MATURATION ALTERS THE PULMONARY ARTERIAL RESPONSE TO HYPOXIA AND INHALED NITRIC OXIDE IN THE PRESENCE OF ENDOTHELIAL DYSFUNCTION

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Surgical intervention in ever younger patients has led to a new appreciation of the unique physiology of the neonate. Specifically, newborn patients may respond very differently to hypoxic episodes and subsequent treatment with inhaled nitric oxide than older infants. In the current study, we examined differences in the pulmonary arterial response to hypoxia and inhaled nitric oxide in 48-hour-old ($n = 8$) and 14-day-old ($n = 8$) Yorkshire pigs in a model of nitric oxide synthase inhibition, as might be seen with endothelial dysfunction. Data were acquired after treatment with the nitric oxide synthase inhibitor *N* ω -nitro-L-arginine during hypoxia (inspired oxygen fraction = 0.10) and during inhalation of nitric oxide (100 ppm). Input mean impedance, reflecting distal arteriolar vasoconstriction, and characteristic impedance, reflecting proximal arterial geometry and distensibility, were calculated. The modulus of elasticity, a measure of the "stiffness" of the proximal vessels, was also calculated. Hypoxia caused a large increase in input mean impedance in both 48-hour-old and 14-day-old pigs (4826 ± 272 versus 8744 ± 488 dyne $\cdot$ cm $\cdot$ sec⁻⁵ and 3129 ± 73 versus 6000 ± 134 dyne $\cdot$ cm $\cdot$ sec⁻⁵, respectively; $p = 0.0078$). Characteristic impedance was not altered in the younger animals (1171 ± 76 dyne $\cdot$ cm $\cdot$ sec⁻⁵) but increased in the older animals (419 ± 15 versus 797 ± 20 dyne $\cdot$ cm $\cdot$ sec⁻⁵, $p = 0.0078$). Older animals also experienced an increase in the modulus elasticity ($1.92\text{E}^{06} \pm 3.2\text{E}^{05}$ versus $1.05\text{E}^{07} \pm 3.9\text{E}^{05}$ dyne/cm², $p = 0.0078$). These data show that inhibited nitric oxide production, as might be seen in endothelial dysfunction, potentiates the profound hypoxic vasoconstriction observed at the level of the distal pulmonary arterioles in both neonatal and infant animals. In contrast, only older animals had a stiffening of the larger, more proximal vessels with hypoxia. In both age groups, inhaled nitric oxide effectively reduced the increases in impedance. (J Thorac Cardiovasc Surg 1997;113:270-7)

Discovery of the endothelium-derived relaxing factor (EDRF) by Furchgott and Zawadzki¹ was the first indication of the importance of the vascular

endothelium in determining both resting hemodynamics and the response to stressors such as hypoxia. However, it is unclear how these responses are affected by the rapid changes occurring in the very early postpartum period. In the first hours and days after birth, pulmonary artery pressure, pulmonary vascular resistance, and impedance fall dramatically, whereas flow rises.² Use of the nitric oxide synthase inhibitor *N* ω -nitro-L-arginine (L-NA) in fetal sheep has been shown to prevent the normal postnatal changes of the pulmonary circuit, indicating the requirement for an intact and functional endothelium for normal maturation.³ One aim of the current experimental series is to determine age-specific differences in the response to hypoxia and inhaled nitric oxide in a model of dysfunctional endothelium. Because both surgical manipulation and car-

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diopulmonary bypass damage the endothelium and inhibit its ability to respond to potential stressors,^{4,5} these studies are of importance in understanding the unique pulmonary arterial circulation of the newborn infant.

Use of hydraulic impedance analysis allows a complete description of the energy contained within the pulmonary arterial pressure and flow waveforms. Energy contained within the pulsatile components of the waveforms can be analyzed and total right ventricular energy expenditure can be defined. Characteristic impedance is determined by the compliance and geometry of the proximal vessels. By measurement of radius and elasticity, it can be determined whether changes in characteristic impedance represent active alterations in the properties of the vessel wall. Input mean impedance is determined by mean terms and represents distal arteriolar vasoconstriction. It is highly analogous to pulmonary vascular resistance.

Materials and methods

All animals received humane care in compliance with the Georgetown Animal Care and Use Committee (GUACUC) and "Principles of Laboratory 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. 85-23, revised 1985).

Surgical preparation. Eight 48-hour-old and eight 14-day-old Yorkshire piglets of either sex were used in this study. The ear vein was catheterized and the animals were anesthetized with intravenous thiopental sodium (25 mg/kg). A half dose of this agent was administered every 20 minutes.

After endotracheal intubation the animals were placed in the supine position and their lungs were ventilated with a pediatric positive-pressure ventilator (Health dyne 105, Marietta, Ga.). Surgical preparation and the collection of baseline data were performed at an inspired oxygen fraction of 1.0; this maintained an arterial oxygen saturation of 95% or greater. Positive inspiratory pressure was preset between 15 and 25 cm H₂O and respiratory rate between 9 and 10 ventilations per minute. These settings achieved an arterial partial pressure of carbon dioxide (Paco₂) of 35 to 45 mm Hg. To prevent atelectasis, a positive end-expiratory pressure of 3 cm H₂O was used.

Pancuronium bromide (0.1 mg/kg intravenously) was given to produce complete muscle relaxation. A titanium clip (Ethicon, Inc., Somerville, N.J.) was used to occlude the ductus arteriosus and separate the pulmonary and systemic circulations. Sonomicrometry crystals for continuous measurement of diameter changes were placed on the lateral aspects of the main pulmonary artery with 4-0 silk sutures. An ultrasonic flow probe (Transonic Systems, Ithaca, N.Y.) was placed around the most proximal portion of the main pulmonary artery. A 6 or 8 mm ultrasonic flow probe (type 65 or 85), chosen for best fit while

avoiding constriction of the vessel, was used. High-fidelity pressure transducers (model SPR-524, Millar Instruments, Inc., Houston, Tex.) were placed into the left atrial appendage and the main pulmonary artery. Each transducer was secured by a 4-0 silk purse-string suture. Premeasurement of the pulmonary artery transducer length in relation to the main pulmonary artery ensured that the tip was positioned 2 mm beyond the flow probe to avoid any perturbation of waveforms. A third transducer was inserted into the right internal carotid artery for measurement of systemic pressures.

Experimental protocol. All animals underwent a 10-minute equilibration period after instrumentation and were then given a 20 mg/kg dose of L-NA (Sigma Chemical Co., St. Louis, Mo.) via the intimal jugular catheter. Baseline data were collected at 3, 5, and 10 minutes after administration of L-NA. Nitrogen was then blended into the ventilator circuit until an inspired oxygen fraction of 0.10 was obtained, and data were again collected at 3, 5, and 10 minutes. While the animals were still hypoxic, nitric oxide infusion was begun through the inspiratory limb of the ventilator at a concentration of 100 ppm (Roberts Oxygen, Rockville, Md.). The dose was chosen on the basis of our own dose-response curves (unpublished data) and the experience of others.⁶ Data collection was repeated at 3-, 5-, and 10-minute intervals. So that the effects of respiratory motion on pulmonary artery pressure and flow could be avoided, ventilation was briefly interrupted during data collection intervals without any observable changes in pulmonary or systemic parameters.⁷ Arterial blood oxygen (Pao₂), Paco₂, and pH were determined with a CIBA Corning analyzer (model 278, Medfield, Mass.) at each "10-minute" interval. Alveolar gas concentration (Pao₂ and Paco₂) and arterial oxygen saturation were constantly monitored (POET II, Criticare Systems, Waukesha, Wis.). Nitric oxide levels were measured in the ventilator tubing as it entered the endotracheal tube with an electrochemical monitor (Pac II NO monitor, Dräger, Inc., Chantilly, Va.). Data analysis and instrument calibration have been previously described.⁸

The dose of L-NA used was determined in a separate series of 48-hour-old ($n = 8$) and 14-day-old ($n = 8$) animals. The response to acetylcholine, an endothelium-dependent vasodilator (10 μ g in 1 ml normal saline solution, given intravenously) and sodium nitroprusside, an endothelium-independent vasodilator (5 μ g in 1 ml normal saline solution, given intravenously) was determined before and after L-NA administration. Response to both drugs was demonstrated by a rise in pulmonary artery flow and a fall in input mean impedance and pulmonary vascular resistance. Blockade of endothelial nitric oxide was considered complete at the dose sufficient to ablate the response to acetylcholine. Specificity of the blockade was demonstrated by preservation of the smooth muscle response to nitroprusside.

Hemodynamic calculations

Pulmonary vascular resistance, input mean impedance, and characteristic impedance. Pulmonary vascular resistance was calculated in the usual fashion:

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

Table I. Response to hypoxia and inhaled nitric oxide

	48-hour-old piglets			14-day-old piglets		
	Baseline	Hypoxia	Nitric oxide	Baseline	Hypoxia	Nitric oxide
PAP	17.4 ± 1.5	30.6 ± 1.5*	16.1 ± 1.1†	12.6 ± 0.7	28.0 ± 0.9†	16.5 ± 1.0†
PAF	5.8 ± 0.9	5.3 ± 0.7	5.2 ± 0.9	7.0 ± 0.5	6.6 ± 0.5	7.3 ± 0.6*
LAP	5.1 ± 0.2	5.3 ± 0.2	5.4 ± 0.3	3.6 ± 0.1	3.8 ± 0.2	3.5 ± 0.2
PVR	3866 ± 239	7683 ± 505†	3541 ± 226†	1504 ± 79	4998 ± 163†	1626 ± 106†
AoP	77.8 ± 3.3	73.9 ± 7.1	70.3 ± 7.6†	86.8 ± 4.1	88.7 ± 6.0	83.9 ± 6.2
O ₂ sat	99.7 ± 0.1	30.8 ± 2.8†	33.5 ± 2.6	98.6 ± 1.1	25.0 ± 1.4†	29.9 ± 3.0
PaO ₂	412.8 ± 42.6	18.3 ± 1.2†	20.2 ± 1.4	364.1 ± 46.0	17.2 ± 0.5†	25.9 ± 3.2†
R _{mn}	3.82 ± 0.21	3.97 ± 0.13	3.91 ± 0.14	4.52 ± 0.21	4.94 ± 0.20†	4.89 ± 0.20

Values presented as mean ± standard error of the mean. PAP, Pulmonary artery pressure (mm Hg); PAF, pulmonary artery flow (ml/sec); LAP, left atrial pressure (mm Hg); PVR, pulmonary vascular resistance (dyne · cm · sec⁻⁵); AoP, aortic pressure (mm Hg); O₂ sat, translingual arterial oxygen saturation; PaO₂, arterial partial pressure of oxygen (mm Hg); R_{mn}, radius of main pulmonary artery at mean pulmonary arterial pressure (mm).

*P < 0.05 versus preceding intervention.

†P < 0.01 versus preceding intervention.

where P_{PA} is the mean pulmonary artery pressure, P_{LA} is the mean left atrial pressure, and Q_{PA} is the mean pulmonary artery flow. Pulmonary arterial impedance calculations were based on a Fourier analysis of pressure and flow waves as previously described.⁸ Data collection periods were 30 seconds long, and 8 to 16 random heartbeats were analyzed for each data interval for each pig. Ten harmonics were calculated for each heartbeat. Total pulmonary flow is expressed as follows:

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

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

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

where P_m is the mean pressure, P_n is the amplitude of the nth harmonic, and β is the phase angle of the nth harmonic. Dividing mean pressure by mean flow produces the input impedance to flow at the 0th harmonic. Similarly, the division of each of the sinusoidal terms gives the input impedance for the nth harmonic. The corresponding phase angle (θ_n) was calculated from subtraction of the flow phase angle from the pressure phase angle. Characteristic impedance is defined as the impedance in the absence of wave reflections and was calculated between 3 and 10 Hz.

Derivation of instantaneous elasticity measurements. Wave velocity (C₀) was calculated for the main pulmonary artery assuming the relationship Womersley⁹ derived between characteristic impedance (Z₀), wave velocity, and radius (R) of a strongly tethered elastic tube:

$$Z_0 = \frac{\rho C_0}{\pi R^2 \sqrt{1 - \sigma^2}} \cdot \frac{1}{M_{10}} \cdot e^{-j\epsilon/2}$$

where ρ = 1055 gm/ml, which is the density of blood; σ = 0.5, which is Poisson's ratio, and j = √-1. M₁₀ and ε are functions of Womersley's nondimensional parameter α:

$$\alpha = R \sqrt{\omega \frac{\rho}{\mu}}$$

where μ = 0.04 poise, which is fluid viscosity.

With the use of the calculated Womersley wave velocity given earlier, a value for the elastic modulus (E_y) is determined by substitution into the Moens-Korteweg equation⁸:

$$C_0 = \sqrt{\frac{E_y h}{\rho 2R}}$$

where h = wall thickness. A paired two-tailed nonparametric test (Mann-Whitney U test) was used to determine statistical significance within groups. A p value equal to or less than 0.05 was considered statistically significant.

Results

Hypoxia. Hypoxia significantly altered baseline pulmonary arterial hemodynamics in both groups of animals. Hypoxia caused large increases in pulmonary artery pressure in both age groups. Pulmonary artery flow and left atrial pressure were not altered in either group. The net result was a large increase in pulmonary vascular resistance in each group (Table I). The increase in input mean impedance in 48-hour-old and 14-day-old animals (81% vs 92%, p = 0.94) was similar. Characteristic impedance, modulus of elasticity, and the pulmonary artery radius at mean pulmonary artery pressure were unchanged in the 48-hour-old piglets. In contrast, characteristic impedance increased by 90%, and modulus of elasticity increased by nearly 400% in the older animals (Fig. 1). This was accompanied by

Fig. 1. Impedance and modulus of elasticity response to hypoxia and nitric oxide. Groups represent input mean impedance (*Zm*), characteristic impedance (*Zo*), and modulus of elasticity (*Ey*). Double asterisks indicate $p < 0.01$ versus preceding intervention. Vertical error bars represent plus or minus standard error of the mean. Baseline data were obtained after L-NA administration.

a significant, but smaller (9%) increase in the radius at mean pulmonary artery pressure in the older animals (Table I). Baseline values for pH (7.42 ± 0.03 , 48-hour-old animals) and (7.39 ± 0.03 , 14-day-old animals) and $Paco_2$ (35.9 ± 1.8 mm Hg, 48-hour-old animals) and (37.2 ± 1.5 mm Hg, 14-day-old animals) were not altered. Aortic pressure was not changed, whereas oxygen saturation and Pao_2 fell with hypoxia in both age groups (Table I).

Nitric oxide. Inhaled nitric oxide lowered pulmonary artery pressure and pulmonary vascular resistance in both age groups. Pulmonary artery flow was not altered by nitric oxide in the 48-hour-old animals but increased in the older animals. Left atrial pressure was not altered in either group (Table I). Nitric oxide caused input mean impedance to fall to

baseline levels in 48-hour-old animals and to fall significantly below baseline values (2449 ± 54 vs 3129 ± 73 , $p = 0.016$) in older animals. There was no alteration in characteristic impedance, modulus of elasticity, or the radius at mean pulmonary artery pressure in the 48-hour-old animals. In 14-day-old animals nitric oxide decreased characteristic impedance and modulus of elasticity to baseline levels. However, the radius at mean pulmonary artery pressure was not altered by nitric oxide inhalation. Hypoxia caused Pao_2 and oxygen saturation to fall, whereas aortic pressure was unaltered (Table I). Neither pH (7.40 ± 0.03 , 48-hour-old animals; 7.38 ± 0.03 , 14-day-old animals) nor $Paco_2$ (38.1 ± 2.3 mm Hg, 48-hour-old animals; 39.9 ± 1.9 mm Hg, 14-day-old animals) was altered.

Fig. 2. Impedance moduli and corresponding phase angles for 48-hour-old piglets at baseline (after L-NA), during hypoxic inhalation, and during nitric oxide (NO) inhalation. Mean moduli plus or minus standard error of the mean at selected frequencies are shown. Input mean impedance is at 0 Hz and characteristic impedance is between 3 and 10 Hz.

Discussion

Current understanding of the importance of the vascular endothelium stems from the pioneering work of Furchgott and Zawadzki¹ in 1980. Their discovery that acetylcholine-induced vasodilatation required an intact endothelium was attributed to the presence of a labile humoral factor released from the endothelial cell. EDRF was subsequently identified as nitric oxide or a closely related compound.¹⁰ Its synthesis from the amino acid arginine and the ability to inhibit its production with analogs of L-arginine (i.e., L-NA) represent a powerful, easily exploited system for studying the role of endogenously produced nitric oxide in modulating resting hemodynamics and pharmacologic responses.^{11, 12}

Hypoxia and its potential for precipitating pulmonary hypertensive crises in neonates has long been appreciated by cardiac surgeons.¹³ Less clear is how the events associated with normal maturation affect this response. Several *in vitro* studies have indicated

that the endothelium is not fully responsive at birth but rather matures to a fully functional state during the first few days after birth. Abman and associates¹⁴ showed that pulmonary artery rings from fetal sheep have less EDRF activity than do the rings of post-natal animals.¹⁴ Zellers and Vanhoutte¹⁵ demonstrated in piglet pulmonary artery rings an increase in endothelium-dependent contractions with increasing age in the early postnatal period. These investigators and others have also shown that the response to sodium nitroprusside (an endothelium-independent vasodilator) is not affected by age, indicating early and complete function of the vascular smooth muscle. This is confirmed in our intact animal preparation as inhaled nitric oxide, an endothelium-independent vasodilator, decreased pulmonary vascular resistance and input mean impedance in both age groups despite inhibition of nitric oxide synthase. This response suggests the guanylate cyclase enzyme in the vascular smooth muscle is functional and capable of producing cyclic

Fig. 3. Impedance moduli and corresponding phase angles for 14-day-old piglets at baseline (after L-NA), during hypoxic inhalation, and during nitric oxide (NO) inhalation. Mean moduli plus or minus standard error of the mean at selected frequencies are shown. Input mean impedance is at 0 Hz and characteristic impedance is between 3 and 10 Hz.

guanosine monophosphate regardless of age. Therefore, maturational differences in the guanylate cyclase/cyclic guanosine monophosphate system are unlikely to be responsible for the age-related differences observed in the characteristic impedance response.

The study of pulmonary vascular impedance allows for a more complete analysis of the energy contained within the pulmonary arterial pressure and flow waveforms. Interpretation of these frequency-dependent measurements allows for a regional analysis of the vascular bed being studied. Input mean impedance occurs at 0 Hz and is therefore highly analogous to the nonpulsatile term *pulmonary vascular resistance*. Both groups underwent large increases in input mean impedance with hypoxia, indicating vasoconstriction at the level of the distal arterioles (Figs. 2 and 3). On exposure to inhaled nitric oxide, both groups experienced arteriolar vasodilation. Input mean impedance and pulmonary vascular resistance re-

turned to prehypoxic levels, indicating an ability of the exogenously applied nitric oxide to functionally replace the endogenous nitric oxide. In 14-day-old animals the input mean impedance actually fell below prehypoxic levels (see Fig. 1). This may represent a tone-dependent difference in the response to inhaled nitric oxide, with older animals being more responsive. A second explanation is suggested by the work reported by Moncada and associates¹⁶ in rat aortic rings. In these experiments an acute and very rapid supersensitivity to exogenously administered nitric oxide occurred after inhibition of endothelium-derived nitric oxide. This phenomenon has been described during exposure to an exogenous mediator after removal of the endogenous form and is primarily a result of a specific supersensitivity at the level of the effector protein—soluble guanylate cyclase in our experiments. This would explain not only the large fall in input mean impedance in the 14-day-old animals, but also the

observation by other investigators using intact animal preparations that inhaled nitric oxide does not vasodilate the nonhypoxic/nonhypercapnic pulmonary arterial circulation.¹⁷ The presence of a functional endothelium in these animals precludes the development of supersensitivity and may be reflected in the relative unresponsiveness to inhaled nitric oxide.

Characteristic impedance, that portion of the impedance modulus graph between 3 and 10 Hz, is not influenced by reflected waves and therefore more accurately reflects changes in the geometry and compliance of the larger, more proximal pulmonary vessels. In our experiments, 14-day-old animals underwent a near doubling of characteristic impedance with hypoxia (Fig. 1), representing either a "stiffening" (increased modulus of elasticity) of the vessels or true vasoconstriction, in which the radius of the vessel is decreased. These older animals, in fact, underwent an increase in radius caused by the increased transmural distending pressure represented by the elevated pulmonary artery pressure. The younger animals, operating at a higher resting tone, with a less compliant vessel wall, do not undergo this passive distention. The increase in characteristic impedance seen in the older animals is therefore explained by the increase in the vessel wall stiffness (modulus of elasticity) that we measured. This represents a disadvantageous coupling between the right ventricle and the main pulmonary arteries. The importance of this effect can be seen when nitric oxide is applied and the fall in characteristic impedance and modulus of elasticity are accompanied by a significant improvement in pulmonary artery flow. These changes are not observed in the already elevated characteristic impedances of the neonatal animals and likely represent a maturational phenomenon related to the normal functional and structural changes that occur in this period of development.

These findings hold important implications in the management of very young patients requiring cardiac surgery. The presence of congenital cardiac defects and pulmonary hypertension have been shown to cause pulmonary artery endothelial cell abnormalities in human beings.¹⁸ In addition, both surgical manipulation and cardiopulmonary bypass further damage the endothelium.⁵ Differences in the response of neonatal and infant piglets with a dysfunctional endothelium in this study serve to emphasize the disparity in very young patients and in patients only hours or days

older. Our studies indicate that older animals undergo an increase in stiffness of the proximal pulmonary arteries not seen in the neonatal animals. This additional deleterious effect of hypoxia in the older animals may explain the observation by Hanley and associates¹⁸ that infants with truncus arteriosus older than 30 days at the time of surgical repair are more likely to have postoperative courses complicated by pulmonary hypertension. Future studies may define the differences in pathways responsible for these unique responses and allow tailoring of treatment for patients of different physiologic ages.

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