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

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Spöhrl, Fabian, Annemiek J. M. Cornelissen, Cornelius Busch, Martha M. Gebhard, Johann Motsch, Eike O. Martin, and Jörg Weimann. Role of endogenous nitric oxide in endotoxin-induced alteration of hypoxic pulmonary vasconstriction in mice. Am J Physiol Heart Circ Physiol 289: H823–H831, 2005.—Pulmonary vasconstriction in response to alveolar hypoxia (HPV) is frequently impaired in patients with sepsis or acute respiratory distress syndrome or in animal models of endotoxemia. Pulmonary vasodilation due to overproduction of nitric oxide (NO) by NO synthase 2 (NOS2) may be responsible for this impaired HPV after administration of endotoxin (LPS). We investigated the effects of acute nonspecific (N³-nitro-L-arginine methyl ester, 1-NNAME) and NOS2-specific [1-N²-(1-iminoethyl)lysine, 1-NIL] NO inhibition and congenital deficiency of NOS2 on impaired HPV during endotoxemia. The pulmonary vasconstrictor response and pulmonary vascular pressure-flow (P-Q) relationship during normoxia and hypoxia were studied in isolated, perfused, and ventilated lungs from LPS-pretreated and untreated wild-type and NOS2-deficient mice with and without 1-NNAME or 1-NIL added to the perfusate. Compared with lungs from untreated mice, lungs from LPS-challenged wild-type mice constricted less in response to hypoxia (69 ± 17 vs. 3 ± 7%, respectively, P < 0.001). Perfusion with 1-NNAME or 1-NIL restored this blunted HPV response only in part. In contrast, LPS administration did not impair the vasconstrictor response to hypoxia in NOS2-deficient mice. Analysis of the pulmonary vascular P-Q relationship suggested that the HPV response may consist of different components that are specifically NOS isoform modulated in untreated and LPS-treated mice. These results demonstrate in a murine model of endotoxemia that NOS2-derived NO production is critical for LPS-mediated development of impaired HPV. Furthermore, impaired HPV during endotoxemia may be at least in part mediated by mechanisms other than simply pulmonary vasodilation by NOS2-derived NO overproduction.

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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-NOS2<sub>−/−</sub>; 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 intubated, and the lungs were ventilated with 21% O<sub>2</sub>-5% CO<sub>2</sub>-74% N<sub>2</sub> (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 cmH<sub>2</sub>O. 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-Analogue roller pump; Laboratoriumstechnik, Wertheim-Mondfeld, Germany) at a constant flow (Q) of 50 ml/min. For hypoxic ventilation, the ventilator was connected to a hypoxic gas mixture (containing 1% O<sub>2</sub>). Pilot experiments (n = 5) revealed that variations of perfusate PO<sub>2</sub> within this range did not affect hypoxic pulmonary vasoconstrictor response.

**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). Mouse 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<sup>−1</sup>·min<sup>−1</sup> 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<sup>−1</sup>·min<sup>−1</sup>, and after another 3 min of baseline perfusion, the lungs were ventilated with the hypoxic gas mixture (containing 1% O<sub>2</sub>). 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 (∆PAP) 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<sup>−1</sup>·min<sup>−1</sup>, normoxic ventilation was reestablished, and PAP was allowed to return to baseline value. In all experiments, perfusate 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 N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich Chemie), a nonselective NOS inhibitor, or 10 μM L-N<sup>ω</sup>-(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 ΔPAP was similar in LPS-pretreated animals perfused with 10 or 100 μM L-NIL but that 100 μM L-NAME augmented the HPV response in untreated control animals, suggesting that this dose of L-NAME 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 vasoconstrictor response (ΔPAP) 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-NAME 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_{LIN}) and extrapolated pressure axis intercept at zero flow (PZF) of a linear regression line (32) of the form

$$\text{PAP} = R_{\text{LIN}} \cdot Q + P_{ZF}$$

where PAP is pulmonary artery pressure (perfusion pressure in our model) and Q is flow, R_{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, PZF 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 PZF in turn were assumed to result from changes in resistance of alveolar, collapsible vessels (16, 48).

However, several limitations regarding the interpretation of R_{LIN} and PZF 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, 53). Although these models involve a large number of variables that may not be obtained in common experimental setups, in our study we utilized an elegant simple one-compartment distensible vessel model developed by Linehan et al. (28). This model uses a nonlinear regression analysis (see Ref. 28, Eq. 12) of the form

$$\text{PAP} = \frac{(1 + \alpha \cdot \text{LAP})^5 + 5 \alpha R_0 Q^5}{\alpha} - 1$$

where $R_0$ describes the pulmonary vascular resistance that would exist if the vessels were at their respective diameter at zero vascular pressure and $\alpha$ is the vascular distensibility factor describing the relationship between vessel diameter and pressure (P) when the diameter ($D_i$) is normalized to the diameter at zero pressure ($D_{0i}$) given by (Ref. 28, Eq. 5)

$$\frac{D_i}{D_{0i}} = 1 + \alpha P$$

Figure 2 shows a typical example of the linear and nonlinear regression curves fitted to the four pressure-flow data points obtained during normoxic and hypoxic ventilation in an isolated, perfused lung of an untreated wild-type mouse (not perfused with a NOS inhibitor added to the perfusate). Note that the pressure axis intercept at zero flow is 2 mmHg under both normoxic and hypoxic conditions in the nonlinear regression curve because LAP was kept constant at this value.

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_{LIN} and PZF values and $\alpha$ and $R_0$ values for each single experiment, respectively. These data as well as ΔPAP 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 \(F_{1O2}\) of 0.21) to hypoxic ventilation (\(F_{1O2}\) = 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 \(P_{ZF}\) 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 \(R_{LIN}\), \(P_{ZF}\), 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

Nonselective 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 nonselective 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).

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The 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 $\Delta R_{L IN}$ and $\Delta R_0$ in untreated mice that was absent in lungs of LPS-challenged mice (Figs. 4B and 5B). In contrast, nonspecific NOS inhibition did not affect $\Delta R_{L IN}$ and $\Delta \gamma$ in untreated mice but augmented the hypoxia-induced changes in $R_{L IN}$ and $\gamma$ in LPS-pretreated mice. The extent of this augmentation reflected the observed partial restoration of $\Delta P_{ZF}$ in septic mice by L-NAME perfusion (Figs. 4A and 5A).

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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 1-NAME or 1-NIL, we did not detect any difference in ΔPAP as well as in ΔR_{LIN}, ΔP_{ZF}, Δα, and ΔR_{0} between untreated and LPS-pretreated NOS2-deficient mice (Figs. 3–5). Although ΔPAP, ΔP_{ZF}, Δα, and ΔR_{0} were similar in lungs obtained from untreated wild-type and NOS2-deficient mice, we detected a smaller hypoxia-induced increase in R_{LIN} in NOS2-deficient than in wild-type mice. This difference was absent when lungs were perfused with 1-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 hypoxic 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_{LIN} and P_{ZF} (Fig. 4) consistent with data obtained in isolated pig lungs (17, 47, 48). However, other authors found an increase in P_{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 α 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 Nelin et al. (35), who found an increase in R_{0} but no decrease in α 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_{LIN}, P_{ZF}, and R_{0} were abolished in LPS-pretreated compared with untreated mice (Figs. 4 and 5B). Of interest, the decrease in α 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-α, IL-1β, 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).

Non-specific NOS inhibition by 1-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_{IN}) and intercept (P_{ZF}) were not affected by non-specific 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 1-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 non-specific NOS inhibition by 1-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 1-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 α) 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 α.

Inhibition of NOS2 by 1-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 1-NAME but not by 1-NIL augmented the pulmonary HPV response (ΔPAP) in untreated wild-type and NOS2-deficient mice (Fig. 3), sug-
gesting that NOS3-derived NO counteracts HPV under physiological conditions as described previously (18). Of interest, this was reflected only by an augmentation of $\Delta R_{0}$, but not $\Delta R_{LIN}$ or $\Delta R_0$ (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 $R_0$ (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 ($n = 70$).]
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 $(r^2 = 0.77, P < 0.001; \text{Fig. } 6)$. In contrast, $R_{\text{LIN}}$ 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 vasconstrictor response may consist of different components that may be modulated NOS isoform specifically in untreated and LPS-treated mice.

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