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Selective type 5 phosphodiesterase inhibition alters pulmonary hemodynamics and lung liquid production in near-term fetal lambs

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Abstract

Nitric oxide causes dilation of the pulmonary circulation and reduction in net lung liquid production in the fetal lamb, two critical perinatal events. Phosphodiesterase inhibition alone causes similar changes and also enhances the effects of nitric oxide. To better define the cyclic guanosine 5'-monophosphate (GMP) pathway in these events, we studied the effects of a specific phosphodiesterase inhibitor, E4021, on pulmonary arteries and veins isolated from near-term fetal lambs, as well as in intact, chronically instrumented late-gestation fetal lambs. In the in vitro experiments, both pulmonary arteries and veins relaxed to E4021 in a dose-dependent manner, although pulmonary veins were significantly more sensitive to E4021. Pretreatment with *N*^G-nitro-L-arginine (L-NNA) abolished this response in arteries but not in veins. In both arteries and veins, pretreatment with β -phenyl-1, *N*2-etheno-8-bromoguanosine-3',5'-cyclic monophosphorothionate blunted relaxations to E4021. In the in vivo experiments, E4021 infusion into either the pulmonary artery or central venous circulation increased pulmonary blood flow and decreased pulmonary vascular resistance, and these responses were blunted by pretreatment with L-NNA. Net lung liquid production, measured by a dye-dilution technique using blue dextran, decreased when E4021 was infused directly into the pulmonary artery and this effect was not altered by L-NNA. There was no effect on lung liquid production when E4021 was infused into the central venous circulation. Taken together, these results suggest that the pulmonary hemodynamic effects of E4021 involve the cyclic GMP pathway and are primarily nitric oxide synthase dependent. In contrast, the effects on E4021 on net lung liquid production appear to be independent of nitric oxide synthase, suggesting that these two critical perinatal events might be modulated independently.

Keywords

guanosine-5'-monophosphate; phosphodiesterase; fetus; pulmonary circulation; birth transition

The Fetal Lung is Distended with fluid that is actively secreted by the pulmonary epithelium (46). Before effective breathing can occur in the newborn, this fluid must be rapidly removed and net luminal secretion must cease (5). Failure to adequately remove this luminal fluid can result in respiratory distress that lasts from hours to days (2). There is increasing evidence that this reversal from net liquid secretion to net resorption is an active process under hormonal control (3,6,7,16–18,33,49,51) and is dependent on active sodium transport (13).

At birth, equally dramatic changes must occur within the pulmonary circulation to enable the lung to function as the organ of gas exchange. In the fetus, pulmonary blood flow is minimal because of high vascular resistance (37,38). The pulmonary transition from fetal to newborn life is accompanied by a decrease in pulmonary vascular resistance that results in a several-fold increase in pulmonary blood flow and a decrease in pulmonary arterial pressure (25). Several mediators, including prostaglandins and nitric oxide (NO), are involved (1,10,15,22, 31,32,38,42). Others and we have shown that certain prostaglandins and NO decrease net lung liquid production (J_v) (17,18,28), suggesting a relationship between these two critical perinatal events.

The vascular effects of NO are mediated by the production of the second messenger guanosine-3',5'-cyclic monophosphate (cGMP). Phosphodiesterases, by metabolizing intracellular cyclic nucleotides, play an important role in intracellular cGMP and cAMP signal transduction. Eleven subtypes of phosphodiesterases have been described that differ in primary structure, relative affinity for cAMP vs. cGMP, response to specific effectors and inhibitors, and mechanisms of regulation (4,39). The type 5 phosphodiesterase (PDE5) both binds and hydrolyzes cGMP with high specificity relative to cAMP. Specific inhibitors of phosphodiesterase, such as zaprinast, E4021, and sildenafil, have been reported to dilate the pulmonary circulation (8,14,20,52) and enhance the effect of NO (26,27,41,43,48,53). We have also found the pulmonary hemodynamic effects of E4021 are equivalent to 50 parts/ million of inhaled NO in a lamb model of persistent pulmonary hypertension (20).

Although phosphodiesterases are likely important in vascular endothelial intracellular signaling in the transitional pulmonary circulation, their role in pulmonary epithelial cell signaling and lung liquid production is less clear. We have recently shown that zaprinast enhances the effect of NO on J_v (19) suggesting that phosphodiesterases may modulate not only the transitional circulation but also the clearance of lung fluid during the perinatal period. In addition, PDE5 activity, protein, and message appear to be regulated during perinatal development and have peak activity at the time of birth (24,40), suggesting a role in transitional perinatal events.

To better understand the functional effects of phosphodiesterase inhibition, we studied the effects of another specific phosphodiesterase inhibitor, E4021, on pulmonary arteries and veins isolated from near-term fetal lambs. We also measured J_v and hemodynamic effects in response to intravascular infusions of E4021 in near-term instrumented fetal lambs. The role of NO synthase in these responses was also determined.

METHODS

Isolated Vessel Preparation

All procedures and protocols in this study were approved by the Laboratory Animal Care Committee at the State University of New York at Buffalo. At 135–136 days gestation (term = 146 days), time-dated pregnant mixed-breed ewes underwent a Cesarean section. Under anesthesia induced with 20 ml of a 5% solution of thiopental sodium and maintained with 1.5–2.0% halothane, the fetus was delivered and the umbilical cord was clamped. Before the first breath, lambs were killed by rapid exsanguination through a direct cardiac puncture. Our laboratory has described the subsequent techniques previously (20,44,45). Briefly, the heart and lungs were removed en bloc from the thorax immediately after death and placed in Krebs-Ringer solution (in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.5 NaHCO₃, 5.6 glucose, and 0.026 calcium disodium ethylenediamine-tetraacetate). Fifth-generation intralobar pulmonary arteries and veins (50) with inside diameters of 0.5–1.5 mm were isolated, dissected with care to preserve the integrity of the endothelium, and cut into rings ~2 mm wide and 0.7–2.6 mg in weight. Wet tissue weights were obtained at the end of

each experiment after blotting the rings dry on gauze pads. The force of contraction was normalized by the weight of each ring and expressed as grams per gram of tissue (g/g). Vessel rings were mounted on stainless steel hooks and placed in water-jacketed chambers. Tissues were bathed with 6 ml of the Krebs-Ringer solution, which was maintained at 37°C and aerated with a gas mixture of 94% O₂-6% CO₂ to maintain a pH of 7.40, a PCO₂ of 38 Torr, and a PO₂ of > 500 Torr. A continuous recording of isometric force generation was obtained by tying each vessel ring to a force-displacement transducer (Statham UC 2; Statham Instruments) that was connected to an oscillographic recorder. Once mounted, the vessel rings were allowed to equilibrate for 20 min in the bathing solution. A micrometer was then used to stretch the tissues repeatedly in small increments over the next 45 min until resting tone remained stable at a passive tension of 0.8 g for pulmonary arteries and 0.6 g for pulmonary veins. Previous experiments determined that this results in an optimal length for generation of active tone in response to exogenous norepinephrine (NE) (45). In this study, optimal lengths ranged from 1.24 ± 0.18 mm (mean $\pm$ SE) for pulmonary arteries and 2.11 ± 0.11 mm for pulmonary veins.

The following pharmacological agents were used: sodium 1-[6-chloro-4-(3,4-methylenedioxybenzyl)-aminoquinazolin-2-yl]piperidine-4-carboxylate sesquihydrate (E4021), indomethacin, L-NE, DL-propranolol hydrochloride, β -phenyl-1,N2-etheno-8-bromoguanosine-3',5'-cyclic monophosphorothionate (Rp-8-bromo-PET-cGMPS), and *N*-nitro-L-arginine (L-NNA). E4021, manufactured by Eisai Tsukuba Research Laboratory, was a gift from the Animal Health Trust (Newmarket, Suffolk, UK). Rp-8-bromo-PET-cGMPS was purchased from Biolog Life Sciences (La Jolla, CA). All other drugs were purchased from Sigma (St. Louis, MO). All drugs were dissolved in distilled H₂O, except for indomethacin, E4021, and Rp-8-bromo-PET-cGMPS, which were dissolved in ethanol, 0.01 N NaOH, and DMSO, respectively. Preliminary experiments determined that the addition of the vehicle alone had no vasoactive effects. Drugs were made fresh daily.

Experimental Protocol: Isolated Vessels

All vessels were pretreated for 20 min with 10^{-6} M propranolol to block β -adrenergic receptors and with 10^{-5} M indomethacin to prevent the formation of vasoactive prostaglandins. They were pre-constricted with an EC₅₀ concentration of NE (10^{-6} M) as determined from preliminary studies in which cumulative concentration-response curves for NE (10^{-8} to 10^{-5} M) were developed. Once the response to NE had reached a steady level, cumulative concentration-response curves to E4021 (10^{-9} to 10^{-5} M) were obtained by increasing its bath concentration in successive steps. The next concentration was added only when the response to the prior concentration had reached a plateau. For experimental protocols using L-NNA (10^{-5} M), the vessels were incubated for 30 min with L-NNA before constriction with NE. Vessel rings were used for one experimental protocol and then discarded.

Intact Fetal Preparation

Fetal sheep instrumentation—By methods previously described, we prepared nine fetal lambs for chronic vascular access and measurement of J_v (17,18). Time-dated pregnant ewes (mixed breeds) were operated on at 127 or 128 days gestation. Anesthesia was induced and maintained as described above with thiopental and halothane. A hysterotomy was performed through a midline abdominal incision that exposed the fetal head, neck, and left forearm. A thoracotomy was performed in the left third intercostal space, and a 6.0-mm ultrasonic transit time flow transducer was placed around the left pulmonary artery (LPA) just beyond the bifurcation from the main pulmonary artery. Sufficient room was left between the probe and vessel to allow for growth. Polyvinyl catheters were inserted into the LPA and the left atrium. The catheters and lead to the transducer were then secured to the exterior of the chest, and the chest was closed. Polyvinyl catheters were inserted in the right carotid artery and the right jugular vein; the venous catheter was advanced into the superior vena cava. We then made an

incision along the side of the trachea and inserted a Foley-type balloon-tipped catheter (Fuji Systems, Tokyo, Japan) into the proximal trachea, ~4–5 cm above the carina. The tracheal incision was closed tightly around the catheter. With the balloon deflated, liquid was free to move from the fetal lung to the upper airway. Through a second tracheal incision distal to the first, a small polyvinyl catheter was inserted and its tip was positioned well below the Foley balloon, for later measurement of intratracheal pressure. After the neck incision was closed, a catheter was sutured to the fetal skin for later monitoring of amniotic liquid pressure. Skin incisions were closed, and all catheters were tunneled through the uterus and the abdominal walls, which were doubly oversewn to prevent fluid leakage. A pouch was sewn to the maternal flank to prevent the ewe from damaging the catheters and the transducer head.

We injected antibiotics into the amniotic sac (700 mg of ampicillin and 20 mg of gentamicin) and fetal vein (300 mg of ampicillin) at the time of surgery and daily thereafter. The ewe also received a daily intramuscular injection of 100 mg of ampicillin (Polyflex) and 40 mg of gentamicin. Vascular catheters were flushed with isotonic saline and filled with heparin solution (1,000 U/ml) daily.

Measurements—Phasic LPA, left atrial, aortic, airway, and amniotic pressures were measured by Gould Statham physiological pressure transducers (P-23 XL, Gould Electronics, Cleveland, OH), which were calibrated at the start of each experiment with a mercury column manometer. Left pulmonary blood flow was measured by a 6.0-mm ultrasonic transit time flow transducer (Transonic Systems, model T 101, Ithaca, NY) around the LPA and processed by a digital flowmeter. These data were recorded continuously on an eight-channel physiological amplifier recorder (Gould Electronics). Vascular and tracheal pressures were referenced to liquid pressure within the amniotic sac. Pulmonary vascular resistance (PVR) was calculated as: $PVR = (LPAP - LAP) / \text{pulmonary blood flow}$ and reported in units of $(\text{mmHg} \cdot \text{min} \cdot \text{ml}^{-1})$, where LPAP is LPA pressure and LAP is left atrial pressure. Aortic blood was collected for measurement of pH, PO_2 , PCO_2 , hemoglobin, and base deficit (Acid-Base Laboratory 3; Radiometer Medical, Copenhagen, Denmark).

Lung liquid production measurement—We measured lung liquid production by a dye-dilution technique previously described (9,19). Briefly, at the beginning of each experiment, the tracheal balloon was inflated (3 ml) to occlude the trachea and isolate the fetal lung lumen from the amniotic cavity. Lung liquid was withdrawn into a 30-ml warmed syringe to permit mixing and withdrawal of samples. Production rates were calculated from the rate of dilution of the impermeant tracer blue dextran (molecular weight 2,000,000) (Sigma). Nine milliliters (50 mg/ml) of blue dextran was instilled into the luminal liquid and mixed well by gently withdrawing and re-instilling liquid several times over a 30- to 40-min period. Thereafter, we removed 1-to 2-ml samples of lung liquid every 8–10 min for the duration of the experiment. Lung liquid was aspirated gently and returned between samplings to ensure mixing. The size of each liquid sample was adjusted to keep luminal volume nearly constant.

Samples were run in duplicate, and changes in light absorption at 240 nm were measured with a Bio-Rad Laboratories photometer (model 3550 ultraviolet microplate reader). With the use of a series of standards, the concentration of blue dextran was determined for each sample (Beer's Law). Fetuses that were studied more than once had samples of lung liquid taken at the start of each subsequent experiment to measure the amount of blue dextran in the lung liquid. After instillation of fresh blue dextran, samples taken during the course of the experiment were then adjusted by subtraction of the background dye concentration.

Experimental protocol: fetal lamb—Animals were allowed to recover from surgery for at least 72 h before experiments began. Experiments were done on unanesthetized fetuses that were judged to be healthy by arterial pH and blood gas tensions. The fetuses were studied while

their ewes stood upright in a cage, with free access to food and water. When repeat studies were done on the same fetus, a rest period of at least 48 h between experiments was observed.

J_v was measured during a 1- to 2-h baseline period and throughout the protocol. E4021 was dissolved in 0.01 N NaOH, and LNA was dissolved in warm normal saline (2 mg/ml). Preliminary experiments infusing the vehicles alone at similar rates produced no significant hemodynamic effect. The infusion site of E4021 was selected at random for delivery into the LPA or the superior vena cava at 31 $\mu\text{g}/\text{min}$ for 20 min at a rate of 6 ml/h. Baseline hemodynamics were measured before the infusion of E4021 was started, every 10 min during the infusion, and every 15 min after completion of the infusion until hemodynamics returned to baseline. This protocol was repeated in the same animal using the other (LPA or superior vena cava) infusion site. In the last protocol of the day, 30 mg of L-NNA was infused via the LPA over 30 min followed immediately by 31 $\mu\text{g}/\text{min}$ of E4021 for 20 min via the LPA. In preliminary experiments, this amount of L-NNA completely blocked the hemodynamic response to acetylcholine. Arterial blood gasses were measured before and after each experimental protocol.

Data Analysis

All data are expressed as means $\pm$ SE, with n representing the number of animals studied. Statistical analysis was performed with the Statview software package (Abacus Concepts, Berkley, CA). Statistical comparisons for normally distributed data within groups were performed using ANOVA for repeated measures, followed if necessary by Student-Newman-Keuls post hoc testing for multiple comparisons. Between-group comparisons were made using unpaired t -tests. Wilcoxon's signed rank test was used to compare groups of data that were not normally distributed. A one-way ANOVA was performed to determine differences in hemodynamic responses between groups of intact lambs. A P value of < 0.05 was considered significant.

RESULTS

Isolated Vessel Experiments

Plateau contractile responses to 10^{-6} M NE were similar in pulmonary arteries and pulmonary veins. Both pulmonary arteries and pulmonary veins relaxed to E4021 in a concentration-dependent manner, although pulmonary veins were significantly more sensitive to E4021 (Fig. 1). L-NNA abolished relaxations to E4021 in pulmonary arteries (Fig. 1, *top*). In contrast, relaxations to E4021 in pulmonary veins were not altered by pretreatment with L-NNA. In both pulmonary arteries and veins, pretreatment with the cGMP kinase inhibitor Rp-8 bromo-PET-cGMPS blunted relaxations to E4021 (Fig. 1, *bottom*).

Intact Fetal Preparation

E4021 infusion into the central venous circulation—In 10 experiments (9 fetuses, 134 ± 1 gestational days), systemic intravenous infusion of E4021 significantly increased pulmonary arterial blood flow and decreased pulmonary vascular resistance; in eight of these experiments J_v was measured and was not affected by E4021 (Table 1). There were no significant changes in pulmonary or systemic arterial blood pressure or left atrial pressure (Table 1), or systemic arterial pH, PO_2 , or PCO_2 (data not shown). Heart rate increased from baseline but was not statistically significant.

E4021 infusion into the LPA—In 10 experiments (9 fetuses, 134 ± 1 gestational days), left pulmonary arterial infusion of E4021 significantly increased pulmonary arterial blood flow and decreased pulmonary vascular resistance (Table 1 and Fig. 2); in eight of these experiments J_v was measured and significantly decreased after E4021 infusion (Table 1 and Fig. 3). There

were no significant changes in pulmonary or systemic arterial blood pressure or left atrial pressure (Table 1) or systemic arterial pH, PO₂, or PCO₂ (data not shown). Heart rate increased significantly from baseline after left pulmonary arterial infusion of E4021 (Table).

L-NNA infusion into the LPA—In nine experiments (8 fetuses, 135 ± 1 gestational days), left pulmonary arterial infusion of L-NNA significantly increased left pulmonary and systemic arterial pressure, tended to decrease pulmonary arterial blood flow, and thus significantly increased pulmonary vascular resistance (Table 1). In six of these experiments, J_v was measured and was not affected by L-NNA infusion (Table 1 and Fig. 3). There were no significant changes in left atrial pressure or heart rate (Table 1) or systemic arterial pH, PO₂, or PCO₂ (data not shown).

E4021 infusion following blockade with LNA—In nine experiments (8 fetuses, 135 ± 1 gestational days), left pulmonary arterial infusion of L-NNA blunted the pulmonary hemodynamic effects to the subsequent infusion of E4021. Although pulmonary blood pressure and pulmonary vascular resistance remained significantly higher than baseline, pulmonary blood flow did not increase above baseline (Table 1). In six of these experiments, J_v was measured; the effects of E4021 on J_v after LNA were similar to the effects of E4021 alone on J_v (Fig. 3). There were no significant changes in systemic arterial blood pressure, left atrial pressure, or heart rate (Table 1), or systemic arterial pH, PO₂, or PCO₂ (data not shown).

DISCUSSION

The present study shows that phosphodiesterase inhibition with the selective PDE5 inhibitor E4021 relaxes pulmonary vessels. Pulmonary veins were significantly more sensitive to E4021 and completely relaxed at a concentration of 10⁻⁷ M, whereas pulmonary arteries had maximal relaxations of 57% at a concentration of 10⁻⁵ M E4021 (Fig. 1). Differential responses to agonists for NO synthase and soluble guanylate cyclase in pulmonary arteries vs. veins have previously been reported (23,44) and were evident again in the present study. For example, relaxations to E4021 in pulmonary arteries were blocked by pretreatment with the NO synthase inhibitor L-NNA, whereas L-NNA had no effect on relaxations to E4021 in pulmonary veins (Fig. 1, *top*). Pretreatment of both vessel types with the selective inhibitor of cGMP-dependent protein kinase Rp-8-Br-PET-cGMP shifted the EC₅₀ for E4021 significantly to the right (Fig. 1, *bottom*), indicating that cGMP production is responsible for at least a portion of the relaxations in both vessel types.

Because all of our vessel bath experiments were performed in the presence of indomethacin, the NO-independent relaxation in pulmonary veins was not due to production of vaso-active prostaglandins. Another potential mechanism for the production of cGMP in pulmonary veins involves the natriuretic peptide-particulate guanylate cyclase pathway. Preliminary studies have demonstrated the presence of particulate guanylate cyclase B receptors and C-type natriuretic peptide in pulmonary venous endothelial cells and vascular smooth muscle cells (29), indicating the potential for cGMP production. Potential mechanisms for the production of cGMP in pulmonary veins, other than the prostaglandin pathway, were not investigated in the present study.

In fetal lambs, intravascular infusions of E4021 significantly increased pulmonary blood flow and decreased pulmonary vascular resistance (Fig. 2 and Table 1). The pulmonary hemodynamic response was significantly greater when the infusion was administered via the LPA compared with the superior vena cava (Table 1). The difference in pulmonary hemodynamics and the lack of effect on lung liquid production when infusing E4021 into the superior vena cava (Table 1) can be explained based on lower concentrations of E4021 in the pulmonary circulation following venous infusions. In the fetus, the majority of blood returning

to the right atrium from the superior vena cava is shunted across the ductus arteriosus to the systemic circulation and placenta (47) rather than to the pulmonary circulation.

The pulmonary hemodynamic effects of E4021 were almost completely blocked by infusing L-NNA before E4021 (Fig. 2 and Table 1), indicating that the pulmonary hemodynamic effects of E4021 are primarily NO synthase dependent. Because L-NNA is a relatively nonspecific NO synthase inhibitor, we are unable to say whether the hemodynamic effects of E4021 are due to cGMP production from the endothelial, neuronal, or inducible NO synthase isoform or some combination. Neuronal and inducible NO synthase isoforms have recently been shown to play a role in altering pulmonary vascular resistance in late-gestation fetal lambs (35,36).

Ours is the first study in which E4021 was given to a fetus. We and others have previously shown that infusions of less-potent and selective PDE5 inhibitors, such as zaprinast and dipyridamole, have significant pulmonary hemodynamic effects in the fetus (41,52). Our results are similar to those of Ziegler et al. (53), who found that the hemodynamic effects of phosphodiesterase inhibition with dipyridamole were completely blocked in the fetal lamb after pretreatment with L-NNA.

E4021 infused in the LPA significantly decreased J_v (Fig. 3 and Table 1). On first examination, this response is similar to multiple other studies in which infusing an agent that decreases pulmonary vascular resistance has a net drying effect on the lung. What is unique about our study is that we were able to block the hemodynamic effects of E4021 by inhibiting NO synthase without changing its effects on J_v . This means that the effects of E4021 on J_v are likely independent of NO synthase. Our present studies support the notion that two critical transitional events of birth, lung liquid resorption and pulmonary vasodilatation, may be modulated independently.

Catecholamines also decrease J_v (21,30,49). In the present study, infusion of E4021 in the LPA increased heart rate and decreased J_v . An increase in endogenous catecholamines as a direct effect of E4021 could explain the changes in heart rate and lung liquid production. However, we think this is unlikely since fetuses pretreated with L-NNA had a similar decrease in lung liquid production but had no change in heart rate. Another possibility is that the pulmonary hemodynamic effects of E4021 produced a steal of flow from the systemic circulation and thereby increased heart rate secondary to an increase in endogenous catecholamines. If this were the case, we would expect a greater decrease in lung liquid production in the fetuses not pretreated with L-NNA.

In the present study, we chose the dye dilution technique using blue dextran for estimating J_v . Recently, the techniques of estimating lung liquid volume in late-gestation fetal lambs using blue dextran and radio-iodinated serum albumin as volume tracers were compared (34). In that study, there was a discrepancy between the two estimates of lung liquid volume in late gestation (142 day) but not in younger (124 day) fetuses. The authors suggested that this might be due to increased lung tissue binding of blue dextran in the near-term fetuses. We believe our estimates of lung liquid production are accurate for several reasons. First, we studied fetuses at 134–136 days gestation, significantly before term, when measurement of lung volume by the two techniques yields similar results. Second, we typically instill at least 50% more blue dextran, which should be sufficient to saturate all binding sites; if all binding sites are saturated with tracer, accurate estimates of lung liquid production can be made since we calculate production rates not from absolute volumes but from relative changes in volume over time. Third, we (unpublished data) and others (11,12) have used both techniques for several years and have consistently found similar estimates of lung liquid production when comparing the two.

The clearance of fetal lung liquid and decrease in pulmonary vascular resistance at birth is a complicated process involving multiple mediators. The process is essential in the transition from placental gas exchange as a fetus to pulmonary gas exchange as a newborn. Our study demonstrates that cGMP produced endogenously is an important mediator in the transition and that the particulate guanylate cyclase pathway may be an additional source of cGMP. Although the changes observed in pulmonary blood flow were significantly elevated from baseline, the response was still only a fraction of the increase that occurs at birth. Cyclic nucleotide phosphodiesterases are likely one of many mediators along with catecholamines, prostaglandins, NO, and others that play an intricate role in the cardiopulmonary transition that occurs at birth.

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Fig. 1.

Relaxation of pulmonary arteries (PA; circles) and pulmonary veins (PV; squares) isolated from near-term fetal lambs in response to increasing concentrations of E4021 before (closed symbols) and after (open symbols) pretreatment with *N*-nitro-L-arginine (L-NNA; *top*) or Rp-8-bromo-PET-cGMPS (PET; *bottom*). All vessels were pretreated with indomethacin and propranolol. Relaxations are expressed as a percentage of the plateau norepinephrine (NE) constriction. Number of lambs for each data point at *top* is 6, at *bottom* is 7 for PA and 9 for PV. Data are means $\pm$ SE. $^*P < 0.05$ compared with untreated vessels of same type. $^\dagger P < 0.05$ veins compared with arteries. Note the relaxation to E4021 in PA is completely blocked by pretreatment with L-NNA but unaltered in PV.

Fig. 2. Left pulmonary artery (LPA) blood flow in near-term fetal lambs after an LPA infusion of E4021 at 31 $\mu\text{g}/\text{min}$ for 20 min with (circles) and without (squares) pretreatment with 30 mg of L-NNA (1 mg/min). Data are means $\pm$ SE; $n = 9$ lambs for E4021 alone and $n = 8$ lambs for LNA followed by E4021. * $P < 0.05$ vs. baseline.

Fig. 3. Net lung liquid production in near-term fetal lambs following LPA infusion of E4021 at 31 $\mu\text{g}/\text{min}$ for 20 min with (circles) and without (squares) pretreatment with 30 mg of L-NNA (1 mg/min). Data are means $\pm$ SE; $n = 8$ lambs for E4021 alone and $n = 6$ lambs for L-NNA followed by E4021. * $P < 0.05$ vs. baseline.

Table 1
Data from groups of fetal lambs in each experimental protocol

	Venous E4021		LPA E4021		L-NNA + LPA E4021		
	Baseline	E4021	Baseline	E4021	Baseline	L-NNA	E4021
Pulmonary artery pressure, mmHg	53 ± 1	50 ± 1	51 ± 2	49 ± 2	51 ± 1	65 ± 1 [*]	62 ± 1 [*]
Carotid artery pressure, mmHg	51 ± 2	49 ± 2	50 ± 1	47 ± 2	49 ± 1	61 ± 1 [*]	60 ± 1 [*]
Left atrial pressure, mmHg	2 ± 1	1 ± 1	2 ± 1	2 ± 1	2 ± 1	3 ± 1	2 ± 1
Heart rate, beats/min	156 ± 5	172 ± 8	154 ± 7	175 ± 12 [*]	151 ± 6	141 ± 7	145 ± 6
Left pulmonary artery blood flow, ml/min	60 ± 5	78 ± 7 [*]	56 ± 4	87 ± 7 [*]	57 ± 6	50 ± 6	57 ± 7
PVR	0.90 ± 0.08	0.68 ± 0.07 [*]	0.94 ± 0.08	0.58 ± 0.05 [*]	0.95 ± 0.13	1.48 ± 0.29 [*]	1.31 ± 0.26 [*]
%Change in PVR		-24.7 ± 13.0		-37.6 ± 11.3 [†]			
J _v	23 ± 1	22 ± 4	23 ± 1	8 ± 5 [*]	22 ± 1	23 ± 5	-2 ± 9 [*]

Values are means ± SE. LPA, left pulmonary artery; L-NNA, left pulmonary vascular resistance; PVR, pulmonary vascular resistance; J_v, net lung liquid production.

^{*} $P < 0.05$ vs. baseline.

[†] $P < 0.03$ vs. venous infusion.