

Recent Advances in the Pathogenesis and Treatment of Persistent Pulmonary Hypertension of the Newborn

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Key Words

Persistent pulmonary hypertension of the newborn • Nitric oxide • Cyclic GMP • Pulmonary hypertension • Rho-kinase • Fasudil • Superoxide dismutase • Sildenafil • BAY 41-2272 • Lung development • Endothelin-1 • Vascular endothelial growth factor

Abstract

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome characterized by failure of the lung circulation to achieve or sustain the normal drop in pulmonary vascular resistance (PVR) at birth. Past laboratory studies identified the important role of nitric oxide (NO)-cGMP signaling in the regulation of the perinatal lung circulation, leading to the development and application of inhaled NO therapy for PPHN. Although inhaled NO therapy has improved the clinical course and outcomes of many infants, pulmonary hypertension can be refractory to inhaled NO, suggesting the need for additional approaches to severe PPHN. To develop novel therapeutic strategies for PPHN, ongoing studies continue to explore basic mechanisms underlying the pathobiology of PPHN in experimental models, including strategies to enhance NO-cGMP signaling. Recent studies have demonstrated that impaired vascular endothelial growth factor (VEGF) signaling may contribute to the pathogenesis of PPHN. Lung VEGF expression is markedly decreased in an experimental model of PPHN in sheep; inhi-

bition of VEGF mimics the structural and functional abnormalities of PPHN, and VEGF treatment improves pulmonary hypertension through upregulation of NO production. Other studies have shown that enhanced NO-cGMP activity through the use of cGMP-specific phosphodiesterase inhibitors (sildenafil), soluble guanylate cyclase activators (BAY 41-2272), superoxide scavengers (superoxide dismutase), and rho-kinase inhibitors (fasudil) can lead to potent and sustained pulmonary vasodilation in experimental PPHN. Overall, these laboratory studies suggest novel pharmacologic strategies for the treatment of refractory PPHN.

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Introduction

At birth the pulmonary circulation must undergo a marked fall from its high resistance state in utero to a low resistance circuit within minutes after delivery to ensure survival of the newborn. This postnatal fall in pulmonary vascular resistance (PVR) allows for the 8-fold increase in pulmonary blood flow, and allows the lung to become an organ for gas exchange. Several mechanisms contribute to the normal fall in PVR at birth, including increased oxygen tension, ventilation, and shear stress [1–5]. These physiologic stimuli lower PVR directly and through changes in the production of several vasoactive products, including increased release of potent endogenous dilators,

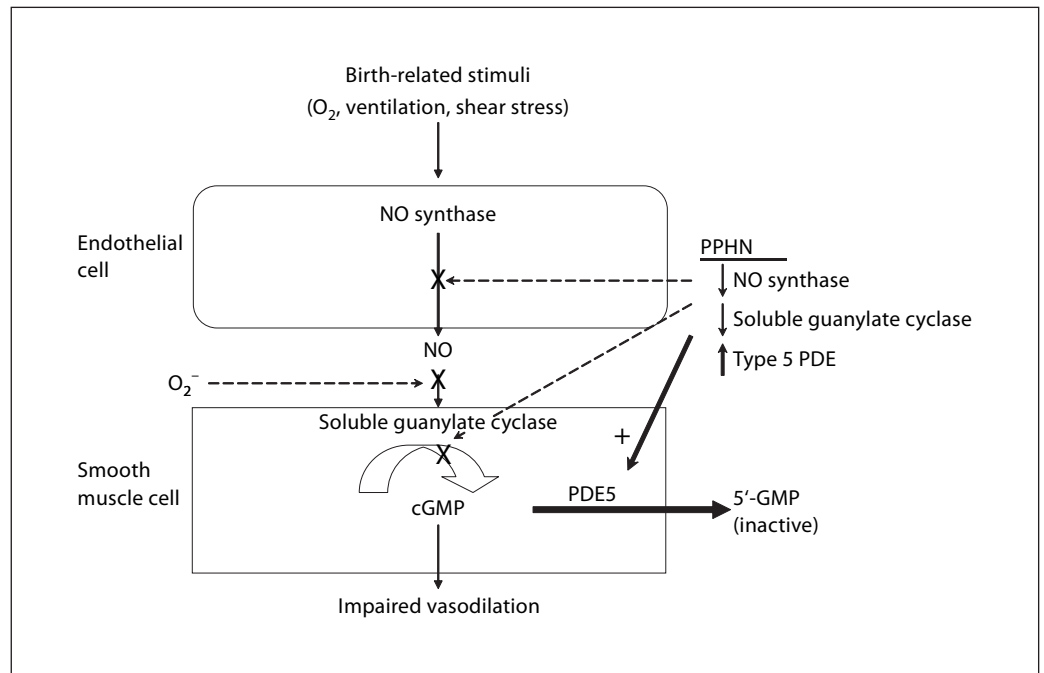


Fig. 1. Abnormalities in the NO-cGMP signaling pathway in PPHN. Experimental models have shown that downregulation of eNOS, sGC and PDE5 contribute to the inability to sustain pulmonary vasodilation. In addition, high superoxide (O_2^-) generation impairs NO bioavailability, further limiting vascular responsiveness.

including nitric oxide (NO) and prostacyclin (PgI_2), and decreased activity of vasoconstrictors, such as endothelin-1 (ET-1) [6–8]. Within minutes of delivery, high pulmonary blood flow abruptly increases shear stress and distends the vasculature causing a structural reorganization of the vascular wall that includes flattening of the endothelium and thinning of smooth muscle cells and matrix [9, 10]. Thus, the ability to accommodate this marked rise in blood flow requires rapid functional and structural adaptations to ensure the normal postnatal fall in PVR.

Some infants fail to achieve or sustain the normal decrease in PVR at birth, leading to severe respiratory distress and hypoxemia, which is referred to as persistent pulmonary hypertension of the newborn (PPHN). PPHN is a major clinical problem contributing significantly to high morbidity and mortality in both full-term and premature neonates [11, 12]. Newborns with PPHN are at risk of severe asphyxia and its complications including death, neurologic injury and other problems. Studies over the past two decades have clearly shown the critical role of NO-cGMP signaling in the regulation of the fetal and neonatal pulmonary circulation, and that disruption of the NO-cGMP cascade during the perinatal period leads

to PPHN [13, 14] (fig. 1). The ability of the newborn lung to produce NO is dependent upon adequate endothelial NO synthase (eNOS) expression, its ability to sustain NO production, NO bioavailability and related factors. Of several enzymes that govern NO production and its activities in the developing lung circulation, soluble guanylate cyclase (sGC) and the cGMP-specific (type 5) phosphodiesterase (PDE5) play especially important roles.

This review briefly discusses the normal developmental physiology of NO-cGMP in the pulmonary circulation, evidence suggesting that impaired NO-cGMP activity and related signaling pathways contribute to the pathobiology of PPHN, and potential implications for novel approaches to refractory PPHN.

NO-cGMP Signaling in the Fetal Pulmonary Circulation

PVR is high throughout fetal life and as a result the fetal lung receives less than 3–8% of combined ventricular output, with most of the right ventricular output crossing the ductus arteriosus to the aorta. Pulmonary

artery pressure and blood flow increase with advancing gestational age along with increasing lung vascular growth [15–17]. Despite this increase in vascular surface area, PVR increases with gestational age when corrected for lung or body weight, suggesting that vascular tone actually increases during late gestation and is high prior to birth. Studies of the human fetus support physiologic observations from fetal lambs [17].

Several mechanisms contribute to high basal PVR in the fetus including low oxygen tension, relatively low basal production of vasodilator products (such as PgI₂ and NO), increased production of vasoconstrictors (including ET-1) and altered smooth muscle cell reactivity (such as enhanced myogenic tone). In addition to high PVR the fetal pulmonary circulation is also characterized by progressive changes in responsiveness to vasoconstrictor and vasodilator stimuli (or ‘vasoreactivity’). In the ovine fetus, the pulmonary circulation is initially poorly responsive to vasoactive stimuli during the early canalicular period and responsiveness to several stimuli increases during late gestation [13]. As observed in the sheep fetus, human studies also demonstrate maturational changes in the fetal pulmonary vascular response to increased PaO₂ [17]. Maternal hyperoxia increases pulmonary blood flow in fetuses >31 weeks’ gestation but does not cause pulmonary vasodilation from 20 to 26 weeks’ gestation. Thus, the developing lung circulation undergoes functional maturation which parallels maturational changes in NO-cGMP signaling.

Mechanisms that contribute to progressive changes in pulmonary vasoreactivity during development are uncertain but include maturational changes in endothelial cell function, especially with regard to NO production [18–22]. Lung eNOS (type III) mRNA and protein are present in the early fetus and increase with advancing gestation in utero and during the early postnatal period [19–21]. A burst of lung eNOS content immediately precedes and parallels changes in the capacity to respond to endothelium-dependent vasodilators as assessed by in vivo and in vitro studies as well as marked capillary proliferation. The ability of the endothelium to produce or sustain production of NO in response to specific stimuli during maturation lags behind the capacity of fetal pulmonary smooth muscle to relax to NO [22]. This may account for clinical observations that extremely premature newborns are highly responsive to inhaled NO [23–25].

eNOS expression and activity are affected by multiple factors including oxygen tension, hemodynamic forces, hormonal stimuli (e.g., estradiol), paracrine factors (including vascular endothelial growth factor (VEGF)), sub-

strate and cofactor availability, superoxide production (which inactivates NO) and others [26–37]. Recent studies suggest that impaired VEGF signaling may contribute to the pathogenesis of PPHN and that this may in part be related to decreased NO-cGMP activity. Experimentally VEGF acutely releases NO and causes pulmonary vasodilation in vivo [29]. Lung VEGF is dramatically decreased in experimental PPHN, and chronic inhibition of VEGF receptors downregulates eNOS and induces pulmonary hypertension in the late gestation fetus [30]. In addition, treatment with recombinant human VEGF protein increases eNOS expression and activity and improves pulmonary hypertensive remodeling in experimental PPHN [31]. Clinical studies of VEGF in blood and tracheal fluid aspirates of ventilated infants further suggest that VEGF may be decreased in human PPHN [38], further supporting the concept that VEGF is a key modulator of pulmonary vascular function in PPHN.

In addition to transcriptional and translational regulation, NO production is modulated through altered NOS activity. NOS is a heterodimer with both reductase and oxygenase domains. When there is an abundance of availability of substrates such as L-arginine and the pteridine cofactor tetrahydrobiopterin, NADPH oxidation and NO synthesis remain coupled and NO production is favored. When concentrations of one or more factors are decreased eNOS is uncoupled and generates *superoxide*. Under certain conditions, NOS may generate reactive oxygen species (e.g., superoxide) rather than NO. The balance of NO versus superoxide production depends on numerous factors, and eNOS uncoupling may account for impaired vasodilation in PPHN [32–34] (fig. 2). Steinhorn et al. [35] have demonstrated that increased superoxide may impair pulmonary vasodilation in experimental PPHN and that this may be partly related to decreased NO activity [36]. Heat shock protein 90 (Hsp90), a molecular chaperone molecule, has been recently been described as a factor that associates with NOS facilitating NO release. Konduri et al. [37] have shown that association of Hsp90 with NOS is required for NO production in response to ATP in pulmonary arteries isolated from late-gestation fetal lambs, and may be decreased in experimental PPHN.

Vascular responsiveness to endogenous or exogenous NO is also dependent upon several smooth muscle cell enzymes including sGC, cGMP-specific phosphodiesterase (PDE5) and cGMP kinase [39–44]. NO stimulates sGC by binding to the prosthetic heme of the enzyme, causing up to a 400-fold activation of the purified enzyme. Several studies have shown that sGC, which pro-

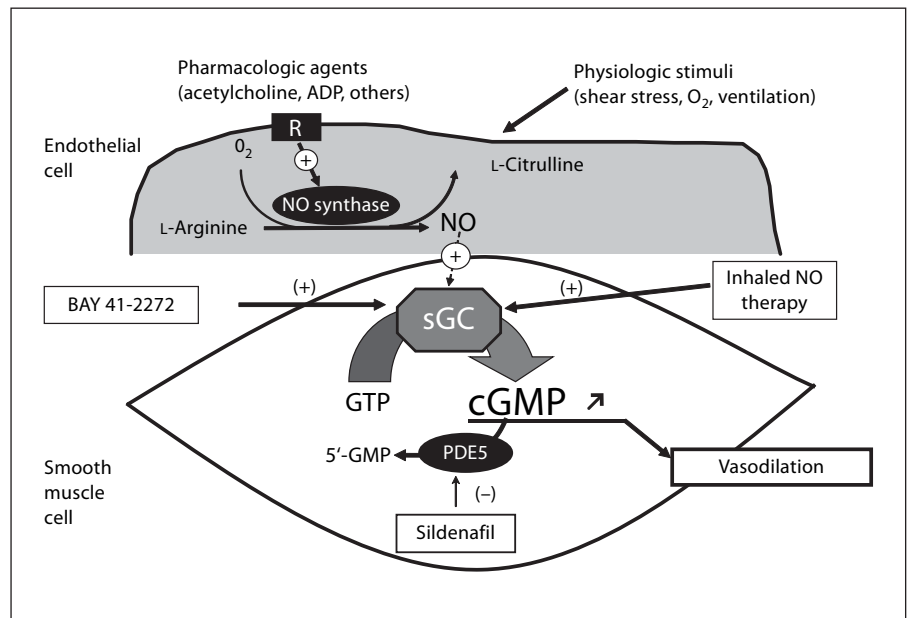


Fig. 2. Schematic illustrating the potential therapeutic targets regarding the NO-cGMP pathway in severe PPHN.

duces cGMP in response to NO activation, is active before 0.7 of term gestation in the ovine fetal lung, and direct pharmacologic stimulation of sGC with BAY 41-2272 causes dramatic pulmonary vasodilation [41]. Similar to the pattern of expression for eNOS, sGC levels are high late in gestation and are greater than those observed in the adult lung [43, 44].

Cyclic nucleotide phosphodiesterases (PDEs) constitute the only known pathway for the hydrolysis of cGMP and control the intensity and duration of its signal transduction. At least 13 families of PDE isoenzymes have been identified and several PDE isoenzymes have been identified in human pulmonary artery. PDE5, a cGMP-binding and cGMP-specific isoform, is found in especially high concentrations in the fetal lung and actively maintains high PVR [39, 41]. In the fetal lung, PDE5 expression has been localized to vascular smooth muscle and, similar to NOS and sGC, PDE5 activity is high in comparison with the postnatal lung [39]. Biochemical studies have shown that lung PDE5 activity is markedly elevated during fetal life and then rapidly falls at birth. Infusions of selective PDE5 antagonists including zaprinast, dipyridamole, E4021, DMPP0 and sildenafil cause potent and sustained fetal pulmonary vasodilation [45, 46]. Thus, PDE5 activity appears to play a critical role in pulmonary vasoregulation during the perinatal period and must be accounted for in assessing responsiveness to endogenous NO and related vasodilator stimuli. In addition, the post-

natal fall in PDE5 activity suggests a major role in sustaining low PVR during postnatal life. While most studies have focused on PDE5, there are many PDE families and isoforms that vary in their specificity for binding or metabolizing cGMP, cAMP or both. PDEs are likely important mediators of 'cross-talk' between cGMP and cAMP signaling pathways and other PDE isoforms may be important in the response to NO.

Functionally the NO-cGMP cascade plays several important physiologic roles in vasoregulation of the fetal pulmonary circulation [13]. These include: (1) modulation of basal PVR in the fetus [3]; (2) mediating the vasodilator response to specific physiologic and pharmacologic stimuli [3, 5], and (3) opposing the strong myogenic tone in the normal fetal lung [47]. Since eNOS protein is present at a stage of lung development when blood flow is absent or minimal, it has been hypothesized that NO may potentially contribute to angiogenesis during early lung development [20]. Recent laboratory studies have provided ample evidence that impaired NO-cGMP activity impairs lung vascular growth and subsequent alveolarization during development, especially in response to neonatal lung injury [48–51]. These studies suggest that therapeutic administration of inhaled NO or sildenafil can enhance lung growth in neonatal models, suggesting a potential role for the treatment of premature newborns at risk of chronic lung disease and pulmonary hypertension [52–54].

Endothelin-1 and PPHN

In addition to impaired vasodilator mechanisms, the potential role of several vasoconstrictor products has been implicated in perinatal pulmonary vasoregulation, especially ET-1. ET-1, a potent vasoconstrictor and co-mitogen that is produced by vascular endothelium, has been demonstrated to play a key role in fetal pulmonary vasoregulation [8, 55]. PreproET-1 mRNA (the precursor to ET-1) was identified in fetal lungs early during gestation, and high circulating ET-1 levels are present in umbilical cord blood. Although ET-1 causes an intense vasoconstrictor response *in vitro*, its effects in the intact pulmonary circulation are complex. Brief infusions of ET-1 cause transient vasodilation but PVR progressively increases during prolonged treatment [56]. The biphasic pulmonary vascular effects during pharmacologic infusions of ET-1 are explained by the presence of at least two different ET receptors. The ET B receptor, localized to the endothelium in the sheep fetus, mediates the ET-1 vasodilator response through the release of NO [57]. A second receptor, the ET A receptor, is located on vascular smooth muscle, and when activated causes marked constriction. Although capable of both vasodilator and constrictor responses, ET-1 is more likely to play an important role as a pulmonary vasoconstrictor in the normal fetus.

Upregulation of ET-1 contributes to the pathophysiology of PPHN. Circulating levels of ET-1, a potent vasoconstrictor and co-mitogen for vascular smooth muscle cell hyperplasia, are increased in human newborns with severe PPHN [58]. In a sheep model of PPHN, lung ET-1 mRNA and protein content are markedly increased and the balance of ET receptors are altered favoring vasoconstriction and smooth muscle cell proliferation [59]. Chronic inhibition of the ET A receptor attenuates the severity of pulmonary hypertension, decreases pulmonary artery wall thickening and improves the fall in PVR at birth in this model [60]. Thus, strong experimental data support the potential role for ET antagonists in the treatment of PPHN as currently used as a major therapy for older patients with chronic pulmonary hypertension.

Several studies have shown that NO and ET-1 regulate each other through autocrine feedback loops. NO decreases ET-1 production via a cGMP-dependent mechanism in cultured endothelial cells [61]. In addition, ET-1 can increase superoxide production which may further impair NO-mediated vasodilation [62]. Complex interactions between the NO-cGMP and ET systems have been described in experimental studies and insights into these pathways may lead to novel strategies to treat PPHN.

Rho-Kinase Activity and PPHN

Recent advances in vascular biology have identified the small GTPase RhoA and its effector protein, rho-kinase, as key regulators of vascular tone and structure [63]. In vascular smooth muscle cells, rho-kinase phosphorylates and inactivates myosin light chain phosphatase thereby promoting vasoconstriction. Previous studies have shown that prolonged treatment with rho-kinase inhibitors prevents the development of pulmonary hypertension caused by monocrotaline and hypoxia in adult rats [64, 65]. Recently, Parker et al. [66] demonstrated that rho-kinase inhibitors, fasudil and Y-27632, cause potent and sustained pulmonary vasodilation in fetal sheep, suggesting that high rho-kinase activity maintains elevated PVR *in utero*. These agents also prevented pulmonary vasoconstriction caused by inhibition of NO production, suggesting close interactions between NO and rho-kinase signaling pathways in the perinatal lung. Additional studies have further demonstrated similar findings in experimental PPHN, suggesting that rho-kinase inhibitors may provide a future therapy for severe PPHN.

Therapeutic Strategies

Management of the newborn with PPHN initially includes aggressive management of systemic hemodynamics with volume and cardiotoxic therapy (dobutamine, dopamine and milrinone) in order to enhance cardiac output and systemic O₂ transport. Increasing systemic arterial pressure itself often improves oxygenation by reducing right-to-left extrapulmonary shunting, the hallmark of PPHN physiology [67]. Pulmonary vasodilator therapy with inhaled nitric oxide (iNO) has been clearly shown to improve oxygenation and decrease the need for ECMO therapy in patients with diverse causes of PPHN [68–72]. Multicenter clinical trials support the use of iNO in near-term (>34 weeks' gestation) and term newborns, whereas the use of iNO in infants <34 weeks' gestation remains less certain. Although iNO may be an effective treatment for PPHN, it should be considered only as part of an overall clinical strategy that cautiously manages parenchymal lung disease, cardiac performance and systemic hemodynamics.

Although clinical improvement during iNO therapy occurs with many disorders associated with PPHN, not all neonates with acute hypoxemic respiratory failure and pulmonary hypertension respond to iNO. Several mechanisms may explain the clinical variability in responsive-

Table 1. New mechanisms and potential therapies for PPHN

Mechanism	Specific therapy
Increased superoxide generation	rhSOD
High PDE5 activity	PDE5 inhibitors (sildenafil)
Impaired/oxidized sGC	sGC activators/stimulators (BAY 58-2667; BAY 41-2272)
Impaired VEGF signaling	rhVEGF
Increased ET-1	ET receptor antagonists (Bosentan)
Altered PgI ₂ production	Prostacyclin analogs
High rho-kinase activity	Rho-kinase inhibitors (fasudil)

ness to iNO therapy. An inability to deliver NO to the pulmonary circulation due to poor lung inflation is the major cause of poor responsiveness. In addition, poor NO responsiveness may be related to myocardial dysfunction or systemic hypotension, severe pulmonary vascular structural disease and unsuspected or missed anatomic cardiovascular lesions (such as total anomalous pulmonary venous return, coarctation of the aorta, alveolar capillary dysplasia and others). Prolonged need for iNO therapy without resolution of disease should lead to a more extensive evaluation to determine whether previously unsuspected anatomic lung or cardiovascular disease is present (e.g., pulmonary venous stenosis, alveolar capillary dysplasia, severe lung hypoplasia or others) [73, 74].

As described extensively in experimental models of PPHN, other mechanisms of poor responsiveness to therapy may be related to abnormalities in endothelial and smooth muscle cell function. Currently, sildenafil, a selective PDE5 inhibitor, has been shown to improve oxygenation in infants with PPHN, especially at centers lacking inhaled NO [75, 76], and has been used extensively for the treatment of pulmonary hypertension in other settings. Despite extensive use of ET receptor antagonists in older patients with severe chronic pulmonary hypertension, there is limited experience with its use in infants, and whether it is effective in the acute setting is less clear.

New studies indicate that scavengers of reactive oxygen species such as superoxide dismutase (SOD), sGC activators and rho-kinase inhibitors can cause pulmonary vasodilation and augment responsiveness to iNO in the laboratory, suggesting a future role for these strategies in neonates who fail to respond to other therapies (table 1).

Conclusions

Experimental studies have clearly demonstrated the critical roles of endogenous NO-cGMP signaling in the regulation of the pulmonary circulation during development and at birth and that impaired NO-cGMP signaling contributes to PPHN. Recent laboratory studies suggest that multiple pharmacologic strategies, including the use of cGMP-specific phosphodiesterase inhibitors, sGC activators, superoxide scavengers (SOD) and rho-kinase inhibitors cause potent and sustained pulmonary vasodilation in experimental PPHN. Overall, these laboratory studies suggest novel pharmacologic strategies for the treatment of refractory PPHN in the clinical setting. More work is needed to expand our therapeutic repertoire in order to further improve the outcome of the sick newborn with severe hypoxemia, especially in patients with lung hypoplasia and advanced structural vascular disease.

References

- 1 Dawes G, Mott JC, Widdicombe JG: Changes in the lungs of the newborn lamb. *J Physiol* 1953;121:141–162.
- 2 Heymann MA, Soifer SJ: Control of fetal and neonatal pulmonary circulation; in Weir EK, Reeves JT (eds): *Pulmonary Vascular Physiology and Pathophysiology*. New York, Dekker, 1989, pp 33–50.
- 3 Abman SH, Chatfield BA, Hall SL, McMurry IF: Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. *Am J Physiol* 1990;259:H1921–H1927.
- 4 Cassin S: Role of prostaglandins, thromboxanes and leukotrienes in the control of the pulmonary circulation in the fetus and newborn. *Semin Perinatol* 1987;11:53–63.
- 5 Cornfield DN, Chatfield BA, McQueston JA, et al: Effects of birth-related stimuli on L-arginine-dependent pulmonary vasodilation in the ovine fetus. *Am J Physiol* 1992;262:H1474–H1481.
- 6 Cornfield DN, Reeves HL, Tolarova S, et al: Oxygen causes fetal pulmonary vasodilation through activation of a calcium-dependent potassium channel. *Proc Natl Acad Sci USA* 1996;93:8089–8094.

- 7 Velvis H, Moore P, Heymann MA: Prostaglandin inhibition prevents the fall in pulmonary vascular resistance as the result of rhythmic distension of the lungs in fetal lambs. *Pediatr Res* 1991;30:62-67.
- 8 Ivy DD, Kinsella JP, Abman SH: Physiologic characterization of endothelin A and B receptor activity in the ovine fetal lung. *J Clin Invest* 1994;93:2141-2148.
- 9 Allen K, Haworth SG: Impaired adaptation of intrapulmonary arteries to extrauterine life in newborn pigs exposed to hypoxia. An ultrastructural study. *J Pathol* 1986;150:205-212.
- 10 Haworth SG, Reid LM: Persistent fetal circulation. Newly recognized structural features. *J Pediatr* 1976;88:614-620.
- 11 Levin DL, Heymann MA, Kitterman JA, et al: Persistent pulmonary hypertension of the newborn. *J Pediatr* 1976;89:626-633.
- 12 Kinsella JP, Abman SH: Recent developments in the pathophysiology and treatment of PPHN. *J Pediatr* 1995;126:853-864.
- 13 Abman SH, Kinsella JP, Parker TA, Storme L, Le Cras TD: Physiologic roles of NO in the perinatal pulmonary circulation; in Weir EK, Archer SL, Reeves JT (eds): *Fetal and Neonatal Pulmonary Circulation*. New York, Futura, 1999, pp 239-260.
- 14 Abman SH, Shanley PF, Accurso FJ: Failure of postnatal adaptation of the pulmonary circulation after chronic intrauterine pulmonary hypertension in fetal lambs. *J Clin Invest* 1989;83:1849-1858.
- 15 Rudolph AM, Heymann MA, Lewis AB: Physiology and pharmacology of the pulmonary circulation in the fetus and newborn; in Hodson W (ed): *Development of the Lung*. New York, Dekker, 1977, pp 497-523.
- 16 Morin FC, Egan EA, Ferguson W, Lundgren CEG: Development of pulmonary vascular response to oxygen. *Am J Physiol* 1988;254:H542-H546.
- 17 Rasanen J, Wood DC, Debbs RH, Cohen J, Weiner S, Huhta JC: Reactivity of the human fetal pulmonary circulation to maternal hyperoxygenation increases during the second half of pregnancy. A randomized study. *Circulation* 1998;97:257-262.
- 18 Abman SH, Chatfield BA, Rodman DM, Hall SL, McMurtry IF: Maturation-related changes in endothelium-dependent relaxation of ovine pulmonary arteries. *Am J Physiol* 1991;260:L280-L285.
- 19 North AJ, Star RA, Brannon TS, Ujiie K, Wells LB, Lowenstien CJ, Snyder SH, Shaul PW: NO synthase type I and type III gene expression are developmentally regulated in rat lung. *Am J Physiol* 1994;266:L635-L641.
- 20 Halbower AC, Tudor RM, Franklin WA, Pollock JS, Forstermann U, Abman SH: Maturation-related changes in endothelial NO synthase immunolocalization in the developing ovine lung. *Am J Physiol* 1994;267:L585-L591.
- 21 Parker TA, Le Cras TD, Kinsella JP, Abman SH: Developmental changes in endothelial NO synthase expression in the ovine fetal lung. *Am J Physiol* 2000;278:L202-L208.
- 22 Kinsella JP, Ivy DD, Abman SH: Ontogeny of NO activity and response to inhaled NO in the developing ovine pulmonary circulation. *Am J Physiol* 1994;267:H1955-H1961.
- 23 Abman SH, Kinsella JP, Schaffer MS, Wilkening RB: Inhaled nitric oxide therapy in a premature newborn with severe respiratory distress and pulmonary hypertension. *Pediatrics* 1993;92:606-609.
- 24 Van Meurs K, Rhine WD, Asselin JM, et al: Response of premature infants with severe respiratory failure to inhaled NO. *Pediatr Pulmonol* 1997;24:319-323.
- 25 Peliowski A, Finer NN, Etches PC, Tierney AJ, Ryan CA: Inhaled NO for premature infants after prolonged rupture of membranes. *J Pediatr* 1995;126:450-453.
- 26 Parker TA, Kinsella JP, Galan HL, Richter G, Abman SH: Prolonged infusions of estradiol dilate the ovine fetal pulmonary circulation. *Pediatr Res* 2000;47:89-96.
- 27 Parker TA, Afshar S, Kinsella JP, Ivy DD, Shaul PW, Abman SH: Effects of chronic estrogen receptor blockade on the pulmonary circulation in the late gestation ovine fetus. *Am J Physiol* 2001;281:H1005-H1014.
- 28 MacRitchie AN, Jun SS, Chen Z, et al: Estrogen upregulates endothelial NO synthase gene expression in fetal pulmonary artery endothelium. *Circ Res* 1997;81:355-362.
- 29 Grover TR, Zenge JP, Parker TA, Abman SH: VEGF causes pulmonary vasodilation through activation of the PI₃-kinase-nitric oxide dependent pathway in the late gestation ovine fetus. *Pediatr Res* 2002;52:907-912.
- 30 Grover TR, Parker TA, Zenge JP, Markham NE, Abman SH: VEGF inhibition impairs endothelial function and causes pulmonary hypertension in the late gestation ovine fetus. *Am J Physiol* 2002;284:L508-L517.
- 31 Grover TR, Parker TA, Markham NE, Abman SH: rhVEGF treatment preserves pulmonary vasoreactivity and structure in an experimental model of pulmonary hypertension in fetal sheep. *Am J Physiol* 2005;289:L315-L321.
- 32 Schmidt HHHW, et al: No NO from NO synthase. *Proc Natl Acad Sci USA* 1996;93:14492-14497.
- 33 Pritchard KA Jr, Ackerman AW, Gross ER, Stepp DW, Shi Y, Fontana JT, Baker JE, Sessa WC: Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric oxide synthase. *J Biol Chem* 2001;276:17621-17624.
- 34 Konduri GG, Bakhtashvili I, Eis AL, et al: Oxidant stress from uncoupled NOS impairs vasodilation in fetal lambs with persistent pulmonary hypertension. *Am J Physiol* 2007 (in press).
- 35 Steinhorn RH, Albert G, Swartz DD, et al: Recombinant human SOD enhances the effect of inhaled NO in PPH. *Am J Respir Crit Care Med* 2001;164:834-839.
- 36 Lakshminrusimha S, Russell JA, Wedgwood S, et al: Superoxide dismutase improves oxygenation and reduces oxidation in neonatal pulmonary hypertension. *Am J Respir Crit Care Med* 2006;174:1370-1377.
- 37 Konduri GG, et al: Decreased association of Hsp90 impairs endothelial nitric oxide synthase in fetal lambs with persistent pulmonary hypertension. *Am J Physiol* 2003;285:H204-H211.
- 38 Lasso P, Turanlahti M, Heikkilä P, et al: Pulmonary VEGF and flt-1 in fetuses, in acute and chronic lung disease, and in persistent pulmonary hypertension of the newborn. *Am J Respir Crit Care Med* 2001;164:1981-1987.
- 39 Hanson KA, Burns F, Rybalkin SD, Miller J, Beavo J, Clarke WR: Developmental changes in lung cGMP phosphodiesterase-5 activity, protein and message. *Am J Respir Crit Care Med* 1995;158:279-288.
- 40 Cohen AH, Hanson K, Morris K, Fouty B, McMurtry IF, Clarke W, Rodman DM: Inhibition of cGMP-specific phosphodiesterase selectively vasodilates the pulmonary circulation in chronically hypoxic rats. *J Clin Invest* 1996;97:172-179.
- 41 Deruelle P, Grover TR, Storme L, Abman SH: Effects of BAY 41-2272, a soluble guanylate cyclase activator, on pulmonary vascular reactivity in the ovine fetus. *Am J Physiol* 2005;288:L727-L733.
- 42 Deruelle P, Grover TR, Abman SH: Pulmonary vascular effects of NO-cGMP augmentation in a model of chronic pulmonary hypertension in fetal and neonatal sheep. *Am J Physiol* 2005;289:L798-L806.
- 43 Tzao C, Nickerson PA, Russell JA, Noble BK, Steinhorn RH, et al: Paracrine role of soluble guanylate cyclase and type III nitric oxide synthase in ovine fetal pulmonary circulation: a double labeling immunohistochemical study. *Histochem Cell Biol* 2003;119:125-130.
- 44 Bloch KD, Filippov G, Sanchez LS, Nakane M, de la Monte SM, et al: Pulmonary soluble guanylate cyclase, a nitric oxide receptor, is increased during the perinatal period. *Am J Physiol* 1997;272:L400-L406.
- 45 Thusu KG, Morin FC, Russell JA, et al: The cGMP phosphodiesterase inhibitor zaprinast enhances the effect of NO. *Am J Respir Crit Care Med* 1995;152:1605-1610.
- 46 Ziegler JW, Ivy DD, Fox JJ, Kinsella JP, Clarke WR, Abman SH: Dipyridamole, a cGMP phosphodiesterase inhibitor, causes pulmonary vasodilation in the ovine fetus. *Am J Physiol* 1995;269:H473.
- 47 Storme L, Rairhig RL, Abman SH: In vivo evidence for a myogenic response in the ovine fetal pulmonary circulation. *Pediatr Res* 1999;45:425-431.

- 48 Lin YJ, Markham NE, Balasubramaniam V, et al: Inhaled NO enhances distal lung growth after exposure to hyperoxia in neonatal rats. *Pediatr Res* 2005;58:22–29.
- 49 Tang JR, Markham NE, Lin YJ, et al: Inhaled NO attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor. *Am J Physiol* 2004;287:L344–L351.
- 50 Balasubramaniam V, Tang JR, Maxey A, et al: Mild hypoxia impairs alveolarization in the endothelial nitric oxide synthase deficient mouse. *Am J Physiol* 2003;284:L964–L971.
- 51 Ladha F, Bonnet S, Eaton F, et al: Sildenafil improves alveolar growth and pulmonary hypertension in hyperoxia-induced lung injury. *Am J Respir Crit Care Med* 2005;172:750–756.
- 52 Schreiber MD, Gin-Mestan K, Marks JD, et al: Inhaled nitric oxide in premature infants with the respiratory distress syndrome. *N Engl J Med* 2003;349:2099–2107.
- 53 Ballard RA, Truog WE, Cnaan A, et al: Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. *N Engl J Med* 2006;205:343–353.
- 54 Kinsella JP, Cutter GR, Walsh WF, et al: Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med* 2006;205:354–364.
- 55 Ivy DD, Abman SH: Role of endothelin in perinatal pulmonary vasoregulation; in Weir EK, Archer SL, Reeves JT (eds): *Fetal and Neonatal Pulmonary Circulation*. New York, Futura, 1999, pp 279–302.
- 56 Chatfield BA, McMurry IF, Hall SL, Abman SH: Hemodynamic effects of endothelin-1 on the ovine fetal pulmonary circulation. *Am J Physiol* 1991;261:R182–R187.
- 57 Ivy DD, Parker TA, Abman SH: Prolonged endothelin B receptor blockade causes pulmonary hypertension in the ovine fetus. *Am J Physiol* 2000;297:L758–L765.
- 58 Rosenberg AA, Kennaugh J, Koppenhafer SL, et al: Increased immunoreactive endothelin-1 levels in persistent pulmonary hypertension of the newborn. *J Pediatr* 1993;123:109–114.
- 59 Ivy DD, LeCras TD, Horan MP, Abman SH: Increased lung prepro-endothelin-1 and decreased endothelin B receptor gene expression after chronic pulmonary hypertension in the ovine fetus. *Am J Physiol* 1998;274:L535–L541.
- 60 Ivy DD, Parker TA, Ziegler JW, et al: Prolonged endothelin A receptor blockade attenuates chronic pulmonary hypertension in the ovine fetus. *J Clin Invest* 1997;99:1179–1186.
- 61 Kourembanas S, McQuillan LP, Leung GK, et al: Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest* 1993;92:99–104.
- 62 Wedgwood S, McMullan DM, Bekker JM, Fineman JR, Black SM et al: Role for endothelin-1-induced superoxide and peroxynitrite production in rebound pulmonary hypertension associated with inhaled nitric oxide therapy. *Circ Res* 2001;89:357–364.
- 63 Somlyo AP, Wu X, Walker LA, Somlyo AV: Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases. *Rev Physiol Biochem Pharmacol* 1999;134:201–234.
- 64 Fagan KA, Oka M, Bauer NR, et al: Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of rho-kinase. *Am J Physiol* 2004;287:L656–L664.
- 65 Nagaoka T, Fagan KA, Gebb SA, et al: Inhaled rho kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension. *Am J Respir Crit Care Med* 2005;171:494–499.
- 66 Parker TA, Roe G, Grover TR, Abman SH: Rho kinase activation maintains high PVR in the ovine fetal lung. *Am J Physiol* 2006;291:L976–L982.
- 67 Kinsella JP, Abman SH: Clinical approach to inhaled NO therapy in the newborn. *J Pediatr* 2000;136:717–726.
- 68 Kinsella JP, Neish S, Shaffer E, Abman SH: Low dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992;340:819–820.
- 69 Roberts JD, Polaner DM, Lang P, Zapol WM: Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992;340:818–819.
- 70 Clark RH, Kueser TJ, Walker MW, et al: Low-dose inhaled NO treatment of PPHN. *N Engl J Med* 2000;342:469–474.
- 71 Neonatal Inhaled NO Study Group: Inhaled NO in full-term and nearly full-term infants with hypoxic respiratory failure. *N Engl J Med* 1997;336:597–604.
- 72 Roberts JD, Fineman JR, Morin FC, et al: Inhaled NO and PPHN. *N Engl J Med* 1997;336:605–610.
- 73 Goldman AP, Tasker RC, Haworth SG, et al: Four patterns of response to inhaled NO for PPHN. *Pediatrics* 1996;98:706–713.
- 74 Hintz SR, Vincent JA, Pitlick PT, Fineman JR, Steinhorn RM, Kim GE, Benitz WE: Alveolar capillary dysplasia: diagnostic potential for cardiac catheterization. *J Perinatol* 1999;19:441–446.
- 75 Kinsella JP, Troug W, Walsh W, et al: Randomized multicenter trial of inhaled nitric oxide and high frequency oscillatory ventilation in severe PPHN. *J Pediatr* 1997;131:55–62.
- 76 Baquero H, Soliz A, Neira F, et al: Oral sildenafil in infants with PPHN: a pilot randomized blinded study. *Pediatrics* 2007;117:1077–1083.