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The Effect of N$^{\omega}$-Nitro-L-Arginine Methylester on Hypoxic Vasoconstriction in the Neonatal Pig Lung

LEIF D. NELIN AND CHRISTOPHER A. DAWSON

Departments of Pediatrics [L.D.N.] and Physiology [C.A.D.] Medical College of Wisconsin, Milwaukee, and Research Service [L.D.N., C.A.D.], Zablocki VA Medical Center, Milwaukee, Wisconsin 53295

ABSTRACT. This study was carried out to determine the influence and site of action of N$^{\omega}$-nitro-L-arginine methylester, an L-arginine analogue, on basal pulmonary vascular tone and hypoxic vasoconstriction in neonatal pig lungs. We studied isolated lungs from pigs, age 14.5 ± 0.5 (SD) d and weight 3.6 ± 0.7 kg, perfused with autologous blood at a constant flow rate. The arterial-venous occlusion method was used to determine sites of action upstream and downstream of the double occlusion pressure (Pd) during baseline, infusion of acetylcholine, and ventilation of the lung with a hypoxic gas mixture. The measurements were then repeated during the three conditions described above after adding N$^{\omega}$-nitro-L-arginine methylester, a competitive inhibitor of nitric oxide synthase, to the blood. During control conditions, the vascular resistance was almost evenly divided upstream and downstream of Pd. Infusion of acetylcholine resulted in downstream dilation, and hypoxia resulted in an increase in both upstream and downstream resistance. After adding N$^{\omega}$-nitro-L-arginine methylester to the blood, there was an increase in both upstream and downstream resistances; acetylcholine infusion resulted in an increase in total vascular resistance, which was entirely due to upstream constriction; and the hypoxia response was much larger both upstream and downstream of Pd. These results suggest that nitric oxide synthase helped maintain a low level of basal pulmonary vascular tone both upstream and downstream of Pd in these neonatal pig lungs; that the vascular effect of acetylcholine was changed from downstream dilation to upstream constriction by N$^{\omega}$-nitro-L-arginine methylester; and that nitric oxide synthase activity modulated both the upstream and downstream vasomotor response to hypoxia. (*Pediatr Res* 34: 349–353, 1993)

Abbreviations

Ach, acetylcholine
NO, nitric oxide
NAME, N$^{\omega}$-nitro-L-arginine methylester
Pa, pulmonary artery pressure
Pd, double occlusion pressure
Pv, left atrial pressure

The release of NO from the endothelial cell has been implicated in the maintenance of the normally low resting pulmonary vascular resistance (1–5), but some studies have found no change in pulmonary vascular resistance during basal conditions when the action of NO is inhibited (6, 7). Similarly, the role of NO during the pulmonary vasoconstrictor response to hypoxia remains unclear, with studies suggesting hypoxia-induced inhibition of NO release (4, 8–11) or stimulation of NO release (5–7, 12, 13). There is currently no available information on how NO synthase inhibitors, such as NAME, influence the arterial venous distribution of pulmonary vascular resistance or the arterial venous site of action of pulmonary vasomotor stimuli. These questions are of particular importance in the neonate, where the pulmonary vasculature appears to be particularly reactive (14). Furthermore, recent studies (2, 15, 16) have suggested a role for NO in modulating pulmonary vascular resistance in the early neonatal period. Thus, the present study was designed to examine these questions by addressing the following three objectives. The first was to examine the impact of NAME on the pulmonary vascular resistance of lungs from newborn pigs. The second was to determine the impact of NAME on hypoxic vasoconstriction in these lungs, and the third was to determine whether there was a difference in response to NAME upstream and downstream of the Pd.

MATERIALS AND METHODS

The isolated perfused lung preparation previously described (17) was used. Briefly, 2-wk-old pigs were anesthetized with acepromazine (1.5 mg/kg) and ketamine (30 mg/kg). A polyethylene catheter was placed in a carotid artery and 1500 units/kg heparin were given. A sample of arterial blood was obtained for blood gas and hematocrit measurements, the results of which were P_{O_2}, 10.5 ± 2.4 (SD) kPa; P_{CO_2}, 5.9 ± 0.9 kPa; pH, 7.30 ± 0.07; and hematocrit, 0.164 ± 0.045 (16.4 ± 4.5%). The pig was then exsanguinated. During the bleeding process, 10 to 15 mL/kg 10% dextran (molecular weight 40 000) in saline were given. A midline thoracotomy was performed, and rigid cannulas were placed in the main pulmonary artery, left atrium, and trachea. The lungs and heart were removed *en bloc* and placed in a heated, humidified perfusion chamber. Perfusion was initiated using the autologous blood with a resultant hematocrit of 0.128 ± 0.025 (12.8 ± 2.5%). The perfusion circuit consisted of a venous reservoir, a heat exchanger, and a Masterflex roller pump. The blood was pumped into the pulmonary artery at a rate of 100 $mL \cdot min^{-1} \cdot kg^{-1}$. The left atrial pressure was set at 0.4 kPa (3 mm Hg). An infusion port was located proximal to the arterial cannula. Pa and Pv were continuously recorded. To determine the arterial and venous distribution of the vascular resistance during the various conditions studied, a vascular occlusion procedure was followed as previously described (18). At the appropriate time during the condition under study, the arterial inflow and venous outflow were simultaneously occluded for 5 to 8 s. During the occlusion maneuver, the Pa and Pv equilibrate at Pd,

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Correspondence requests: Leif D. Nelin, M.D., Research Service 151, Zablocki VA Medical Center, 5000 W. National Ave., Milwaukee, WI 53295.
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which divides the Pa-Pv pressure difference into two segments: Pa-Pd, upstream from Pd, and Pd-Pv, downstream from Pd. The Pd is generally considered to be the microvascular pressure during the perfusion (19). Thus, changes in the upstream and downstream pressure drops during constant flow perfusion are considered to represent changes in the arterial and venous resistances, respectively.

The lungs were ventilated with a piston-type ventilator with a tidal volume of 75 ± 13 mL, an end-expiratory pressure of 0.2 ± 0.1 kPa (1.5 ± 1 mm Hg), and a frequency of 12 breaths/min. These settings resulted in a peak inspiratory pressure of 1.0 ± 0.1 kPa (7.5 ± 0.8 mm Hg) and a lung compliance, calculated as tidal volume divided by the difference between peak inspiratory pressure and end-expiratory pressure, of 12.8 ± 3.6 mL/mm Hg. The ventilating gas mixture contained approximately 15% O_2, 6% CO_2, and balance N_2, which is hereafter referred to as normoxia. The normoxic gas mixture resulted in blood gas tensions of P_{O_2}, 15.1 ± 0.8 kPa; P_{CO_2}, 5.5 ± 0.4 kPa; and pH, 7.45 ± 0.03.

To determine the utility of the NO synthase inhibitor NAME for studying the NO synthase pathway in this neonatal pig lung preparation, we examined its effect on Pa. In five isolated perfused lungs from 2-wk-old pigs (age 14.4 ± 0.9 d, weight 3.0 ± 0.5 kg), the responses to NAME and L-arginine were determined. During steady state conditions, 54 mg of NAME (2 mL of 10^{-1} M NAME) were added to the reservoir with a recirculating volume of approximately 150 mL. After a new steady state was reached, 421 mg L-arginine (2 mL of 1 M L-arginine) were added to the reservoir.

To systematically determine the influence of NAME on the hypoxic response and the site of action of reduced PO_2, Ach, and NAME, we studied eight pigs, age 14.5 ± 0.5 (SD) d, weight 3.6 ± 0.7 kg. An occlusion maneuver was carried out during baseline conditions and then repeated during the infusion of 10 μg/min Ach. Preliminary studies revealed that an infusion of 10 μg/min Ach resulted in a decrease in Pa with no change in airway compliance, and this response could be blocked by adding 2.7 mg of NAME (1 mL of 10^{-3} M NAME) to the blood. The ventilating mixture was then changed to a reduced P_{O_2} mixture consisting of approximately 11% O_2, 6% CO_2, and balance N_2, which is hereafter referred to as hypoxia. The hypoxic gas mixture resulted in blood gas tensions of P_{O_2}, 11.2 ± 0.7 kPa; P_{CO_2}, 5.3 ± 0.3 kPa; and pH, 7.43 ± 0.03. This submaximal hypoxic stimulus was used because in preliminary studies a maximal hypoxic stimulus resulted in an excessive response after NAME treatment, with Pa reaching approximately 120 mm Hg, and that led to gross edema formation within minutes. When the Pa was steady, an occlusion maneuver was performed. The ventilating gas mixture was then returned to the normoxic mixture. When the Pa had returned to baseline levels, 2.7 mg of NAME (1 mL of 10^{-3} M NAME) were added to the reservoir (containing approximately 150 mL of blood). An occlusion maneuver was performed when the Pa was stable and then repeated as described above during Ach infusion and ventilation with the hypoxic gas mixture. Additionally, in a preliminary study in five isolated neonatal (age 12 ± 6 d) pig lungs with a range of hypoxic stimuli (P_{O_2}, 4.0 to 10.7 kPa), the hypoxic response at constant flow and Pv was compared for two repeated challenges with no intervention between challenges to the same hypoxic stimulus. The mean increases in Pa were 9.0 ± 5.4 mm Hg and 8.6 ± 5.7 mm Hg for the first and second challenge, respectively, indicating that in the relevant time period, time and previous history of exposure to hypoxia had no significant impact on the response.

The data are expressed as mean ± SEM unless otherwise indicated. Comparisons between groups were carried out using the paired t test. Differences between groups were considered significant when $p < 0.05$. The study was approved by the institutional committee on animal investigation of the Zablocki VA Medical Center.

RESULTS

The addition of NAME to the reservoir resulted in a significant increase in Pa, from 18.4 ± 2.6 mm Hg baseline to 31.8 ± 4.6 mm Hg NAME ($p < 0.05$). When L-arginine was added, the Pa immediately fell back to 20.9 ± 2.6 mm Hg, which was not significantly different from baseline. This suggests that NAME exerted its effect on the pulmonary vasculature via its NO synthase inhibitory action.

The results in Table 1 also reveal that NAME resulted in an increase in pulmonary vascular resistance during normoxic conditions. The increase occurred in both the upstream and downstream pressure drops, and both Pa and Pd pressures were increased by NAME (Fig. 1).

Ach infusion resulted in vasodilation during control conditions (Fig. 2) and in vasoconstriction after treatment with NAME (control response different from NAME response, $p < 0.01$). The vasodilator site of action of Ach was downstream as shown by

Table 1. *Hemodynamic data**

	Pa	Pa-Pd	Pd-Pv
Control			
Baseline	15.5 ± 2.8	6.4 ± 1.7	6.3 ± 1.0
Ach	14.1 ± 2.6	6.1 ± 1.7	5.2 ± 0.9†
Hypoxia	18.5 ± 3.0†	8.1 ± 1.7†	7.6 ± 1.2†
NAME			
Baseline	22.1 ± 2.9‡	9.7 ± 1.8‡	9.5 ± 1.1‡
Ach	25.0 ± 4.1‡	13.3 ± 2.9†‡	8.8 ± 1.4†
Hypoxia	35.8 ± 3.0†‡	19.0 ± 2.0†‡	13.8 ± 1.1†‡

* Pv was 3 mm Hg, and flow rate was 100 $mL \cdot min^{-1} \cdot kg^{-1}$ in all cases.
† Different from corresponding baseline, $p < 0.03$.
‡ Different from corresponding control condition, $p < 0.04$.

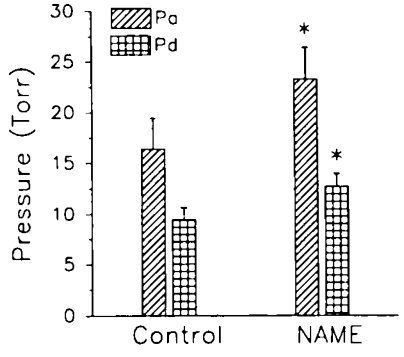

Fig. 1. The effect of NAME treatment on Pa and Pd. *Asterisk* indicates significantly different from control ($p < 0.03$) Torr = mm Hg.

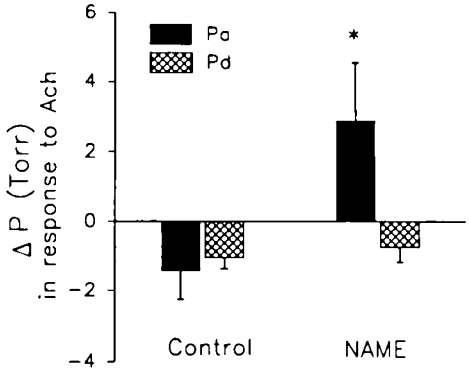

Fig. 2. The change in Pa and Pd due to the infusion of 10 μg/min Ach during control and after NAME. A positive change indicates that Ach caused vasoconstriction, and a negative change indicates that Ach caused vasodilation. *Asterisk* indicates significantly different from control ($p < 0.007$). Torr = mm Hg.

the significant decrease in the downstream pressure drop in Table 1 with no change in the upstream pressure drop. However, after NAME, the vasoconstrictor response to Ach occurred upstream (Table 1).

The mild hypoxic stimulus resulted in a small but significant increase in Pa before NAME treatment. The hypoxic response was greatly augmented by NAME (Fig. 3). The hypoxic response occurred both upstream and downstream of Pd, as shown in Figure 3 and Table 1, and the augmentation produced by NAME occurred both upstream and downstream as well (Table 1). However, this augmentation was greater upstream as evidenced by the 5.5-fold increase in the upstream response compared with the 2.5-fold increase in the downstream response.

DISCUSSION

We found that in the isolated perfused neonatal pig lung, NAME increased vascular resistance. This suggests that NO synthase activity was playing a modulatory role under the control conditions established in these experiments. This observation is consistent with those of Fineman *et al.* (1, 2), who reported that the i.v. infusion of either N^{ω}-nitro-L-arginine, another NO synthase inhibitor, or methylene blue, a guanylate cyclase inhibitor, led to increased pulmonary vascular resistance in neonatal lambs. In our study, the nearly identical increase in upstream and downstream pressure drops after NAME treatment suggests that the basal release of NO is a factor controlling both the arterial and venous vascular resistance in the neonatal pig lung. The concept that vasodilator mechanisms help maintain the low pulmonary vascular resistance in neonatal animals has another precedent as well. Redding *et al.* (20) found that in the neonatal pig lung (<12 d old) the injection of meclofenamate resulted in an increase in Pa, suggesting that another endothelium-derived factor, prostacyclin, was involved in the maintenance of the low basal pulmonary vascular resistance. Thus, in the neonatal pig lung, it appears that the maintenance of the normally low basal vasomotor tone may depend, at least in part, on the basal release of NO and prostacyclin countering some tone-inducing mechanism.

At least one component of the endothelium-dependent vasodilator action of Ach has been found to be an NO synthase-mediated response (21–24). We found that Ach was a vasodilator in this preparation during control conditions but a vasoconstrictor after NAME was added. The pulmonary vascular response to Ach has been found to be vascular tone dependent in rabbit (25) and cat (26), with Ach producing vasodilation when tone was elevated by alveolar hypoxia or U46619 infusion. In our study, the level of vasomotor tone was greater after NAME administration than under normal control conditions. The fact that NAME resulted in both elevated vasomotor tone and a vasoconstrictor response to Ach suggests that it was the NO synthase inhibitory action of NAME that was the dominant influence of NAME on the Ach response in this preparation.

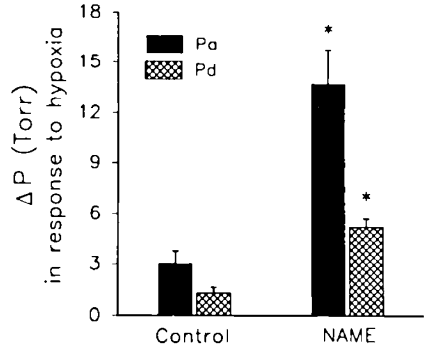

Fig. 3. The increase in Pa and Pd due to hypoxia during control and after NAME. *Asterisk* indicates significantly different from control ($p < 0.004$). Torr = mm Hg.

Although other mechanisms for Ach-induced pulmonary vasodilation also occur (24, 27), the implied NO synthase–mediated response in the present study is consistent with the study by Cherry and Gillis (25) in isolated perfused rabbit lungs in which treatment with Hb and quinacrine, substances that interfere with the NO pathway, changed the action of Ach from dilation to constriction. The reversal of the Ach response by NAME and of the NAME reversal by L-arginine are consistent with the concept that NAME did inhibit NO synthase activity in this preparation.

Examination of Table 1 reveals that Ach acted differently upstream and downstream of Pd in this preparation. Upstream, Ach caused a small dilation during control conditions and a marked constriction after NAME treatment. Downstream, Ach-induced dilation was unaffected by the dose of NAME used. The Ach-induced vasodilation may have occurred via different mechanisms upstream compared with downstream, but because the Ach response appears to have been the net result of both dilator and constrictor mechanisms, it may be that the contribution of the two mechanisms varies upstream and downstream or that the inhibition was more complete upstream. The observation of differing responses upstream and downstream is comparable to those in isolated perfused canine lungs in which Ach-induced responses in the arteries and veins were affected differently by methylene blue (28). In isolated canine femoral arterial and venous rings, N^{ω}-monomethyl-L-arginine (another L-arginine analogue capable of blocking NO synthase) inhibited Ach-induced relaxation in arteries but not in veins, whereas a different L-arginine analogue, N^{ω}-nitro-L-arginine, inhibited Ach-induced relaxation in both arteries and veins (29). These findings lead the author to conclude that in the veins synthesis of NO may be associated with several pools of L-arginine or isozymes of NO synthase with which analogues of L-arginine compete and bind differentially (29).

The addition of NAME to the blood led to an increase in Pa during the normoxic ventilation. This indicates that the resistance vessels were at a smaller diameter during normoxia after adding NAME. Therefore, the question arises: Did the increase in the hypoxic response reflect a greater increase in wall tension of the resistance vessels after NAME treatment or was the larger increase in resistance simply because of the vessels starting from a smaller diameter? To answer this question, we can relate the changes that occur in vessel resistance (R) on going from diameter d_n under normoxic conditions to diameter d_h under hypoxic conditions using Poiseuille's law to obtain:

$$\left(\frac{R_n}{R_h}\right)^{0.25} = \left(\frac{d_h}{d_n}\right) \quad (1)$$

The vessel transmural pressure (P) is related to wall tension (T) and diameter by Laplace's law:

$$P = \frac{2T}{d} \quad (2)$$

If, when going from d_n to d_h, the increase in vessel transmural pressure is proportional to the increase in Pa, we then can relate the change in wall tension that occurs going from normoxia to hypoxia by combining equations 1 and 2:

$$\frac{T_h}{T_n} = \left(\frac{Pa_h}{Pa_n}\right)\left(\frac{R_h}{R_n}\right)^{-0.25} \quad (3)$$

When the data shown in Table 1 are used in equation 3, the calculated fractional increase in average wall tension of the resistance vessels would be about 13% going from normoxia to hypoxia without NAME, whereas the calculated increase would be about 42% from normoxia to hypoxia with NAME. This suggests that an increase occurred in the force of the vascular smooth muscle contraction in response to hypoxia after NAME treatment.

There are two basic views on the role of NO synthase in the

hypoxic pulmonary vasoconstrictor response. One is that the hypoxic response is due to a decrease in NO release leading to vasoconstriction. For example, studies on rat (9) and calf (10) pulmonary artery rings have suggested that endothelium-dependent relaxation was preferentially inhibited by hypoxia and that a decrease in endothelial NO production due to hypoxia may contribute to hypoxic vasoconstriction. In isolated pulmonary arterial rings, it was found that hypoxia and inhibition of NO synthase were associated with decreased production of the second messenger of NO, cGMP (8). In cat lungs, the infusion of methylene blue changed the response to hypoxia from constriction to dilation (4). In the rabbit, NO could be detected in the expired air, and both NAME and hypoxia decreased the concentration of NO in the expired air (11). These observations also can be interpreted as consistent with the concept that hypoxic pulmonary vasoconstriction is due to decreased NO release (4, 8–10). The alternative view is that the basal release of NO has simply a modulating effect on the hypoxic vasoconstrictor response or that NO production is stimulated by the hypoxia or its sequelae (3, 5–7, 12, 13). This view appears to be consistent with our findings in the neonatal pig in which the hypoxic response was significantly increased after treatment with NAME. Treatment with NAME has been associated with an increase in the hypoxic response in arterial rings from rat lungs (27) and in rabbit lungs (3, 5). In isolated rat lungs, treatment with either N^{ω}-monomethyl-L-arginine or methylene blue was associated with an increase in the hypoxic pressor response (6, 7, 13). The opposite response in the cat (4) and the rat (6, 7, 13) to the same inhibitor of the NO pathway, methylene blue, suggests that species differences might reconcile the apparently contradictory views.

The site of action of hypoxia was mostly in the vessels upstream of the Pd, a finding that is in agreement with previous studies in adult lungs (18, 30–32). However, there was a significant increase in the resistance downstream of the Pd (Table 1). This is compatible with micropuncture studies in isolated, perfused neonatal pig lungs (33). An increase in downstream resistance might have implications for lung fluid balance. For example, it might contribute to the pathogenesis of postasphyxial lung disease in the neonate.

The neonatal pig lung has been shown to have a similar anatomy and anatomical development compared with the human lung (34), but over a much shorter time course. Therefore, the neonatal pig is a useful model for studying the regulation of the pulmonary circulation during the developmental period, a time when the pulmonary vasculature appears to be very reactive (14). This reactivity may in part be due to alterations in NO synthase activity. We have found that the basal release of NO may be involved in the maintenance of the normally low pulmonary vascular resistance. Furthermore, NO synthase probably played a role in modulating the hypoxic vasoconstrictor response by opposing the vasoconstriction and thereby reducing the magnitude of the response. The action of NAME occurred both upstream and downstream of Pd during control and hypoxic conditions. These results suggest that it may be fruitful to examine the possibility that alterations in the control of NO synthase activity may be an etiologic factor in pulmonary vascular diseases of the newborn and that therapies aimed at NO synthase activity may be beneficial in their treatment. Because the endogenous release of NO can apparently attenuate the hypoxic response, these results are also consistent with the concept that the exogenous administration of NO may be useful for reversing the hypoxic response in instances where it is a contributing factor in persistent pulmonary hypertension of the newborn (14, 35, 36).

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