0031-3998/03/5401-0044
PEDIATRIC RESEARCH

Vol. 54, No. 1, 2003
Printed in U.S.A.

Role of the Alpha$_2$-Adrenoceptors on the Pulmonary Circulation in the Ovine Fetus

ERIC MAGNENANT, SOPHIE JAILLARD, PHILIPPE DERUELLE, VÉRONIQUE HOUFFLIN-DEBARGE, YVON RIOU, SERGE KLOSOWSKI, AND LAURENT STORME

Department of Perinatal Medicine [E.M., S.J., P.D., V.H.-D., Y.R., S.K., L.S.], and EA 1049, Department of Biophysics [S.J., Y.R., L.S.], Centre Hospitalier et Universitaire, Lille, France.

ABSTRACT

Recent *in vitro* studies reported that nitric oxide release and pulmonary vasorelaxation can be mediated by endothelial α_2-adrenoceptor activation. As norepinephrine (α_1-,α_2-, and β_1-adrenoceptor agonist) was found to induce pulmonary vasodilation in the ovine fetus, we hypothesized that α_2-adrenoceptors may modulate basal pulmonary vascular tone and mediate the vascular effect of norepinephrine during fetal life. To determine the role of α_2-adrenoceptors and the mechanisms of norepinephrine-mediated vasodilation in the fetal pulmonary circulation, we tested, in chronically prepared late-gestation fetal lambs, the hemodynamic response to *1*) yohimbine (α_2 antagonist); *2*) UK 14,304 (α_2 agonist) with and without L-nitro-arginine (nitric oxide synthase inhibitor); and *3*) norepinephrine infusion with and without yohimbine. We found that yohimbine increased mean pulmonary artery pressure by 15% ($p < 0.05$), decreased pulmonary flow by 22% ($p < 0.01$), and increased pulmonary vascular resistance by 51% ($p < 0.01$). UK 14,304 increased pulmonary flow by 145% ($p < 0.01$) and decreased pulmonary vascular resistance by 58% ($p < 0.01$). L-Nitro-arginine abolished the UK 14,304-mediated pulmonary vasodilation. Norepinephrine (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) increased both pulmonary flow by 61% ($p < 0.01$) and pulmonary arterial pressure by 13% ($p < 0.01$) and decreased pulmonary vascular resistance by 33% ($p < 0.01$). Yohimbine abolished the norepinephrine-induced pulmonary vasodilation. This study suggests that *1*) a basal α_2-adrenoceptor activation-induced pulmonary vasodilation exists during fetal life; *2*) the pulmonary vascular effects of α_2-adrenoceptor activation are related at least in part to nitric oxide production; and *3*) the norepinephrine-mediated pulmonary vasodilation involves α_2-adrenoceptor activation. As a surge of norepinephrine exists at birth, we speculate that norepinephrine and endothelial α_2-adrenoceptor activation may play a significant role in pulmonary vasodilation at birth. **(*Pediatr Res* 54: 44–51, 2003)**

Abbreviations

NO, nitric oxide
PAP, mean pulmonary artery pressure
$\dot{Q}$p, pulmonary flow
PVR, pulmonary vascular resistance
LPA, left pulmonary artery
L-NA, L-nitro arginine
NOS, NO synthase
Pao$_2$, pulmonary arterial oxygen pressure
AoP, aortic pressure
LAP, left atrial pressure

High resistance and low blood flow characterize the fetal pulmonary circulation. PVR decreases dramatically during the normal transition from the fetal to neonatal circulation at birth. Three main factors contribute to the increase of $\dot{Q}$p during this transition: ventilation of the lung (1), increased O_2 (2), and hemodynamic forces, such as increased shear stress (3). Vasoactive mediators released from the endothelium, such as NO, play a major role in the regulation of acute changes in vascular tone in the perinatal lung, and in many cases, modulate the pulmonary vascular response to these birth-related stimuli (4). Inhibition of NO synthesis can attenuate the postnatal adaptation of the pulmonary circulation (5). The vasodilator action of several substances, such as acetylcholine, bradykinin, or ADP, and shear stress are dependent, at least in part, on NO release (6). More recently, *in vitro* studies reported that NO release and pulmonary vasorelaxation can also be mediated by endothelial α_2-adrenoceptor activation (7–9). However, little is known about the role of α_2-adrenoceptors on the basal pulmonary vascular tone during fetal life and on the adaptation of the pulmonary circulation at birth.

Increase in plasma catecholamine concentration has been observed at the end of gestation, with the predominant circulating catecholamine being norepinephrine (10, 11). Birth in-

Received June 24, 2002; accepted December 20, 2002.
Correspondence: Laurent Storme, M.D., Service de Médecine Néonatale, Hôpital Jeanne de Flandre, Centre Hospitalier et Universitaire, 59037 Lille Cedex, France; e-mail: lstorme@chru-lille.fr
Research support granted by Centre Hospitalier Régional Universitaire de Lille, Cardio-Vascular Committee of Fondation de France, and Université de Lille II.

DOI: 10.1203/01.PDR.0000065726.43910.91

duces a marked surge in catecholamine secretion (12). Increased catecholamines at delivery promote lung fluid reabsorption, surfactant release, and systemic circulatory adaptation (13, 14). However, the effects on the pulmonary circulation are uncertain. Although norepinephrine is commonly known as a vasopressor agent, *in vitro* studies have shown that norepinephrine dilates some systemic (15) and pulmonary (16) vessels in newborn animals. We reported that norepinephrine infusion induces a potent NO-dependent pulmonary vasodilation in fetal lambs (17). As α_2-adrenoceptor antagonists were found to inhibit norepinephrine-mediated relaxation of pulmonary (16, 18) or systemic arteries (15), or enhance norepinephrine-mediated pulmonary vasoconstrictive response (8, 9), norepinephrine-mediated pulmonary vasodilation may result from activation of endothelial α_2-adrenoceptors.

Accordingly, the main hypotheses tested in this study were that *1*) α_2-adrenoceptors are involved in the control of basal pulmonary vascular tone during perinatal life; *2*) the effects of α_2-adrenoceptors activation are related to NO production; and *3*) the pulmonary vascular effects of norepinephrine are at least in part mediated by α_2-adrenoceptor activation. To test these hypotheses, we studied, in chronically prepared late-gestation fetal lambs, the hemodynamic response to *1*) selective α_2-adrenoceptor activation, with and without NOS inhibition, and selective α_2-adrenoceptor blockade; and *2*) norepinephrine associated with selective α_2-adrenoceptor blockade.

METHODS

Animal Preparation

All animal procedures and protocols used in this study were reviewed and approved by the French "Ministère de l'Agriculture, de la Pêche et de l'Alimentation" before the studies were conducted. Mixed-breed (Columbia-Rambouillet) pregnant ewes between 126 and 128 d gestation (term, 147 d) were fasted for 48 h before surgery. Ewes were sedated with i.v. pentobarbital sodium (total dose, 2–4 g) and anesthetized with 1% bupivacaine hydrochloride (4 mg) by lumbar puncture. Ewes were kept sedated but breathed spontaneously throughout the surgery. Under sterile conditions, the fetal lamb's left forelimb was delivered through a uterine incision. A skin incision was made under the left forelimb after local infiltration with lidocaine (2 mL, 1% solution). Polyvinyl catheters (20-gauge) were advanced into the ascending aorta and the superior vena cava after insertion in the axillary artery and vein. A left thoracotomy exposed the heart and great vessels. Catheters were inserted into the LPA (22-gauge), main pulmonary artery (20-gauge), and left atrium (20-gauge) by direct puncture through pursestring sutures and secured as described (19). An ultrasonic flow transducer (size 6; Transonic Systems, Ithaca, NY, U.S.A.), was placed around the LPA to measure blood flow. The uteroplacental circulation was kept intact, and the fetus was gently replaced in the uterus. An additional catheter was placed in the amniotic cavity to measure pressure. Amoxicillin (500 mg) was added to the amniotic cavity before closure of the hysterotomy. The flow transducer and catheters were exteriorized through a subcutaneous tunnel to an external flank pouch. Catheters were maintained by daily infusions of 2 mL of heparinized saline (10 U/mL). Catheter positions were verified at autopsy. Studies were performed after a minimum recovery time of 48 h. Estimated weight of the fetal lambs was 3000 g.

Physiologic Measurements

The flow transducer cable was connected to an internally calibrated flowmeter (T201; Transonic Systems), for continuous measurements of left $\dot{Q}p$. The output filter of the flowmeter was set at 30 Hz. The absolute value of flow was determined from the mean of phasic blood flow signals (at least 30 cardiac cycles), with zero blood flow defined as the measured flow value immediately before the beginning of systole (20). Main pulmonary artery, aortic, left atrial, and amniotic catheters were connected to blood pressure transducers (Merlin monitor, Hewlett-Packard, U.S.A.). Pressures were referenced to the amniotic cavity pressure. Calibration of the pressure transducers was performed with a mercury manometer. Heart rate was determined from the phasic $\dot{Q}p$ signal. PVR in the left lung was calculated as the difference between PAP and LAP divided by mean left $\dot{Q}p$. Blood samples from the main pulmonary artery catheter were used for blood gas analysis and oxygen saturation measurements (OSM 3 hemoximeter and ABL 520, Radiometer, Copenhagen, Denmark).

Experimental Design

Five different experimental protocols were included in this study: *1*) the effect of α_2-adrenoceptor blockade on basal pulmonary vascular tone; *2*) pulmonary hemodynamic response to α_2-adrenoceptor activation; *3*) pulmonary hemodynamic response to α_2-adrenoceptor activation after NOS inhibition; *4*) pulmonary hemodynamic response to norepinephrine; and *5*) effect of α_2-adrenoceptor blockade on the hemodynamic response to norepinephrine.

Protocol 1. Effect of α_2-adrenoceptor blockade on basal pulmonary vascular tone. Saline (6 mL/h) was infused into the LPA for at least 30 min. Starting 30 min after stable baseline measurements, yohimbine was infused into the LPA at a rate of 0.75 mg/kg for 10 min, followed by a continuous infusion of 0.75 mg/kg for 110 min (6 mL/h). Preliminary tests showed that lower doses (0.1, 0.2, or 0.5 mg/kg) or bolus alone did not provide sustained α_2-adrenoceptor blockade. The LPA catheter was then infused with saline (6 mL/h) for 30 min.

Protocol 2. Pulmonary hemodynamic response to α_2-adrenoceptor activation. Saline (6 mL/h) was infused into the LPA for at least 30 min. Starting 30 min after stable baseline measurements, UK 14,304 was infused into the LPA at a rate of 0.8 mg/kg (6 mL/h) for 120 min. Preliminary tests showed that lower doses (0.2 and 0.4 mg/kg) did not induce major changes in PVR. Conversely, higher doses decreased AoP and caused severe acidosis. The LPA catheter was then infused with saline (6 mL/h) for 30 min.

Protocol 3. Pulmonary hemodynamic response to α_2-adrenoceptor activation after NOS inhibition. Protocol 2 was repeated after L-NA infusion. Saline (6 mL/h) was infused into the LPA for at least 30 min. Starting 30 min after stable

baseline measurements, L-NA (30 mg for 10 min) was infused into the LPA (from 30 min to 40 min). This dose was selected from past studies that have demonstrated effective blockade of NOS activity during acetylcholine- and flow-induced vasodilation for at least 4 h (5). Then the pulmonary artery catheter was flushed with normal saline at a constant rate of 6 mL/h during 20 min, before starting the UK 14,304 infusion (from 60 to 180 min).

Protocol 4. Pulmonary hemodynamic response to norepinephrine. Saline (6 mL/h) was infused into the venous catheter for at least 30 min. Starting 30 min after stable baseline measurements, norepinephrine was infused for 120 min into the venous catheter at a rate of 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (6 mL/h). The catheter was then infused with saline (6 mL/h) for 30 min.

Protocol 5. Effect of α_2-adrenoceptor blockade on the hemodynamic response to norepinephrine. Saline (6 mL/h) was infused into the LPA and into the venous catheter for at least 30 min. After 30 min of stable baseline measurements, yohimbine was infused into the LPA at a rate of 0.75 mg/kg for 10 min, followed by a continuous infusion of 0.75 mg/kg for 110 min (6 mL/h). Norepinephrine infusion (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 120 min, 6 mL/h) was infused into the venous catheter and started 20 min after the beginning of yohimbine infusion. Both catheters were then infused with saline (6 mL/h) for 30 min.

Main PAP, LAP, mean AoP, amniotic pressure, left $\dot{Q}p$, and heart rate were recorded at 10-min intervals in each protocol. PVR in the left lung was calculated.

Drug Preparation

Norepinephrine (Aguettant, Lyon, France), and yohimbine hydrochloride (Sigma Chemical Co, St Quentin Fallavier, France) were diluted in normal saline to a concentration of 15 μg/mL, and 2 mg/mL, respectively. UK 14,304 (Sigma Chemical Co., St Quentin Fallavier, France) was diluted in 2 drops of DMSO, then further diluted in normal saline to a concentration of 360 μg/mL. L-NA (30 mg) was dissolved in a few drops of 1 M HCl, and diluted in 1 mL of normal saline. NaOH was then used to titrate the pH to 7.40. All drugs except norepinephrine were infused in the LPA.

Data Analysis

The results are presented as mean ± SD. The data were analyzed using repeated-measures and factorial ANOVA. Intergroup differences were analyzed with the Fisher's, Scheffé's, and Bonferroni/Dunn's least significant test (StatView for PC; Abacus Concepts, Berkeley, CA, U.S.A.). A $p < 0.05$ was considered as statistically significant.

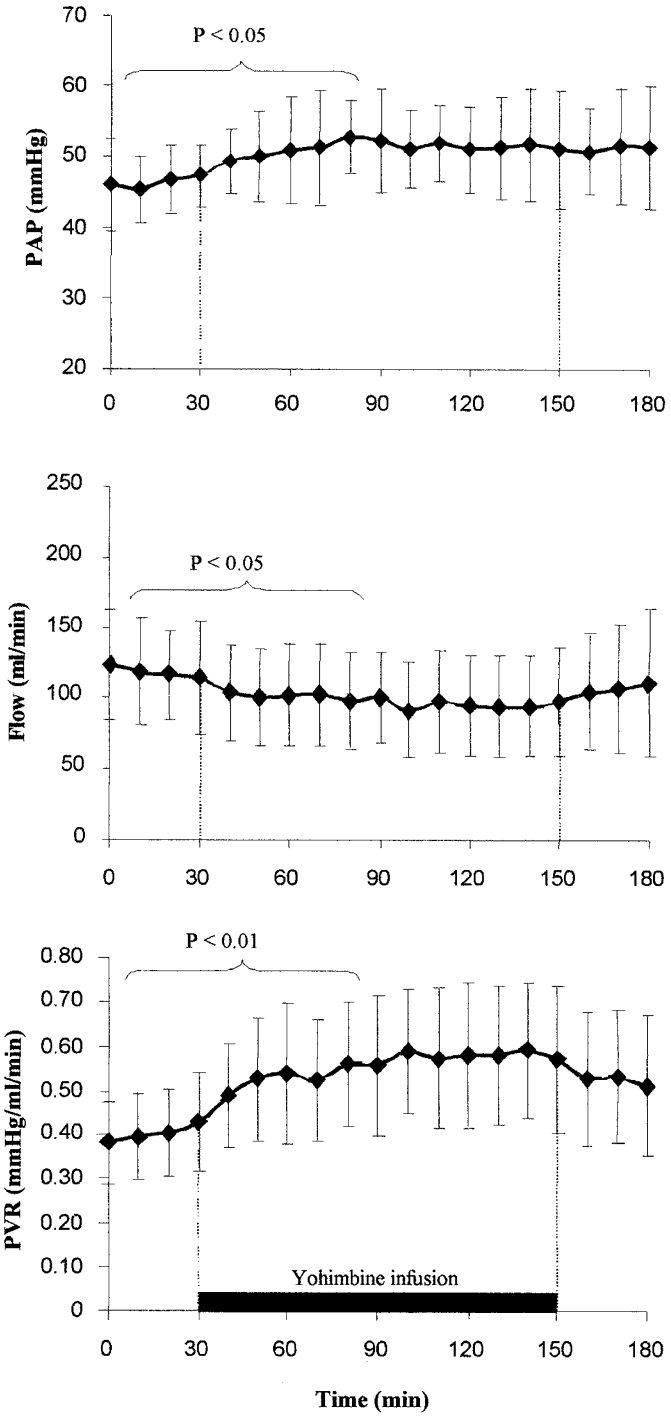

Figure 1. Pulmonary hemodynamic response to yohimbine (α_2 -adrenoceptor antagonist) infusion (0.75 mg/kg for 10 min, followed by 0.75 mg/kg for 2 h; $n = 8$). Yohimbine induced a reduction of $\dot{Q}p$ ($p < 0.05$) and an increase in PVR ($p < 0.05$). Values are expressed as mean ± SD.

RESULTS

Protocol 1: Effect of α_2-adrenoceptor blockade on the basal pulmonary vascular tone. Yohimbine infusion increased mean PAP by 15% (from 46 ± 5 to 53 ± 5 mm Hg; $p < 0.05$; $n = 8$), and decreased left $\dot{Q}p$ by 22% (from 119 ± 36 to 92 ± 33 mL/min; $p < 0.05$; Fig. 1). PVR increased by 51% (from 0.39 ± 0.1 to 0.59 ± 0.14 mm Hg·min·mL^{-1}; $p < 0.01$; Fig. 1). Mean AoP increased by 13 ± 5% (from 44 ± 1 to 50 ± 2 mm Hg; $p < 0.05$). Mean LAP before infusion was 2 ± 1 mm Hg and did not change during the study period. Heart rate did not change during the infusion. Pulmonary arterial P_{O_2} did not change significantly during the study period (Table 1).

Protocol 2: Pulmonary hemodynamic response to α_2-adrenoceptor activation. UK 14,304 infusion did not alter basal mean PAP, but increased left $\dot{Q}p$ by 145% (from 102 ±

Table 1. *Blood gas, heart rate, and mean AoP, before and at the end of each protocol*

	pH	$Paco_2$ (mm Hg)	Pao_2 (mm Hg)	HR (beats/min)	AoP (mm Hg)
Norepinephrine					
Before	7.36 ± 0.02	46.3 ± 1.65	16.4 ± 0.95	170 ± 2.2	45 ± 2
After	7.36 ± 0.02	46.1 ± 1.8	17.1 ± 1.1	174 ± 3.2	50 ± 3*
Yohimbine					
Before	7.35 ± 0.01	47 ± 2.2	16.7 ± 0.7	164 ± 5.3	44 ± 1.4
After	7.34 ± 0.03	48.2 ± 1.9	15.2 ± 0.8	178 ± 4.2	50 ± 2.1*
Yohimbine + NE					
Before	7.37 ± 0.01	47.7 ± 2.3	16.3 ± 1.2	170 ± 4.4	48 ± 1.6
After	7.34 ± 0.03	52.8 ± 4.7	17.8 ± 1.3	182 ± 4.4	52 ± 1.7
UK 14,304					
Before	7.30 ± 0.06	45 ± 1.7	17 ± 1.7	165 ± 4.5	49 ± 1.3
After	7.23 ± 0.03	48 ± 9.5	17 ± 1.2	171 ± 6.2	51 ± 1.1
L-NA + UK14,304					
Before	7.38 ± 0.05	48 ± 3.7	15.2 ± 1.2	163 ± 5.2	53 ± 1.3
After	7.34 ± 0.05	52 ± 4.4	14.7 ± 1.8	180 ± 4.5	65 ± 3*

Mean ± SD. * $p < 0.05$.
HR, heart rate; NE, norepinephrine.

23 to 250 ± 70 mL/min; $p < 0.01$; $n = 5$; Fig. 2). PVR decreased by 58% (from 0.51 ± 0.13 to 0.21 ± 0.05 mm Hg·min·mL^{-1}; $p < 0.01$) during the study period (Fig. 2). Mean LAP before infusion was 2 ± 1 mm Hg and did not change during the study period. AoP, heart rate, and pulmonary arterial Po_2 did not change during the infusion (Table1).

Protocol 3. Pulmonary hemodynamic response to α_2-adrenoceptor activation after NOS synthase inhibition. L-NA infusion did not significantly change mean PAP and $\dot{Q}p$. However, mean PVR increased by 15% (from 0.54 ± 0.06 to 0.63 ± 0.09 mm Hg·min·mL^{-1}; $p < 0.01$; $n = 5$). After L-NA infusion, UK 14,304 infusion increased both mean PAP and mean PVR by 29% and 19%, respectively (Fig. 3). $\dot{Q}p$ did not change during UK 14,304 infusion (Fig. 3). Heart rate and blood gas did not change during the study period (Table 1).

Protocol 4: Pulmonary hemodynamic response to norepinephrine infusion. Norepinephrine infusion at 0.5 μg/kg/min increased mean PAP by 13% (from 48 ± 5 to 54 ± 5 mm Hg) after 20 min ($p < 0.01$; $n = 11$), and progressively increased left $\dot{Q}p$ by 61% (from 100 ± 28 to 161 ± 40 mL/min, $p < 0.01$; Fig. 4). During norepinephrine infusion, PVR decreased by 33% (from 0.48 ± 0.1 to 0.32 ± 0.08 mm Hg·min·mL^{-1}; $p < 0.01$; Fig. 4). Mean AoP increased by 10 ± 1% (from 45 ± 2 to 50 ± 3 mm Hg; $p < 0.05$; Table 1). Mean PAP, left $\dot{Q}p$, and mean AoP progressively returned to baseline value after the end of drug infusion. Mean PVR returned to baseline 30 min after the end of drug infusion. Heart rate and blood gas did not change significantly during the study period (Table 1).

Protocol 5. Effect of adrenoceptor blockade on the hemodynamic response to norepinephrine. Norepinephrine infusion at 0.5 μg·kg^{-1}·min^{-1} during yohimbine infusion increased mean PAP by 10% (from 50 ± 3 to 55 ± 3 mm Hg; $p < 0.05$; $n = 8$; Fig. 5). Both left $\dot{Q}p$ and PVR did not change significantly during concomitant norepinephrine and yohimbine infusion (Fig. 5). Mean AoP, heart rate, and blood gas did not change during the study period (Table 1).

DISCUSSION

In this *in vivo* experimental study, we examined the role of the α_2-adrenoceptors on the pulmonary circulation during fetal life and the mechanisms underlying fetal pulmonary vasodilation induced by norepinephrine. We studied the response to both a selective α_2-adrenoceptor antagonist and agonist on the basal pulmonary vascular tone and the effect of a selective α_2-adrenoceptor antagonist on the norepinephrine-induced pulmonary vasodilation in chronically prepared late-gestation fetal lambs. We found that yohimbine (α_2-adrenoceptor antagonist) induces a pulmonary vasoconstriction, whereas UK 14,304 (α_2-adrenoceptor agonist) induces a potent pulmonary vasodilation. NOS inhibition prevents UK 14,304-mediated pulmonary vasodilation. Furthermore, yohimbine abolishes the norepinephrine-mediated pulmonary vasodilation.

This study provides new information regarding the mechanisms that regulate pulmonary vascular tone during fetal life. Low fetal Pao_2 (21, 22), lack of a gas–liquid interface (1), and production of vasoconstrictor mediators such as leukotrienes (23, 24) and endothelin-1 (25) contribute to maintain high PVR in the fetus. However, endogenous release of NO modulates the vasoconstrictor tone in the late-gestation fetus (4, 5) and mediates pulmonary vasodilation to several physiologic and pharmacologic stimuli such as acetylcholine, shear stress, and oxygen (4, 5, 26, 27). NO production also contributes to the decrease in PVR at the time of birth (4, 28–30). Inhibition of NOS with L-arginine analogs increases basal PVR and selectively blocks endothelium-dependent vasodilation to many stimuli (4). As selective α_2-adrenoceptor blockade by yohimbine increases PVR, a basal α_2-adrenoceptor activation–induced vasodilation exists in the fetal lung. The potent pulmonary vasodilation observed with UK 14,304 further indicates that selective activation of α_2-adrenoceptors decreases PVR. As observed with most pulmonary endothelium-dependent vasodilator stimuli in the fetus (acetylcholine, prostaglandins, shear stress, bradykinin), the pulmonary vascular response to UK 14,304 was transient (2, 3). The mechanisms opposing prolonged pulmonary vasodilation are uncertain but may include time-dependent decreased ability to sustain production or effectiveness of endogenous vasodilators or enhanced production of vasoconstrictors. Previous *in vitro* studies clearly showed that α_2-adrenoceptor activation-induced vasodilation results from NO release (7–9). Our results show-

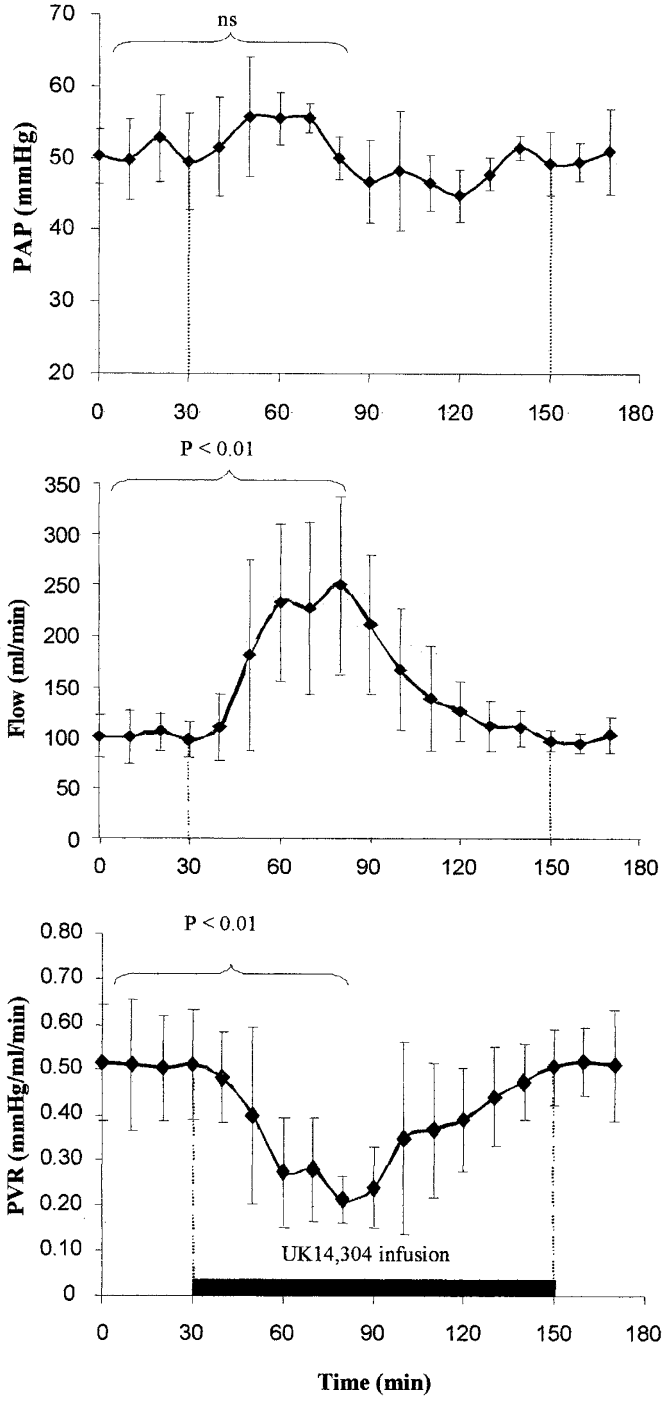

Figure 2. Pulmonary hemodynamic response to UK 14,304 (α_2-adrenoceptor agonist) infusion (0.8 mg/kg for 2 h; $n = 5$). UK 14,304 increased left $\dot{Q}p$ ($p < 0.01$) and decreased PVR ($p < 0.01$). Values are expressed as mean ± SD.

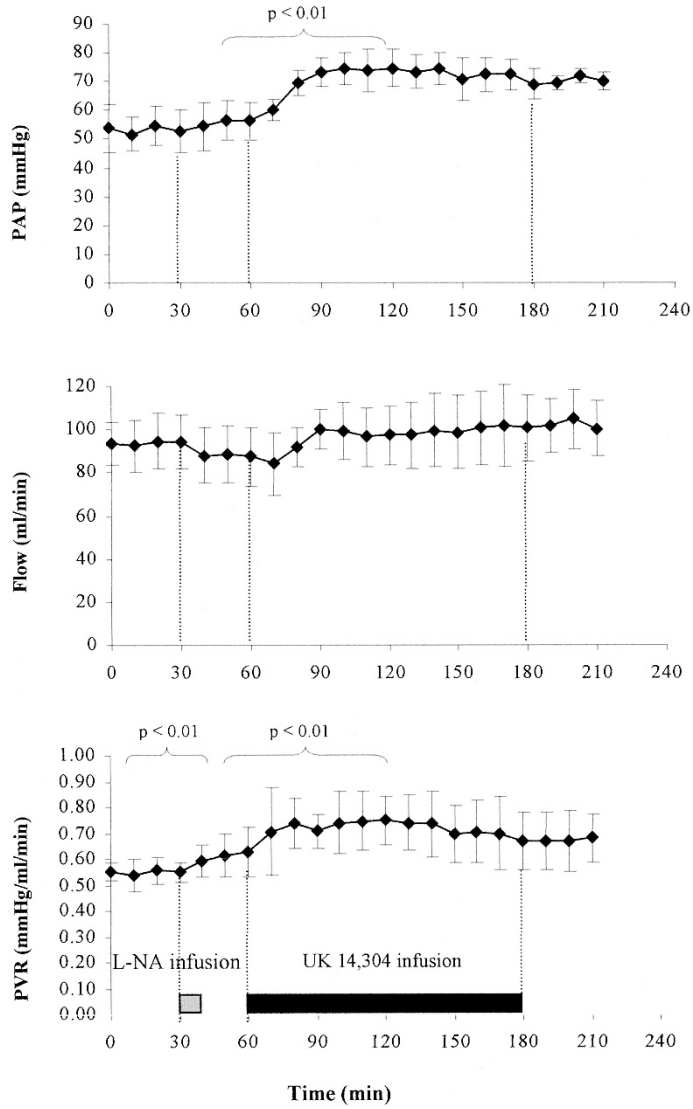

Figure 3. Pulmonary hemodynamic response to UK 14,304 infusion after NOS inhibition ($n = 5$). L-NA infusion increased mean PVR ($p < 0.01$). Then, whereas $\dot{Q}p$ did not change, both mean PAP and PVR increased during UK 14,304 infusion. Values are expressed as mean ± SD.

ing that NO inhibition completely abolishes the UK 14,304-mediated pulmonary vasodilation further support the hypothesis that α_2-adrenoceptor activation results in pulmonary vasodilation through increased NO release. Taken together, these findings indicate that α_2-adrenoceptors may modulate basal pulmonary vascular tone in the developing lung and that NO release mediates the α_2-adrenoceptor activation–induced pulmonary vasodilation.

Endogenous α_2-adrenoceptor agonists are uncertain, but may include norepinephrine. *In vitro* studies have reported that relaxant effects of norepinephrine observed in some systemic (15) and pulmonary (16) vessels in newborn animals result from endothelial α_2-adrenoceptor activation. Our *in vivo* studies further support the hypothesis that norepinephrine may activate α_2-adrenoceptors: *1*) norepinephrine induces a potent pulmonary vasodilation in fetal lambs (17); *2*) norepinephrine-induced pulmonary vasodilation is mediated by NO production (17); *3*) selective α_2-adrenoceptor blockade abolishes the pulmonary vasodilator response to norepinephrine; and *4*) the α_2-agonist UK 14,304 mimics the effects of norepinephrine on the pulmonary circulation. High circulating norepinephrine levels are found during fetal life (10, 11). Thus, the increase in PVR observed in our study during yohimbine infusion may result from inhibition of norepinephrine-induced α_2-adrenoceptor activation. However, we cannot rule out that

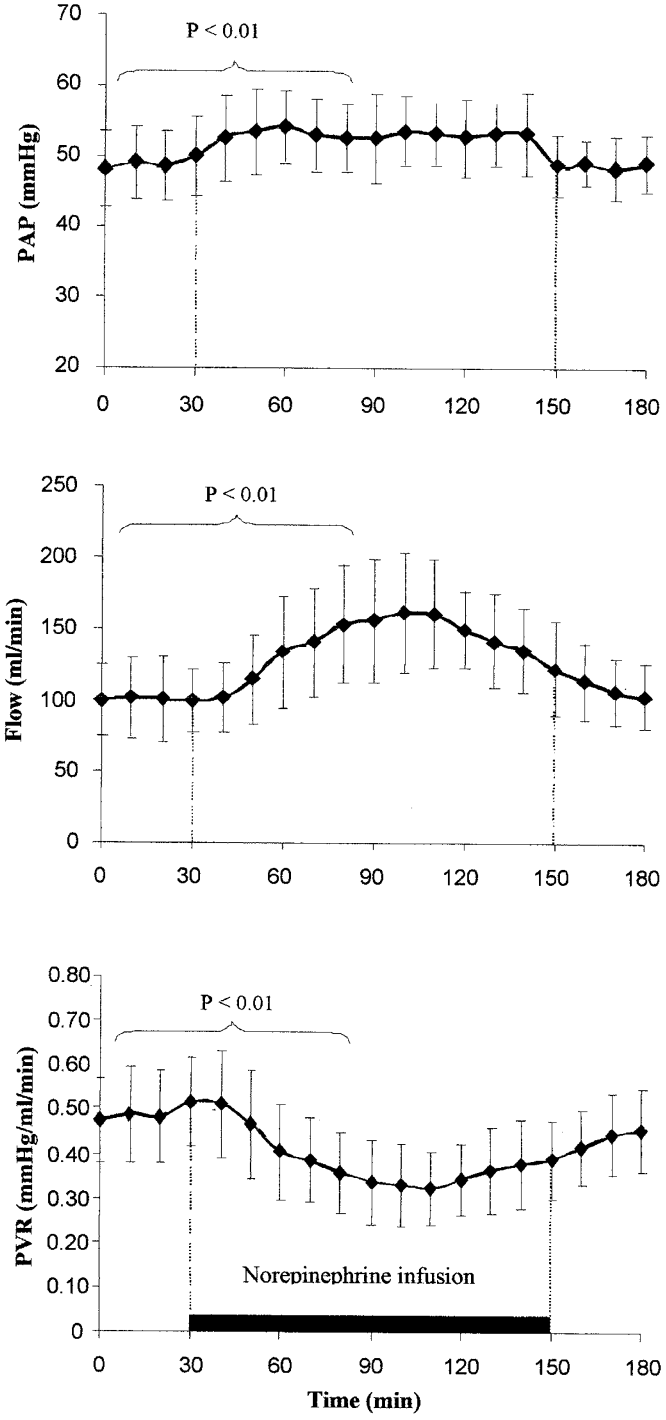

Figure 4. Pulmonary hemodynamic response to norepinephrine infusion (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$; $n = 11$). Norepinephrine increased left $\dot{Q}p$ and PAP ($p < 0.05$) and decreased PVR ($p < 0.05$). Values are expressed as mean ± SD.

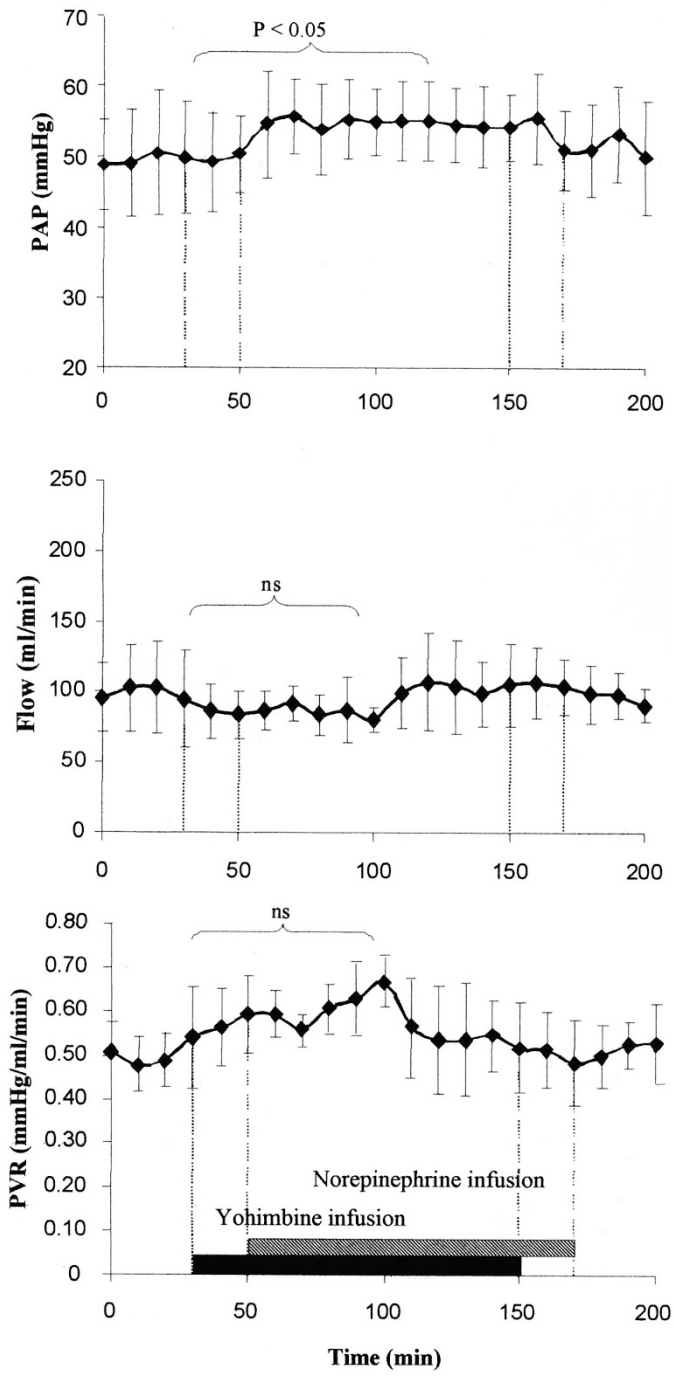

Figure 5. Effect of concomitant infusion of yohimbine (α_2-adrenoceptor antagonist) and norepinephrine ($n = 8$). Norepinephrine infusion did not alter the left $\dot{Q}p$ or PVR during yohimbine infusion. Values are expressed as mean ± SD.

other endogenous α_2-adrenoceptor agonists exist in the fetal circulating blood.

Vascular α-adrenoceptors are divided into α_1 and α_2 subtypes. In contrast to larger arteries, which generally have only α_1-adrenoceptors mediating contraction, both α_1- and α_2-adrenoceptors are involved in the modulation of tone of the resistance arteries. α_2-Adrenoceptors can be found both on vascular smooth muscle cells and on the endothelium, where they exert opposing effects on vascular tone. Several lines of evidence suggest that α_2-adrenoceptors participate in the control of pulmonary vascular tone. Removal of the endothelium causes a shift to the left of the concentration-response curves to norepinephrine and the selective α_2-adrenoceptor agonist UK 14,304 in canine pulmonary vessels (8). In that study, norepinephrine and UK 14,304 induced relaxation of precontracted ring vessels in the presence of α_1-adrenoceptor blockers. These vasorelaxant effects are abolished by α_2-adrenoceptor antagonists and by endothelium removal (8). Moreover, α_2-

adrenoceptor agonists mediate relaxation of precontracted porcine pulmonary arteries when endothelium is intact (7). Inhibition of NOS enhances the contractile response to norepinephrine and UK 14,304 in rabbit isolated pulmonary artery (9). Our results are in accordance with these *in vitro* studies. Whereas UK 14,304 decreased PVR, an increase in pulmonary vascular tone was observed with UK 14,304 after L-NA infusion, indicating that NOS inhibition not only abolishes the pulmonary vasodilator effects of α_2-adrenoceptors agonists but also unmasks their pulmonary vasoconstrictive properties. As α_2-adrenoceptor agonists induce pulmonary smooth muscle cell contraction (7–9), pulmonary vascular response to α_2-adrenoceptor blockade and activation in our study may result from the balance between activation of α_1- and α_2-adrenoceptor–induced vasoconstriction and endothelial α_2-adrenoceptor–mediated pulmonary vasodilation.

There are four potential limitations to our study. First, the drug infusions (UK 14,304, yohimbine, norepinephrine, L-NA) may have caused changes in ductus arteriosus tone. As ductus arteriosus compression may induce pulmonary vasodilation (3), norepinephrine- and UK 14,304-mediated pulmonary vasodilation may result from ductus arteriosus constriction. However, this hypothesis is unlikely as the pressure gradient between the pulmonary artery and aorta did not change during drug infusion, suggesting a lack of significant effect on basal tone of the ductus arteriosus. Second, we cannot rule out that the changes of PVR observed during UK 14,304 or yohimbine infusion may result, at least in part, from activation or blockade of the presynaptic α_2-adrenoceptors. Indeed, activation of prejunctional α_2-adrenoceptors may inhibit stimulation-evoked neurotransmitter release such as norepinephrine from both sympathetic nerves and CNS (31). Conversely, α_2-adrenoceptor antagonists may enhance neuronal norepinephrine release (31). Such changes in sympathetic outflow may modulate pulmonary vascular tone. However, our previous *in vivo* study does not support this hypothesis as norepinephrine reduces PVR in the fetal lamb (17). Third, changes in pulmonary vascular tone observed during norepinephrine or yohimbine infusion may result from systemic or centrally mediated reflex events. Indeed, both norepinephrine and yohimbine increase pulmonary and systemic arterial pressure. As an increase in PAP elevates $\dot{Q}p$ (3), norepinephrine-induced vasodilation may be caused by the mechanical increase in shear stress. Furthermore, shear stress–mediated pulmonary vasodilation is also NO dependent. However, norepinephrine-mediated increase in PAP was lower (mean increase in PAP = 6 mm Hg) than the increase in PAP required to induce pulmonary vasodilation (usually 15 mm Hg) (3). On the other hand, the similar increase in PAP during yohimbine infusion (mean increase in PAP = 7 mm Hg) was associated with decreased $\dot{Q}p$. Thus, it is unlikely that the pulmonary vascular effects of norepinephrine or yohimbine may be exclusively related to an increase in PAP. Fourth, at baseline, mean fetal Pa_{O_2} was low in this study. As fetal Pa_{O_2} is a potent modulator of pulmonary vascular tone (32), low Pa_{O_2} may have increased pulmonary vascular tone or pulmonary vascular reactivity. However, Pa_{O_2} did not change during the experiments. Thus, the pulmonary vascular responses to drug infusion cannot be related to altered fetal Pa_{O_2}. Moreover, such levels of Pa_{O_2} have been previously reported in fetal lambs (32, 33). Finally, in our study, basal PVRs were in the same range as those measured in previous studies (17, 32, 33).

CONCLUSION

In this study, we showed that α_2-adrenoceptors are involved in the control of basal pulmonary vascular tone and in the pulmonary vasodilator effect of norepinephrine during fetal life. Especially, α_2-adrenoceptor activation induces a potent NO-dependent pulmonary vasodilation. As a surge of norepinephrine exists at birth, we speculate that norepinephrine and α_2-adrenoceptor activation may play a significant role in the pulmonary vasodilation at birth, along with other stimuli such as shear stress, increased Pa_{O_2}, tidal ventilation, or bradykinin. The respective role of each factor, which may vary with time (late gestation, birth, immediate postnatal period), remains to be determined.

Acknowledgment. The authors gratefully acknowledge Steven H. Abman, M.D., for help in developing the experimental ovine model.

REFERENCES

1. Teitel DF, Iwamoto H, Rudolph AM 1987 Effects of birth-related events on central blood flow patterns. Pediatr Res 22:557–566
2. Assali NS, Kirschbaum TH, Dilts PV 1968 Effects of hyperbaric oxygen on *in utero* placental and fetal circulation. Circ Res 22:573–588
3. Abman SH, Accurso FJ 1989 Acute effects of partial compression of ductus arteriosus on fetal pulmonary circulation. Am J Physiol 257:H626–H634
4. Abman SH, Chatfield BA, Hall SL, McMurtry IF 1990 Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. Am J Physiol 259:H1921–H1927
5. Cornfield DN, Chatfield BA, McQueston JA, McMurtry IF, Abman SH 1992 Effects of birth-related stimuli on L-arginine-dependent pulmonary vasodilation in ovine fetus. Am J Physiol 262:H1474–H1481
6. Rairigh RL, Storme L, Parker TA, LeCras TD, Kinsella JP, Jakkuli M, Abman SH 1999 Inducible (type II) nitric oxide synthase attenuates shear stress-induced pulmonary vasodilation in the late gestation ovine fetus. Am J Physiol 276:L513–L521
7. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Ridden C, Kealey T 1993 Alpha-adrenergic stimulation of porcine pulmonary arteries. Eur J Pharmacol 235:169–175
8. Miller VM, Vanhoutte PM 1985 Endothelial α_2-adrenoceptors in canine pulmonary and systemic blood vessels. Eur J Pharmacol 118:123–129
9. MacLean MR, McCulloch KM, McGrath JC 1993 Influences of the endothelium and hypoxia on α_1 and α_2-adrenoceptor-mediated responses in the rabbit isolated pulmonary artery. Br J Pharmacol 108:155–161
10. Eliot RJ, Klein AH, Glatz TH, Nathanielsz PW, Fisher DA 1981 Plasma norepinephrine, epinephrine, and dopamine concentrations in maternal and fetal sheep during spontaneous parturition and in premature sheep during cortisol induced parturition. Endocrinology 108:1678–1682
11. Peleg D, Arbogast LA, Peleg E, Ben-Jonathan N 1984 Predominance of L-dopa in fetal plasma and the amniotic fluid during late gestation in the rat. Am J Obstet Gynecol 15:880–883
12. Agata Y, Hiraishi S, Misawa H, Han JH, Oguchi K, Horiguchi Y, Fujino N, Takeda N, Padbury JF 1995 Hemodynamic adaptations at birth and neonates delivered vaginally and by cesarean section. Biol Neonate 68:404–441
13. Padbury J, Agata Y, Ludlow J, Ikegami M, Baylen B, Humme J 1987 Effect of fetal adrenalectomy on catecholamine release and physiological adaptation at birth in sheep. J Clin Invest 80:1096–1103
14. Smolich JJ, Cox HS, Berger PJ, Walker AM, Eisenhofer G, Esler MD 1997 Left ventricular norepinephrine and epinephrine kinetics at birth in lambs. Circ Res 81:438–447
15. Nishina H, Ozaki T, Hanson MA, Poston L 1999 Mechanisms of noradrenaline-induced vasorelaxation in isolated femoral arteries of the neonatal rat. Br J Pharmacol 127:809–812
16. Wilson LE, Levy M, Stuart-Smith K, Haworth SG 1993 Postnatal adrenoreceptor maturation in porcine intrapulmonary arteries. Pediatr Res 34:591–595
17. Jaillard S, Houfflin-Debarge V, Riou Y, Rakza T, Klosowski S, Lequien P, Storme L 2001 Effects of catecholamines on the pulmonary circulation in the ovine fetus. Am J Physiol 281:R607–R614
18. Tulloh RM, Dyamenahalli U, Stuart SK, Haworth SG 1994 Adrenoceptor-stimulated endothelium-dependent relaxation in porcine intrapulmonary arteries. Pulm Pharmacol 7:299–303

19. Abman SH, Accurso FJ, Ward RM, Wilkening RB 1986 Adaptation of fetal pulmonary blood flow to local infusion of tolazoline. Pediatr Res 20:1131–1135
20. Anderson DF, Bissonette JM, Farber JJ, Thornburg KL 1981 Central shunt flows and pressures in the mature fetal lamb. Am J Physiol 241:H60–H66
21. Rudolph AM, Heymann MA 1972 Pulmonary circulation in fetal lambs. Pediatr Res 6:341–347
22. Lewis AB, Heymann MA, Rudolph AM 1976 Gestational changes in pulmonary vascular responses in fetal lambs *in utero.* Circ Res 39:536–541
23. Soifer SJ, Loitz RD, Roman C, Heymann MA 1985 Leukotriene end-organ antagonists increase pulmonary blood flow in fetal lambs. Am J Physiol 249:H570–H576
24. Cassin S, Gause G, Davis T, TerRiet M, Baker R 1988 Do inhibitors of lipoxygenase and cyclooxygenase block neonatal hypoxic pulmonary vasoconstriction? J Appl Physiol 66:1779–1784
25. Ivy DD, Kinsella JP, Abman SH 1994 Physiologic characterization of endothelin A and B receptor activity in the ovine fetal pulmonary circulation. J Clin Invest 93:2141–2148
26. McQueston JA, Cornfield DN, McMurtry IF, Abman SH 1993 Effects of oxygen and exogenous L-arginine on EDRF activity in the fetal pulmonary circulation. Am J Physiol 264:H865–H871
27. Shaul PW, Farrar MA, Zellers TM 1992 Oxygen modulates endothelium-derived relaxing factor production in fetal pulmonary arteries. Am J Physiol 262:H355–H364
28. McQueston JA, McMurtry IF, Abman SH 1992 Effects of birth-related stimuli on L-arginine-dependent pulmonary vasodilation in the ovine fetus. Am J Physiol 262:H1363–H1368
29. Tiktinsky MH, Morin III FC 1993 Increasing oxygen tension dilates fetal pulmonary circulation via endothelium-derived relaxing factor. Am J Physiol 265:H376–H380
30. Le Cras TD, Xue C, Rengasamy A, Johns RA 1996 Chronic hypoxia upregulates endothelial and inducible NO synthase gene and protein expression in rat lung. Am J Physiol 270:L164–L170
31. Starke K, Göthert M, Kilbinger H 1989 Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol Rev 69:864–989
32. Abman SH, Accurso FJ, Wilkening RW, Meschia G 1987 Persistent pulmonary hypoperfusion following acute hypoxia. Am J Physiol 253:H941–H948
33. Storme L, Rairigh RL, Parker TA, Kinsella JP, Abman SH 1999 Acute pulmonary hypertension *in utero* impairs endothelium-dependent vasodilation. Pediatr Res 45:575–581