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Inhibition of Nitric Oxide Synthase Prevents Hyporesponsiveness to Inhaled Nitric Oxide in Lungs from Endotoxin-challenged Rats

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Background: **Inhalation of nitric oxide (NO) selectively dilates the pulmonary circulation and improves arterial oxygenation in patients with adult respiratory distress syndrome (ARDS). In approximately 60% of patients with septic ARDS, minimal or no response to inhaled NO is observed. Because sepsis is associated with increased NO production by inducible NO synthase (NOS2), the authors investigated whether NOS inhibition alters NO responsiveness in rats exposed to gram-negative lipopolysaccharide (LPS).**

Methods: **Sprague-Dawley rats were treated with 0.4 mg/kg *Escherichia coli* 0111:B4 LPS with or without dexamethasone (inhibits NOS2 gene expression; 5 mg/kg), L-NAME (a nonselective NOS inhibitor; 7 mg/kg), or aminoguanidine (selective NOS2 inhibitor; 30 mg/kg). Sixteen hours after LPS treatment, lungs were isolated-perfused; a thromboxane-analog U46619 was added to increase pulmonary artery pressure (PAP) by 5 mmHg, and the pulmonary vasodilator response to inhaled NO was measured.**

Results: **Ventilation with 0.4, 4, and 40 ppm NO decreased the PAP less than in lungs of LPS-treated rats (0.75 ± 0.25, 1.25 ± 0.25, 1.75 ± 0.25 mmHg) than in lungs of control rats (3 ± 0.5, 4.25 ± 0.25, 4.5 ± 0.25 mmHg; $P < 0.01$). Dexamethasone treatment preserved pulmonary vascular responsiveness to NO in LPS-treated rats (3.75 ± 0.25, 4.5 ± 0.25, 4.5 ± 0.5 mmHg, respectively; $P < 0.01$ *vs.* LPS, alone). Responsiveness to NO in LPS-challenged rats was also preserved by treatment with L-NAME (3.0 ± 1.0, 4.0 ± 1.0, 4.0 ± 0.75 mmHg, respectively; $P < 0.05$ *vs.* LPS, alone) or aminoguanidine (1.75 ± 0.25, 2.25 ± 0.5, 2.75 ± 0.5 mmHg, respectively; $P < 0.05$ *vs.* LPS, alone). In control rats, treatment with dexamethasone, L-NAME, and aminoguanidine had no effect on inhaled NO responsiveness.**

Conclusion: **These observations demonstrate that LPS-mediated increases in pulmonary NOS2 are involved in decreasing responsiveness to inhaled NO. (Key words: Aminoguanidine; dexamethasone; pulmonary circulation.)**

INHALATION of nitric oxide (NO) selectively dilates the pulmonary circulation and increases arterial oxygenation by enhancing the matching of perfusion and ventilation in patients with adult respiratory distress syndrome (ARDS).[1,2] In approximately 30% of patients with ARDS, minimal or no pulmonary vasodilator or oxygenation response to inhaled NO is observed.[2] The mechanisms of hyporesponsiveness to inhaled NO are unknown, but the evidence suggests that gram-negative bacteremia and endotoxemia, the most common clinical causes of ARDS, contribute to NO hyporesponsiveness. In patients with septic ARDS, 60% had little or no vasodilator or oxygenation response to inhaling 18 or 36 parts per million (ppm) NO.[3]

Nitric oxide is a lipid-soluble free radical molecule with a short half-life in biological fluids. In the presence of oxygen, NO is produced from L-arginine by nitric oxide synthase (NOS) enzymes. NOS enzymes are divided into two classes, constitutive and inducible. The activities of the constitutive isoforms, neuronal NOS (NOS1) and endothelial NOS (NOS3), are dependent on

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intracellular calcium levels. Inducible NOS (NOS2) expression is stimulated by endotoxin and cytokines and inhibited by glucocorticoids.[4,5] In pulmonary vascular smooth muscle cells, NO activates soluble guanylate cyclase, an enzyme responsible for the conversion of guanosine triphosphate (GTP) to guanosine 3′,5′-cyclic monophosphate (cGMP).[6] cGMP activates cGMP-dependent protein kinases, leading to decreased pulmonary vascular smooth muscle tone.

We previously used an isolated-perfused rat lung model to investigate the mechanisms regulating pulmonary vascular responsiveness to inhaled NO.[7] We observed that responsiveness to inhaled NO was decreased in lungs from rats pretreated with endotoxin-lipopolysaccharide (LPS). In this study, we hypothesized that in LPS-treated rats increased pulmonary NO production associated with induction of NOS2 expression contributes to the development of hyporesponsiveness to inhaled NO. To test this hypothesis, the effects of NOS inhibition on the ability of inhaled NO to induce pulmonary vasodilation was examined in isolated, perfused, and ventilated lungs from LPS-treated rats. We report here that agents that inhibit endotoxin-stimulated NO production—dexamethasone, N^G-L-arginine methyl ester (L-NAME), and aminoguanidine—prevented the development of hyporesponsiveness to inhaled NO caused by LPS exposure.

Materials and Methods

These investigations were approved by the Subcommittee for Research Animal Studies of the Massachusetts General Hospital, Boston, Massachusetts.

Isolated, Perfused Rat Lungs

Lungs obtained from rats were isolated, perfused, and ventilated as described previously.[7] Briefly, adult Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 450–550 g, were killed by intraperitoneal injection of sodium pentothal (100 mg/kg body weight). After a midline thoracotomy, the pulmonary artery and left atrium were cannulated. Lungs were perfused with Hank's balanced salt solution containing 5% dextran, 5% bovine serum, 580 μM L-NAME, and 30 μM indomethacin, using a roller pump (Cardiovascular Instrument, Wakefield, MA) at a pulsatile flow of 0.03 $ml \cdot g\ body\ weight^{-1} \cdot min^{-1}$ in a recirculating system at 37°C. The perfusate was continuously aerated with 95% O_2 and 5% CO_2 in the presence of $NaHCO_3$ to adjust and maintain pH, P_{CO_2}, and P_{O_2} between 7.35–7.40, 35–40 mmHg, and 150–250 mmHg, respectively.

Pulmonary artery pressure (PAP) and left atrial pressure were measured *via* small catheters (PE-50 tubing) placed within the lumen of the inflow and outflow perfusion catheters, respectively. Left atrial pressure was set at 4 mmHg. PAP measurements were recorded in increments of 0.25 mmHg.

Measurement of Pulmonary Vasoreactivity to Inhaled Nitric Oxide

In isolated-perfused lungs, the stable thromboxane analog U46619 (Cayman Chemical, Ann Arbor, MI) was administered to increase PAP by 5 mmHg. Next, the infusion rate was adjusted to infuse the minimum quantity required to maintain a stably elevated PAP. The lungs were ventilated with 0.4, 4, and 40 ppm NO in random order for periods of 3 min. After each period of NO ventilation, the PAP was allowed to increase to the elevated baseline.

Nitric oxide gas was obtained from Airco (Hingham, MA) as a mixture of 800 ppm NO in pure N_2. Variable concentrations of NO were mixed with 21% O_2 and balanced N_2 just before entering the ventilator. NO levels were measured during each administration by chemoluminescence analysis (Eco Physics CLD 700AL, Dürnten, Switzerland).

Pulmonary Vasoreactivity to Inhaled NO in Rats Treated with Lipopolysaccharide, Dexamethasone, L-NAME, and Aminoguanidine

Eight groups of rats were studied. In four groups (A, B, C, D), the animals were injected intraperitoneally 16 h before the lung perfusion experiments with 0.4 mg/kg *Escherichia coli* 0111:B4 LPS (Difco Laboratories, Detroit, MI). The other four groups remained untreated as controls (E, F, G, H). Two groups of rats (B, n = 9; F, n = 10) were injected intraperitoneally with dexamethasone (5 mg/kg) 1 h before LPS-injection. Groups C (n = 6) and G (n = 7) received L-NAME (7 mg/kg) by intraperitoneal injection 2 h after LPS treatment. Groups D (n = 7) and H (n = 5) received aminoguanidine (30 mg/kg) by intraperitoneal injection 4 h after LPS treatment. Group A (n = 8) and Group E (n = 6) received no additional treatment. Sixteen hours after LPS injection, the lungs were isolated, perfused, and ventilated. Then pulmonary vasoreactivity to inhaled NO was measured as described previously.

Nitrite and Nitrate Levels in Perfusate of Isolated Lungs from Rats Treated with Aminoguanidine

Rats were pretreated with an intraperitoneal injection of 0.5 mg/kg LPS (n = 12) or were untreated control rats (n = 9). Four hours later, LPS-treated (n = 7) and control rats (n = 4) received an intraperitoneal injection of aminoguanidine (30 mg/kg). After 12–14 h, lungs were isolated-perfused as described previously, except that in these studies, L-NAME was omitted from the perfusate. Perfusate samples were taken after 30 min of perfusion and nitrite–nitrate levels determined as described previously.[8] In brief, 100 μl of the perfusate was diluted with 500 μl phosphate buffer (pH, 7.5). Nitrate was reduced to nitrite using nitrate reductase (50 μl; 1 U/ml; Sigma, Deisenhofen, Germany) and NADPH (50 μl; 1.8 mM) was added. After 2 h of incubation, excess NADPH was oxidized by adding 50 μl phenazine methosulfate (80 μM). Then, 100 μl zinc acetate (0.5 M) and 100 μl NaOH (0.5 M) were added to deproteinate the solution. Nitrite was measured in the supernatant by Griess assay adding 250 μl sulfanilamide (0.1 M in 1.5 M phosphoric acid) and 250 μl naphthylethylenediamine (8 mM). Colorimetric absorption at 540 nm was linearly correlated with nitrite concentration.

Pulmonary Vasoreactivity to Isoproterenol

Rats were treated either with an intraperitoneal injection of LPS (n = 19) or remained untreated as control rats (n = 9). Four hours later, six LPS-treated rats and four control rats received aminoguanidine (30 mg/kg) by intraperitoneal injection. Twelve to 14 h later, lungs were isolated-perfused and preconstricted with the thromboxane analog U46619. Then, isoproterenol (10 μM) was added to the perfusate, and the reduction of pulmonary artery pressure (PAP) was measured.

Pulmonary Vasoreactivity to Inhaled NO after Prolonged Breathing of NO

Three rats were placed in a 40-l acrylic chamber and exposed to 80 ppm NO, as previously described.[9] The $F_{I_{O_2}}$ and concentrations of NO, nitrogen dioxide, and nitrogen with higher oxidation states (NOx) were measured continuously. After 19–24 h, lungs were isolated, perfused, and ventilated, and the pulmonary vasoreactivity to 4 ppm NO was measured.

Statistical Analysis

The measurements of the pulmonary vasodilator response to inhaled NO were the absolute changes in PAP caused by ventilation with three concentrations of NO gas. Multivariate analysis of variance (ANOVA) with repeated-measures techniques was used to examine the effects of LPS (controls *vs.* LPS-treated), specific treatment (dexamethasone, L-NAME, and aminoguanidine *vs.* none) and NO dose simultaneously. The treatment difference (treatment *vs.* none) between the LPS-treated rats and control rats was examined by testing the interaction term (treatment + LPS) in the model. All data are expressed as mean ± SD. Statistical significance was reached when $P < 0.05$.

Results

Dexamethasone Prevents Hyporesponsiveness to Inhaled Nitric Oxide

To begin to investigate whether NOS2 induction contributes to NO hyporesponsiveness, we measured pulmonary vasoreactivity to inhaled NO in rats treated or untreated with dexamethasone 1 h before LPS-exposure. In control lungs, 0.4, 4, and 40 ppm NO dilated the pulmonary circulation by 56 ± 8, 79 ± 3, and 86 ± 2%, respectively, of the vasoconstriction induced by U46619 (fig. 1). In lungs of animals treated with LPS, 0.4, 4, and 40 ppm NO vasodilated less (15 ± 5, 26 ± 5, and 34 ± 5%, respectively; overall $P < 0.01$ *vs.* control lungs). Administration of dexamethasone 1 h before LPS treatment completely prevented the hyporesponsiveness to inhaled NO: inhalation of 0.4, 4, and 40 ppm NO decreased PAP by 71 ± 3, 82 ± 3, and 89 ± 1%, respectively (overall $P < 0.01$ *vs.* LPS-treated rats without dexamethasone). In lungs from control rats, dexamethasone had no effect on the vasodilator activity of inhaled NO (70 ± 7, 79 ± 6, and 82 ± 4%, respectively).

Nonselective NOS Inhibition Improves Nitric Oxide Responsiveness in Lungs from LPS-treated Rats

To determine whether inhibition of pulmonary NO production prevents the development of hyporesponsiveness to inhaled NO, we administered L-NAME, a nonselective inhibitor of NOS activity, 2 h after LPS exposure. Compared with lungs from rats exposed to LPS alone, treatment with L-NAME of LPS-challenged rats improved the ability of inhaled NO to dilate the pulmonary circulation (fig. 2): 0.4, 4, and 40 ppm NO decreased PAP by 41 ± 11, 59 ± 11, and 58 ± 8%, respectively (overall $P < 0.05$ values differ compared

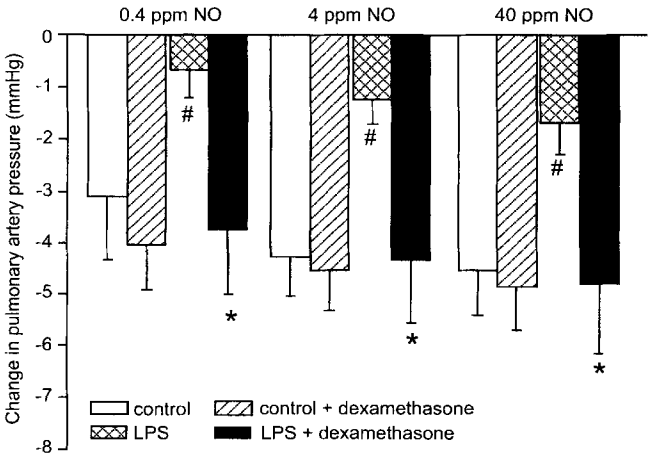

Fig. 1. Pretreatment with dexamethasone prevents hyporesponsiveness to inhaled NO in lungs from LPS-treated rats. Two groups of rats were treated with LPS (0.4 mg/kg, n = 6) or remained untreated as control rats (n = 8). An additional 19 rats received an injection of dexamethasone (5 mg/kg), of which 9 received LPS 1 h later. Sixteen hours after LPS-injection, the lungs were isolated, perfused, and ventilated. The PAP was increased by 5 mmHg using U46619, and decreases in PAP during ventilation with 0.4, 4, and 40 ppm NO were measured. In LPS-treated lungs, PAP decreased less than in lungs from control rats (# $P < 0.01$ LPS differs from controls). Pretreatment with dexamethasone completely prevented hyporesponsiveness to inhaled NO in lungs from LPS-treated rats (*$P < 0.01$ when comparing LPS-treated rats with and without dexamethasone at each NO level).

with LPS-treated rats without L-NAME). Administration of L-NAME to control rats had no effect on responsiveness to ventilation with 0.4, 4, and 40 ppm NO (43 ± 2, 69 ± 8, and 85 ± 6%, respectively).

Treatment with Aminoguanidine Improves Responsiveness to Inhaled Nitric Oxide

To further examine whether the inhibition of pulmonary NO production associated with increased NOS2 activity contributes to NO hyporesponsiveness, we administered aminoguanidine, a selective inhibitor of NOS2, 4 h after exposure to LPS (fig. 3). Aminoguanidine improved the ability of lungs obtained from LPS-treated rats to vasodilate in response to inhalation of 0.4, 4, and 40 ppm NO with PAP reduced by 34 ± 6, 44 ± 6, and 52 ± 6%, respectively (overall $P < 0.05$ *vs.* LPS-treated rats without aminoguanidine). In lungs from control rats, aminoguanidine treatment had no effect on pulmonary vasoreactivity to 0.4, 4, and 40 ppm NO (56 ± 5, 67 ± 6, and 75 ± 6%, respectively).

Aminoguanidine Decreases Release of Nitrite-Nitrate into the Perfusate of Lungs Obtained from Rats Treated with LPS

To demonstrate that aminoguanidine inhibits endogenous NO production in lungs from rats exposed to LPS, we measured nitrite and nitrate levels in the perfusate of isolated lungs obtained from rats with and without LPS pretreatment (fig.4). Compared with LPS-treated rats without aminoguanidine, total perfusate levels of nitrite and nitrate were lower in the perfusate of lungs from LPS-treated rats given aminoguanidine (16 ± 7 μM *vs.* 7 ± 2 μM; $P < 0.05$). Aminoguanidine did not alter the combined nitrate and nitrite levels in perfusate from the lungs of rats not exposed to LPS.

Lipopolysaccharide Does Not Affect the Pulmonary Vasoreactivity to Isoproterenol

To determine whether LPS induced a nonspecific change of pulmonary vascular responsiveness, we measured pulmonary vasoreactivity to isoproterenol, a β-agonist, in control and LPS-treated rats. The pulmonary vasodilator response to isoproterenol did not differ in control lungs and in lungs obtained from LPS-treated rats (59 ± 20% *vs.* 36 ± 17%; P = NS). Moreover, aminoguanidine did not affect the response to isoproterenol in

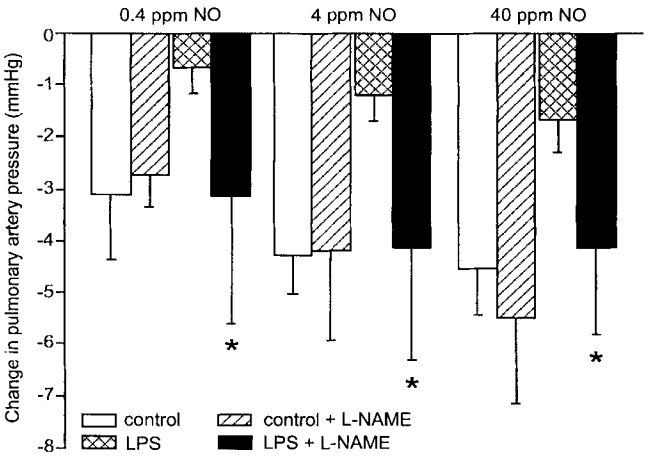

Fig. 2. Treatment with the NOS inhibitor L-NAME improves responsiveness to inhaled NO in lungs of LPS-treated rats. Untreated control rats (n = 7) and rats treated with LPS 2 h earlier (n=6) received a bolus injection of L-NAME (7 mg/kg). Fourteen hours after L-NAME administration, the lungs were isolated and perfused. The PAP was increased by 5 mmHg using U46619, and decreases in PAP during ventilation of 0.4, 4, and 40 ppm NO were measured. Early administration of L-NAME improved responsiveness to inhaled NO in lungs from LPS-treated rats (*$P < 0.05$ values differ when comparing LPS-treated rats with and without L-NAME at each NO level).

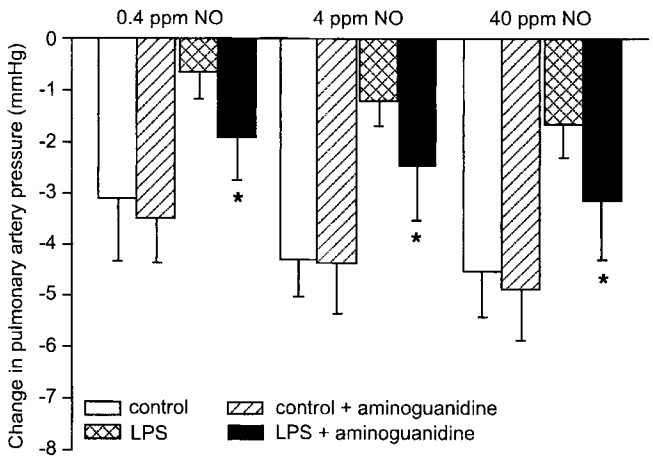

Fig. 3. Treatment with the NOS2-selective inhibitor aminoguanidine improves responsiveness to inhaled NO in lungs of LPS-treated rats. Rats were treated with LPS (n = 7) or remained untreated control rats (n = 5). After 4 h, rats received a bolus injection of aminoguanidine (30 mg/kg), and 12 h later, the lungs were isolated, perfused, and ventilated. The PAP was elevated using U46619, and the vasodilator response to ventilation with 0.4, 4, and 40 ppm NO was measured. Compared to lungs from LPS-treated rats without aminoguanidine (n = 6), there was an improved response to inhaled NO in lungs from rats treated with LPS and aminoguanidine (*$P < 0.05$ LPS with aminoguanidine differs from LPS alone at each level of NO).

either control rats (61 ± 29%) or LPS-treated rats (39 ± 9%).

Pulmonary Vasoreactivity to Inhaled NO in Rats after Prolonged Breathing of Nitric Oxide

To examine whether increased pulmonary NO levels, in the absence of LPS, can decrease pulmonary vasoreactivity to acutely inhaled NO, rats (n = 3) breathed 80 ppm NO in air for approximately 20 h. Isolated-perfused lungs from these rats vasodilated modestly less in response to ventilation with 4 ppm NO than lungs from control rats (67 ± 2% *vs.* 79 ± 8%, $P < 0.005$).

Discussion

Inhalation of NO selectively dilates the pulmonary circulation and improves arterial oxygenation in patients with ARDS. However, up to 60% of patients with septic ARDS do not respond or respond only minimally to inhaled NO with improved oxygenation.[3] Because sepsis, the most common cause of ARDS, promotes increased NO production by NOS2,[5,10-12] we studied the effects of inhibitors of NOS2 gene expression and enzyme activity on responsiveness to inhaled NO using isolated, perfused, and ventilated lungs obtained from LPS-treated rats. We previously observed that, in isolated-perfused lungs from rats exposed to LPS, the ability of inhaled NO to induce pulmonary vasodilation is impaired.[7] Systemic administration of LPS stimulates NOS2 synthesis in a variety of cell types, including pulmonary vascular cells.[4,5,13] We hypothesized that increased pulmonary NO levels in LPS-treated rats contribute to impaired pulmonary vascular responsiveness to inhaled NO.

Glucocorticoids block LPS-induced synthesis of NOS2 and the associated increase in NO levels.[12,14,15] Pretreatment with dexamethasone completely preserved the ability of inhaled NO to decrease PAP in lungs obtained from LPS-treated rats (fig. 1). These results suggest that, in our model, NOS2 synthesis induced by administration of LPS contributes to the development of hyporesponsiveness to inhaled NO. These findings are supported by a study of Tsuchida *et al.* who observed that, in rat aortic strips incubated *in vitro* with LPS, impaired vasodilation in response to sodium nitroprusside (SNP) was prevented by coadministration of dexamethasone with

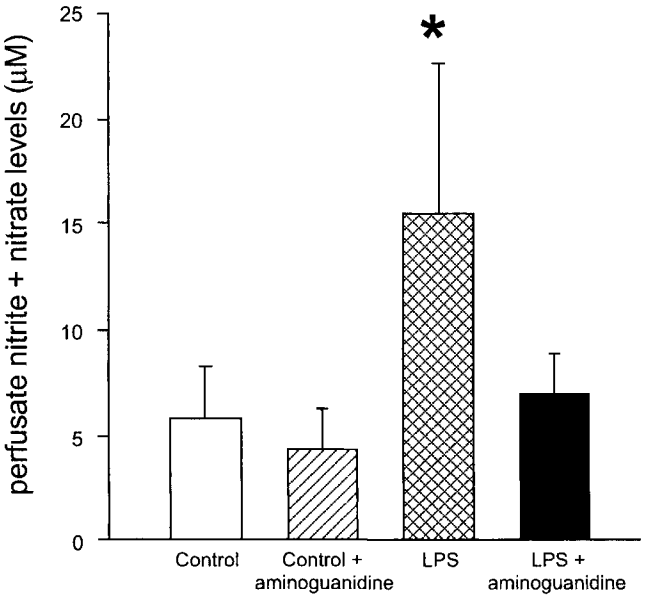

Fig. 4. Aminoguanidine decreases release of nitrite and nitrate into the perfusate of lungs obtained from rats treated with LPS. Rats were pretreated with an intraperitoneal injection of 0.5 mg/kg LPS (n = 12) or were untreated control rats (n = 9). Four hours later, seven of the LPS-treated rats and four of the control rats received an intraperitoneal injection of aminoguanidine (AG; 30 mg/kg). After 12–14 h, the lungs were isolated and perfused, and perfusate samples were taken after 30 min of perfusion. Compared with LPS-treated rats without aminoguanidine, the combined nitrite and nitrate levels were less in the perfusate of lungs from LPS-treated rats given aminoguanidine (*$P < 0.05$ values differ).

LPS.[16] However, dexamethasone modulates the induction of a variety of enzymes in addition to NOS2, which may contribute to the development of hyporesponsiveness to inhaled NO.

To further investigate whether increased NO production by NOS in LPS-treated rats alters pulmonary vascular responsiveness to inhaled NO, we used L-NAME, an L-arginine analog, to inhibit NOS activity. L-NAME inhibits NOS activity in endotoxin-treated animals, as reflected by decreased tissue cGMP levels and plasma nitrate and nitrite levels.[14-17] In our study, we observed that injection of L-NAME 2 h after LPS treatment preserved pulmonary vascular responsiveness to inhaled NO (fig. 2). These results indicate that inhibition of LPS-induced pulmonary NO production prevents the development of inhaled NO hyporesponsiveness. Once again, our results are supported by the observation of Tsuchida *et al.*, who reported that, in isolated rat aortic strips incubated with LPS *in vitro,* impaired ability of SNP to induce relaxation was restored by incubation with N^G-nitro-L-arginine for 30 min.[16]

We next sought to determine if LPS-stimulated NO production by the inducible NOS isoform, NOS2, contributes to the development of NO hyporesponsiveness in LPS-treated rats. A number of studies have demonstrated that aminoguanidine (15-45 mg/kg) selectively inhibits NOS2 but not NOS3 activity.[18-21] Griffiths *et al.* observed that, in pulmonary arteries obtained from rats exposed to LPS, aminoguanidine selectively inhibits NOS2.[18] In our model, administration of aminoguanidine 4 h after LPS administration preserved pulmonary vascular responsiveness to inhaled NO (fig. 3) and decreased nitrite and nitrate release into the perfusate of isolated lungs (fig. 4). Of note, aminoguanidine administration did not alter nitrite and nitrate release into the perfusate of lungs isolated from rats that were not exposed to LPS, suggesting that aminoguanidine was selective for LPS-induced NOS activity (likely attributable to NOS2). These results suggest that increased NO production associated with NOS2 synthesis contributes to the development of hyporesponsiveness to inhaled NO in LPS-treated rats. However, aminoguanidine has also been demonstrated to scavenge peroxynitrite, which could contribute to the development of hyporesponsiveness to inhaled NO: in cultured macrophages, aminoguanidine inhibited peroxynitrite-induced benzoate hydroxylation and 4-hydroxyphenylacetic acid nitration.[20]

A number of studies have demonstrated that administration of nonselective NOS inhibitors augments tissue damage and increases the mortality rate in rodents and dogs exposed to LPS.[22-24] In contrast, selective inhibition of NOS2 has been shown to improve survival in rodent models of sepsis.[25,26] These considerations suggest that early treatment with a selective NOS2 inhibitor, similar to aminoguanidine, may preserve responsiveness to inhaled NO in those with ARDS associated with sepsis.

The observations presented herein suggest that, in an animal model of sepsis, enhanced endogenous NO production by NOS2 is required for the development of hyporesponsiveness to inhaled NO. However, it is uncertain whether increased pulmonary NO concentrations alone are sufficient to produce hyporesponsiveness to inhaled NO. Combs *et al.* reported that in isolated-perfused lungs from rats breathing 30 ppm NO for 48 h during normoxic conditions, vasodilation to SNP was preserved.[27] Frank *et al.* recently reported that responsiveness to inhaled NO was not altered in isolated-perfused lungs from rats exposed to 20 ppm NO for up to 3 weeks.[28] In our study, the vasodilator effect of 4 ppm NO on the isolated-perfused lungs obtained from rats exposed to 80 ppm NO for approximately 20 h was only 15% less than that observed in control rat lungs. In contrast, the pulmonary vasodilator effect of 4 ppm NO was 67% less in lungs from LPS-treated rats than that in lungs from untreated rats. These results suggest that increased pulmonary NO levels alone are insufficient to induce hyporesponsiveness to inhaled NO. It is likely that other sepsis-induced factors act together with NO, leading to the development of NO hyporesponsiveness. One example of such a sepsis-induced factor may be superoxide, which can react with NO to form the potentially toxic metabolite peroxynitrite.[29] Furthermore, under some conditions, NOS can generate superoxide, a process that is inhibitable by arginine analogs and that could contribute to the development of hyporesponsiveness to inhaled NO.[30]

In summary, the inhibition of either NOS2 gene expression or NOS2 enzyme activity preserves the ability of inhaled NO to dilate the pulmonary circulation of rats exposed to LPS. Preservation of NO responsiveness by inhibition of NOS2 in the rat exposed to LPS suggests that increased pulmonary NO production may contribute at least in part to the development of NO hyporesponsiveness in patients with ARDS due to sepsis. These observations suggest that treatment with a selective NOS2 inhibitor may prevent the development of inhaled NO hyporesponsiveness in patients with ARDS associated with sepsis.

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