Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth

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ABMAN, STEVEN H., BARBARA A. CHATFIELD, STACIA L. HALL, AND IVAN F. McMURTRY. Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. Am. J. Physiol. 259 (Heart Circ. Physiol. 28): H1921-H1927, 1990.—To examine the potential role of endothelium-derived relaxing factor (EDRF) in regulation of the perinatal pulmonary circulation, we studied the hemodynamic effects of a selective inhibitor of EDRF production, nitro L-arginine (L-NA), on pulmonary vascular tone and dilator reactivity in the late-gestation ovine fetus and on the pulmonary vasodilation that normally occurs at birth. L-NA infusion decreased pulmonary blood flow from 78 ± 8 to 65 ± 6 ml/min (P < 0.01) and increased pulmonary artery pressure from 48 ± 2 to 54 ± 3 mmHg (P < 0.002, n = 8 animals). To study the selectivity of L-NA on vasodilator responses to endothelium-dependent (acetylcholine) and -independent (atrial natriuretic factor) stimuli, we measured responses to brief infusions of each dilator before and after L-NA treatment. Acetylcholine increased pulmonary blood flow during the control period but not after L-NA treatment. In contrast, L-NA had little effect on the vasodilator response to atrial natriuretic factor. To study the role of EDRF in the transition of the pulmonary circulation from fetal to neonatal conditions, we infused L-NA into the left pulmonary artery immediately before cesarean-section delivery. In comparison with control animals, the rise in pulmonary blood flow at 1 h after delivery was reduced in the L-NA-treated animals (331 ± 28 in control vs. 185 ± 16 ml/min in treated, P < 0.001). We conclude that L-NA causes fetal hypertension, selectively inhibits endothelium-dependent pulmonary vasodilation in the late-gestation fetus, and attenuates the rise in pulmonary blood flow at delivery. These findings suggest that EDRF activity is present in the ovine fetal lung and may contribute to postnatal adaptation of the pulmonary circulation at birth.

dontrictors, including endothelin (7). The endothelium responds to multiple physiological and pharmacological stimuli by releasing vasoactive mediators, thereby providing a local mechanism for regulating vascular tone. For example, changes in hemodynamic forces, including increases in flow or shear stress, can stimulate prostacyclin (35) or EDRF (34) release, leading to vasodilation. In addition, vasodilator responses to many pharmacological agents, including acetylcholine, require the presence of an intact endothelium (16), whereas other drugs, including atrial natriuretic factor, do not (30). Recent work has identified EDRF as nitric oxide, or a nitric oxidelike compound (32), produced by a soluble NADPH- and Ca²⁺-dependent enzyme that converts L-arginine to L-citrulline (31). Enantiomeric analogues of L-arginine, including N⁰-monomethyl-L-arginine and N⁰-nitro-L-arginine (L-NA), can competitively inhibit this reaction and decrease EDRF production (19, 27, 28, 33).

Although several studies have demonstrated the role of EDRF in the adult circulation, little is known about the potential role of EDRF in the perinatal setting (12). In the normal fetus, blood flow to the lung is low, constituting <10% of combined ventricular output (13). With the onset of birth, the pulmonary circulation undergoes a dramatic transition, with blood flow increasing up to 10-fold and pulmonary arterial pressure falling from systemic levels in the fetus to one-half systemic values within hours (9, 10, 13). Mechanisms that contribute to postnatal adaptation of the pulmonary circulation are incompletely understood. Increases in oxygen tension, establishment of a gas-liquid interface, ventilation, and release of vasoactive mediators play important roles in the transition (8-11, 20, 22, 23). The pulmonary circulation also undergoes a dramatic "structural adaptation," as the endothelium appears mechanically flattened or stretched within minutes of birth (18), probably secondary to vascular dilation, distension, and recruitment. Whether alterations in endothelial function, such as an augmented release of EDRF, accompany these structural changes, or even if the fetal lung is capable of EDRF activity, are not known.

In light of the dramatic increase in flow and structural...
changes in pulmonary endothelium at birth, we hypothesized that EDRF release may contribute to the transition of the pulmonary circulation from its fetal to newborn state. To approach these questions, we studied the hemodynamic effects of EDRF inhibition with an analogue of L-arginine, L-NA (19, 27, 28). In particular, we asked the following questions. First, does endogenous EDRF influence the basal tone of the pulmonary circulation in the late-gestation fetus? Second, is EDRF activity increased by a putative endothelial-dependent dilator in the fetal pulmonary circulation? Finally, does EDRF contribute to the decline in pulmonary artery pressure and the rise in pulmonary blood flow at birth?

**Methods**

**Surgical Preparation**

All procedures and protocols used in these studies were previously reviewed and approved by the Animal Care Committee at the University of Colorado Health Sciences Center. Fourteen mixed-breed pregnant ewes (Columbia-Rambouillet) between 125 and 142 days gestation (full term, 147 days) were fasted for 48 h before surgery. Ewes were sedated with pentobarbital sodium (total dose ranged between 2.3 and 4 g iv, depending on the length of the procedure) and anesthetized with 1% tetracaine hydrochloride (3 mg) by lumbar injection. Ewes were maintained deeply sedated but capable of spontaneous respiration, as monitored by a technician not performing the surgery. A hysterotomy was performed under sterile conditions, and the fetal left forelimb was externalized. A skin incision under the left forelimb was made after local infiltration with lidocaine (3–6 ml of a 1% solution). Polyvinyl catheters were advanced into the ascending aorta and superior vena cava via the axillary artery and vein under direct visualization. The heart and great vessels were exposed through a left thoracotomy. Catheters were inserted into the left and main pulmonary arteries by direct puncture through purse-string sutures, as previously described (1, 4, 5). Left atrial catheters were placed in three animals. A cuff-type electromagnetic flow transducer (C and C Instruments, Culver City, CA) was placed on the left pulmonary artery to measure blood flow to the left lung. The diameters of the transducers ranged between 3.5 and 6.0 mm and were selected to allow sufficient contact with the left pulmonary artery while avoiding significant compression, as judged by the quality of the intraoperative phasic blood flow signal. A catheter was placed in the amniotic cavity to measure pressure.

**Physiological Measurements**

Left pulmonary artery blood flow was measured after attaching the transducer cables to an internally calibrated flowmeter (Micron Instruments, Los Angeles, CA), providing a continuous measurement of flow. Calculations for the absolute values for flow were determined from the phasic flow signal tracing, as previously demonstrated (24). Main pulmonary artery, aortic, left atrial, and amniotic catheters were connected to a Gould-Statham P23 ID pressure transducer. Pressures were reference to amniotic cavity pressure. Calibrations of pressure transducers were performed with a mercury column manometer. Heart rate was determined from phasic pulmonary blood flow tracings. Arterial PO₂, PCO₂, and pH were measured in samples obtained from the main pulmonary artery and aorta (Radiometer, model BM53, Mark 2, Copenhagen, Denmark). Oxygen saturations were measured by oximeter (Radiometer, OSM-2).

**Experimental Design**

**Protocol 1.** Hemodynamic effects of intrapulmonary infusion of L-NA in late-gestation fetal lambs (n = 8 animals; gestational ages, 125-142 days). After at least 90 min recovery from fetal surgery, hemodynamic, blood gas tension and pH, and hematocrit values were measured during a 30-min baseline period. L-NA (Aldrich, Milwauk ee, WI) was dissolved immediately before use for each experiment and was infused into the left pulmonary artery at 1–3 mg/min for 10 min. This dose was determined empirically, based on preliminary studies in fetal lambs. Hemodynamic and arterial blood gas tension and pH measurements were repeated 10 min after the L-NA infusion was completed. In three animals, measurements were continued for up to 120 min to examine the duration of hypertension after L-NA infusion (1 mg/min for 30 min). L-Arginine (30 mg) was infused into the left pulmonary artery after L-NA to determine if the effects of L-NA were reversible (n = 3 fetuses). In a single fetus, nitro-D-arginine (D-NA, 30 mg; Bachem, Torrance, CA) was infused into the left pulmonary artery over a 30-min period to test the specificity of the enantiomeric analogue of the arginine analogue on the hemodynamic response.

**Protocol 2.** Effects of L-NA treatment on fetal pulmonary vascular responses to intrapulmonary infusions of acetylcholine and atrial natriuretic factor (n = 6 animals; gestational ages, 129–142 days). After at least 30 min of stable baseline measurements, acetylcholine (1.5 μg/min, Sigma Chemical) or atrial natriuretic factor (0.3 μg/min. Sigma) was infused into the left pulmonary artery at 0.1 ml/min for 15 min. All drugs were prepared immediately before study. Selection of doses was based on past studies demonstrating an approximate doubling of left pulmonary arterial blood flow without significant systemic hemodynamic changes (2, 5). After at least 30 min of recovery, the second vasodilator was infused. L-NA (1 mg/min for 10 min) was infused, as described above, and then repeat infusions of acetylcholine and atrial natriuretic factor were given. No tachyphylaxis is observed with 30–60 min recovery times between short infusions of either acetylcholine or atrial natriuretic factor (2, 5). The order of drug infusion was random.

**Protocol 3.** Hemodynamic effects of L-NA treatment on the response to cesarean-section delivery (n = 7 control and 6 treated animals; gestational age, 139–145 days). During the 90-min recovery period after placement of fetal catheters and transducer (as described above), the umbilical-placental circulation was left undisturbed and the fetuses were kept warm (>39°C, rectal). Serial arterial blood gas tensions and pH and continuous hemodynamic measurements, including phasic and mean left pulmonary artery blood flow, and pulmonary and aortic
pressures, were measured during the recovery, baseline, and study periods. Pressures were referenced with the transducer at midchest level. After a 30-min baseline period, saline (control) or L-NA (1 mg/min for 30 min) was infused at 0.1 ml/min into the left pulmonary artery. After pancuronium bromide (1 mg, axillary vein) was administered, the head was withdrawn from the uterus and a 5.0 cuffed endotracheal tube was placed by tracheostomy. Tracheal fluid was allowed to drain by gravity. Ventilation was initiated with a time-cycled, pressure-limited ventilator, at the following settings: peak inspiratory pressure, 24–28 cmH₂O; positive end-expiratory pressure, 4 cmH₂O; rate, 20 breaths/min; inspiratory to expiratory ratio, 1:3; and fraction of inspired oxygen concentration (FiO₂), 1.00. The umbilical cord was clamped and ligated after the initiation of ventilation. Hemodynamics, arterial blood gas tensions, and pH were measured for 1 h. Ventilator settings and FiO₂ were adjusted to maintain PaO₂ > 70 and PaCO₂, between 35 and 45 Torr. Sodium bicarbonate was not administered to adjust arterial pH. Animals were killed with high-dose pentobarbital or a euthanasia compound (T-61; Hoechst, Somerville, NJ) after completion of the studies.

Statistical analysis was performed by paired t tests (protocol 1) and one- or two-factor repeated-measures analysis of variance (for protocols 2 and 3). P values < 0.05 were considered significant.

RESULTS

L-NA (1–3 mg/min for 10 min) decreased left pulmonary artery blood flow and increased pulmonary and systemic arterial blood pressure (Fig. 1). Elevations of mean pulmonary artery and aortic pressures were observed in each animal. As a group, mean pulmonary artery pressure increased from 48 ± 2 to 54 ± 3 mmHg (P < 0.002) and left pulmonary artery blood flow decreased from 78 ± 8 to 65 ± 6 ml/min (P < 0.01) by 10 min after stopping L-NA administration. Changes in aortic pressure paralleled the rise in pulmonary artery pressure, and the gradient between mean pulmonary arterial and aortic pressures did not change. This suggests that L-NA did not significantly increase tone of the ductus arteriosus. Initial arterial blood gas tensions and pH (PaO₂, 21 ± 4 Torr; PaCO₂, 46 ± 2 Torr, and pH, 7.35 ± 0.02 U) did not change during or after L-NA infusion. At 10 min after the infusion, heart rate was not different from baseline values (193 ± 8 beats/min).

As illustrated in Fig. 1, L-NA infusion into the left pulmonary artery caused a slow, progressive rise in pulmonary and aortic pressures. The hypertension was sustained, with pulmonary and systemic pressures remaining elevated up to 120 min after 30 min infusions of L-NA (1 mg/min, n = 3 animals). In these animals, pulmonary artery pressure progressively increased from 45 ± 4 (baseline) to 62 ± 5 mmHg at 120 min. In a single 134-day gestation fetus, infusion of D-NA (1 mg/min for 30 min) had no effect on pulmonary artery and aortic pressures. Although L-arginine (30 mg) infusions had no effect on baseline pressures and flow, L-arginine rapidly attenuated the hypertension and increased blood flow when administered after infusion of L-NA (n = 2 fetuses, data not shown).

As illustrated in Fig. 2, L-NA attenuated fetal pulmonary vasodilation to the endothelium-dependent stimulus acetylcholine (1.5 μg/min for 15 min) but not to the endothelium-independent dilator atrial natriuretic factor (0.3 μg/min). Initially, acetylcholine increased left pulmonary artery blood flow without altering pulmonary artery and aortic pressure (Fig. 3, top). L-NA infusion inhibited the response to acetylcholine, with no vasodilation observed during its repeat infusion. Mean pulmonary artery pressure, which increased after L-NA treatment, did not change during the second acetylcholine infusion. Atrial natriuretic factor increased baseline flow (Fig. 3, top), and, unlike the inhibition of acetylcholine induced vasodilation, the response of pulmonary blood flow to the repeat infusion of atrial natriuretic factor was not significantly decreased. Mean pulmonary artery and aortic pressures, arterial blood gas tensions, and pH were
not different during the first and second atrial natriuretic factor infusions.

The hemodynamic effects of L-NA after cesarean-section delivery of fetal lambs are shown in Fig. 4. In seven control animals, pulmonary artery pressure decreased from 48 ± 5 to 32 ± 8 mmHg (P < 0.001), mean aortic pressure increased from 46 ± 5 to 52 ± 4 mmHg (P < 0.05), and left pulmonary artery blood flow increased from 71 ± 6 to 331 ± 28 ml/min (P < 0.001) by the end of the study period (1 h after delivery). In the L-NA-treated animals (n = 6), mean pulmonary artery pressure increased from 50 ± 2 to 58 ± 4 mmHg (P < 0.05) and aortic pressure rose from 48 ± 2 to 56 ± 4 mmHg (P < 0.05) after L-NA infusion but before delivery. With delivery and ventilation, mean pulmonary artery pressure decreased to 36 ± 4 mmHg (P < 0.001 vs. before delivery), and aortic pressure was 61 ± 6 mmHg (P = NS vs. before delivery). Although pulmonary blood flow increased from 75 ± 7 to 184 ± 17 ml/min at 60 min in the L-NA-treated animals, this was only 56% of the
DISCUSSION

Based on previous studies demonstrating selective inhibition of EDRF by enantiomeric-specific analogues of L-arginine (19, 27, 28, 33), we examined the effects of L-NA on the ovine fetal and transitional pulmonary circulations. When administered as a brief infusion under basal conditions, L-NA caused the gradual onset of sustained pulmonary and systemic hypertension and decreased pulmonary blood flow. In addition, L-NA blocked fetal pulmonary vasodilation to an endothelium-dependent stimulus acetylcholine but had little effect on the endothelium-independent agent atrial natriuretic factor.

Also, pretreatment with L-NA attenuated the rise in pulmonary blood flow that accompanies normal delivery and ventilation. These findings support the hypothesis that the late-gestation fetal lung is capable of releasing EDRF under basal and stimulated (acetylcholine-induced) conditions and that EDRF may contribute significantly to the regulation of vascular tone and reactivity in the normal fetus and newborn.

This study is the first in vivo demonstration of EDRF activity on fetus. Although most previous studies of EDRF have been in adult circulations, EDRF activity has been previously demonstrated in vitro in pulmonary artery rings from the ovine fetus (3) and newborn piglets (12), suggesting that it could contribute to control of fetal pulmonary vascular tone and reactivity and to the transition of the pulmonary circulation at birth. Past studies have shown fetal pulmonary vasodilation to several pharmacological agents, including acetylcholine, bradykinin, and histamine (6, 8, 13, 20). In other experimental settings these agents have been found to require an intact endothelium to mediate vasodilation (16). If EDRF activity in the developing ovine fetal lung parallels the vasodilator response to acetylcholine (24), it appears the capacity to release or respond to EDRF increases with advancing gestational age. Although changes in EDRF activity with maturation are poorly understood, in vitro studies of intralobar (conducting) pulmonary arteries from fetal, neonatal, and adult sheep suggest the capacity to release EDRF also increases during postnatal life (3). In comparison with newborn (1–4 wk) and adult pulmonary arteries, fetal arteries had similar responses to the endothelium-independent dilator sodium nitroprusside but markedly diminished relaxation to the endothelium-dependent stimuli acetylcholine, adenosine diphosphate, and A23187. In addition, augmentation of phentolamine by the EDRF inhibitor hemoglobin was less in fetal than postnatal pulmonary arteries. Mechanisms contributing to these apparent age-related differences and their physiological relevance to control of fetal and newborn pulmonary vascular tone and reactivity are unclear. Interestingly, there is diminished EDRF activity in adult arteries exposed to low Po2 (21) and hypertension (25) and augmented EDRF activity with chronic elevation of flow (26). Whether these same mechanisms are operative in the normal fetal pulmonary circulation, which is characterized by low Po2, high pressure, and low blood flow, has not been studied.

It has been suggested that establishment of an air-liquid interface, ventilation, increased Po2, increased flow, and/or the release of chemical mediators, such as bradykinin and prostacyclin, contribute to the decline in pulmonary vascular resistance at birth (6, 8–11, 13, 18, 20, 22, 23). We observed that L-NA attenuated the rise in pulmonary blood flow after delivery, suggesting EDRF also contributes to the transition of the pulmonary circulation from its fetal to neonatal state. Whether more complete inhibition of the transition would have been observed with higher doses of L-NA is not known. In addition, it is not known which of the physiological stimuli at birth might directly stimulate EDRF activity. With the marked change in endothelial cell shape (18) and dramatic increase in flow or shear stress (26, 34) at
 born, it is not surprising that endothelium-derived mediators are released. Because hypoxia impairs EDRF release and/or action in adult pulmonary arteries (27), it is also possible that the increased PO₂ in the neonate contributes to enhanced EDRF.

Previous in vivo studies of adult animals report decreased heart rate and presumably decreased cardiac output secondary to the hypertensive response to Nω-monomethyl-L-arginine (33). Although decreased cardiac output secondary to the hypertension may have contributed to the abnormal transition with delivery after L-NA treatment in our study, heart rate, but not pulmonary blood flow, was not different from controls at 1 h after delivery.

Our findings suggest that although EDRF activity is stimulated at birth, it is not solely responsible for the decline in pulmonary resistance. Previous work suggests the release of another endothelium-derived product, prostacyclin (PGI₂), contributes to transition of the pulmonary circulation in newborn lambs and goats (11, 22, 23). Davidson (11) and Leffler et al. (23) found that cyclooxygenase inhibition attenuated the normal decline in pulmonary vascular resistance at birth, but the relative contribution of PGI₂ in comparison with other dilator stimuli at birth is uncertain, because the latter study demonstrated a greater physiological role for PGI₂ than the former. Our study suggests that EDRF activity may contribute to these pulmonary vascular changes as well. We speculate the release of EDRF and PGI₂ may act synergistically to augment vasodilation to specific stimuli such as increased flow (26, 34, 35) or ventilation (22). Other endothelium-derived products, such as endothelium-derived hyperpolarizing factor, endothelin, and platelet-activating factor, may also participate in the regulation of the perinatal pulmonary circulation.

These findings support the hypothesis that in the late-gestation ovine fetal lung, endogenous EDRF activity can influence basal vascular tone, can be stimulated by a pharmacological agent, and contributes to the transition of the pulmonary circulation at birth. Therefore, we hypothesize that perinatal pulmonary vascular injury due to asphyxia, hyperoxia, hypertension (4, 29), or other adverse stimuli can alter endothelial function, causing sustained hypertension, diminished reactivity to dilator stimuli, and the failure of postnatal adaptation. Whether endothelial cell injury contributes to the pulmonary vascular abnormalities characteristic of newborns with persistent pulmonary hypertension remains speculative.

The authors are grateful for the technical support of Kristy Diamond and Y.-Da Fan and the support and encouragement of Drs. Frederick C. Battaglia and Giacomo Moschera.

This work was supported in part by grants from the National Institutes of Health Studies in Prematurity (HD-00781, HD-01869), Adaptation to Hypoxia (HL-14985), Critical Care Medicine, Dept. of Pediatrics, Box B-395, Children's Hospital, 1056 E. 19th Ave., Denver, CO 80210-1086.

Received 17 July 1990; accepted in final form 27 August 1990.

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Received 17 July 1990; accepted in final form 27 August 1990.
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