In acute lung injury (ALI) pulmonary hyporesponsiveness to inhaled nitric oxide (iNO) still represents an unresolved clinical challenge. In septic ALI patients the incidence of hyporesponsiveness to iNO is increased; therefore, endotoxemia appears to play a major role. Experimental data suggest that endotoxemia, e.g., induced by lipopolysaccharides (LPS), contribute to the hyporesponsiveness to iNO. Guanosine 3',5'-cyclic monophosphate (cGMP) is metabolized by phosphodiesterases (PDE). The role of PDE in reduced pulmonary vascular response in experimental endotoxemia is still not known. Here, we hypothesized that PDE activity modulates initial pulmonary responsiveness to iNO in ALI following systemic endotoxin exposure. Rats were treated with LPS or used as controls. Lungs were isolated-perfused 0–36 h after LPS injection and the synthetic thromboxane analogue U46619 was added to increase pulmonary artery pressure by 6–8 mmHg \((n = 47)\). Then, the pulmonary vasodilatory response to 3 doses of iNO (0.4, 4 and 40 ppm) was measured. Furthermore, lungs were prepared as described previously, and 2, 10, and 18 h after LPS the change in pulmonary artery pressure in response to two different inhibitors of PDE, one of which is PDE sensitive (8-Br-cGMP) and one is PDE stable (8-pCPT-cGMP), was determined \((n = 43)\). Serum nitrite/nitrate levels started to increase 4 h after LPS, with a maximum at 18 h. In contrast, decreased pulmonary vasoreactivity in response to iNO developed as early as 2 h later and remained depressed up to 18 h. The pulmonary vasoreactivity to the PDE-sensitive 8-Br-cGMP after LPS-stimulation was lower than that in lungs treated with the PDE-stable 8-pCPT-cGMP. In rats pretreated with LPS, hyporesponsiveness of pulmonary vessels to iNO is time-limited and associated with increased serum nitrite/nitrate levels, and appears to be attributed in part to increased pulmonary PDE activity. © 2008 Elsevier Inc. All rights reserved.

Key Words: phosphodiesterase; inhaled nitric oxide; isolated lung; hyporesponsiveness.

INTRODUCTION

Gram-negative sepsis is a major cause of death in intensive care units [1, 2]. Indeed, severe infection is one of the conditions most commonly associated with the development of the acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) [3], and pulmonary hypertension and severe arterial hypoxemia represent unresolved challenges in treating septic lung injury [4].

Despite the controversial role that nitric oxide (NO) plays in the pathogenesis of acute lung injury [5–7], the therapeutic inhalation of nitric oxide (iNO) selectively dilates the pulmonary vessels and improves arterial oxygenation in patients with ARDS [8]. Interestingly, in approximately 60% of septic patients with ARDS, only a minimal or no pulmonary vasodilator or oxygenation response to iNO is observed [8]. Such variable responses to iNO were found in septic children suffering from persistent pulmonary hypertension of the newborn [6, 9] and in adult septic ARDS patients [6, 10]. The underlying mechanisms of hyporesponsiveness to iNO in patients with ARDS are still unknown. Although recently published clinical studies did not show a benefit of iNO on morbidity and mortality in patients with severe lung injury [11], NO inhalation serves as a rescue therapy in critically ill patients with severe respiratory failure [12]. Therefore, it is neces-
sary to know which group of patients may benefit at which time from this treatment.

The majority of pathologic features of human ARDS have also been observed in experimental animal models in response to systemic infusion of live bacteria or endotoxin of gram-negative bacteria [13]. Experimental data suggest that gram-negative bacteremia and endotoxemia, the most common clinical causes of ARDS, contribute to the clinically observed hyporesponsiveness to iNO [13–15]. In pulmonary vascular smooth muscle cells, NO activates soluble guanylate cyclase (sGC), an enzyme responsible for converting guanosine triphosphate to Guanosine 3′,5′-cyclic monophosphate (cGMP). cGMP activates cGMP-dependent protein kinases, which decrease pulmonary vascular smooth muscle tone. A family of enzymes known as the phosphodiesterases (PDE) inactivate cGMP by converting it to GMP [16]. The impact of PDE activity in reduced pulmonary vascular response to iNO in experimental endotoxemia is actually not completely understood. Recently, we demonstrated that subthreshold doses of PDE5 inhibitors improved responsiveness to iNO [13,15]. In this study, we hypothesized that ultra-low iNO in an isolated, perfused lung model [17]. In addition, nothing is yet known about the role of the pulmonary PDE in septic ARDS.

We previously demonstrated hyporesponsiveness to iNO in isolated, perfused lungs from rats 18 h after LPS injection [13]. In this study, we hypothesized that administering a bolus of LPS in vivo reversibly reduces the pulmonary vascular response to iNO in isolated, perfused lungs after treatment with LPS. To clarify the role of pulmonary PDE in the development of modulated responsiveness to iNO, we tested the vasodilatory capacity of PDE-sensitive and PDE-stable analogues in isolated lungs from LPS-challenged rats.

We report here for the first time that a single bolus injection of LPS induces a time-limited hyporesponsiveness to iNO in an isolated, perfused lung model over 36 h. The impaired vascular dilatory capacity of the PDE-sensitive cGMP analogue after LPS stimulation appears to rely on an increased pulmonary PDE activity. Especially in early endotoxemia, this mechanism could play a significant role in the development of hyporesponsiveness to iNO.

MATERIALS AND METHODS

These investigations were approved by the Committee for Research Animal Studies at the University of Heidelberg, Germany.

Isolated, Perfused Rat Lungs

Lungs obtained from rats were isolated, perfused, and ventilated as described previously [13, 15]. Briefly, adult Sprague Dawley rats (Charles-River-line, Crl CD (SD) Br; Charles River, Sulzfeld, Germany) weighing 350 to 400 g were killed by intraperitoneal injection of sodium pentothal (100 mg/kg body weight) (Narcoren; Merial GmbH, Hallbergmoos, Germany) and positioned on a warm touch pad heated to 37°C (Thermosteck TK24; Beurer GmbH and Co., Ulm, Germany). After a midline thoracotomy, the pulmonary artery and left atrium were cannulated. Lungs were perfused in situ with Hanks’ balanced salt solution (HBSS; Life Technologies Inc., Paisley, Scotland) containing 5% Dextran (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 5% bovine serum (Serva Electrophoresis GmbH, Heidelberg, Germany), and 30 μM indomethacin (Sigma-Aldrich Chemie GmbH), using a roller pump at a pulsatile flow of 0.03 mL g body weight−1 min−1 in a recirculating system at 37°C. The perfusate was continuously aerated with 95% O2 and 5% CO2 in the presence of NaHCO3, to adjust and maintain pH, pCO2, and pO2 between 7.35–7.40, 35–40 mmHg, and 150–250 mmHg, respectively. By installing a tracheal cannula, the rats could be ventilated with an animal respirator (RUS-1300; FMI Föhr Medical Instruments GmbH, Seeheim/Ober-Beerbach, Germany). 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. The sensitivity for measuring PAP in our experiment was 0.01 mmHg.

Measurement of Pulmonary Vasoreactivity to iNO

To demonstrate the vasodilatory effects of iNO in isolated, perfused lung specimens, the pulmonary vessels must be preconstricted ([13, 15]. Therefore, in isolated, perfused lungs, the stable thromboxane analogue U46619 (Cayman Chemical, Montigny le Bretonneux, France) was administered to increase PAP by 6–8 mmHg. Next, the infusion rate was adjusted to infuse the minimum quantity of U46619 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 5 min and the decrease in PAP was measured. After each period of NO ventilation, the PAP was allowed to increase to the elevated baseline. The variation with respect to the formerly increased levels of PAP was within a 5% deviation range or less than 0.3 mmHg.

NO gas was obtained as a mixture of 800 ppm NO in pure N2 (Linde Gas, Höllriegelskreuth, Germany). Variable concentrations of NO were mixed with 21% O2 and balanced N2 just before entering the ventilator. NO levels were measured by chemoluminescence analysis each time it was administered (Eco Physics CLD 700AL, Dürnten, Switzerland).

Pulmonary Vasoreactivity to iNO in Rats Treated with LPS

A total of 47 rats were studied to determine the pulmonary vasoreactivity to NO. The animals were injected intraperitoneally with 0.5 mg Escherichia coli 011:B4 LPS per kg body weight 2–36 h before the lung perfusion experiments (LPS, lipopolysaccharide E. coli 0111: B4; Difco Laboratories, Detroit, MI) as described previously [13, 15], or were not treated and used as controls. Two to 36 h after LPS injection, the lungs were isolated, perfused, and ventilated. Then, pulmonary vasoreactivity to iNO was measured after inducing pulmonary hypertension by U46619 as described previously.

Serum Levels of Nitrite/Nitrate in Isolated Lungs

In these rats, blood samples were taken (n = 47) before preparing the lung specimens. 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 M). Then, 100 μL zinc acetate (0.5 M) and 100 μL NaOH (0.5 M) were added to deprotonate the solution. Nitrite was measured in the supernatant by using the Griess assay and 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 the nitrite concentration.
Dose Response of 8-bromo-cGMP and 8-pCPT-cGMP in Control Rats Preconstricted with U46619

In untreated controls, the vasodilatory capacity of 10^{-7}–10^{-5} M of the PDE-sensitive 8-bromoguanosine 3',5'-cyclic monophosphate (8-Br-cGMP; Sigma-Aldrich Chemie GmbH) and the PDE-stable 8-4-parachlorophenylthioguanosine-3',5'-cyclic monophosphate (8-pCPT-cGMP, Sigma-Aldrich Chemie GmbH) cGMP analogue was measured to assess the dose response of the two cGMP analogues. Lungs were isolated, perfused, and PAP was elevated as described previously. The change in PAP in response to 10^{-7}–10^{-5}M of 8-bromo-cGMP (n = 8) or 8-pCPT-cGMP (n = 8) given in the modified Hanks' perfusion solution was measured after perfusing the lungs for 3 min at each concentration.

Pulmonary Vasoreactivity to Different cGMP Analogues in Isolated Lungs

Two to 18 h after LPS challenge, the vasodilatory capacity of the PDE-sensitive 8-Br-cGMP and the PDE-stable 8-pCPT-cGMP were measured. Study rats were treated with an intraperitoneal injection of 0.5 mg/kg body weight LPS; some were not treated and taken as controls (n = 43). After 0–18 h, lungs were isolated, perfused, and PAP was elevated as described previously. The change in PAP in responses to 10^{-6} M and 10^{-5} M of 8-bromo-cGMP (n = 21) or 8-pCPT-cGMP (n = 22) given in the modified Hanks’ perfusion solution were measured after perfusing the lungs for 3 min at each concentration.

Statistical Analysis

The data are expressed as percent change in elevated PAP in response to NO inhalation. Multivariate analysis of variance (ANOVA) with repeated-measures techniques was used to examine the effects of LPS (control versus LPS-treated). Scheffe’s test was used to examine the results of specific cGMP analogue treatments. The vasodilatory capacity of both cGMP analogues were measured as the percent change in PAP caused by the administering the cGMP analogues to the perfusate. The nitrite/nitrate levels were expressed as μmol in the serum. All data are expressed as mean ± standard deviation (SD). Statistical significance was set at P < 0.05.

RESULTS

Time-Limited Hyporesponsiveness to iNO in LPS-Treated Rats

To investigate the pulmonary vasoreactivity to iNO during endotoxemia, we measured the vascular response of isolated, perfused lungs from LPS-challenged rats at different time points up to 36 h after LPS injection. Compared with untreated controls, the pulmonary vasodilation to 0.4, 4, and 40 ppm NO was reduced in isolated, perfused lungs from LPS-treated rats (Fig. 1). The hyporesponsiveness to iNO started as early as 2 h after LPS injection (4.8% ± 5.2%, 25.1% ± 6.4%, and 34.7% ± 8.8% versus 34.7% ± 11.7%; P < 0.05) and was observed up to 24 h for 0.4 ppm NO (12.8% ± 9.8% versus 34.7% ± 11.7%; P < 0.05) and up to 30 h for 4 and 40 ppm NO, respectively (43.4% ± 7.8% and 70% ± 6.1% versus 79.2% ± 6.5% and 93.8% ± 5.8%; P < 0.05). Thirty-six hours after LPS injection, responsiveness to iNO was completely restored (Fig. 1).

Modulation of Nitrite/Nitrate Levels in Controls and LPS-Challenged Rats

To investigate whether this reversible, reduced pulmonary vascular response to iNO was associated with increased endogenous NO production, we measured sera levels of nitrite/nitrate. Compared with untreated controls, serum levels of nitrite/nitrate started to increase after 4 h in rats treated with LPS (Fig. 2). Peak levels were measured 18 h after LPS treatment and...
nitrite/nitrate levels did not reach control values 36 h after LPS treatment (Fig. 2).

8-bromo-cGMP and 8-pCPT-cGMP Decreased PAP in a Dose-Dependent Manner in Control Rats Preconstricted with U46619

To determine effective doses of 8-bromo-cGMP and 8-pCPT-cGMP, we performed a dose-response experiment with three different doses of each cGMP analogue.

At concentrations of $10^{-6}$ and $10^{-5}$ M both cGMP analogues increased pulmonary vasodilatation, whereas $10^{-7}$ M concentrations did not ($-17.9 \pm -5.5$ and $-17.6 \pm -5.3\%$ versus pulmonary hypertension at $10^{-5}$, $-7.1 \pm -3.6$ and $-7.9 \pm -5.6\%$ versus pulmonary hypertension at $10^{-4}$, $P < 0.05$ versus pulmonary hypertension; $-1.3 \pm -2.7$ and $-1.5 \pm -2.2\%$ versus pulmonary hypertension at $10^{-3}$, $P = ns$) (Fig. 3).

8-pCPT-cGMP But Not 8-bromo-cGMP Dilated the Pulmonary Vessels in LPS-Treated Rats

After assessing the dose response to investigate whether reversible hyporesponsiveness to iNO could be attributed to increased pulmonary PDE activity, we applied two different cGMP analogues to induce pulmonary vasodilatation in isolated-perfused lungs of LPS-treated rats. 8-Bromo-cGMP, a PDE-sensitive cGMP analogue, did not cause pulmonary vasodilatation in isolated-perfused lungs from LPS treated rats at $10^{-6}$ M or at $10^{-5}$ M, compared with untreated controls ($P = ns$) (Figs. 4 and 5). In contrast, PDE-stable 8-pCPT-cGMP dilated the pulmonary vessels of isolated, perfused lungs from rats treated with LPS after 2, 10, and 18 h by $-6.4\% \pm 4\%$, $-7.8\% \pm 4.8\%$, and $-7.8\% \pm 3.9\%$ at $10^{-6}$ M, and by $-23.1\% \pm 8.3\%$, $-22.2\% \pm 8.1\%$, and $-19.5\% \pm 3.2\%$ at $10^{-5}$ M (n.s. versus control, $P < 0.05$ versus 8-bromo-cGMP) (Figs. 4 and 5). In controls, both 8-bromo-cGMP and 8-pCPT-cGMP decreased PAP in isolated lungs pre-constricted with U46619 in a dose-dependent manner ($P < 0.05$) (data not shown).

DISCUSSION

The main findings of this study are (1) bolus LPS challenge in vivo produced a time-limited decrease in
the pulmonary vasoreactivity to iNO in situ up to 30 h after LPS stimulation. (2) Time-limited hyporesponsiveness to iNO was associated with a delayed increase in serum levels of nitrite/nitrate. (3) Time-limited hyporesponsiveness appears to rely on sustained increase of pulmonary PDE activity.

ALI and ARDS are frequent complications in patients with sepsis and both represent serious clinical problems that are associated with high mortality [1]. Inhalation of NO selectively dilates the pulmonary vessels in patients with hypoxic respiratory failure associated with pulmonary hypertension [10]. However, in some, especially septic patients, only minimal pulmonary vasoreactivity to iNO is observed [8–10, 14]. The precise mechanisms responsible for hyporesponsiveness to iNO are unknown, but evidence suggests that gram-negative bacteremia and/or the stage of the underlying disease are related to NO hyporesponsiveness [9, 13, 14]. In gram-negative bacterial sepsis, the most common cause of acute respiratory failure, endotoxin (LPS) induces the release of inflammatory mediators, thereby promoting vascular occlusion and constriction; this results in pulmonary hypertension which, when severe, is associated with a poor prognosis [18]. The finding that injection of LPS in animals induces acute noncardiogenic pulmonary edema and other features of human ARDS suggest that LPS plays a central role in the pathogenesis of sepsis-associated lung injury [19].

**FIG. 4.** Different pulmonary vascular responses to $10^{-5}$ M 8-Br-cGMP or 8-pCPT-cGMP in isolated lungs from endotoxin challenged rats. Animals were divided in random order into two groups receiving the PDE-sensitive (8Br-cGMP) ($n = 21$) or the PDE-stable (8-pCPT-cGMP) cGMP analogue ($n = 22$). Rats were injected with 0.5 mg/kg body weight LPS or not treated and used as controls. Two, 10, and 18 h after LPS injection, the lungs were isolated, perfused, and ventilated. After elevation of the pulmonary pressure by about 6–8 mmHg after infusion of U46619, the pulmonary vascular response to either $10^{-5}$ M 8-Br-cGMP or 8-pCPT-cGMP was measured. ($^*P < 0.05$ versus control; mean ± SD).

**FIG. 5.** Different pulmonary vascular response to $10^{-6}$ M 8-Br-cGMP or 8-pCPT-cGMP in isolated lungs from endotoxin challenged rats. Animals were divided in random order into two groups receiving the PDE-sensitive (8-Br-cGMP) ($n = 21$) or the PDE-stable (8-pCPT-cGMP) cGMP analogue ($n = 22$). Rats were injected with 0.5 mg/kg body weight LPS or not treated and used as controls. Two, 10, and 18 h after LPS injection, the lungs were isolated, perfused, and ventilated. After elevation of the pulmonary pressure by about 6–8 mmHg after infusion of U46619, the pulmonary vascular response to either $10^{-6}$ M 8-Br-cGMP or 8-pCPT-cGMP was measured. ($^*P < 0.05$ versus control; mean ± SD).
Our model does not simulate continuous endotoxemia and sepsis-induced organ failure; however, this model has been used to study the effects of LPS-induced damage to the lungs, assuming that such damage resembles that observed in sepsis-induced ALI. ALI/ARDS secondary to sepsis cannot be compared with experimental endotoxemia or bolus LPS treatment, but by using isolated organs the number of confounding variables can be reduced.

NO is a well-known, lipid-soluble free radical and secondary messenger molecule that, in the presence of oxygen, is synthesized by a group of hemoproteins known as NO synthase (NOS1–3). NOS2 is the inducible form whose expression is stimulated in a variety of cells by endotoxin and cytokines and can be inhibited by specific NOS2 inhibitors [20]. Inhibition of NOS2 is effective in mitigating endotoxemic changes and lung damage in isolated, perfused lungs of LPS-challenged rats [21]. NO-activated soluble guanylate cyclase is responsible for converting guanosine triphosphate to cyclic guanosine monophosphate. Cyclic guanosine monophosphate activates cGMP-dependent protein kinases, decreasing pulmonary vascular smooth muscle tone. A family of enzymes known as the phosphodiesterases inactivates cGMP by converting it to guanosine monophosphate (GMP).

We previously demonstrated that the pulmonary vasoreactivity to inhaled NO is impaired 18 h after LPS treatment [13]. This diminished vasodilatory response to iNO was associated with decreased NO-stimulated cGMP release into the perfusate, and LPS increased pulmonary PDE activity by 40% [13]. The PDE-sensitive cGMP analogue 8-Br-cGMP vasodilated lungs from LPS-pretreated rats to a lesser degree than lungs from control rats. In contrast, the PDE-stable 8-pCPT-cGMP vasodilated lungs from both groups equally.

The aim of this study was to demonstrate that hyporesponsiveness to iNO in isolated lungs from LPS-challenged rats is time-dependent and reversible. Administration of a single bolus of endotoxin represents a model of sepsis response in experimental animal studies but less is known about the duration and specific time gap of intervention. After bolus administration of LPS in vivo hyporesponsiveness to iNO developed rapidly in isolated lungs of LPS-treated rats after 2 h and remained depressed for up to 30 h. LPS increases NOS2 expression in several cell types with a peak expression at 4 h after LPS stimulation [22]. Hayashi et al. demonstrated in lungs of LPS-challenged rats that the increase in NOS2 expression and NOS2 activity peaked at 6 h after LPS injection and decreased thereafter up to 20 h after LPS treatment [23]. Furthermore, they found a high correlation between plasma nitrite/nitrate levels and NOS activity in the lung, suggesting that NOS activity in the lungs can be estimated from plasma nitrite/nitrate levels in septic disorders. Despite the 20-fold higher dose of LPS in the study of Hayashi et al. (10 mg/kg versus 0.5 mg/kg), the nitrite/nitrate levels are in line with those in our study. These findings suggest that at least 6 h after LPS treatment the time course of pulmonary NOS2 expression and activity seems to be similar to that of modulation of pulmonary vasoreactivity to iNO in isolated, perfused lungs of LPS-challenged rats.

There is evidence that inducing NOS2 may play a harmful role by directly causing tissue damage and through formation of peroxynitrite [7, 24]. Additionally, the notion is evolving that excessive NO production mainly produced by peroxynitrite mediates acute tissue injury in the lung [7, 27]. However, the induction of NOS2 associated with massive NO production alone cannot be responsible for the development of the hyporesponsiveness to iNO in our model because the onset of hyporesponsiveness can be detected 2 h after LPS treatment, i.e., before nitrite/nitrate levels are elevated, which is after 4 h. Notably, Hayashi et al. first measured NOS2 expression and activity and nitrite/nitrate after 6 h [23]. Additionally, Lee et al. demonstrated elevated nitrite/nitrate levels in rats 3 h after LPS injection [28]. This effect was reduced by treatment with two different NOS2 inhibitors but was not completely inhibited, suggesting an additional role of NOS1, for example, in this model.

In this study, we further investigated whether pulmonary PDE is responsible for hyporesponsiveness to iNO. Therefore, we tested the vasodilatory capacity of one PDE-sensitive and one PDE-stable cGMP analogue in isolated lungs from LPS-challenged rats. One of the main findings in this study was that pulmonary vasoreactivity to the PDE-sensitive cGMP analogue was reduced, whereas the PDE-stable cGMP analogue induced a pulmonary response comparable to that in controls, indicating a significant role of pulmonary PDE in the development of hyporesponsiveness to iNO in endotoxemic rats. These findings suggest that increased pulmonary PDE activity demotes a large amount of pulmonary cGMP. Mullershausen et al. recently demonstrated that that the functional responsiveness to NO correlates with the relative abundance of guanylate cyclase and PDE5 in aortic and bronchial tissue, respectively [29]. In rats treated with LPS pentoxifylline, a PDE inhibitor attenuates LPS-induced lung injury [30]. It is worthy of note that pentoxifylline
decreases lung neutrophil infiltration, intercellular adhesion molecule-1 expression, and nuclear factor-kappa B activation alike and may explain the lung-protective effects.

An increased pulmonary PDE can contribute to the hyporesponsiveness to iNO by inactivating pulmonary cGMP induced by exogenous delivered nitric oxide. In an animal model of guinea pigs exposed to inhaled LPS, an increased PDE activity was observed, 278% compared with baseline [31]. Additionally, the guanylate cyclase activity similarly increased by up to 210% compared with baseline levels. Furthermore, 48 h after LPS inhalation guanylate cyclase activity was still raised whereas the PDE activity declined by 68% compared with 48 h after vehicle treatment [31]. In contrast, Holzmann et al. found that in isolated, perfused lungs of LPS-challenged, sGC activity did not differ compared with untreated controls [13]. The difference in species and stimulating agents may explain this controversial finding of sGC activity. In cultured rat pulmonary artery smooth muscle cells, Scott and Nakayama found that NO has important regulatory effects on cGMP synthesis at the level of enzyme activity and mRNA abundance. NO caused an immediate synthesis of large amounts of cGMP. With prolonged exposure, sGC enzyme activity decreases and cGMP production drops [32].

In summary, despite the lack of benefit of iNO on morbidity and mortality in patients with severe lung injury in several clinical trials, iNO may serve as a rescue therapy in critically ill patients with severe respiratory failure. We show here in an isolated lung model of endotoxemic rats that hyporesponsiveness to iNO is a time-dependent phenomenon similar to the clinically observed intra- and interindividual response. The time-dependent pulmonary vasoreactivity to iNO is probably not merely mediated by increased endogenous NO levels or modulated function of sGC. The increased activity of the pulmonary PDE contributes to the time-dependent impaired pulmonary vasoreactivity to iNO in our model and may support the clinical assumption of inter- and intra-individually different responses to NO inhalation in patients with septic acute lung injury. It appears to be important to challenge severe arterial hypoxemia in ARDS patients with inhaled NO over a longer period of time because non-responders may then respond to treatment.

REFERENCES


