ($P_{\text{ZF}}$) were not affected by nonspecific NOS inhibition under normoxic baseline conditions (Table 1). These findings are supported by other investigators using isolated lung preparations who showed that the response to NO inhibition may vary among species (8) and the composition of the perfusate (44, 51). However, by analyzing the pulmonary vascular P-Q relationship in terms of the distensible vessel model (28), we could detect a significantly elevated $R_0$ in isolated lungs of untreated mice of both genotypes perfused with l-NAME during normoxia (Table 1), which is consistent with the concept of endogenous NO production lowering pulmonary vascular tone under resting conditions.

We found that nonspecific NOS inhibition by l-NAME led to an increase in vessel distensibility in both wild-type and NOS2-deficient mice. Vessel distensibility was not affected by NOS2 inhibition with l-NIL (Table 1). This may suggest that endogenous NOS3-derived NO reduces vessel distensibility in the normal lung, a finding that would not fit into the concept of NO eliciting vasodilation. However, one could speculate that NOS inhibition increases resistance in more proximal vessels (increase in $R_0$, see above), which in turn may result in a decrease in pressure in the distal vessels (i.e., capillaries and venules) that are much more compliant (39). Then, because of the nonlinear pressure-volume relationship, overall vessel distensibility (as described by $\alpha$) would increase. However, one should be aware of the fact that vessel distensibility depends on both active vascular tone and the hierarchical structure of the vasculature, complicating the interpretation of changes in $\alpha$.

Inhibition of NOS2 by l-NIL or the absence of the gene encoding NOS2 did not affect baseline perfusion pressure or the pulmonary vascular P-Q relationship in untreated mice under normoxic conditions, a finding consistent with other reports in vivo (50) and in vitro (11, 53).

Inhibition of endogenous NO production by l-NAME but not by l-NIL augmented the pulmonary HPV response ($\Delta P_{\text{AP}}$) in untreated wild-type and NOS2-deficient mice (Fig. 3), sug-
suggesting that NOS3-derived NO counteracts HPV under physiological conditions as described previously (18). Of interest, this was reflected only by an augmentation of $\Delta P_{ZF}$ and $\Delta R_0$, but not $\Delta R_{LIN}$ or $\Delta R$ (Figs. 4 and 5), suggesting that NOS3-derived NO may modulate a static component rather than a dynamic component (i.e., vessel distensibility) of the HPV response.

**Effects of NOS Inhibition on Impaired HPV During Endotoxemia**

Acute inhibition of pulmonary NO synthesis by L-NAME augmented but did not fully restore HPV in LPS-pretreated wild-type mice (Fig. 3). Because perfusion of lungs from LPS-challenged wild-type mice with the NOS2-specific inhibitor L-NIL caused augmentation of HPV similar to that observed with L-NAME, the main NOS isoform responsible for endogenous NO production partly counteracting HPV during endotoxemia may be NOS2 rather than NOS3. Similar observations were reported in awake sheep following endotoxin administration (36) or infusion of Pseudomonas aeruginosa (12). However, Ullrich et al. (50) could not detect any change in blood flow redistribution toward the right lung after left main stem bronchus occlusion by administration of 5 mg/kg L-NIL intravenously in either untreated or LPS-pretreated anesthetized mice (50).

Furthermore, partial restoration of the HPV response in septic wild-type mice by perfusion with L-NIL or L-NAME was reflected only by an augmented hypoxia-induced increase in $R_{LIN}$ (Fig. 4A) but not in $\alpha$ (Fig. 5A). Moreover, augmentation of the hypoxia-induced change in $P_{ZF}$ or $R_0$ by L-NAME as observed in lungs from untreated wild-type mice was absent in lungs of LPS-treated mice (Figs. 4B and 5B). Therefore, our data analyzing the pulmonary P-Q relationship reveal that certain features of the pulmonary vascular response to hypoxia may be differentially modulated by NOS2- and NOS3-derived endogenous NO under physiological or pathological circumstances such as endotoxemia. One explanation for this phenomenon may be a variable longitudinal distribution of NOS isoform expression that may be changed by LPS treatment (10). Alternatively, the NO-cGMP pathway may be altered during endotoxemia, resulting in impaired pulmonary vasodilation in response to NO (53).

NOS2 deficiency completely prevented the LPS-induced alteration in HPV (Fig. 3). Moreover, the hypoxia-induced changes in the pulmonary vascular P-Q relationship were not found to differ between LPS-pretreated and untreated NOS2-deficient mice (Figs. 4 and 5). Therefore, the expression of NOS2 is required for the production of LPS-mediated alteration in HPV. This finding is supported by a study of Ullrich et al. (50) showing preserved pulmonary blood flow distribution in NOS2-deficient mice following LPS-challenge.

NOS2 activity varies over the course of endotoxemia. Kristof et al. (25) showed that NOS2 protein in lung homogenates is expressed maximally at 6 h, weakly at 12 h, and not detectably at 24 h after LPS injection in mice (25). Moreover, they found that lung NOS activity largely parallels this time course. Thus, during the late phase of sepsis [at 18 h in our model and at 22 h in the model of Ullrich et al. (50) following LPS injection] when impaired HPV can be detected, NOS2 activity may have already returned to near baseline values, producing only fair amounts of NO. This is supported by the finding that the NOS2-specific inhibitor L-NIL augments, but does not completely restore, HPV responsiveness in LPS-treated compared with untreated mice during that late phase of endotoxemia in vivo (50) and in our model. In contrast, when the early LPS-induced upregulation of NOS2 is prevented, either by inherited NOS2 deficiency (this study) or by treating mice 1 and 8 h after LPS challenge with a single dose of the short-acting selective NOS2 dimerization inhibitor BBS-2 (22), HPV responsiveness is preserved. This suggests that NOS2-derived NO formation during the early phase of the inflammatory response (including NOS2 induction) leads to septic pulmonary vascular dysfunction found later in the course of sepsis. The underlying mechanism, however, is not known, but may include NO-mediated regulation of gene transcription, alteration of protein function by S-nitrosylation, and interaction with other cytotoxic radicals (such as reactive oxygen species) (14, 26, 41).

**Criticism of Experimental Setup and Mathematical Models**

In this study, we used an isolated, perfused lung model to study pulmonary vasoreactivity in response to alveolar hypoxia in mice challenged with LPS. Several limitations of this experimental setup have to be considered when extrapolating our data to the situation to humans. A single LPS injection may not reflect the septic syndrome observed in patients, which is rather characterized by a continuous inflammatory process until the focus of infection is controlled. However, the present approach represents a highly reproducible model of systemic inflammation that, moreover, has been shown previously to include impaired reactivity of the pulmonary vasculature in response to vasoactive stimuli (50, 53). Species differences in the response to lung inflammation have been considered elsewhere (56). In contrast to in vivo models, the isolated, perfused lung model allows us to generate and study pulmonary vascular P-Q curves in a controlled way, which may provide information regarding the properties of the pulmonary vasculature that cannot be obtained by simply studying pulmonary arterial pressure at a single given flow.

To learn more about the hypoxia-induced changes in the properties of the pulmonary vasculature and how these are altered during endotoxemia, we studied the pulmonary vascular P-Q relationship by applying different mathematical models, a linear regression analysis based on the collapsible vessel model by Permutt et al. (37) and a nonlinear regression anal-

![Fig. 6. Correlation between the hypoxia-induced $\Delta P_{ZF}$ and $\Delta R_0$ according to linear regression analysis ($r^2 = 0.77, P < 0.001$; solid line). Data were pooled from all experiments ($n = 70$).](http://ajpheart.physiology.org/)
ysis based on a distensible vessel model proposed by Linehan et al. (28). According to the collapsible vessel model, $P_{ZF}$ basically describes the upper limit of a pressure range, with the backpressure being the lower limit (in our study LAP). The collapsible vessel model assumes that within this range of pressures there is no flow through the lungs. Furthermore, the distensible vessel model calculates static resistance ($R_0$) as the resistance that would exist if the vessels were at their respective diameters at zero vascular pressure. Thus, with $P_{ZF}$ and $R_0$, both models introduce parameters that describe features of the pulmonary vascular P-Q relationship that are independent of the response of vessel diameter to changes in pressure or flow. Of interest, the pattern of between-group differences in the hypoxia-induced change in $P_{ZF}$ (Fig. 4B) was largely mirrored by the respective changes in $R_0$ (Fig. 5B), and we found a strong linear correlation between $\Delta P_{ZF}$ and $\Delta R_0$ when data from all 70 experiments were pooled ($r^2 = 0.77, P < 0.001$; Fig. 6). In contrast, $R_{LIN}$ and $\alpha$ may be interpreted to describe dynamic vessel wall properties, since they are calculated as a change in pressure in response to change in flow and as a change in diameter in response to change in pressure, respectively.

In general, one has to be aware of the fact that both mathematical models do not account for longitudinal pressure, volume, and distensibility distribution as well as for asymmetric branching within the pulmonary vascular bed (9, 24). However, we could demonstrate the usefulness of analyzing the pulmonary vascular pressure-flow relationship by combining the collapsible vessel model and the distensible vessel model. The differential modulation of the HPV response by NOS isoform-specific endogenous NO production as suggested by our data would have remained undetected if we had only measured changes in perfusion pressure at a single given flow in this study.

In summary, NOS2-derived NO production is critical for LPS-mediated development of impaired HPV in mice. The observation that acute NOS2 inhibition did not fully restore HPV in septic mice suggests that impaired HPV during endotoxemia is mediated at least in part by mechanisms other than simply pulmonary vasodilation by NO overproduction. Analysis of the pulmonary vascular P-Q relationship under normoxic and hypoxic conditions with the use of two different mathematical models suggests that the hypoxic pulmonary vasocconstrictor response may consist of different components that may be modulated NOS isoform specifically in untreated and LPS-treated mice.

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