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Effect of Nitric Oxide Synthase Inhibition during Group B Streptococcal Sepsis in Neonatal Piglets

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ABSTRACT

Nitric oxide (NO), an important vasodilatory modulator of systemic and pulmonary vascular tone, is synthesized from L-arginine by the enzyme NO synthase in vascular endothelial and smooth muscle cells. L-Arginine analogs, such as N^{ω}-nitro-L-arginine methyl ester (L-NAME), are competitive antagonists of NO synthase and inhibit NO synthesis. Group B streptococcus (GBS) causes pulmonary hypertension, hypoxemia, lung vascular injury, and reduced cardiac output in both human newborns and neonatal piglets. Lung vascular injury associated with prolonged GBS infusion in piglets may attenuate NO production and thus promote severe pulmonary hypertension. We studied the effect of the NOS inhibitor, L-NAME and the precursor of NO, L-arginine, on pulmonary and systemic hemodynamics during late-phase GBS sepsis in the piglet model. Neonatal piglets were anesthetized, ventilated with room air, and randomized to receive a continuous infusion of saline (n = 5) or GBS (n = 5) for 4 h. After 3 h of infusion, both groups received a bolus of L-NAME (3 mg/kg). Hemodynamic and gas exchange indices were measured at baseline, 30 min, and 3 h of infusion, and 30 min and 1 h after L-NAME treatment. L-NAME treatment caused *1*) significant increases in mean pulmonary arterial pressure, pulmonary vascular resistance, mean systemic arterial pressure, and systemic vascular resistance for both groups; *2*) a similar percentage of increase in pulmonary vascular resistance for the two groups; *3*) greater reduction in cardiac output and SV in the GBS compared with the control group; and *4*) no significant alterations in arterial partial pressure of oxygen or the difference between alveolar and arterial partial pressure of oxygen for either group. L-Arginine (1 g/kg) infusion after 3 h of GBS infusion (n = 3) caused no significant changes in any measured hemodynamic or gas-exchange variable. We conclude that *1*) endogenous NO synthesis is ongoing during late-phase GBS-induced pulmonary hypertension in neonatal piglets, and *2*) NO synthesis is not limited by the substrate L-arginine in this model. NO synthase inhibitors alone appear to be contraindicated in the treatment of neonatal GBS sepsis due to worsening pulmonary hypertension and progressive decline in cardiac output. (***Pediatr Res* 36: 776–783, 1994**)

Abbreviations

A-aDo_2, difference between alveolar and arterial partial pressure of oxygen
CO, cardiac output
GBS, group B streptococcus
L-NAME, L-Nω-nitro-L-arginine methyl ester
L-NAA, L-Nω-amino-L-arginine
NO, nitric oxide
NOS, nitric oxide synthase
Pao_2, arterial partial pressure of oxygen
Pcw, mean pulmonary capillary wedge pressure
Ppa, mean pulmonary arterial pressure
ΔP, pulmonary vascular driving pressure (Ppa − Pcw)
Psa, mean systemic arterial pressure
PVR, pulmonary vascular resistance
SVR, systemic vascular resistance
TNF-α, tumor necrosis factor-α
HR, heart rate
SV, stroke volume
$\dot{V}/\dot{Q}$, ventilation-perfusion ratio

NO is an important modulator of systemic and pulmonary vascular tone (1–4). The synthesis of NO from L-arginine is catalyzed by the enzyme NOS (1). NO stimulates the soluble guanylate cyclase in vascular smooth muscle cells, thereby increasing the intracellular concentrations of cyclic GMP and causing vasodilation (1–4). Endothelial cells contain a constitutive, calcium-dependent form of NOS (1–4). An inducible, calcium-independent isoform of NOS has also been identified in several cell types, including vascular endothelial and smooth muscle cells (2, 5–7). Most reports show that the inducible isoform of NOS (2, 5–7) is up-regulated in response to Gram-negative endo-

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toxin and cytokines such as TNF-α. Enhanced NO production contributes to endotoxin-mediated systemic hypotension in adult animals (1, 2, 5–9). There are no published reports on the role of endogenous NO in the control of pulmonary arterial pressure in neonatal animal models of sepsis and pulmonary hypertension.

L-Arginine analogs, such as L-NAME and L-NAA, are competitive antagonists of both isoforms of NOS and inhibit NO synthesis (4, 9). These arginine analogs have been used to determine the extent of ongoing NO production during pathologic conditions. In adult dogs administered a single bolus of endotoxin, L-NAA further augmented the endotoxin-induced increase in PVR, suggesting that NO modulates PVR in this model of Gram-negative sepsis (9). There are no reports on the influence of endogenous NO on pulmonary vascular tone during sepsis produced by Gram-positive organisms.

GBS is a Gram-positive pathogen and the most common cause of neonatal sepsis (10–14). GBS sepsis can produce pulmonary hypertension, reduced cardiac output, hypoxemia, and $\dot{V}/\dot{Q}$ mismatch in human neonates and neonatal animals (10–12). GBS-induced lung vascular injury also occurs as suggested by GBS invasion of lung capillary walls, intraalveolar hemorrhage and protein-rich pulmonary edema in human infants (13, 14), and ultrastructural findings of lung capillary endothelial cell injury in both a piglet and nonhuman primate model of GBS sepsis (10, 15). GBS infusion into piglets is delineated into an early (<1 h) and late phase (2 to 6 h) (11, 12). The late phase is presumably more clinically relevant and is associated with GBS-induced pulmonary hypertension and lung vascular injury, hypoxemia, reduced cardiac output, and increased serum levels of thromboxane B_2, prostacyclin, and TNF-α (11, 12). We reported that exogenous administration of inhaled NO reverses late-phase GBS-induced pulmonary hypertension (16), showing that the pulmonary vascular smooth muscle is not sufficiently injured to impair its response to inhaled NO. However, it is unknown whether sustained GBS infusion injures the pulmonary vasculature sufficiently to reduce endogenous NO production and thereby contribute to GBS-induced pulmonary hypertension. Alternatively, GBS may induce increased NOS activity and endogenous NO production and thereby minimize the degree of pulmonary hypertension.

To determine whether endogenous NO synthesis persists during late-phase GBS sepsis and pulmonary hypertension, we studied the effect of the NOS inhibitor, L-NAME, on hemodynamics and gas exchange in the piglet model. We also studied the effect of L-arginine infusion on late-phase GBS-induced pulmonary hypertenison to determine whether the NOS substrate was a limiting factor.

METHODS

This study was approved by the University of Washington Animal Care Committee.

Animal preparation. Healthy, mixed-strain piglets (13 ± 3 d, 3.1 ± 1.0 kg) were anesthetized with pentobarbital (30 mg/kg), paralyzed with pancuronium bromide (0.3 mg/kg), heparinized (1000 IU), and mechanically ventilated via a tracheostomy tube with a Harvard ventilator (Harvard Apparatus Co., South Natick, MA). The piglets were ventilated to maintain $PaCO_2$ between 35 and 45 torr. During the 1-h study period after L-NAME was infused, no ventilator adjustments were made. Catheters were placed in: *1*) the aorta, to measure Psa and to sample arterial blood for pH and blood gas tensions; *2*) the left external jugular vein, to infuse GBS; and *3*) the pulmonary artery, to measure Ppa, Pcw, and CO (in triplicate by thermodilution using an Edwards 9520A cardiac output computer; Edwards Laboratories, Santa Ana, CA) and to sample mixed venous blood for pH and blood-gas tensions (5F Swan-Ganz thermodilution catheter). Anesthesia and paralysis were maintained by a continuous infusion of pentobarbital (3 mg/kg/h) and hourly doses of pancuronium bromide (0.3 mg/kg). Vascular and airway pressures were measured using Hewlett-Packard 1280 transducers (Hewlett-Packard, Waltham, MA) referenced to midchest. Vascular pressure measurements were recorded at end-expiration. Piglet body temperatures were maintained at 38.5 ± 0.5°C by a radiant heat source.

GBS preparation. A clinical isolate of type III group B β-hemolytic streptococci (COH-1) was prepared as previously described (15). Bacteria were incubated in Todd-Hewitt broth for 18 h before each experiment. The broth culture was then centrifuged (1000 × *g*) at 4°C for 10 min, washed, and resuspended in nonbacteriostatic normal saline. Final concentration of bacteria was determined by OD, using a previously determined plot associating OD with bacteria colony-forming units/mL.

Experimental protocol. In pilot animals infused with GBS for 3 h (n = 3), we tested a range of L-NAME bolus infusions (0.3 to 30 mg/kg). L-NAME caused no significant hemodynamic effects at a dose of 0.3 mg/kg but caused a sustained increase in PVR and SVR at 3 mg/kg, and doses of 10 or 30 mg/kg caused a rapid and marked decline in CO with subsequent death. An L-NAME dose of 3 mg/kg was selected for the study.

The piglets were randomly assigned to two groups of five each (GBS: 12 ± 3 d of age, 3.0 ± 0.9 kg; control: 13 ± 3 d of age, 3.2 ± 1.0 kg). Measurements taken at each time point included: Psa, Ppa, Pcw, HR, and CO. PVR, SVR, and SV were calculated at each time point (PVR = Ppa − Pcw/CO; SVR = Psa/CO; SV = CO/HR). Arterial and mixed–venous-blood gases were sampled at each time point. Measurements were taken at baseline, 5, 180, 185, 210, and 240 min of GBS or normal saline infusion to study the late phase of GBS sepsis; the measurement at 5 min was to establish the acute pulmonary hypertensive response and document the sustained nature of GBS-induced pulmonary hypertension into the late phase. After baseline measurements, the GBS group received a GBS infusion (1.25×10^9 colony-forming units/kg/h) for

240 min. The control group received a 240-min saline infusion at the same volume as that which the GBS animals had received. A fraction of inspired oxygen of 0.21 was maintained for the 240 min of GBS or saline infusion. After 180 min of GBS or saline infusion, a 3-mg/kg bolus of L-NAME was given. After 240 min of GBS or saline infusion, the piglets were killed by an overdose of pentobarbital followed by a KCl infusion.

To test whether L-arginine substrate limitation contributes to GBS-induced pulmonary hypertension, L-arginine (1 g/kg i.v. bolus diluted in 10 mL normal saline, Sigma Chemical Co., St. Louis, MO) was infused at 3 h in four additional piglets (GBS: $n = 3$, 2.9 ± 1.3 kg, 9.6 ± 3.2 d of age; saline: $n = 1$, 3.2 kg, 10 d of age). The protocol for these animals was otherwise identical.

Statistical analysis. Data are presented as mean ± SD. To determine the effect of L-NAME, immediate pretreatment (3-h time point), and posttreatment (30 min and 1 h after L-NAME) values of hemodynamics and gas-exchange data were compared by paired t test (SPSS/PC+ v.4.0, SPSS Inc., Chicago, IL). To determine whether the PVR responses to L-NAME were similar or different in the two experimental manipulations (GBS and saline), the percentages of change in immediate pre- and posttreatment with L-NAME were compared by unpaired t test (SPSS/PC+ v.4.0). To determine that baseline hemodynamics and gas exchange values were similar in study and control piglets, intergroup baseline values were compared by unpaired t test (SPSS/PC+ v.4.0). A p value of < 0.05 was considered significant.

RESULTS

Effect of L-NAME on pulmonary hemodynamic indices. Saline infusion (control) caused no significant increases in Ppa, ΔP, or PVR above baseline values during the 3-h infusion (Fig. 1, Table 1). As expected, GBS infusion caused both acute and sustained increases in Ppa, ΔP, and PVR during the 3-h infusion. The increase in PVR for the GBS group was caused by an increase in ΔP and a decrease in CO (Table 1). Compared with values at 3 h for each group, L-NAME treatment of control and GBS piglets caused significant increases in Ppa, ΔP, and PVR at 30 and 60 min after L-NAME (Fig. 1, Table 1). At 30 min after L-NAME treatment, the control-group Ppa increased by 1.6 ± 0.4 kPa, ΔP increased by 1.5 ± 0.3 kPa, and PVR increased by 3.5 ± 1.1 kPa/L/min (159 ± 31%); for the GBS group, Ppa increased by 1.2 ± 0.5 kPa, ΔP increased by 1.0 ± 0.4 kPa, and PVR at 30 min after L-NAME increased by 10.6 ± 3.9 kPa/L/min (171 ± 35%). The percentage of increase in PVR caused by L-NAME was not significantly different for the two groups. GBS, but not saline, caused a significant decrease in CO during the 3-h infusion (Table 1). L-NAME treatment of control piglets caused a significant decrease in CO, and L-NAME treatment of GBS piglets caused a further significant decline in CO compared with values at 3 h. Therefore, the increase in PVR after L-NAME treatment in the GBS and control groups was due to both a decrease in CO and an increase in ΔP, as evidence for active pulmonary vasoconstriction.

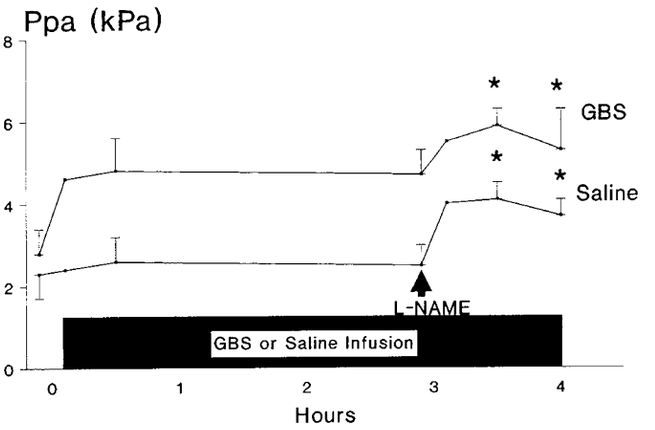

Figure 1. A plot of Ppa before and after L-NAME treatment for both the control and GBS groups. The data are presented as the means ± SD. The *solid black bar* represents the 4-h continuous infusion of saline or GBS. The *black arrow* denotes the the time of the L-NAME infusion. * denotes $p < 0.05$ compared with intragroup value after 3 h of infusion.

Effect of L-NAME on systemic hemodynamic indices. Neither saline nor GBS infusions caused significant changes in Psa or SVR above baseline values during the 3-h

Table 1. *Pulmonary hemodynamics**

Group	Baseline	30 min	3 h	30 min L-NAME	1 h L-NAME
Control					
Ppa (kPa)	2.3 ± 0.6	2.6 ± 0.6	2.5 ± 0.5	4.1 ± 0.4†	3.7 ± 0.4†
Pcw (kPa)	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.3	0.7 ± 0.3	0.5 ± 0.3
Ppa-Pcw (kPa)	1.8 ± 0.4	2.0 ± 0.4	1.9 ± 0.4	3.4 ± 0.3†	3.2 ± 0.4†
CO (L/min)	0.92 ± 0.30	0.85 ± 0.27	0.88 ± 0.31	0.60 ± 0.22†	0.63 ± 0.22†
PVR (kPa/L/min)	2.0 ± 0.4	2.3 ± 0.5	2.2 ± 1.0	5.7 ± 1.3†	5.1 ± 1.0†
GBS					
Ppa (kPa)	2.8 ± 0.6	4.8 ± 0.8	4.7 ± 0.6	5.9 ± 0.4†	5.3 ± 1.0†
Pcw (kPa)	0.8 ± 0.3	0.9 ± 0.5	1.2 ± 0.5	1.4 ± 0.5	1.3 ± 0.5
Ppa-Pcw (kPa)	2.0 ± 0.6	3.8 ± 0.6	3.5 ± 0.6†	4.5 ± 0.4†	4.0 ± 0.7†
CO (L/min)	0.85 ± 0.26	0.73 ± 0.33	0.57 ± 0.25	0.27 ± 0.17†	0.27 ± 0.17†
PVR (kPa/L/min)	2.3 ± 1.1	5.4 ± 2.4	6.2 ± 2.6	16.8 ± 5.5†	14.8 ± 6.3†

* Values are mean ± SD. 30 min = 30 min of saline or GBS infusion; 3 h = 3 h of saline or GBS infusion; 30 min L-NAME = 3.5 h of saline or GBS infusion and 30 min after L-NAME treatment; 1 h L-NAME = 4 h of saline or GBS infusion and 1 h after L-NAME treatment.

† $p < 0.05$ compared with intragroup value at 3 h of saline or GBS infusion.

infusion (Figs. 2 and 3). L-NAME treatment of control and GBS piglets at 3 h caused significant increases in both Psa and SVR at 30 and 60 min after L-NAME.

GBS, but not saline, caused a significant increase in HR and a significant decrease in SV during the 3-h infusion (Table 2). L-NAME treatment of control piglets caused a significant decrease in SV with no significant change in HR. L-NAME treatment of GBS piglets caused a further decline in CO and SV and a further increase in HR. L-NAME caused a significantly greater reduction in SV in the GBS compared with control piglets.

Effect of L-NAME on the PVR/SVR ratio. GBS, but not saline, caused a significant increase in the PVR/SVR ratio during the 3-h infusion (Fig. 4). L-NAME treatment caused a mild but significant increase in the PVR/SVR ratio 30 min after L-NAME in the control group. L-NAME caused no further increase in the PVR/SVR ratio in the GBS group. This suggests that during late-phase GBS sepsis there are similar levels of NO production from the systemic and pulmonary vascular beds.

Effect of L-NAME on pulmonary gas exchange. GBS, but not saline, caused a reduction in pH, Pa_{O_2}, and Pv_{O_2} during the 3-h infusion (Table 3). L-NAME treatment caused no significant changes in arterial or mixed–venous-blood gas tensions in the control group. In the GBS group, L-NAME treatment caused no further decrease in Pa_{O_2} but did cause further reductions in mixed venous P_{O_2} and pH. GBS, but not saline, caused a significant increase in $A\text{-}aD_{O_2}$ values during the 3-h infusion (Table 2). L-NAME treatment caused no significant changes in $A\text{-}aD_{O_2}$ values for either group compared with 3-h pretreatment values, suggesting that L-NAME caused no significant alterations in ventilation-perfusion matching in control or GBS piglets.

Effect of L-arginine on hemodynamics and gas exchange. Three neonatal piglets were infused with GBS for 3 h as above and then administered 1 g/kg L-arginine by i.v. infusion. L-Arginine caused no significant changes in any measured hemodynamic or gas-exchange variable (Table 4). In a single control, saline-infused piglet, 1 g/kg L-arginine, caused no significant changes in any measured variable. We conclude that endogenous NO production is not limited by L-arginine concentrations during late-phase GBS-induced pulmonary hypertension.

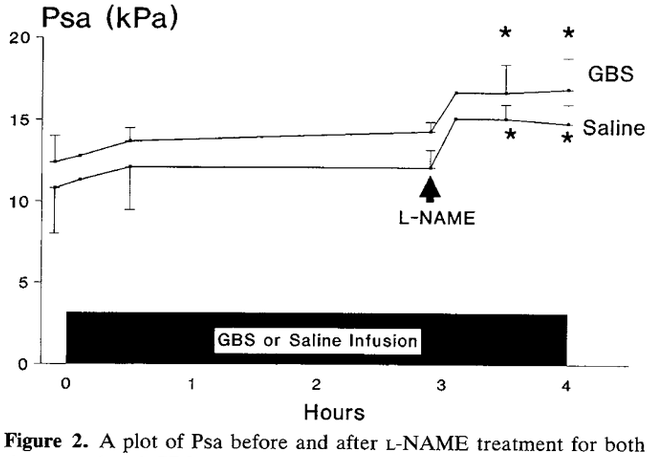

Figure 2. A plot of Psa before and after L-NAME treatment for both the control and GBS groups. The data are presented as the means ± SD. The *solid black bar* represents the 4-h continuous infusion of saline or GBS. The *black arrow* denotes the time of the L-NAME infusion. * denotes $p < 0.05$ compared with intragroup value after 3 h of infusion.

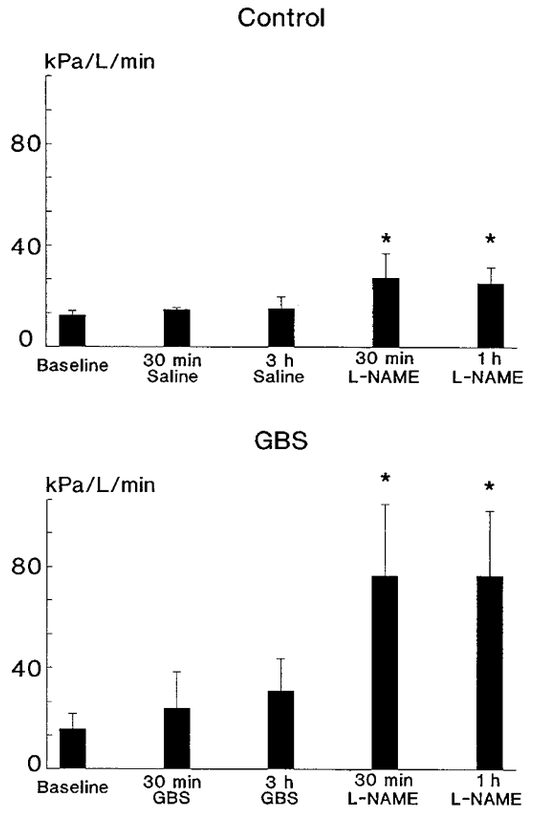

Figure 3. Histogram plots of SVR before and after L-NAME treatment for both the control and GBS groups. The data are expressed as the means ± SD. *30 min*, 30 min of saline or GBS infusion; *3 h*, 3 h of saline or GBS infusion; *30 min L-NAME*, 3.5 h of saline or GBS infusion and 30 min after L-NAME treatment; *1-h L-NAME*, 4 h of saline or GBS infusion and 1 h after L-NAME treatment. * denotes $p < 0.05$ compared with intragroup values at 3 h.

DISCUSSION

In a neonatal piglet model of sustained GBS sepsis, inhibition of NOS by L-NAME caused *1*) further increases in Ppa, ΔP, PVR, and SVR, with no change in the PVR/SVR ratio; *2*) further reductions in CO and SV; and *3*) a reduction in mixed–venous-oxygen tension without a change in ventilation-perfusion matching. L-NAME treatment caused similar increases in ΔP and PVR in GBS and control piglets. These data provide indirect evidence that endogenous NO production persists during sustained GBS infusion in piglets and that endogenous NO reduces pulmonary vascular hypertension in this model of late-phase GBS sepsis. However, we cannot make conclusions about the degree of endogenous NO synthesis during sustained GBS infusion, *i.e.* increased, decreased, or unchanged. We did not include an additional control group of sustained GBS infusion for 4 h without L-NAME

Table 2. *Effect of L-NAME on cardiac function**

Group	Baseline	30 min	3 h	30 min L-NAME	1 h L-NAME
Control					
CO (L/min)	0.92 ± 0.35	0.85 ± 0.27	0.88 ± 0.37	0.60 ± 0.22†	0.63 ± 0.22†
HR (beats/min)	225 ± 44	226 ± 43	212 ± 35	219 ± 32	220 ± 30
Stroke vol (mL)	4.3 ± 2.0	4.0 ± 1.7	4.4 ± 2.8	2.9 ± 1.4†	3.0 ± 1.3†
GBS					
CO (L/min)	0.85 ± 0.26	0.73 ± 0.38‡	0.57 ± 0.35§	0.27 ± 0.17†	0.27 ± 0.17†
HR (beats/min)	201 ± 32	215 ± 34	228 ± 24‡	241 ± 21†	271 ± 38‖
Stroke vol (mL)	4.3 ± 1.2	3.5 ± 1.8‡	2.5 ± 1.5§	1.1 ± 0.8‖	1.1 ± 0.9‖

* Values are as mean ± SD. Abbreviations same as for Table 1.
† $p < 0.05$ compared with intragroup 3-h data.
‡ $p < 0.05$ compared with intragroup baseline value.
§ $p < 0.05$ compared with intragroup baseline value and comparable intergroup value.
‖ $p < 0.05$ compared with intragroup 3-h data and intergroup value.

treatment at 3 h, because, in using the same model, we previously reported no significant changes in pulmonary hemodynamics between 3 and 4 h of sustained GBS infusion (16). In addition, the rapid hemodynamic responses to L-NAME in both the control and GBS groups (<5 min) suggests that the alterations in pulmonary hemodynamics are due to the L-NAME bolus and not the continuation of GBS infusion. Rudinsky *et al.* (17) have published a preliminary report on the effect of L-N^G-nitro-L-arginine in a piglet model of acute GBS sepsis. The hemodynamic effects were similar to our study, inasmuch as GBS + L-N^G-nitro-L-arginine caused a marked increase in Ppa and a marked decrease in CO compared with GBS alone. In our study, L-arginine infusion after 3 h of sustained GBS infusion did not cause any significant changes in hemodynamic or gas-exchange variables. Rudinsky *et al.* (18) have also published preliminary data that L-arginine treatment (300 mg/kg) of acute GBS sepsis in piglets causes partial attenuation of GBS-induced pulmonary hypertension and mild reduction of the PVR/SVR ratio with no effect on CO or Psa. These findings differ from our observation of L-arginine treatment in a model of late-phase, sustained GBS pulmonary hypertension. The reason for the discrepancy is uncertain, with possibilities including *1*) increased duration of GBS infusion and increased number of vasoconstrictive mediators in the late-phase model (11, 12, 16) and *2*) differing GBS doses. We conclude that *1*) endogenous NO synthesis persists during late-phase GBS-induced pulmonary hypertension in neonatal piglets, and *2*) NO production is not limited by L-arginine concentrations during late-phase GBS-induced pulmonary hypertension.

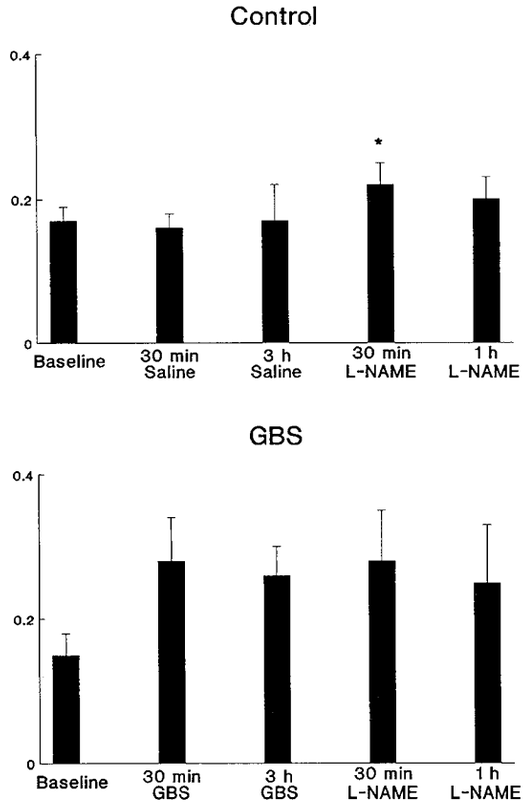

Figure 4. Histogram plots of the PVR/SVR ratio before and after L-NAME treatment for both the control and GBS groups. The data are expressed as the means ± SD. *30 min*, 30 min of saline or GBS infusion; *3 h*, 3 h of saline or GBS infusion; *30 min* L-NAME, 3.5 h of saline or GBS infusion and 30 min after L-NAME treatment; *1 h L-NAME*, 4 h of saline or GBS infusion and 1 h after L-NAME treatment. * denotes $p < 0.05$ compared with intragroup values at 3 h.

The lack of direct measurement of NO-related parameters, such as plasma nitrates, is a limitation of our study. In the absence of these measures, we cannot be certain that the dose of L-NAME used in our study (3 mg/kg) caused complete inhibition of NOS activity in this piglet model of GBS-induced pulmonary hypertension. L-NAME doses of 1 to 3 mg/kg have previously been reported to markedly inhibit NO production in rats and cats *in vivo*, with doses of 10 to 30 mg/kg causing little additional hemodynamic alterations (4, 5, 19). We selected an L-NAME dose of 3 mg/kg based on the literature and our pilot studies in piglets in which doses > 3 mg/kg caused hemodynamic instability and death before completing the 4-h protocol. If the dose of L-NAME used in our study caused submaximal inhibition of NOS activity, this would only underestimate the contribution of endogenous NO to late-phase GBS-induced pulmonary hypertension. This hypothetical scenario would not alter our conclusion that endogenous NO synthesis persists during late-phase GBS-induced pulmonary hypertension.

Table 3. *Effect of L-NAME on gas exchange**

Group	Baseline	30 min	3 h	30 min L-NAME	1 h L-NAME
Control					
pH	7.46 ± 0.02	7.45 ± 0.03	7.44 ± 0.06	7.44 ± 0.04	7.45 ± 0.03
$Paco_2$ (kPa)	4.5 ± 0.3	4.9 ± 0.3	5.1 ± 0.5	4.9 ± 0.5	4.9 ± 0.4
Pao_2 (kPa)	10.4 ± 1.2	10.3 ± 1.1	10.5 ± 1.5	10.0 ± 1.1	10.1 ± 1.2
Pvo_2 (kPa)	5.1 ± 1.3	5.1 ± 1.0	5.1 ± 1.0	4.5 ± 0.8	4.7 ± 0.6
A-aDo_2 (kPa)	4.0 ± 0.6	3.7 ± 0.4	3.6 ± 0.5	4.0 ± 0.7	3.9 ± 0.5
GBS					
pH	7.44 ± 0.02	7.45 ± 0.04	7.44 ± 0.04	7.41 ± 0.07†	7.34 ± 0.06‡
$Paco_2$ (kPa)	4.8 ± 0.6	5.2 ± 1.3	4.8 ± 0.6	4.8 ± 0.5	5.3 ± 0.9‡
Pao_2 (kPa)	10.0 ± 1.0	9.1 ± 2.0§	8.7 ± 1.9§	8.5 ± 1.7§	8.3 ± 1.6§
Pvo_2 (kPa)	5.3 ± 0.8	4.7 ± 0.7†	4.4 ± 0.8§	3.1 ± 0.3‖	3.3 ± 0.5‖
A-aDo_2 (kPa)	4.1 ± 0.7	4.5 ± 0.9	5.6 ± 0.8†	5.6 ± 0.6§	5.3 ± 0.7§

* Values are mean ± SD. Abbreviations same as for Table 1.
† $p < 0.05$ compared with intragroup baseline value.
‡ $p < 0.05$ compared with intragroup 3-h data.
§ $p < 0.05$ compared with intragroup baseline value and comparable intergroup value.
‖ $p < 0.05$ compared with intragroup 3-h data and intergroup value.

Analogs of L-arginine have been used to treat endotoxin-mediated shock in animal models and humans (2, 5–8, 20, 21). The reports are conflicting as to their benefit, with some discrepancies possibly due to the degree of NOS inhibition and animal model used. Some studies report lethal complications with marked decline in CO and SV (9), whereas others report reversal of shock (8) or increased survival in a murine model of *Escherichia coli* sepsis (22). L-NAA treatment of adult control dogs resulted in similar findings to L-NAME treatment of our control piglets. These effects include depressed CO and reduced SV with no change in HR (9). The effect of L-NAME on cardiac function was more deleterious during late-phase GBS, with greater reduction in CO and SV and an increase in HR. The cause of the progressive cardiac dysfunction produced by NOS inhibition may be multifactorial, including *1*) coronary artery vasoconstriction with myocardial ischemia and reduced contractility (23–26), *2*) reduced endocardial cell production of NO with potential decreased myocardial performance (27), and *3*) increased cardiac afterload with the acute increase in PVR and SVR (28, 29). The neonate seems particularly susceptible to acute cardiac afterload stress (28, 29), and the combination of GBS sepsis with limited cardiac reserve and an acute afterload stress may have precipitated the marked decline in CO.

There are limited studies on the effect of NOS inhibitors on pulmonary gas exchange (9, 30, 31). In adult dogs, L-NAA treatment caused a reduction in Pvo_2 with no other significant changes in gas exchange (9). In an adult animal model of oleic acid lung injury with marked intrapulmonary shunt and ventilation-perfusion mismatch, N^G-nitro-L-arginine treatment caused no changes in Pao_2, venous Po_2, or intrapulmonary shunt (30). L-NAME caused no significant alterations in A-aDo_2 in control or GBS piglets but did reduce venous Po_2 in the GBS piglets, presumably secondary to progressive decrease in systemic O_2 delivery due to decreased CO. These prior studies and our data suggest that inhibition of NOS causes no significant changes in ventilation-perfusion matching in animal models of sepsis with pulmonary hypertension. However, in an adult sheep model of endotoxic shock with mild hypoxemia and increased intrapulmonary shunt, L-NAME caused return to baseline values for Pao_2 and intrapulmonary shunt fraction (31). The reason for this discrepancy is uncertain, but factors may include: *1*) age of animals (32, 33); *2*) species (6); *3*) L-NAME dose; and, perhaps most importantly, *4*) the minimal intrapulmonary shunt (1 to 3%) in our neonatal piglet model at baseline or during GBS infusion (16). Inhibition of NO production by L-NAME may result in redistribution of pulmonary blood flow away from shunt and low $\dot{V}/\dot{Q}$ regions. We speculate that the minimal intrapulmonary shunt and hypoxemia observed in our piglet model of GBS-induced pulmonary hypertension did not provide

Table 4. *Effect of L-arginine infusion during GBS infusion**

	pH	$Paco_2$ (kPa)	Pao_2 (kPa)	Pvo_2 (kPa)	Psa (kPa)	HR (beats/min)	Ppa (kPa)	CO (L/min)	PVR (kPa/L/min)
Baseline	7.42 ± 0.03	4.7 ± 0.3	11.0 ± 1.2	5.5 ± 0.8	11.5 ± 2.4	207 ± 25	2.4 ± 0.5	0.87 ± 0.31	2.0 ± 0.4
30′ GBS	7.43 ± 0.06	4.8 ± 0.4	9.5 ± 1.4	4.8 ± 0.8	11.2 ± 2.7	220 ± 35	4.4 ± 1.1	0.75 ± 0.40	4.5 ± 0.6
3 h GBS	7.40 ± 0.05	5.1 ± 0.3	8.9 ± 0.9	4.5 ± 1.0	10.4 ± 3.1	216 ± 28	4.3 ± 0.9	0.60 ± 0.27	5.4 ± 0.8
30′ ARG	7.42 ± 0.04	5.1 ± 0.3	8.8 ± 1.1	4.4 ± 1.1	10.5 ± 2.5	224 ± 32	4.5 ± 0.9	0.58 ± 0.19	5.7 ± 0.9
1 h ARG	7.39 ± 0.06	5.2 ± 0.4	8.3 ± 1.2	4.4 ± 0.9	10.3 ± 2.9	218 ± 36	4.1 ± 1.2	0.61 ± 0.23	4.9 ± 1.0

* Values are mean ± SD. There were no significant changes in any parameter after L-arginine infusion. 30′ GBS = 30 min GBS infusion; 3 h GBS = 3 h GBS infusion; 30′ ARG = 3.5 h GBS infusion and 30 min after 1 g/kg L-arginine i.v. bolus; 1 h ARG = 4 h GBS infusion and 1 h after L-arginine infusion.

enough low $\dot{V}/\dot{Q}$ regions for L-NAME to modulate regional pulmonary blood flow.

Endotoxin treatment both *in vitro* and *in vivo* causes increased NOS activity and enhanced NO production, according to most reports (2, 5–9). However, others have observed endotoxin treatment *in vivo* caused decreased endothelial-dependent relaxation of adult canine or guinea pig systemic arterial rings (34, 35). The reports that endotoxin reduces NO production may be due to the down-regulation of the constitutive isoform of NOS acutely, and the reports showing that endotoxin increases NO production may be due to up-regulation of the inducible isoform of NOS after more prolonged endotoxin exposure. In addition to the dose and duration of endotoxin treatment, other factors that may alter NO production include vascular endothelial or smooth muscle injury, species, and the assays used to measure endothelial-derived relaxation factor activity. Some reports have shown organ-specific patterns of NOS induction by endotoxin, with the lung being a major source of inducible NOS (5, 6). Endotoxin treatment *in vivo* can increase the inducible isoform of NOS by 3 h (5, 6). Most studies of NOS inhibitors in models of sepsis have focused on systemic hemodynamics and did not report changes in pulmonary hemodynamics caused by NOS inhibition (2, 5, 8). However, after endotoxin treatment of adult dogs, a continuous infusion of LNAA caused a sustained and progressive increase in PVR and SVR and further reductions in CO, HR, and SV (9). In this same study, there were no pulmonary hemodynamic data presented for a group of saline-infused control dogs treated with L-NAA, and no direct statistical comparisons were made between the saline- and endotoxin-treated groups. In our study using GBS, a Gram-positive pathogen, NOS inhibition caused no change in the PVR/SVR ratio, suggesting a similar effect on the pulmonary and systemic vascular beds. We did not directly measure NOS activity or NO metabolites and can only conclude that NO contributes to both pulmonary and systemic vascular tone during GBS sepsis. Possible explanations for the different response to NOS inhibition include *1*) age of animals (32, 33), *2*) species-specific responses to NOS inhibition (36), and *3*) Gram-positive pathogen compared with Gram-negative endotoxin.

A recent study detected increased circulating NO metabolites in neonates with both Gram-positive and Gram-negative sepsis, and the plasma nitrate levels were highest in those patients with shock (37). Continuous NO synthesis by vascular endothelial or smooth muscle cells appears to modulate pulmonary and systemic vascular tone in this piglet model of neonatal sepsis and pulmonary hypertension. Late-phase GBS sepsis in neonatal piglets is also associated with increased serum levels of the potent vasodilator, prostacyclin, a potent vasoconstrictor, thromboxane A_2, and a proinflammatory and vasoactive cytokine, TNF-α (11, 12). The degree of late-phase GBS-induced pulmonary hypertension is presumably an integrated function of these multiple vasoactive stimuli, regional alveolar hypoxia, and pulmonary blood flow. The multiple circulating vasoconstrictive stimuli in this model of GBS sepsis may in part explain the higher pre- and post-L-NAME values for PVR and SVR in the GBS group compared with the control group. Endogenous NO production has been measured to be 8 to 15 parts per billion of exhaled gas in humans and animals (38). These endogenous concentrations are not sufficient to override multiple vasoconstrictive stimuli and normalize pulmonary artery pressure during late-phase GBS sepsis.

Previous studies have suggested that the NOS substrate, L-arginine, is not a limiting factor for NOS-induced vasodilation under basal conditions (39, 40). However, there are limited studies on the use of L-arginine infusions in *in vivo* models of neonatal sepsis and pulmonary hypertension (18). In our study, L-arginine infusion at 1 g/kg had no affect on late-phase GBS-induced pulmonary hypertension. These data suggest that L-arginine is not a limiting factor for NOS-induced vasodilation in this model of neonatal sepsis and pulmonary hypertension. One potential explanation is that there are adequate levels of L-arginine in plasma to saturate the NOS of both endothelial and vascular smooth muscle cells (41). Further study of the signals for NO synthesis may lead to more potent stimulators of endogenous NO for the treatment of pulmonary hypertension. Despite the reports of increased NO production in neonatal sepsis with shock (37), NOS inhibitors were deleterious in this model of neonatal sepsis due to progressive decline in cardiac output and worsening pulmonary hypertension. Combining inhaled NO with systemic NOS inhibitors may reduce the untoward effect of worsening pulmonary hypertension. However, the use of NOS inhibitors alone appears to be contraindicated in the treatment of human newborns with GBS sepsis.

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