GENERAL THORACIC SURGERY

THE ROLE OF Nω-NITRO-L-ARGININE IN MODULATION OF PULMONARY VASCULAR TONE IN THE MATURING NEWBORN PIG

Current therapeutic modalities for treatment of newborn pulmonary hypertensive crisis include but are not limited to the administration of nitric oxide (endothelium-derived relaxing factor). However, few data are available on the role of endogenously produced endothelium-derived relaxing factor in the modulation of pulmonary vascular tone in the neonate. In the current study, we investigated the acute effects of Nω-nitro-L-arginine (a potent competitive inhibitor of endothelium-derived relaxing factor synthase) on the pulmonary vasculature of anesthetized open-chest 48-hour-old ($n = 8$) and 2-week-old ($n = 7$) Yorkshire pigs. After baseline data were acquired, all animals received a 10 mg/kg per minute infusion of Nω-nitro-L-arginine for 10 minutes. To discern distal and proximal pulmonary arterial vessel changes, input mean and characteristic impedance were respectively determined. Pulmonary vascular resistance was also calculated (units determined in dyne · sec · cm^{-5} plus or minus the standard error of the mean). Results showed Nω-nitro-L-arginine infusion did not significantly alter baseline pulmonary arterial pressure (22,370 ± 1473 dyne/cm^2), pulmonary vascular resistance (5171 ± 805 dyne · sec · cm^{-5}), input impedance (6343 ± 806 dyne · sec · cm^{-5}), or characteristic impedance (2073 ± 418 dyne · sec · cm^{-5}) in 48-hour-old pigs. In 2-week-old pigs, infusion of Nω-nitro-L-arginine elevated pulmonary arterial pressure (18,162 ± 1415 dyne/cm^2 versus 23,838 ± 1810 dyne/cm^2, $p = 0.015$), pulmonary vascular resistance (810 ± 137 dyne · sec · cm^{-5} versus 1519 dyne · sec · cm^{-5}, $p = 0.030$), and input impedance (2302 ± 251 dyne · sec · cm^{-5} versus 2900 ± 255 dyne · sec · cm^{-5}, $p = 0.018$). Characteristic impedance was not altered in 2-week-old pigs. These data indicate that Nω-nitro-L-arginine infusion resulted in pulmonary arteriolar vasoconstriction in 2-week-old pigs, but not in 48-hour-old pigs. This finding suggests that endothelium-derived relaxing factor does not modulate basal pulmonary arteriolar tone during the early newborn period, but does play a significant role in 2-week-old pigs. These data also suggest that the functional role for endothelium-derived relaxing factor is confined to the distal arteriolar pulmonary bed and does not extend to the larger proximal arterial vessels. (J THORAC CARDIOVASC SURG 1995;110:1486-92)

Patrick W. Domkowski, PhD (by invitation), John T. Cockerham, MD (by invitation), Peter A. Kot, MD (by invitation), Jeff L. Myers, MD (by invitation), Robert B. Wallace, MD, and Richard A. Hopkins, MD, *Washington, D.C.*

From Georgetown University School of Medicine, Departments of Surgery, Physiology and Biophysics, and Pediatrics, Washington, D.C.

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Address for reprints: Richard A. Hopkins, MD, Georgetown University Hospital, 3800 Reservoir Rd., N.W., Washington, DC 20007.

Endothelium-derived relaxing factor (EDRF), first reported by Furchgott and Zawadzki,[1] has long been appreciated to be a potent endogenous and exogenous modulator of both pulmonary and systemic vascular tone in many different species.[1, 2] However, its precise role in modulating the rapid

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maturational changes that occur in the newborn pulmonary circulation remains poorly understood. The newborn mammalian pulmonary circulation undergoes a plethora of significant morphologic and hemodynamic alterations during the first few weeks of extrauterine life. We and others have documented the rapid decline in pulmonary vascular resistance (PVR) and characteristic impedance (Z_o) that occur during the first 2 weeks of extrauterine life.[3, 4] However, the role of endogenously released compounds such as EDRF and its contribution toward these developmental changes remain enigmatic.

Some in vitro studies have demonstrated age-dependent responses to nitric oxide in the piglet[5]; however, few investigations have focused on the intact animal. The purpose of the present study was to determine whether endogenously produced EDRF contributes to the basal level of pulmonary arterial tone in the maturating newborn pig at two distinct age intervals. Specifically, the hemodynamic effects of Nω-nitro-L-arginine (L-NA), a potent competitive inhibitor of nitric oxide synthase, were determined in 2-day-old and 2-week-old neonatal pigs. In addition, to discern differences between proximal and distal pulmonary arterial alterations by L-NA treatment, pulmonary vascular impedance was determined. Calculation of input hydraulic impedance by measurement and analysis of pulsatile pressure and flow waveforms defines total right ventricular energy expenditure, which is dependent on proximal arterial geometry and compliance, as well as the resistance of the distal arteriolar-capillary bed traditionally assessed by PVR.

Methods

Eight 48-hour-old (±4 hours, mean weight 1.5 to 2.8 kg) and seven 2-week-old (mean weight 4.0 to 5.0 kg) Yorkshire pigs of either sex were anesthetized with intravenous thiopental sodium (25 mg/kg). Additional anesthetic was administered as needed. Thiopental sodium did not significantly alter PVR or Z_o or alternatively affect the hemodynamic activity of L-NA. All animals underwent endotracheal intubation and were placed in the supine position. The ear vein was catheterized and an adequate amount of pancuronium bromide (0.1 mg/kg intravenously) was administered to produce complete muscle relaxation. The lungs were mechanically ventilated with an inspired oxygen fraction of 1.0 with a pediatric positive pressure ventilator (Health Dyne model 105, Marietta, Ga.). To avoid the effects of respiratory motion on pulmonary artery pressure and flow, ventilation was briefly interrupted during data collection intervals without any observable changes in pulmonary or systemic hemodynamic parameters. A positive end-expiratory pressure of 3 cm H_2O was maintained intraoperatively to prevent atelectasis.

Surgical techniques and instrumentation of the animal have previously been described in detail.[3, 6] Briefly, after a median sternotomy, the main pulmonary artery was dissected free from the aorta. An ultrasonic flow probe (Transonic Systems, Ithaca, N.Y.) was fitted around the main pulmonary artery. A medium-sized titanium clip (Ethicon, Rochester, N.Y.) was used to occlude the ductus arteriosus. A high-fidelity Millar pressure transducer (model MPC-500, Millar Inc., Houston, Tex.) was passed into the left atrial appendage and a second high-fidelity Millar pressure transducer (model SPC-320) was inserted into the main pulmonary artery. Premeasurement of the catheter length in relation to the main pulmonary artery ensured that the transducer tip was positioned beyond the ultrasonic flow probe. A third high-fidelity pressure transducer was inserted into the right internal carotid artery for measurement of systemic arterial blood pressure. Data analysis and instrument calibration have been described previously.[3, 6]

L-NA (Sigma Chemical Co., St. Louis, Mo.) was solubilized in 0.9% sterile NaCl and administered via the right internal jugular vein. All animals received a 10-minute intravenous infusion of L-NA of 10 mg/kg per minute. On the basis of dose responses reported in other hemodynamic studies, a maximal dose of L-NA was used to saturate the nitric oxide synthase enzyme system. Hemodynamic measurements were collected at 3, 5, and 10 minutes. Data from these three times were averaged inasmuch as there was no significant difference in the response to L-NA among these three times. L-NA is a potent inhibitor of endogenous EDRF production in rabbits, rats, dogs, and pigs and is therefore not species specific.[7]

All experiments were preapproved by the Georgetown Animal Care and Use Committee and all animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

PVR was calculated in the usual fashion:

$$\bar{P}_{PA} - \bar{P}_{LA}/\bar{Q}_{PA}$$

where $\bar{P}_{PA}$ is mean pulmonary artery pressure, $\bar{P}_{LA}$ is mean left atrial pressure, and $\bar{Q}_{PA}$ is mean pulmonary artery flow. Pulmonary arterial impedance calculations were based on Fourier analysis of pressure and flow waves as previously described.[8, 9] Data-collection periods were 30 seconds, and 6 to 10 random heartbeats were analyzed for each period. Ten harmonics were calculated for each heart beat. Total pulmonary flow is expressed as

$$Q_t = Q_m + \sum_{n=1}^{10} Q_n \sin(n\omega t + \theta_n)$$

where Q_m is mean flow, Q_n is amplitude of the nth harmonic, ω is the fundamental angular frequency $2\pi f$ (where f is frequency in hertz), t is the length of the sequence, and θ_n is the phase angle of the nth harmonic. Pressure waveforms are expressed as

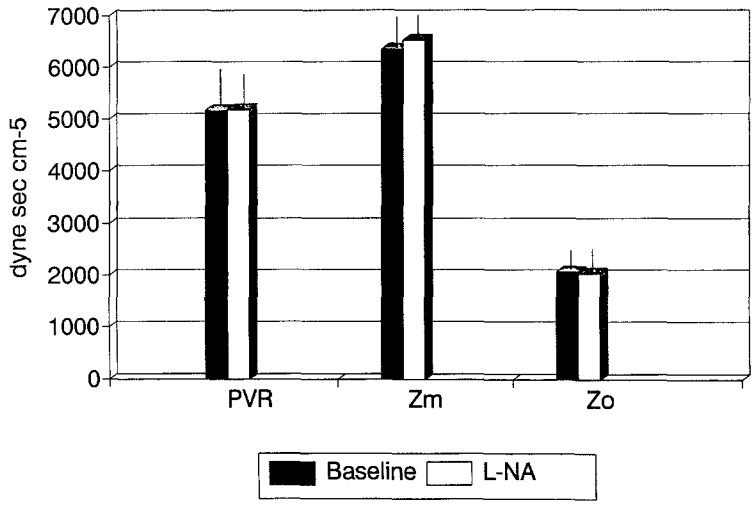

Fig. 1. Bar graph depicting PVR, Z_m, and Z_o at baseline and in response to L-NA infusion in 48-hour-old pigs. Vertical *error bars* represent plus or minus the standard error of mean.

$$P_t = P_m + \sum_{n=1}^{10} P_n \sin(n\omega t + \beta_n)$$

where P_m is mean pressure, P_n is amplitude of the nth harmonic, and β_n is the phase angle of the nth harmonic.

Dividing mean pressure by mean flow (P_m/Q_m) produces the input impedance (Z_m) to mean flow at the zeroth harmonic. Similarly, the division of each of the sinusoidal terms (P_n/Q_n) gives the input impedance for the nth harmonic. The corresponding phase angle (θ_n, depicted in radians in each graph) was calculated from subtraction of the flow phase angle from the pressure phase angle ($\beta_n - \theta_n$). Z_o is defined as the impedance in the absence of wave reflections and was calculated between 2 and 7 Hz. Statistical difference within each group was assessed by a parametric two-tailed Student's *t* test.

Results

Forty-eight-hour-old pigs. L-NA infusion did not significantly alter pulmonary artery pressure (22,370 ± 1473 dyne/cm^2) or pulmonary artery flow (4.0 ml/sec) from baseline values. Mean left atrial pressure was not significantly altered during or after infusion of L-NA. Values of PVR, Z_m, and $Z_{o(2\text{ to }7\text{ Hz})}$ were not changed from baseline during L-NA infusion (Fig. 1). The baseline impedance modulus indicated a first minimum between 2 and 3 Hz with the phase angle becoming positive between 3 and 4 Hz (Fig. 2). During L-NA infusion the first minimum was not significantly altered with no change in magnitude of oscillations throughout the higher frequencies. The phase angle was not altered with L-NA infusion. Heart rate ranged from 1.27 Hz to 3.32 Hz. Aortic pressure increased from baseline values with L-NA infusion (77,025 ± 7429 dyne/cm^2 versus 65,813 ± 4805 dyne/cm^2, $p = 0.007$).

Two-week-old pigs. L-NA infusion elevated pulmonary artery pressure (18,162 ± 1415 dyne/cm^2 versus 23,838 ± 1810 dyne/cm^2, $p = 0.015$) from baseline values by 24%. Pulmonary artery flow (8.4 ± 1 ml/sec) was not significantly altered. Mean left atrial pressure was not significantly changed. PVR and Z_m were augmented during L-NA infusion (Fig. 3). $Z_{o(2\text{ to }7\text{ Hz})}$ was not significantly altered during L-NA infusion (Fig. 3). The baseline impedance modulus indicated a first minimum between 1 and 2 Hz with the phase angle becoming positive between 2 and 3 Hz. During L-NA infusion the first minimum remained between 1 and 2 Hz (Fig. 4). Heart rate ranged from 1.05 Hz to 2.21 Hz. Aortic pressure increased from baseline values significantly during L-NA administration (66,611 ± 7022 dyne/cm^2 versus 51,660 ± 7202 dyne/cm^2, $p = 0.005$).

Discussion

L-NA was first characterized by Ishii and colleagues[10] in 1990 as a potent inhibitor of endogenous EDRF/nitric oxide release. L-NA is an analogue of L-arginine and acts as a competitive inhibitor of nitric oxide synthase. However, L-NA is a significantly more potent EDRF inhibitor compared with N^G-monomethyl-L-arginine.[7, 11] In the current study, infusion of L-NA did not significantly alter pulmonary hemodynamic parameters from baseline values in the 48-hour-old pigs. However, in

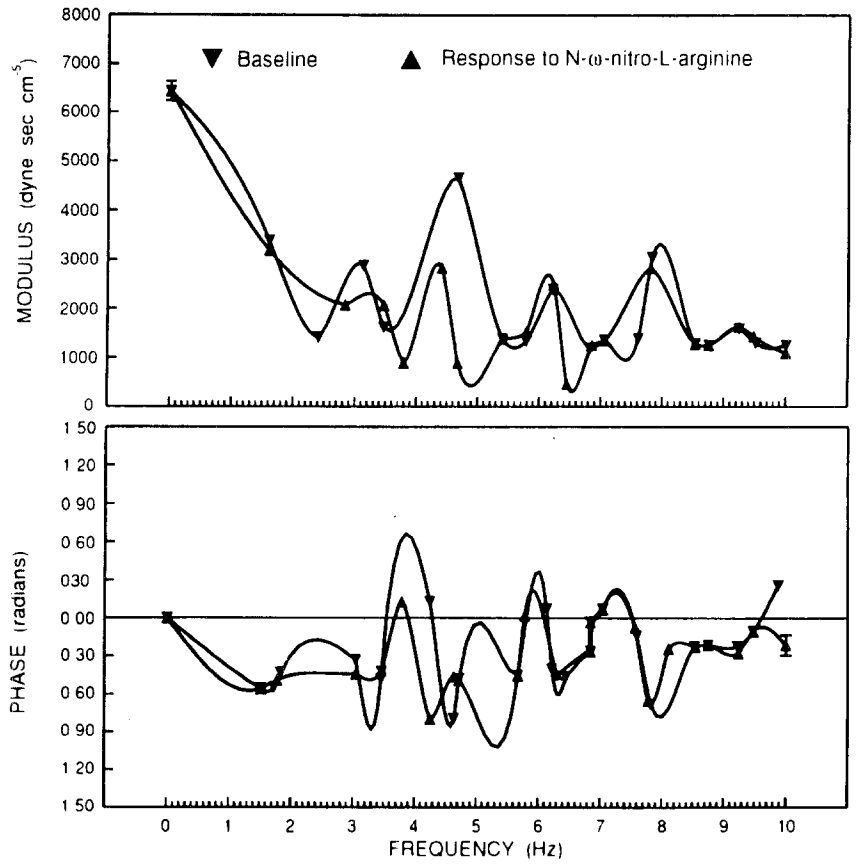

Fig. 2. Impedance moduli and corresponding phase angles for 48-hour-old pigs at baseline and during L-NA infusion. Mean moduli plus or minus standard error of mean at selected frequencies are shown.

2-week-old pigs L-NA administration was associated with pulmonary arteriolar vasoconstriction as reflected by the augmented PVR and Z_m values (Fig. 3). These data indicate that the newborn pulmonary vascular endothelium undergoes significant functional alterations during the first 2 weeks of extrauterine life.

The lack of a vascular response to L-NA at 48 hours of age suggests that EDRF does not contribute to basal pulmonary vascular tone in the very early newborn period. On the other hand, EDRF may be an important modulator of basal vascular tone in the 2-week-old pig pulmonary arterial circulation. Perhaps nitric oxide synthase may not yet be active at birth but becomes functional sometime during the first few weeks of life. Alternatively, the second messenger system activated by EDRF, cyclic guanosine monophosphate via guanylate cyclase, may not yet be fully mature at birth. Zellars and Vanhoutte[5] performed in vitro experiments on isolated newborn pig pulmonary artery rings. They found that endothelium-dependent relaxations to nitric oxide were greater in 30-day-old pig pulmonary artery rings than in 1-day-old pig pulmonary arteries. Our results are consistent with their findings. They hypothesized that during the early newborn period, the pig pulmonary arteries are incapable of synthesizing or releasing EDRF. However, with maturation the production or release of endogenous EDRF is enhanced as manifested by the rise in pulmonary vascular tone after administration of EDRF inhibitors. Unlike the findings reported by Perreault and DeMarte,[12] who demonstrated that L-NA significantly vasoconstricted 1-day-old perfused isolated pig lungs, our data suggest that the basal production and release of EDRF is negligible during the very early newborn period and appears to increase during maturation. The disparity between the data of Perreault and DeMarte[12] and our own data may be explained by the fact that their study was not done in intact newborn pigs. It is not uncommon for in vitro and in vivo data to be

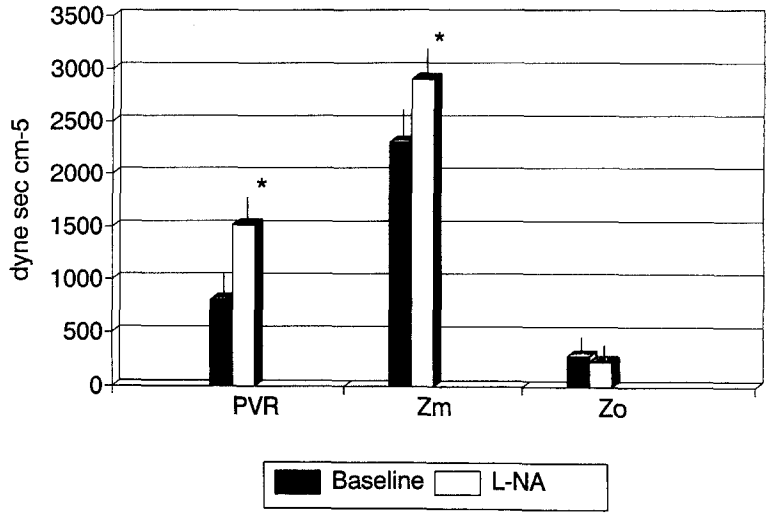

Fig. 3. Bar graph depicting PVR, Z_m, and Z_o at baseline and in response to L-NA infusion in 2-week-old pigs. *Asterisks* indicate $p < 0.05$ versus baseline value. Vertical *error bars* represent plus or minus standard error of mean.

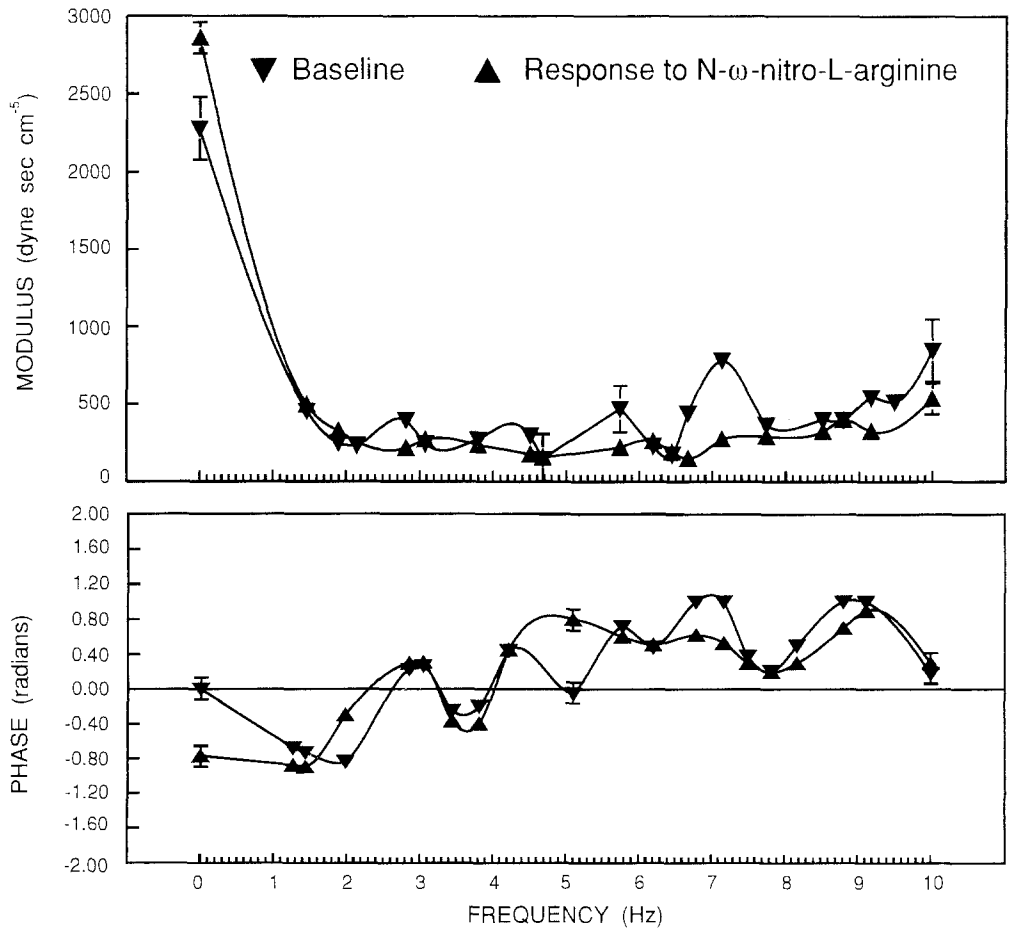

Fig. 4. Impedance moduli and corresponding phase angles for 2-week-old pigs at baseline and during L-NA infusion. Mean moduli plus or minus standard error of mean at selected frequencies are shown.

different, especially in a system like the pulmonary circulation in which there seems to be a multitude of pathways including endothelium-dependent and endothelium-independent mechanisms, as well as more generalized local, regional, and neurohumoral modulation of arteriolar vasomotion.

Although there is increasing evidence that the vascular action of this L-arginine analogue is dependent on the age of the animal, there have been no reports of the effects of L-NA on the pulmonary hydraulic impedance response in either newborn or adult animal models. Unlike PVR measurements, which describe vasomotor activity only in the distal arteriolar region, hydraulic impedance curves also provide information about the larger proximal pulmonary arteries. Impedance analysis considers the pulsatile components of pressure and flow waveforms and thus (see Methods section) is able to distinguish the relative contributions to the morphology of these waveforms by the geometry and viscoelastic properties of the proximal (Z_o) conduit arteries and the distal (Z_m) pulmonary arteriolar-capillary bed.[8] The overall shapes of our baseline impedance moduli and phase angles in both the 48-hour-old and 2-week-old pigs are comparable to those reported in other studies done in both open- and closed-chest animal preparations (Fig. 2 and 4, respectively).[8, 9] However, L-NA by itself had no significant effects on $Z_{o(2\text{ to }7\text{ Hz})}$ or impedance moduli in 48-hour-old pigs. Similarly, infusion of L-NA caused no significant alterations in Z_o or the impedance moduli in 2-week-old pigs. This suggests little or no role for EDRF in modulating tone or geometry of the larger pulmonary arteries in neonates. In contrast, L-NA infusion in both 48-hour-old and 2-week-old pigs in our study resulted in a significant increase in aortic pressure. Thus continuous release of EDRF may be an important modulator of basal vascular tone in the systemic circulation even in the early newborn interval.

In conclusion, L-NA, by itself, did not affect pulmonary hemodynamics in 48-hour-old pigs. However, in 2-week-old pigs L-NA infusion resulted in pulmonary arteriolar constriction. These data also suggest that release of endogenous EDRF does not contribute toward basal pulmonary vascular tone in the early newborn interval (for example, 48-hour-old pigs) but does play a substantial role in regulating basal pulmonary arteriolar tone in older newborn pigs. In addition, this study indicates that the effects of L-NA are mediated through actions that affect predominately the distal arteriolar region and not the proximal pulmonary arteries in 2-week-old Yorkshire pigs. This is supported by the significant increases in PVR and Z_m with no concomitant change in Z_o. Perhaps during the early postpartum interval newborn infants may not be able to endogenously produce, release, or respond to EDRF in the pulmonary circulation and it is only through physiologic maturation of the pulmonary circulation that EDRF activity occurs. With the increasingly frequent use of nitric oxide in newborn patients with pulmonary hypertension, these data suggest that there might be variations in endogenous activity of EDRF in the neonatal circulation that might affect responses to exogenously delivered nitric oxide. Certainly, our data suggest that neonatal immature circulations have diminished endogenous EDFR–mediated vasodilator capacity. It is interesting to speculate that the progressive vasodilation (both geometric and wall compliances changes) characteristic of the maturing neonatal pulmonary circulation is linked to the changing role of EDRF.

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Discussion

Dr. Peter K. Smith *(Durham, N.C.).* I would like to congratulate Dr. Domkowski and his co-authors on a significant addition to their work in characterizing the changes in pulmonary vascular energetics that occur in the maturing lung.

I have several questions. First, these results suggest that the neonatal animal either does not produce EDRF or is not responsive to it. Have the authors exposed neonatal animals to nitric oxide to document that a vasodilatory response is even possible in this model?

Second, there is evidence in isolated neonatal lung systems that nitric oxide synthase inhibition can cause vasoconstriction. Could the authors speculate on the differences in their system that cause this apparent conflict with their data?

Finally, many of the indices that are reported here are related to vascular geometry in a preparation in which the size is doubling in the period studied. Have the authors calculated any geometry-independent indices to more directly demonstrate changes in the vascular elastic properties that occur during the period?

Dr. Domkowski. We have actually in the laboratory looked at exogenous administration of nitric oxide to piglets to see whether this is a maturational-dependent change in the second messenger system or whether in fact the EDRF synthase enzyme is not working. We believe this to be predominantly a situation in which the EDRF synthase enzyme is not yet functional. The reason we believe this is based on preliminary data generated by experiments conducted by Dr. Myers in which he gave nitric oxide at 100 ppm to 48-hour-old pigs and found that in fact there is a significant decrease of approximately 23% in the PVR, which indicates that the second messenger system, cyclic guanosine monophosphate via guanylate cyclase, is in fact intact in 48-hour-old animals and perhaps the EDRF synthase enzyme is not yet functional. The second reason we believe this to be true is based on Zellers and Vanhoutte's data (Pediatr Res 1991;30:176-80) that do demonstrate in isolated segments some degree of relaxation in 3-day-old piglets. Also, however, we did see an increased amount of relaxation in older piglets, which suggests a sensitization or an upregulation perhaps of the second messenger system, cyclic guanosine monophosphate.

With respect to the geometry, we actually have examined the changes, predominantly in the proximal pulmonary vasculature, in both the 48-hour-old animals and the 2-week-old animals, by simultaneously measuring pressure flow and diameter of the main pulmonary artery. We see nearly a 50% increase in the diameter of the pulmonary artery in just over a 12-day period, and this alters the geometry significantly.

Although we saw decreasing Z_o, indicating a decrease in stiffness, we have related these Z_o changes to elastic modulus. We have solved for wave velocity using our Z_o and then, using the Moens-Korteweg equation, solved for the elastic modulus from our wave velocity. We found in fact that in the elastic modulus, the ratio of stress to strain in the vessel, the stress actually went up over this 12-day period just with respect to the baseline value, even though Z_o went down. Thus it seems there are definitely very active changes going on in the proximal pulmonary artery with respect to the collagen-elastin ratio. Perhaps that reflects the increase in the elastic modulus.

As concerns the vasoconstriction seen in some isolated segments in response to nitric oxide, my understanding is that this is also an age-dependent phenomenon and may be reflective of another mechanism that is active in the neonate that is not active in the adult. As a short corollary, we did see age-dependent responses to endothelin, a potent vasoconstrictor, such that it appeared to be not only an initial vasodilator in the 48-hour-old pigs, but also a consistent vasodilator that had an effect throughout the entire period.