Regulation of the Pulmonary Circulation in the Fetus and Newborn

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I. INTRODUCTION

In the developing fetal lung, the pulmonary vasculature undergoes an initial vascular tube formation that is followed by the establishment of a hierarchical vascular system. In humans, although a continuity of the circulation between the heart and the capillary plexus of the lung has been documented at around 34 days of gestation (227, 228), there is no appreciable reactivity of pulmonary vessels at midterm gestation (364, 480). Reactivity of the human fetal pulmonary circulation increases thereafter with advancing gestation, which is characterized by high pulmonary vascular resistance (PVR) and low blood flow to the lung (<20% of combined ventricular output) (480). The thick-walled pulmonary vessels of the fetus (258) as well as the high vasomotor tone in these vessels contribute to the high PVR (250, 501). A major fraction of the cardiac output is diverted away from the lung to other...
organs via the foramen ovale and the ductus arteriosus (331). This process is facilitated by the greater resistance in the fetal pulmonary circulation compared with the systemic circulation (4, 407). Oxygen tension in the fetal pulmonary circulation is lower than in the newborn and the adult, with \( P_{\text{O}_2} \) ranging from 17 to 19 mmHg. However, since fetal blood contains a high concentration of fetal hemoglobin that has a higher oxygen affinity than adult hemoglobin (98, 108, 331), there is sufficient oxygen delivery to the lung to support its growth and metabolic functions (407, 500).

At birth, with the onset of breathing, PVR dramatically decreases and pulmonary blood flow increases such that the entire right ventricular output goes to the lung, as they assume the function of gas exchange (350, 481) (Fig. 1). The decrease in PVR is attributed in part to a rapid reorganization of the structure of the pulmonary vessel walls, recruitment of intra-acinar arteries, and a more gradual vascular remodeling (242, 256–259). An increased release of vasodilators, in particular endothelium-derived nitric oxide (EDNO) and PGL\(_2\), and a decreased release of vasoconstrictors such as platelet activating factor (PAF), as well as changes in their signaling pathways contribute significantly to the fall in PVR (243, 250, 276, 279, 295, 501). These mechanisms are not only involved in the immediate postnatal fall in PVR but also help maintain the low PVR in the newborn and the adult. The hemodynamic changes in the pulmonary circulation prior to, at, and after birth are regulated by various mechanical factors and vasoactive agents in a complicated but coordinated manner (180, 211, 564).

This review discusses the development of the pulmonary vasculature and the regulation of the fetal and neonatal pulmonary circulation. Finally, the condition of persistent pulmonary hypertension of the newborn, an altered regulation of newborn pulmonary circulation, are discussed.

## II. DEVELOPMENT OF THE PULMONARY CIRCULATION

### A. Developmental Stages

Lung development can be divided into five different stages based on the histological appearance, namely, embryonic (1–7 wk gestation), pseudoglandular (5–17 wk gestation), canalicular (16–26 wk gestation), saccular (24–38 wk gestation), and alveolar stage (36 wk gestation onward) (103, 256). The precise timing of the different stages of the lung development varies with species. For instance, sheep possess already alveolarized lung at birth, the newborn human lung contains only a fraction of the adult number of alveoli, and the rat lung is slightly more immature at birth than the human lung (103, 377, 650).

Human lung development starts from the lung bud, which appears as a ventral diverticulum of the foregut during the fourth week of gestation. The most proximal part of the pulmonary circulation is derived from the truncus arteriosus, which becomes divided into the aorta and pulmonary trunk. A continuity of the circulation between the heart and the capillary plexus of the lung exists from at least 34 days of gestation with the artery extending from the outflow tract of the heart and the vein...
connecting to the prospective left atrium. As the circulation develops, a mesenchymal capillary plexus forms between the arteries and veins (25, 227, 244, 621). A similar appearance of a completed circulation has been described in the mouse at embryonic day (E) 10.5 (509) or E13–14 days of gestation (150). At the end of the pseudoglandular stage, all preacinar pulmonary and bronchial arteries are in place and correspond with the bronchial branching pattern in human lung (257, 259, 361).

The canalicular stage marks a great increase in the number of lung capillaries, which are arranged in close apposition to the epithelium to form the first air-blood barrier. During the saccular stage, the last generation of airways is formed, which end with clusters of thin-walled saccules. Capillaries form a bilayer within the cellular inter-saccular septa at this stage. During the period of alveolarization, the interalveolar septa are thinned, the double capillary layer matures into a single layer adult form, and the microvasculature undergoes marked growth and development. It has been always contentious about when the alveolar stage ends. In the classical view of alveolarization, the alveolar stage stops at 2 yr after birth. However, recent studies in mice (415), rats (514), and rhesus monkeys (272) have provided convincing evidence that the alveolar stage does not end until young adulthood, and alveolarization can be divided into two phases: phase one corresponds with classical alveolarization and phase two with late alveolarization (103, 514). In phase one, new alveoli are formed from immature preexisting septa containing a double capillary network, while in phase two, new alveoli develop from mature septa containing a single-layered capillary network. In both phases, the underlying capillary layer initially forms a double-layered capillary network inside the newly formed septum and then matures into a central capillary layer. Alveolarization is a very important stage in the development of the lung. In humans or rats, the gas-exchanging surface area increases ~20-fold from birth to adulthood (103, 256, 514, 546).

Pulmonary macrovascular (arteries and veins) and microvascular (capillaries) segments arise independently and are connected at the pseudoglandular phase of development (149, 150). The main pulmonary veins develop as endothelial invaginations in the cranial portion of the sinus venosus during development (85). After evolving from the sinus venosus, the pulmonary veins become invested with a myocardial sleeve derived from Pitx2-positive cells from the secondary heart field (401). In humans, pulmonary arterial smooth muscle cells (PASMCs) are derived from three sites in a temporally distinct sequence: the earliest cells come from the bronchial smooth muscle, followed by the mesenchyme surrounding the arteries, and finally from the endothelial cells. All venous smooth muscle cells (SMCs), derived directly from the mesenchyme, gradually acquire smooth muscle specific proteins from 56 days of gestation (227, 228).

B. Vasculogenesis and Angiogenesis

Blood vessels form either by vasculogenesis or angiogenesis. Vasculogenesis is a process by which endothelial progenitor cells are recruited and differentiate into mature endothelial cells to form new blood vessels. In angiogenesis, the new vessels arise by sprouting from existing vessels. Three different models have been proposed to describe the roles of vasculogenesis or angiogenesis in the development of lung vasculature. deMello and co-workers (149, 150) proposed that both vasculogenesis and angiogenesis are involved. The distal microvascular networks arise from blood islands where pluripotent mesenchymal cells are differentiated into endothelial cells. The proximal pulmonary arteries and veins arise from proliferation and migration of endothelial cells from existing vessels. The proximal and distal pulmonary vascular segments are connected together at the pseudoglandular phase of development (149, 150). Hall et al. (227, 228) proposed vasculogenesis as the primary mechanism. Their studies suggest that the intrapulmonary arteries and veins are derived from a continuous expansion of the primary capillary plexus within the mesenchyme by vasculogenesis. The arteries are formed by continuous coalescence of endothelial tubes alongside the newly formed airways. The third model by Parera et al. (445) proposes distal angiogenesis as a mechanism for early pulmonary vascular morphogenesis. In their model, capillary networks surrounding the terminal buds expand by formation of new capillaries from preexisting vessels as the lung bud grows (445). A mechanism termed intussusceptive angiogenesis (IA), i.e., “growth within itself,” is likely to have an important role in the expansion of pulmonary capillaries (379). IA was initially observed in postnatal lung capillary beds by Burri and colleagues (104, 449). It is characterized by the splitting and remodeling of preexisting vessels by transcapillary pillar formation. This process is modulated by basic fibroblast growth factor (bFGF) and stimulated by blood flow (379). Since IA does not require extensive cell proliferation and basal membrane degradation as conventional angiogenesis does, it proceeds faster at lower energetic costs and preserves physiological status (104).

C. Specialization Towards Arteries and Veins

Studies demonstrate that ephrinB2 and EphB4 are expressed in cells that will ultimately differentiate into arteries and veins, respectively, preceding the formation of morphologically distinct arteries and veins (316, 559). However, in the human fetus, ephrinB2 and EphB4 ex-
pression in lung tissue does not distinguish the endothelium of presumptive pulmonary arteries and veins as found in systemic vessels in mice (227, 228). In the mouse lung, the endothelial cells do not exhibit selective expression of EphrinB2 or EphB4 until late pseudoglandular stage (E15.5). Interestingly, the vast majority of the endothelial cell population is venous during this stage of lung development, judging by the selective expression of EphrinB2 or EphB4 (513). Increasing evidence indicates that arterial-venous differentiation and patterning are genetically controlled through the coordinated actions of specific transcription factors. Arterial fate is acquired by the combined effect of Fox c1 and c2 and vascular endothelial growth factor (VEGF) signaling while vein identity is regulated by the orphan nuclear receptor COUP-TFII through the repression of Notch1. Studies also show that arterial and venous identity is modulated by hemodynamic forces (262, 316).

D. Branching Pattern and Structure

Pulmonary arteries and veins are divided by Elliott and Reid into two populations: conventional and supernumerary vessels (162, 485). The former runs alongside the airway and its branches while the latter does not accompany airways. Supernumerary vessels occur throughout the length of the pulmonary vascular tree to directly supply the closest pulmonary acinus. These vessels are more numerous toward the lung periphery. It is estimated that the ratio of supernumerary to accompanying terminal arteries is ~2.8:1, while the ratio for venous supernumerary vessels is even higher, at ~3.5:1 (105, 162, 485). During fetal life, as each airway appears, its accompanying conventional and supernumerary arteries also appear, with the same ratio of conventional to supernumerary arteries as that in the adult. The preacinar branching pattern of pulmonary arteries is complete at midterm gestation. Later changes in the arteries are only in dimension and wall structure. In the fetus, the thickness of the arterial wall at any given generation and diameter does not change significantly throughout the second half of the gestation period, and is twice the adult. The smallest arteries have an even thicker vessel wall and may be the site of greatest resistance (100, 258, 598). In fetal lambs from 85 to 140 days of gestation, the medial width/external diameter ratio of resistance arteries remains constant, although the number of vessels per unit volume increases nearly eightfold (361).

The increase in size and number of the pulmonary veins and their branching pattern is similar to that of arteries in the human fetus, although the muscle development in veins lags behind that in arteries. At 20 weeks gestation, there is no muscle found in the wall of even the largest veins in the lung, with the wall consisting of a layer of endothelial cells surrounded by collagen fibers and occasional small elastic fibers. At term gestation, the wall thickness of veins over 300 μm external diameter is the same regardless of the vessel size. In <300 μm veins, the wall thickness increases as the external diameter decreases. For all sized veins, the wall thickness is less than that of the arteries (257, 505, 507).

After birth, there is an immediate decrease in wall thickness of human pulmonary arteries <200 μm in diameter. The rapid decrease in vessel wall thickness may largely result from a reduction in overlap of adjacent SMCs and a reduction in mean SMC diameter. The medial thickness of the larger vessels decreases in the first 10 days and continued to decrease during the first 3 mo of life, after which there is little change. Pulmonary arterial size increases most rapidly during the first 2 mo of life, and the growth rate remains high during the first 4 yr. Arterial number increases rapidly in the first 2 mo and subsequently increases at the same rate as alveoli. The thickness of veins is similar from birth to adolescence and is less than that of pulmonary arteries of the same size. Vein muscle wall thickness is low throughout childhood (17, 245, 256, 257, 259, 595, 597). A similar pattern of postnatal vascular remodeling of porcine pulmonary vasculature has also been reported, although probably occurring faster in the pig than in the human lung (225, 226, 242, 243, 245, 324, 325).

E. Molecular Mechanisms of Pulmonary Vascular Development

The formation of the pulmonary vasculature and the development of the pulmonary circulation is a complicated process, which comprises of sequential differentiation of mesenchymal progenitor cells into hemangioblasts, formation of the primitive vascular plexus, and the remodeling of the vascular network into a hierarchical vascular system. These processes are genetically controlled and regulated by an array of transcription factors and growth factors in an intricate spatio-temporal manner (133, 198, 377). It involves cross-talk of paracrine signals between various cell types (377). Among these regulators, VEGF appears to be absolutely required in the earliest stages of vasculogenesis and that it also continues to play a critical role during subsequent angiogenesis (248, 571, 592). Studies also suggest that bone morphogenetic proteins (117), Fox transcription factors (148), Wnt proteins (132, 146, 195), Notch (262), and angiopoietins (410) may be critically involved in vascular formation in the lung. It should be noted that compared with the current knowledge about the regulation of airway and alveolar formation and the vasculature in general, our understanding of the regulation of the vascularization of the lung is rather scanty and limited (546).
I. VEGF

VEGF belongs to a gene family that includes placental growth factor (PLGF), VEGF-B, VEGF-C, and VEGF-D. Here we focus on the biology of the prototype member, VEGFA (hereinafter referred to as VEGF), a key angiogenic growth factor (172, 592). VEGF is an absolute requirement for vascular development. The loss of a single VEGF allele results in defective vascularization and early embryonic lethality (107, 172, 641). A critical role for VEGF in pulmonary vascular development has been documented in both pre- and postnatal lung. Treatment of 1-day-old newborn rats with a single dose of Su-5416, a VEGFR inhibitor, has been shown to impair pulmonary vascular growth and postnatal alveolarization, and these effects are long term, persisting well into adulthood (353).

The effects of VEGF are mediated by two types of tyrosine kinase receptors, VEGFR1 (Flt-1) and VEGFR2 (KDR/Flik-1). VEGFR2 knockout mice are embryonic lethal with poor vascular formation, indicative of a primary role for VEGFR-2 in angiogenesis (518). VEGFR1 knockout mice die in utero due to lack of structural organization of vessel walls, indicative of an essential role for VEGFR1 in the organization of the embryonic vasculature (186, 187). In embryonic lung, the angiogenic effect of VEGF is likely to result from a coordinate action of VEGFR1 and VEGFR2. When VEGFR2 is predominantly expressed (embryonic day E9.5-E13.5), vascular endothelial cells proliferate less actively; when VEGFR1 expression is enhanced (E14.5-E16.5), these cells proliferate, thereby constituting organized networks (628). The reduced proliferation seems to be due to the inhibitory effect of VEGFR1 by its action as a decoy receptor (592).

VEGF is not only essential for the formation of the pulmonary vasculature but is also essential for epithelial branching morphogenesis (566). In the fetal lung, VEGF is first expressed in lung mesenchyme and epithelium and then becomes increasingly restricted to the epithelium. The temporal and spatial expression of VEGF is likely to be regulated coordinately by FGF9 and sonic hedgehog signaling in lung mesenchyme (617). In the human fetal lung during the late pseudoglandular phase, VEGF is expressed in the epithelium of terminal buds and its receptors are localized to the endothelial cells closely apposed to the developing epithelium (532). Transgenic mice in which the VEGF gene is conditionally inactivated in lung epithelium show almost complete absence of pulmonary capillaries. The pulmonary capillaries may in turn influence epithelial morphogenesis by modulating the expression of hepatocyte growth factor (HGF) whose signaling in respiratory epithelium is necessary for distal lung development (628). Signaling cross-talk between the epithelial and endothelial cells is critical for the development of respiratory epithelial cells and the pulmonary vasculature (546, 592).

Many studies suggest that NO is an important downstream target for the proliferative effects of VEGF (404, 444, 648) and for the differentiation of developing pulmonary artery endothelial cells (42). Endothelial nitric oxide synthase (eNOS) is activated by VEGF via c-Src and phospholipase C-1 (PLC-1) (247). Lungs of eNOS-deficient mice exhibit a striking paucity of distal arteriolar branches and extensive regions of capillaries showing hypoperfusion, together with misalignment of pulmonary veins (231). VEGF also increases PGL2 production by activation of prostacyclin synthase via mitogen-activated protein kinase (MAPK) (247). The effect of PGL2 on the development of the pulmonary vasculature may be inhibitory. Fetal rats treated with indomethacin in utero show an increased medial smooth muscle mass in resistance pulmonary arteries and arterioles (238).

2. Angiopoietins

The glycoprotein growth factors angiopoietins (Ang) include Ang-1, -2, -3, and -4. Angiopoietins and their major receptor Tie-2 (tyrosine kinase with immunoglobulin and EGF-like domains) are involved in survival and migration of endothelial cells and regulation of vascular remodeling, maintenance of vascular integrity, and lymphangiogenesis (410). A lack of Ang-1 or Tie-2 protein leads to severe microvascular defects and subsequent embryonic lethality in mice (507, 558). Shh, an important morphogen known to regulate epithelial-mesenchymal interactions during embryonic development, induces the expression of Ang-1 and -2 (459). In Shh-deficient mice, the pulmonary vascular bed is maldeveloped together with downregulated expression of Ang-1 (584). Transgenic mice overexpressing Ang-2 display phenotypes similar to those observed in Ang-1- and Tie-2-deficient mice. It is postulated that Ang-2 acts as a physiological Ang-1 antagonist (378). Ang-1 is produced by lung mesenchyme and smooth muscle, and Tie-2 is restricted to endothelial expression. Ang-1 promotes interactions between endothelial cells, extracellular matrix, and pericytes that are required for vessel maturation (410, 546).

3. BMP and Smad

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor (TGF)-β superfamily and are critical mediators of early human and mouse embryonic patterning (600). BMPs are involved in organogenesis of the lung (601). Mice deficient in Smad1 or Smad5, the main signaling intermediaries downstream of the BMP receptors, die at E10.5 to E11, with death being attributable to multiple vascular abnormalities, including vascular defects where enlarged blood vessels are surrounded by a decreased number of vascular SMCs (630). In normal adult human lung, BMPR-II is expressed by endothelial cells and by the underlying SMCs (40). Re-
duced expression of BMPR-II (40) and reduced Smad signaling (491, 631) are features of both familial and idiopathic pulmonary arterial hypertension. BMP signaling is mediated by the formation of heterodimeric complexes of serine/threonine kinase receptors, comprising a type I BMP receptor together with BMPR-II. Activation of a type I BMP receptor by the constitutively active BMPR-II triggers intracellular signaling via Smad1, -5, or -8, which, upon phosphorylation, forms a complex with Smad4. The Smad complex then translocates to the nucleus to interact with coactivators and corepressors to modify target gene transcription in a cell- and tissue-specific manner (400). The expression of BMP4 and BMP receptors is temporally and spatially regulated during lung development. BMPR-II expression is highest during the late pseudoglandular and early canalicular stage of lung development, when vascularogenesis is intense. BMPs 2 and 4 induce phosphorylation of Smad1/5 and pulmonary artery endothelial cell migration and proliferation (543).

4. Fox

Foxes are characterized by a conserved 100-amino acid domain called the “forkhead box.” These transcription factors modulate diverse biological processes including cardiovascular development and disease (342, 443). Pulmonary hemorrhage and peripheral microvascular deficits are found in about half of newborn Foxf1(+/-) mice, associated with significantly reduced levels of the Notch-2 receptor and the Notch-2 downstream target hairy enhancer of split-1 (317). Recent studies suggest that Foxc2 and FoxO, members of Fox family, are involved in vascular development such as endothelial cell migration, tube formation, and the correct organization of the vascular system. Their precise role in pulmonary vascular morphogenesis remains to be determined (148, 342, 443).

5. Wnt

Wnt proteins form a family of highly conserved secreted signaling molecules involved in diverse aspects of cardiovascular development. Wnts are broadly categorized into two groups based on their signal transduction pathway. Canonical Wnts transduce their signals through cellular membrane receptors of the frizzled family and cytosolic messenger β-catenin, then translocate into the nucleus where they form a complex with T-cell factor/lymphoid enhancer-binding factor (Tcf/LEF) family members and activate transcription of target genes in the nucleus (132, 195). Noncanonical Wnts signal via either the planar cell polarity (PCP) pathway or the Wnt/Ca2+ pathway may use frizzled receptors or other receptors, including the orphan tyrosine kinase Ror2 (322, 618).

Several Wnt genes have been shown to be expressed in the developing lung including Wnt-2, Wnt-2b, Wnt-7b, Wnt-5a, and Wnt-11 (411). Of these, Wnt7b is expressed at the distal tips of the airway epithelium in an increasing gradient from the proximal-to-distal airway epithelium. Mice with mutated Wnt7b (Wnt7b<sup>lacZ/-/-</sup>) exhibit perinatal death due to respiratory failure associated with defects in early mesenchymal proliferation leading to lung hypoplasia. Wnt7b<sup>lacZ/-/-</sup> embryos and newborn mice exhibit severe defects in the smooth muscle component of the major pulmonary vessels. It is suspected that loss of Wnt7b function results in defects in vascular smooth muscle differentiation and/or survival leading to a hypertrophic response, degradation of the vessel wall, and eventual rupture of the weakened vessels (535). This effect is suspected to be mediated by the canonical Wnt signaling cascade and through forkhead box transcription factors (546). A separate study using a conditional Wnt7b-null mouse found no abnormalities of vascular smooth muscle development. Rather, Wnt7b mutants have decreased replication of epithelium and mesenchyme, which results in a small lung with preserved differentiation and architecture (478).

Wnt5a is an important factor patterning many aspects of early development, including the lung. Pulmonary noncanonical Wnt5a uses Ror2 to control patterning of both distal air and vascular tubulogenesis (alveolarization). Lungs with mis/overexpressed Wnt5a develop with severe pulmonary hypoplasia associated with altered expression patterns of Shh, L-CAM, fibronectin, VEGF, and Flk1. The effect of Wnt5a on vascular patterning appears by affecting fibronectin levels directly, and by affecting the fibronectin pattern of expression through its regulation of Shh (372).

6. Notch

The Notch signaling pathway is a highly conserved cell signaling system that regulates intercellular communication and directs individual cell fate decisions. There are four distinct Notch receptors (Notch1 to -4) and five ligands (Jag1 and -2; Dll1, -3, and -4) that exist in mammals. Since Notch receptors and their ligands are type 1 transmembrane proteins, Notch receptor-ligand interactions require direct contact between cells or should be within the context of the plasma membrane of the same cell. Once activated, the intracellular domain of Notch (or NICD) translocates to the nucleus where it promotes transcription of target genes via CSL-dependent (canonical) and -independent (noncanonical) mechanisms (262, 412). The Notch signaling pathway may act as a downstream target for VEGF during embryonic vascular development. The mRNA transcripts for Notch1–4 and Jagged1 increase progressively from early to later lung development. Immunofluorescence staining revealed the progressive acquisition of Notch1 and Jagged1 proteins by the emerging endothelium. Notch1 and Jag1 are expressed...
initially on larger vessels within the embryonic lung bud and progressively on finer vascular networks (560).

F. Abnormal Development of the Pulmonary Vasculature

The development of the pulmonary vasculature is precisely orchestrated by an array of mitogenic factors in a specific temporospatial order and coordinated by heterogeneous cell types including the epithelial and endothelial cells and SMCs. The process is genetically controlled and also influenced by the uterine environment. Any alteration in such a complex process leads to various diseases or syndromes such as alveolar capillary dysplasia, congenital pulmonary hemangiomatosis, and bronchopulmonary dysplasia.

1. Alveolar capillary dysplasia

Alveolar capillary dysplasia (ACD) is a rare disease first described by Janney et al. in 1981 (309). It is characterized by a decrease in the number of air-blood barriers/capillaries leading to severe hypoxemia and persistent pulmonary hypertension of the neonate, which is often fatal. The alveolar epithelium is surrounded with mesenchyme containing small blood vessels, but no capillaries or capillaries fail to come into apposition with type I pneumocytes. These changes cause a decrease in the blood-air interface surface area. Furthermore, there is marked medial hypertrophy of small pulmonary arteries with muscular extension into intra-acinar vessels and malposition of pulmonary veins adjacent to small pulmonary arteries in ACD patients (166, 197, 220, 224, 309, 393, 517). ACD is likely to be autosomal recessive in some families. It has a high incidence of extrapulmonary abnormalities, with the genitourinary and cardiovascular system most commonly affected (24, 303, 517, 586).

eNOS null mice exhibit a striking paucity of distal arteriolar branches, extensive regions of capillary hypoperfusion, and misalignment of pulmonary veins in the lung, which represent the characteristic features of ACD. A majority of the mice die within the first hours of life from respiratory distress (230, 231). NO may promote angiogenesis through VEGF by enhancing the stability of hypoxia-inducible factor (HIF)-1 following S-nitrosylation of the tumor suppressor genes vHL and p53 (159, 230, 231, 642). NO may also directly augment angiogenesis in a cGMP-dependent manner (230, 231, 457).

The close juxtaposition of the capillary network to lung epithelial cells is essential for gas exchange. A recent study suggested that the growth and patterning of the lung capillary plexus is the result of the temporal and spatial expression of VEGF coordinately regulated by FGF9 and shh signaling. FGF9 and shh signaling to lung mesenchyme, but not necessarily to endothelial cells, are each necessary and together sufficient for distal capillary development (617). In mice there are three major VEGF isoforms (VEGF120, -164, and -188) resulting from alternate mRNA splicing. The three isoforms are known to differ in their solubility (VEGF120 is freely soluble and VEGF188 is completely matrix-bound, while VEGF164 has intermediate properties) and receptor binding properties [VEGF164 does and VEGF120 does not bind to neuropilin-1 (Nrp-1)]. The levels of VEGF120 and -164 increase only slightly during embryonic lung development while that of VEGF188 increases continuously from E13 to before birth (425). In lung of fetal and newborn mice expressing only VEGF120 although the preacinar vessels are similar to that in wild type mice, the most peripheral vessels are more widely separated, with fewer air-blood barriers and a decreased airspace-to-parenchyma ratio. These results suggest that the absence of VEGF164 and -188 isoforms impairs lung microvascular development and delays airspace maturation (199).

2. Congenital diaphragmatic hernia

Congenital diaphragmatic hernia (CDH) is a relatively common disorder occurring in 1 in 2,500–5,000 live births. It is a severe birth defect of fetal chest development accompanied by malformations of the lung, heart, testis, and other organs. About 11–15% of CDH patients also have cardiovascular malformations and have lung hypoplasia and elevated PVR. The mortality of CDH ranges from 20 to 90%, depending on the severity of the persistent pulmonary hypertension in the newborn period (PPHN) (9, 69, 73, 147, 366, 496).

The lungs of CDH infants are hypoplastic. The alveolar-to-arterial ratio is normal, but the alveolar number is markedly decreased. The pulmonary vascular bed is reduced in size. The adventitial and medial walls of peripheral pulmonary arteries are thickened with abnormal muscularization of the small preacinar and intra-acinar arterioles. These aberrant changes lead to a persistence of pulmonary hypertension and are often refractory to diverse medical interventions (9, 88, 329, 330). The pulmonary hypoplasia manifested in CDH was considered to be solely as a result of external compression by herniating abdominal contents into the chest cavity. Increasing evidence indicates that it reflects intrinsic defects in lung development and that the defect in the diaphragm may be secondary (43, 323, 520). The mechanisms responsible for impaired lung growth in CDH remain poorly understood. Recent studies suggest that mutations in genes belonging to one or more important developmental pathways contribute to CDH and its accompanying defects (9, 73, 318).

For instance, in mouse models, Fog2, Gata4, and COUP-TFI have been implicated in CDH, and these three genes are likely to be important in the same developmental pathways involved in development of both the lung and
diaphragm (128, 263, 318, 633). Studies through interference with the retinoic acid pathways either by gene mutations, teratogenic exposures, or the herbicide nitrofen have suggested that genetic abnormalities in the vitamin A pathway may contribute to the most common form of CDH, isolated “Bochdalek” hernia (9, 44, 217, 323).

In the nitrofen-induced CDH model in rats (124, 285), VEGF expression in the lung is reduced (114, 414). Since disruption of angiogenesis can inhibit alveolarization in the developing lung (2, 199, 230, 231, 300), it is postulated that the downregulation of VEGF may contribute to lung hypoplasia in CDH. Indeed, disruption of lung architecture after VEGF receptor blockade has been found to be similar to nitrofen-induced changes (414). VEGF-induced lung angiogenesis is in part mediated by NO. The enhanced lung growth after treatment with NO in nitrofen-exposed lung may result from NO-induced angiogenesis (159, 230, 231, 642).

3. Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) is a disorder of the lung, common in premature babies, occurring in 1 in 2,000–5,000 live births. BPD was described by Northway et al. (431) in 1967 as a syndrome of severe lung injury resulting from oxygen and mechanical ventilation in modestly premature newborns. With advances in perinatal care, the infants with BPD are those born at very early gestational ages, that is, during the canalicular period (16–26 wk). It is now recognized that BPD is less of an injury syndrome and more of a disruption or arrest in lung development with marked decreases in alveolarization and a dysmorphic vascular structure. Recent studies suggest that genetic susceptibility plays a critical role in the pathogenesis of BPD (7, 70, 314, 351, 546, 566).

The pulmonary vasculature in BPD is characterized by reduced arterial number, medial hypertrophy and distal muscularization of small peripheral arteries, and abnormal vasoreactivity. These changes lead to pulmonary hypertension, which is the major cause for morbidity and mortality in children with BPD (2, 129, 220). The expression of VEGF is decreased in lungs of a number of animal models of BPD (381, 382), in lung from human infants dying with BPD (71), and in the tracheal aspirate of infants developing BPD (349). VEGF receptor blockers have been shown to reduce pulmonary vascularization and alveolarization of the neonatal rat and mouse lung (213, 319, 353, 561). Inhaled NO therapy has been shown to improve the pulmonary outcome for premature infants who are at risk for bronchopulmonary dysplasia (48, 52). There is some evidence that inhaled NO can preserve neonatal lung growth in bronchopulmonary dysplasia by enhancing endothelial cell survival after lung injury (41, 563). In a rat pup model of BPD, a cGMP-specific phosphodiesterase (PDE5) inhibitor sildenafil also prevented the injurious effects of hyperoxia on alveolar growth and lung angiogenesis, suggesting that the effect of NO may in part be mediated by cGMP (343).

In addition to VEGF, a vast array of growth/differentiation factors and receptors, transcription factors, matrix components, and remodeling enzymes are thought to play a role in BPD (93, 306, 594), which includes TGF-α (338), TGF-β (151), platelet-derived growth factor (PDGF) (99), and HGF (442). The specific roles of these factors in the pathogenesis of BPD remain to be elucidated.

III. REGULATION OF THE PULMONARY CIRCULATION IN THE FETUS, POSTNATAL TRANSITION, AND EARLY NEONATAL LIFE

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In the human fetus, a continuous circulation between the heart and the capillary plexus of the lung forms by 34 days of gestation (227, 228). The size and number of pulmonary arteries and veins increase as gestation advances. From 20 to 30 wk gestation, blood flow to the lung increases from 13 to 25% of the combined cardiac output, accompanied with a significant decrease in weight-indexed PVR (481). These changes result mainly from the increased pulmonary vascular bed since maternal hyperoxegenation has no effect on the reactivity of the pulmonary circulation in the human fetus during this period (480). From 30 to 38 wk of gestation, blood flow to the lungs decreases slightly from 25 to 21% of combined cardiac output while the weight-indexed PVR significantly increases. Meanwhile, the pulmonary circulation during this gestation period responds to maternal hyperoxegenation with increased blood flow and decreased vascular resistance, indicating that the fetal pulmonary circulation develops vasmotor tone and vasoreactivity at least during the end of the third trimester (480, 481). Compared with the human, pulmonary venous return to the heart in fetal lambs is considerably less. It represents only 3.7 and 7.0% of the combined cardiac output at 0.4 gestation and at 1.0 gestation, respectively. During this period, there is also a progressive decrease in PVR (250, 499–501). Pulmonary arteries and veins of near-term and term fetal lambs exhibit active responses to various vasoactive agents and to changes in oxygen tension (109, 364, 407, 572, 644). Increasing oxygen tension from 24 to 46 mmHg increases pulmonary blood flow by 10-fold in term lambs (407). This suggests that a substantial portion of the high PVR in the mature fetus is maintained by vasoconstriction in an oxygen tension-sensitive manner. Such a notion is supported by studies in a number of species including rabbits, lambs, pigs, primates, and humans (45, 56, 264, 361, 480, 635).

Fetal blood is oxygenated in the placenta and returns to the body through the umbilical veins. Oxygenated blood enters the left atrium from the right atrium through
the foramen ovale. It then enters the left ventricle whence it is pumped into the aorta. The blood received by the right ventricle is largely from the superior and distal inferior vena cava and poorly oxygenated. Of the blood pumped out by the right ventricle, only a smaller portion enters the lung via pulmonary arteries due to the high PVR; a large portion enters the aorta via the ductus arteriosus (331, 481, 499, 536). The oxygen tension in pulmonary arterial blood is \( \sim 18 \text{ mmHg} \) and oxygen saturation \( \sim 50\% \) (331, 499).

At birth, with the onset of breathing, the lung replaces the placenta as the organ of gas exchange. Pulmonary blood flow increases from 21\% of combined ventricle output to the total cardiac output in the human infant (481). After the establishment of respiration, the pulmonary arterial pressure gradually decreases while systemic pressure increases. The mean pulmonary arterial pressure approaches 50\% of mean systemic pressure by the end of the first day and drops to the adult level within the first 2 wk of life (163, 499). As soon as systemic pressure and resistance become greater than that in the pulmonary circulation, the foramen ovale closes. The ductus arteriosus begins to close within the first few hours after birth.

Shunting of blood through the ductus arteriosus either becomes physiologically insignificant or ceases entirely by 15 h of age in the term infant (413, 583).

This dramatic change in the pulmonary circulation of the newborn during the transition from fetal to neonatal life is primarily attributable to the marked decrease in PVR postnatally. It results from various birth-related events that occur concurrently and sequentially, which include ventilation, oxygenation, increasing shear stress of blood flow, changes in the activities of a number of vasoactive agents and their signaling pathways such as EDNO, PGI2, endothelin-1 (ET-1), and PAF (243, 250, 276, 501). These factors contribute not only to the initial rapid decrease in PVR but also to the maintenance of low PVR in the newborn and adult. Other important factors involved include the postnatal vascular reorganization and remodeling that occurs rapidly after birth and vascular remodeling that continues over weeks and months (242, 244, 245, 256). In the following sections, the roles of mechanical factors and vasoactive agents in the regulation of the pulmonary circulation during the fetal, postnatal transition, and neonatal life are discussed.

A. Mechanical Factors

1. Fluid-filled lung

Fetal lungs are filled with liquid in the airspaces that is produced by lung. The liquid leaves via the trachea, where it is either swallowed or enters the amniotic sac (10, 82, 266–268). Fetal lung liquid is formed as a result of chloride secretion by the respiratory epithelium via \( \text{Na}^+\text{K}^+\text{Cl}^- \) cotransport. Chloride transport is driven by \( \text{Na}^+\text{K}^+\text{ATPase} \) (83). Studies with cultured explants of human fetal lung suggest that liquid production by the bronchopulmonary epithelium may occur as early as the sixth week of gestation, with resultant expansion of the lung lumen. In fetal sheep lung, epithelial Cl\(^-\) transport begins as early as mid-gestation. The rate of production varies from 2.7 to 5.5 ml·kg\(^{-1}\)·h\(^{-1}\) during the last third of fetal life (236, 438, 439).

In comparison to neonatal lungs, fetal lungs are hyperexpanded. In late gestation, the volume of liquid retained within the potential airspaces of fetal lambs (\( \sim 45 \) ml/kg) is much greater than functional residual capacity \( \text{FRC} \), the equivalent measurement of lung volume postnatally (265) in newborn lambs (\( \sim 25 \) ml/kg) (268, 591). This probably results in greater extraluminal pressures surrounding the pulmonary vasculature. In fetal lambs, reducing extraluminal pressure by draining the lung liquid markedly increases pulmonary blood flow, while increasing the extraluminal pressure by tracheal obstruction greatly reduces pulmonary blood flow. These observations suggest that the high extraluminal pressure contributes importantly to high PVR in the fetus (461).

Fetal lung liquid also plays an important role in the development of the lung by maintaining them at an appropriate level of expansion (268). In several species including sheep, rabbit, rat, and mice, tracheal occlusion by surgery to prevent egress of lung fluid from the lung accelerates both lung growth and maturation during the canalicular and especially the saccular stages, a period critical to lung development as the functional gas exchange units first begin to appear, characterized by increases in lung volume, increased levels of pseudoalveolar septation, interstitial tissue thinning, and greater microvascular development (236, 268, 328). Tracheal occlusion has been shown to promote the growth of large pulmonary vessels and capillaries in fetuses of sheep, rabbits, and rats (154, 374, 497). Adverse effects of tracheal occlusion have also been reported. In one study, tracheal occlusion at midgestation in fetal sheep caused fetal hydrops and produced changes in lung structure that were not compatible with efficient gas exchange (464).

Decrease in fetal lung distension such as in congenital diaphragmatic hernia, in contrast, causes pulmonary hypoplasia and abnormal pulmonary vascular development that may lead to persistent pulmonary hypertension after birth (360). In mice, increasing fetal lung distension by tracheal occlusion accelerates microvascular growth concomitant with a rapid increase in the expression of VEGF mRNA and VEGFR-1 as well as VEGFR-1 and Ang-1 (127). In fetal rats, tracheal occlusion augments the expression of VEGF protein, while decreasing fetal lung distension by congenital diaphragmatic hernia retards the
normal developmental pattern and decreases VEGF protein (234).

2. Breathing movements

Fetal breathing movements (BM) have been observed in all mammals that have been studied. In rats, characteristically single BMs are observed occasionally at E16, and episodes of clustered BMs commence by E18 and increase in frequency in an age-dependent manner. Episodic BMs commence at week 10 of gestation in humans and later in the first trimester in sheep (81, 235, 252, 333). It appears that fetal BMs of normal frequency and amplitude do not affect mean pulmonary blood flow but do induce small changes in phasic flow (332). In near-term fetal sheep, pulmonary blood flow is increased while PVR is decreased during accentuated increased episodes of BM, possibly as a result of phasic reductions in intrapulmonary pressures (460).

Fetal BMs are necessary for normal growth and structural maturation of the fetal lung. Abolition of BMs by phrenectomy or spinal cord transection leads to pulmonary hypoplasia in fetal rabbits and sheep (11, 14, 173, 237). In mixed pulmonary cell cultures of fetal, neonatal, and adult mouse lung, continuous cycles of stretch-relaxation augment the expression of VEGF proteins. This may be one mechanism for modulation of pulmonary vascular development (416).

3. Ventilation

In near-term fetal lambs, mechanical ventilation without a change in fetal O2 results in an increase in pulmonary blood flow by four- to fivefold within 1 h (135, 577). This effect is reduced by 67% by nitro-L-arginine, suggesting a critical role for EDNO (135). The effect of ventilation is also inhibited by tetraethylammonium (a nonspecific K+ channel blocker) but not by glibenclamide (an ATP-sensitive K+ channel blocker). Therefore, non-ATP-sensitive K+ channels may modulate the transitional changes in the pulmonary circulation caused by ventilation (577).

4. Lung volume

Fetal lungs are hyperexpanded at rest compared with the resting volume or functional residual volume postnatally. The volume of liquid retained within the potential airspaces in late-gestation fetal lambs is ~80% greater than the FRC of air-filled lungs in newborn lambs (591). In near-term fetal sheep, a reduction in fetal lung liquid volume similar to that at birth causes a three- to fourfold increase in pulmonary blood flow and reduction in PVR of a similar magnitude to that at birth, presumably in response to the associated reduction in extraluminal pressure (265, 268, 591).

5. Surface tension-air liquid interface

At birth with the first breath and contraction of the diaphragm, the lungs are being filled with air (171). The normal functional residual capacity of ~30 ml/kg body wt is usually attained within 2–3 h of birth (123, 397). Following the replacement of liquid with air, surface tension at the air-liquid interface is created. Fetal lung liquid drains mainly into the pulmonary microcirculation with subsequent drainage into the systemic circulation. About 10–15% of the luminal liquid drains from the lung via lymphatics into the systemic venous system (83, 565).

Alveolar surface tension may affect pulmonary arterial pressure by counterbalancing the distending force of alveolar pressure. This is reflected in changes in pulmonary arterial pressure during respiration that is mainly due to changes in resistance of alveolar vessels. Studies show that PVR is higher on deflation versus inflation (106, 556). Since alveolar surface tension is lower during deflation, the overall pressure applied to the alveolar capillaries is higher on deflation, and therefore, PVR is higher (556). Changes in surface tension also affect pulmonary capillary volume and compliance (575). Surfactant deficiency may be partially responsible for persistent pulmonary hypertension in neonates with CDH. In the newborn lamb model of CDH, correcting the surfactant deficiency increases pulmonary blood flow, decreases PVR, and reduces right-to-left shunting (441).

6. Shear stress

Shear stress created with the abrupt surge in pulmonary blood flow at birth may further increase blood flow by acutely stimulating eNOS (135, 606) and up-regulating its expression (77, 606). Shear stress-induced upregulation of eNOS seems to result from increased transcriptional activity of c-Jun in fetal but not adult pulmonary arterial endothelial cells (613) and is in part mediated by protein kinase C (PKC) activity (606). PKC consists of 12 members, some of which may have opposing effects on NO signaling. For instance, PKC-δ inhibits the expression of eNOS via STAT3. In fetal lamb lung, pulmonary artery endothelial cells of fetal lambs shear stress decreases PKC-δ activity and thus reduces the inhibitory effect of STAT3 on eNOS promoter (553). In addition to eNOS, shear stress may increase pulmonary blood flow in fetal lambs by stimulating inducible NO synthase, perhaps in SMCs (471). The activation of potassium channels has also been implicated in shear stress-induced fetal pulmonary vasodilatation, which seem to be dependent on Ca2- and voltage-dependent K+ channel activity but not on low-conductance Ca2- and ATP-dependent channel activity (551).
B. EDNO

EDNO is the major endogenous agent regulating both basal and stimuli-induced vasodilatation of the pulmonary vasculature. In the perinatal pulmonary vasculature, the effects of EDNO are primarily mediated by cGMP elevation via soluble guanylyl cyclase (sGC) activation and subsequent activation of cGMP-dependent protein kinase (PKG) (153, 200, 423). EDNO is synthesized in endothelial cells by the conversion of L-arginine to L-citrulline by eNOS. Three distinct isoforms of NO synthase have been identified: neuronal (nNOS), inducible (iNOS), and eNOS are all present in the lung. eNOS is expressed primarily in endothelial cells. nNOS is expressed not only in neurons but also in PASMCs (431, 472, 523, 603). iNOS is present predominantly in SMCs, and its expression is induced by cytokines (51, 130, 188).

In human fetal lung, eNOS immunoreactivity appears in cells of the 2-wk gestation embryo and increases as gestation proceeds. These cells coalesce to form an endothelial layer for pulmonary vessels (626). Immunohistochemical study shows that eNOS is strongly expressed in human lung in the canalicular and saccular stages with comparable intensity and falls sharply in the alveolar stage and further decreases after birth (362). In the baboon lung from 0.7 to 0.8 gestation (term = 175 days), a marked increase was noticed in total NO synthase activity and in the expression of eNOS and nNOS, whereas iNOS expression and activity were minimal. From 0.8 gestation to term, total NO synthase activity remains constant, eNOS and nNOS protein expression decreases, but iNOS rises sharply (523). In sheep lung, lung eNOS protein expression in the fetus rises and peaks at 0.8 gestation, decreases before birth, rises in the newborn period, but is lower in the adult. Lung NO synthase activity also rises and peaks at 118 day gestation in the fetus before falling in late gestation and remaining low in the newborn and adult (447). In the rat, both mRNA and protein of eNOS are detectable in 0.7 gestation fetal lung and increases to maximal levels at 0.9 gestation (term = 22 day). In contrast, nNOS protein increases while its mRNA abundance declines during late fetal life, suggesting that the regulation of pulmonary eNOS may primarily involve alterations in transcription or mRNA stability, whereas nNOS expression in late gestation also involves posttranscriptional modifications (430). In another study, eNOS mRNA and protein levels were detected in rat lung during the late fetal and postnatal periods. The highest levels were detected within 24 h after birth, and elevated mRNA levels persisted for 16 days and then decreased to lower levels in adults (320).

Arginases compete with NO synthases for L-arginine as their common substrate. Two isoforms of arginase, type I and type II, have been identified, and both are expressed in the lung. In rat pulmonary arteries, arginase II content and total arginase activity are highest in the fetus and decrease with age, being lower in newborn and adult rats. In the presence of N^-hydroxy-nor-L-arginine, an arginase inhibitor, pulmonary arterial force generation was significantly reduced in fetal and newborn vessels. Therefore, the higher arginase expression and/or activity may contribute to lower NO production and the maintenance of a high PVR in fetal lung (63).

In near-term and term fetal lambs, infusion of nitro-L-arginine decreases pulmonary artery blood flow and increases pulmonary artery pressure. Pulmonary vasodilatation induced by acetylcholine is also attenuated by nitro-L-arginine (6). These results suggest that EDNO is functional in the fetus and may exert an inhibitory effect on fetal pulmonary vasomotor tone. Since oxygen is a necessary substrate for NO synthesis and fetal pulmonary endothelial cells are in a low oxygen environment (17–19 mmHg), the role of EDNO in opposing pulmonary vasoconstriction is likely to be limited in the fetus (209). In a study in near-term lambs, raising fetal arterial oxygen tension (PaO2) from 25 to 55 Torr by making the pregnant ewe breath hyperbaric 100% oxygen at 3 atmosphere pressure resulted in an increase in the proportion of right ventricular output distributed to the fetal lung from 8 to 50% (408). In very immature lambs (~0.65 gestation), there was no change in the proportion of right ventricular output distributed to the lung when fetal PaO2 was increased from 27 to 174 Torr, indicating that EDNO is not functional at this early stage of gestation. Several studies also demonstrate that heterogeneity exists along the pulmonary vascular tree. In term fetal pigs, N^-nitro-L-arginine methyl ester (l-NAME), an inhibitor of eNOS, attenuated relaxation to the endothelium-dependent vasodilator bradykinin by 50% in conduit pulmonary arteries but almost completely abolished relaxation in resistant pulmonary arteries (87).

In the late-gestation ovine fetus, inhibition of NO synthase not only increases pulmonary arterial pressure and decreases pulmonary blood flow induced by acetylcholine, but it also reduces the rise in pulmonary blood flow at birth (6, 403). The increase in pulmonary blood flow can be induced by an increase in Po2 in fetal pulmonary arterial blood in a manner sensitive to the inhibition of NO synthase, indicating an important role for oxygenation and NO in the transitional changes in the pulmonary circulation (390, 570). Oxygenation may acutely increase EDNO production by acting as an essential substrate for eNOS and increase eNOS activity and NO production by upregulating eNOS expression through transcriptional and posttranscriptional mechanisms (32, 365, 429). The increase in fluid shear stress at birth, resulting from increased pulmonary blood flow, also increases NO production by phosphorylation of eNOS and by increasing eNOS mRNA and protein expression (77, 135, 184, 606, 613). These
mechanisms are likely to be involved in the sustained reduction in PVR.

In primary cultures of ovine fetal pulmonary arterial endothelial cells, shear stress acutely stimulates NO production through reduction in phosphorylation of eNOS at Thr-495 and an increase in phosphorylation of eNOS at Ser-1177s. The increased phosphorylation of eNOS at Ser-1177 may result from an increased Akt signaling due to the inhibition of PKC-δ (340). Shear stress has also been shown to upregulate the expression of eNOS through increased phosphorylated c-Jun levels (613) and through reduction in binding of STAT3 to eNOS promoter (553).

In pulmonary artery rings from neonatal (5 min to 2 h) pigs, the vasodilator response to ACh is negligible, becomes greatest at 3–10 days, and then decreases with age (370). The 90-kDa heat shock protein (Hsp90) is a constitutively expressed molecular chaperone. Its association with eNOS facilitates eNOS activation. In piglet resistance pulmonary arteries, the physical interactions between Hsp90 and eNOS mature over the first weeks of life (35). In perfused lung of newborn piglets, Hsp90 inhibition reduces eNOS: Hsp90 interaction and NO production associated with reduced pulmonary vasodilatation and increased generation of O2− in the endothelial cells in response to ACh. This study suggests that by acting as a modulator of eNOS activity, Hsp90 may contribute to the postnatal fall in PVR and to the changes in agonist-induced pulmonary vascular responses characteristic of the early neonatal period (34).

Although many studies demonstrated that EDNO is predominantly produced by eNOS, the other NO synthase isoforms may be also involved in the regulation of fetal pulmonary vasoreactivity (469–472). In chronically instrumented fetal lambs (~0.85 gestation), selective inhibition of nNOS with 7-nitroindazole resulted in an increase in basal PVR by 37%. Western blot analysis detected nNOS protein in the fetal lung and in large pulmonary vessels. Since nNOS has also been detected in intact and endothelium-denuded vessels, the enzyme may be present in the medial or adventitial layer (472). The iNOS isoform is constitutively expressed predominantly in airway epithelium and vascular smooth muscle in the late-gestation ovine fetal lung. Intrapulmonary infusions of selective iNOS antagonists (aminoguanidine and S-ethylisothiourea) increase basal PVR in late-gestation fetal lambs and attenuate shear stress-induced pulmonary vasodilatation caused by acute compression of the ductus arteriosus, whereas nonselective blockade with nitro-L-arginine completely blocked this response (469, 471, 530). Studies also show that norepinephrine-induced contractions of isolated ovine pulmonary arteries are diminished by the presence of an attached bronchus in 1–2 day neonatal and juvenile lambs but not in those isolated from near-term and term fetuses. The effect was abolished by removal of bronchial epithelium and by inhibition of NO synthase. It is therefore suspected that the neonatal but not fetal bronchus may release a NO-like relaxing factor that facilitates the postnatal pulmonary vasodilatation (344, 345). In normal, nonsmoking adult human individuals, NO in airways appears to be mainly derived from the epithelial NO synthase type II enzyme, and its production is dependent on the O2 concentration in inspired air. The Km O2 (135 μM) for iNOS activity allows for the generation of NO in proportion to the inspired oxygen concentration throughout the physiological range. Therefore, epithelium-derived NO may be modulating pulmonary vasodilatation (161).

1. sGC

NO induces pulmonary vasodilatation mainly by increasing the intracellular level of cGMP resulting from sGC activation. sGC is a heterodimer consisting of α- and β-subunits. The predominant sGC isoform in vascular system is α1β1 (193). Reaction of NO with the heme moiety of sGC induces a conformational change leading to a several hundredfold increase in production of cGMP from GTP (49). In near-term fetal lungs, sGC immunostaining is more pronounced in small pulmonary arteries than in large ones; in veins sGC immunostaining is more pronounced in large than in small vessels (144).

A maturational increase in the mRNA and/or protein expression of sGC has been observed in isolated pulmonary arteries of piglets and in isolated rat lung, which correlated with increased vasorelaxant responses to the physiological sGC activator NO and to the exogenous sGC activator NO or YC-1 (121, 370, 405, 453). In pulmonary arteries of newborn (3–18 h of age) and 2-wk-old piglets, the expression of sGC β1-subunit increases with postnatal age, both at mRNA and protein levels, which correlated with increased vasorelaxant responses to NO and to sGC activator YC-1 (405). Abundant mRNA and protein of α1- and β1-subunit of sGC have also been found in lung of late-gestation fetal and neonatal rats, with markedly reduced levels detected in adult lung. Pulmonary sGC activity stimulated with sodium nitroprusside, a NO donor, is approximately sevenfold greater in 1- and 8-day-old rats than in adult rats (84). In cultured rat PASMCs, the protein level and enzyme activity of sGC are greater in normoxia than in hypoxia. It is not clear whether or not this is the case in perinatal PASMCs (240).

2. PKG

The effects of cGMP are mediated through activation of PKG, nucleotide-gated ion channels, and cGMP-regulated PDEs (189, 261). In perinatal ovine pulmonary vessels, cGMP-mediated relaxation is primarily mediated by PKG (153, 200, 423). cGMP-mediated relaxation of ovine pulmonary arteries is less in fetal than in newborn and adult sheep (334). However, PKG protein expression and
activity are developmentally downregulated (Gao and Raj, unpublished observations). Relaxation of pulmonary arteries and veins of term fetal lambs to 8-Br-cGMP, a cell permeable cGMP analog, is greater after exposure for 4 h to normoxia (PO₂, 140 mmHg) compared with hypoxia (PO₂, 30 mmHg). The decreased relaxation of pulmonary veins to cGMP in hypoxia may result from reduced expression of PKG protein and mRNA as well as posttranscriptional modification of PKG by peroxynitrite and other reactive oxygen species (ROS); in pulmonary arteries, the suppressed response to cGMP in hypoxia seems largely due to a PKG-independent mechanism (201, 423).

3. PDEs
cGMP is degraded by PDEs. Among the 11 PDE subtypes that have been identified, PDE5 specifically hydrolyzes cGMP and is found to be abundant in lung tissues (134). RNA blot hybridization shows that PDE5 mRNA is detectable in fetal rat lung at ~0.5 gestation and reaches maximal levels in neonates. mRNA levels in adult rat lung are markedly lower than in newborn rat lung (505). Rates of hydrolysis of cGMP in ovine pulmonary vessels are greater in the fetus than in the newborn lamb, and rates of hydrolysis of cGMP are greater in pulmonary arteries than in veins. A higher PDE5 activity may contribute to the greater contractility of fetal pulmonary vessels, particularly in the veins (435). In term fetal lambs, inhibition of PDE5 with E4021 causes significant relaxation of intrapulmonary arteries. The effect is blocked by inhibition of NO synthase (158). When treated with sildenafil, a specific PDE5 inhibitor, PVR of term fetal lambs is lower during maternal oxygen inhalation compared with air-breathing ewes (298).

In ovine and mouse lung, within 1 h following birth, PDE5 activity, protein, and mRNA levels decrease in both species, in a manner that correlates with decreases in PVR during the early transition period. In pig pulmonary arteries, however, it was found that PDE5 may not be responsible for the maturational increase in NO-mediated responses during the first days of extraterine life (406). From 4 to 7 days after birth, a secondary decrease in PDE5 activity, protein, and mRNA expression occurs in both ovine and mouse lung. These changes may contribute to the sustained low pulmonary vasomotor tone in postnatal lung (232).

In summary, fetal and newborn pulmonary vasoactivity is regulated by developmental changes in NO-cGMP-PKG signaling. This pathway is mature during late gestation but is suppressed by the hypoxic fetal environment and the low blood flow state. At birth with the onset of breathing, increased production of EDNO results from oxygenation- and shear stress-dependent stimulation of eNOS, which is critically important in the transitional changes of the pulmonary circulation. Increased oxygen tension after birth may also upregulate the expression of eNOS and PKG, which act in concert with the maturational increase in sGC and PKG activities and decrease in PDE activity, to contribute to a low postnatal PVR (Fig. 2).

C. Prostaglandins
PGL₃ (prostacyclin) and PGE₂ are potent dilator prostanoids in pulmonary vessels of the fetus and newborn. They are produced mainly from endothelium, with the production of PGI₂ being dominant (96, 109, 112, 208, 356). Prostaglandins (PGs) are synthesized from arachidonic acid (AA) released from the cell membrane following activation of phospholipase A₂ (PLA₂) by calcium. Released AA is converted by cyclooxygenases (COX) to 15-OH-prostaglandin-9,11-endoperoxide (PGH₃), which is further converted to PGs and thromboxanes (Tx) by their respective synthases. COXs are the rate-limiting enzymes for the production of prostanoids. The enzymes are present as two types, constitutive and inducible, termed...

FIG. 2. Possible mechanisms for vasodilatation of newborn pulmonary arteries induced by endothelium-derived nitric oxide (NO) in response to oxygenation and shear stress. Oxygenation and shear stress may acutely increase NO production by increasing endothelial NO synthase (eNOS) activity and by upregulating eNOS expression (32, 77, 135, 184, 365, 429, 606, 613). Shear stress may acutely stimulate eNOS activity by reducing the inhibitory effect of PKC-δ on Akt-mediated phosphorylation of eNOS (340). Shear stress may upregulate eNOS expression by activating c-Jun and by relieving the inhibitory effect of STAT3 on eNOS mRNA expression (553, 613). Oxygenation may upregulate the expressions and activities of soluble guanylyl cyclase (sGC; Refs. 67, 627) and cGMP-dependent protein kinase (PKG; Refs. 201–203, 423). Oxygenation may also reduce the degradation of cGMP by inhibiting the activity of phosphodiesterase type 5 (PDE5; Refs. 232, 233, 249, 619). The solid lines indicate stimulatory action, and the dashed lines indicate inhibitory action.
COX-1 and COX-2, respectively. Although the expression of COX-2 is induced by inflammatory factors, studies suggest that it may also be present under normal conditions and during development (183).

The synthesis of PGI₂ and PGE₂ are developmentally regulated (96, 525, 527). In intrapulmonary arteries of perinatal lambs from the third trimester to 4 wk of age, the synthesis of PGI₂ continuously increases. PGE₂ synthesis increases during the third trimester but decreases from late gestation to 1 wk of age and remains unchanged postnatally (96, 527). These changes are associated with an increased expression of COX-1 protein. Since PGI₂ synthesis from exogenous PGH₂, a product of COXs, is similar at all ages, the maturation changes in dilator prostaglandin production by perinatal pulmonary vasculature seems mainly due to an increase in activity of COX-1 (95, 96).

In isolated resistance pulmonary arteries and veins of term fetal lambs, the cyclooxygenase inhibitor indomethacin has no effect on arteries preequilibrated at a low Po₂ (~21 mmHg) but induces contraction in arteries exposed to an intermediate (~40 mmHg) or high (~70 mmHg) Po₂ (599). The ineffectiveness of COX inhibitors on fetal pulmonary vascular tone under hypoxic conditions is in part due to inhibition of PGI₂ production by hypoxia. Compared with adult ovine pulmonary vessels, fetal ovine pulmonary arteries and veins are more sensitive to the release of endothelium endothelium-derived TxA₂ (60, 61).

D. ET

ETs are a family of bicyclic 21-amino acid peptides composed of three isoforms: ET-1, ET-2, and ET-3. ET-1 is the major isoform with vasoactive properties. Endothelial cells are the major source of ET-1. When subjected to various stimuli such as shear stress, hypoxia, or ischemia, ET-1 is transcribed, synthesized, and secreted within minutes. Under certain pathophysiological conditions, pulmonary vascular smooth muscle and epithelial cells can also produce ET-1 (284, 512). ET-1 is synthesized first as a 212-amino acid proET-1 peptide, which is subsequently transformed to pro-ET-1 by a signal peptidase, to big ET-1 by a furin convertase, and finally to the vasoactive ET-1 by ET-converting enzyme (ECE). There are three isoforms of ECE that have been identified, namely, ECE-1, ECE-2, and ECE-3. Among them, ECE-1 is the most important physiologically, based on its wide action and expression. ET-1 could also be converted from big ET-1 by chymase and neprilysin (NEP), a member of zinc metalloendopeptidase family, and/or ECE-1 in vivo. NEP is also involved in the degradation of ET-1 (156).

ET-1 is released from endothelial cells predominantly (~75%) towards the abluminal side where it can bind to two receptor types, ETₐ and ETₐ receptors, which are
present in SMCs and induce vasoconstriction. ET-1 also induces vasodilatation through EDNO and prostacyclin by binding to endothelial ET<sub>A</sub> receptors. ET<sub>A</sub> receptors also mediate the pulmonary clearance of circulating ET-1 and the reuptake of ET-1 by endothelial cells (110, 387, 512, 574, 629). In the lung of humans (145, 389) and rats (251, 376, 451, 541), ET<sub>A</sub> receptors are more abundant in the proximal whereas ET<sub>B</sub> receptors are more abundant in the distal arterial SMCs; the expression of ET<sub>B</sub> receptors in the endothelium is higher in proximal than in distal vessels.

Under physiological conditions, ET-1 causes vasoconstriction by elevating intracellular Ca<sup>2+</sup> (Ca<sup>2+</sup>) and sensitizing myofilaments to Ca<sup>2+</sup>. The initial transient increase in Ca<sup>2+</sup> results from Ca<sup>2+</sup> release from sarcoplasmic reticulum mediated by PLC and inositol trisphosphate (IP<sub>3</sub>), which is followed by a sustained increase in Ca<sup>2+</sup> through the activation of a nonselective cation channel and a store-operated Ca<sup>2+</sup> channel (383, 399). ET-1 promotes sensitization of the contractile apparatus through the RhoA and Rho-kinase (ROK) pathway by activation of G protein (G<sub>12/13</sub>) (383). ET-1 is also potent in promoting growth and proliferation of vascular SMCs. Its effects are mediated by mitogen-activated protein kinases and perhaps epidermal growth factor receptor (296).

In human lung, ET-1 expression is greater during the saccular (25–35 wk) and alveolar stages (36–41 wk) than in canalicular stages (16–24 wk). ET<sub>A</sub> expression is strong throughout gestation, while ET<sub>B</sub> receptor expression is weak in the canalicular stage but increases markedly during the saccular and alveolar stages (362). In fetal ovine lung of 0.5–0.9 gestation, the levels of preproET-1 mRNA, ETA mRNA, ETB mRNA, and ET protein surge at 0.9 gestation but decrease before birth; a similar pattern occurs for the mRNA expression of ET<sub>A</sub> and ET<sub>B</sub> receptors (290). In fetal lambs of late gestation, intrapulmonary infusion of ET<sub>A</sub> receptor agonists causes pulmonary vasoconstriction or vasodilatation depending on the gestational age and dose of ET<sub>A</sub> receptor agonist infused while ET<sub>B</sub> blockers cause a moderate change in pulmonary vascular tone (110, 116, 287, 288, 290, 293, 573, 623). Acute blockade of ET<sub>B</sub> Receptors has no effect on basal tension in fetal ovine pulmonary vessels. It is known that the effect of ET<sub>B</sub> on pulmonary vasoreactivity is mainly through activation of eNOS followed by increased production of EDNO. Decreased eNOS activity, due to low oxygen environment of the fetal lung, may account for the ineffectiveness of ET<sub>B</sub> receptors in regulating basal vascular tone in the fetus (290, 292, 623). The ineffectiveness of ET<sub>A</sub> or ET<sub>B</sub> blockers on fetal pulmonary vasoreactivity may also suggest that the amount of ET-1 in fetal lung is not sufficient to affect vessel tone (369, 519, 579).

Chronic treatment with an ET<sub>A</sub> receptor blocker decreases pulmonary artery pressure, right ventricular hypertrophy, and distal muscularization of small pulmonary arteries in the ovine fetus (293). In contrast, prolonged ET<sub>B</sub> receptor blockade increases pulmonary arterial pressure and PVR in fetal lambs, with greater right ventricular hypertrophy, muscularization of small pulmonary arteries, and elevated lung ET-1 levels (292). The mechanisms underlying the effects of chronic blockade of ET<sub>A</sub> or ET<sub>B</sub> receptors are not well understood, but may in part result from their effects on proliferation of pulmonary vascular SMCs. Studies suggest that ET-1 may promote SMC proliferation not only by its mitogenic effect, but more importantly, by its ant apoptotic effect (50, 303, 531, 625).

Plasma ET-1 level goes up immediately after birth in humans (426, 468). In newborn lambs, circulating ET-1 level has also been found to be higher 8 h after delivery (289). There are no significant differences in the levels of preproET-1 mRNA, ETA mRNA, ETB mRNA, and ET protein between the term fetus and 1-day-old newborn lambs (291). In near-term fetal lambs, blockade of ET<sub>B</sub> receptors does not affect PVR under baseline conditions but attenuates the reduction in PVR induced by ventilation and oxygenation, indicating that ET<sub>B</sub> receptor stimulation contributes to pulmonary vasodilatation at birth (291). Increased ET<sub>B</sub>-mediated dilator response was also observed in rabbit pulmonary arteries and porcine pulmonary veins of piglets within 1 day after birth (143, 155). The effect of ET<sub>B</sub> receptor activation seems to be mediated, in part, by EDNO and by ATP-sensitive potassium channels (458, 624). Bosentan, a nonselective ET receptor antagonist, does not significantly affect the increase in pulmonary blood flow or decrease in PVR associated with in utero O<sub>2</sub> ventilation of term fetal lambs, suggesting that both ET<sub>A</sub>-mediated vasoconstriction and ET<sub>B</sub>-mediated vasodilatation may equally regulate fetal pulmonary vascular tone. These studies suggest that endogenous ET-1 activity may not play a major role in the vasodilator response to ventilation with O<sub>2</sub> (620). In pulmonary vessels of rabbits and piglets, there is no change in ET<sub>A</sub>-mediated contraction within 24 h after birth (155, 511). In primary cultures of ovine pulmonary arterial endothelial cells of newborn lambs, ET-1 secretion is inhibited by an NO donor, activator of sGC, and cGMP analog. The decrease in ET-1 secretion occurs in conjunction with a decrease in preproET-1 mRNA, indicating that the increased production of EDNO at birth may inhibit ET-1 production via cGMP pathway (326).

In humans, plasma ET-1 levels gradually decrease after birth (165, 363, 375, 422). Plasma ET-1 concentration in healthy human neonates at birth is more than threefold greater than at 5 or 30 days of age (165). Plasma ET-1 level in piglets within 2 h after birth is also high but decreases from 3 days of age to adulthood (363). In both rabbit pulmonary resistance arteries and porcine pulmonary veins, there is an age-related decrease in ET<sub>A</sub>-mediated...
contraction and increase in ET$_B$-mediated EDNO-dependent relaxation.

In summary, although ET-1 expression peaks at midgestation, it exerts only a moderate influence on vasomotor tone in fetal pulmonary vessels. Results from chronic treatment with ET$_A$ or ET$_B$ antagonists suggest that activation of ET$_A$ promotes while activation of ET$_B$ inhibits the development of pulmonary hypertension, probably through their effect on vascular remodeling. At birth the prevailing effect of ET-1 is to augment pulmonary vasodilatation induced by ventilation and oxygenation. This effect is mainly via ET$_B$-dependent EDNO release. The gradual postnatal reduction in ET-1 production during the newborn period may contribute to the maintenance of low PVR in the newborn.

E. PAF

PAFs are a group of structurally related compounds synthesized from membrane lipid precursors. It possesses a wide range of biological activities including potent vasoconstrictor or relaxant activity and stimulation of pulmonary vascular SMC proliferation (64, 278, 286, 402). Ibe et al. (276) by infusing WEB-2170, a specific PAF receptor antagonist, into fetal lambs in vivo, demonstrated that PAF contributes significantly to maintenance of high tone in the pulmonary circulation in utero (276). The level of PAF in plasma and in lung tissue is higher in the fetus than in newborn lambs (276, 278, 282, 504). It seems that the hypoxic environment is a critical determinant of the high PAF levels in the fetal lung. Hypoxia stimulates PAF synthesis in fetal ovine PASMCs and pulmonary venous SMC (PVSMCs). The expression of PLA$_2$, an important enzyme for PAF synthesis, is also upregulated by hypoxia in these cells (280). PAF is produced in response to specific stimuli by a variety of cell types, such as platelets, macrophages, endothelial cells, and SMCs (64, 278, 282, 402). In fetal lambs, the amount of PAF synthesized by PASMCs is at least 400-fold more than by endothelial cells, a calculation based on equal numbers of cells. Moreover, the production of PAF is augmented by hypoxia in SMCs but not in endothelial cells (278, 578). Therefore, PAF derived from endothelial cells may only play a minor role, compared with PAF derived from pulmonary vascular SMCs and other sources, in the maintenance of high vasomotor tone in fetal lung.

PAF receptor (PAF-R) mRNA expression and PAF-R density are high in fetal lung (283). In fetal ovine PVSMCs, PAF-R density in hypoxia is higher than under normoxia. The difference in PAF-R protein expression between hypoxia and normoxia is largely eliminated by cycloheximide, a protein synthesis inhibitor, indicating translational regulation by oxygen tension. Moreover, PAF-mediated IP$_3$ increase and Ca$^{2+}$ release from intracellular stores are greater in hypoxia, suggesting that the hypoxic fetal environment facilitates PAF-R binding and signaling, thereby promoting PAF-mediated pulmonary vasoconstriction and maintenance of high PVR in utero (279, 280).

PAF is inactivated by acetylhydrolase (PAF-Ah) (402, 538). In fetal lamb, lung activity of PAF-Ah is attenuated by hypoxia (276, 278, 280, 282, 504), indicating that slow enzymatic degradation of PAF in fetal pulmonary vasculature may contribute to a high level of PAF in fetal lung. A high PAF synthesis rate and low PAF catabolism result in high PAF levels available for PAF receptor binding. These factors point to an important role for PAF as an endogenous modulator of increased pulmonary vasomotor tone in the fetus.

Plasma PAF levels in fetal lambs fell from 6.43 to 1.31 ng/ml within 90 min after delivery. PAF-Ah mRNA level and activity in lamb lung are upregulated in the immediate newborn period, thereby facilitating the fall in postnatal PAF levels (278, 282, 504). In both PASMCs and in whole lung, PAF-Ah activity is augmented by oxygenation (278, 280, 282). PAF-R mRNA expression and PAF-R density decrease within 2 h after birth, returning to a new intermediate level within a few weeks of life (279, 280, 283). In fetal ovine PVSMCs, oxygen downregulates PAF receptor binding, PAF-mediated IP$_3$ release, and Ca$^{2+}$ signaling (279). Elevated cGMP and cAMP levels resulting from oxygen-induced release of EDNO and PGI$_2$ may further decrease PAF receptor binding and PAF-stimulated IP$_3$ production in a PKG-dependent manner (275). All these factors contribute to the postnatal changes in the pulmonary circulation (Fig. 3).

D. Ion Channels

1. Potassium channels

At least four classes of potassium channels have been identified in the pulmonary vasculature. They are the inward-rectifier (Kir) channel family including the ATP-dependent (K$_{ATP}$) channels (Kir6.x combined with the sulfonylurea receptor), the voltage-dependent (K$_v$), calcium-sensitive (K$_{Ca}$), and the two-pore (such as TASK) potassium channels (16). PASMCs from conduit vessels are enriched in the large-conductance calcium-sensitive (BK) channels while those from resistance vessels have mainly K$_v$ channels, particularly hypoxia- and 4-aminopyridine (4-AP)-sensitive K$_v$1.5 and K$_v$2.1. channels, implicating an important role for these K$_v$ channels in the resting membrane potential and hypoxic constriction of resistance pulmonary arteries (27, 29, 30, 90, 168). The main extraparenchymal pulmonary veins are structured coaxially with a subendothelial layer of typical SMCs surrounded by a layer of cardiac myocytes. Pulmonary veins express K$_v$, Kir, and BK potassium channels. Kir3.1 is only expressed in veins and not in arteries. Kir channels in
pulmonary venous cardiomyocytes but not in PVSMCs. 

Potassium channels consist of a primary pore-forming α-subunit often associated with auxiliary regulatory subunits. Over 70 different genes encode for the potassium channel subunits in the human genome. Potassium channels can be divided into the 2TM (two transmembrane domain), 4TM, and 6TM families. The 2TM family of potassium channels is the Kir channel family. Both Kv and BK potassium channels belong to the 6TM domain family (16). The 4TM domain family of potassium channels, such as TASK-1, TASK-2, THIK-1, TREK-2, and TWIK-2, have been found to be expressed in both pulmonary arteries and veins (210). Compared with that of Kv blockers, TASK-1 blockers cause only modest contraction of pulmonary arteries, indicating a smaller role for TASK-1 channels in regulating basal tone (26, 637). TASK-1 channels have been found to be oxygen sensitive, which could account for the depolarization of the resting membrane potential in response to hypoxia in resistance pulmonary arteries (223).

Protein and mRNA expression of BK channels in distal pulmonary arteries are greater in the fetus than in the adult. Iberiotoxin (a specific inhibitor of BK channels) caused a marked increase in Ca$^{2+}$ in fetal ovine PASMCs but had no effect in adult PASMCs (484, 490). The greater activity of BK channels seems to result from the hypoxic environment encountered by fetal pulmonary vessels. BK α-subunit mRNA expression and BK protein of cultured fetal ovine PASMCs are increased by hypoxia. Under in vivo conditions, hypoxia for 3 wk increases the expression of BK α- and β-subunit mRNA of rat lung by two- to threefold, in part via a HIF-1-dependent mechanism. Both hypoxia and the HIF-1 mimetic deferoxamine have been shown to significantly increase PASMC BK β1 and β2 reporter expression (486). BK channels have also been implicated as the predominant potassium channels in setting the resting membrane potential of fetal pulmonary arteries. Patch-clamp studies show that the resting membrane potential of fetal ovine PASMCs can be depolarized by blockers of BK channels but not by blockers of Kv channels (137).

Activation of potassium channels is involved in the pulmonary hemodynamic changes triggered by ventilation, oxygenation, and shear stress at birth (137, 463, 506, 551, 577). In fetal ovine PASMC, oxygenation increases potassium current and reduces the intracellular calcium level, an effect that is sensitive to BK channel blockers (137, 463). Pulmonary vasodilatation in fetal lambs induced by oxygenation is blunted not only by BK channel blockers but also by guanylate cyclase antagonists andPKG inhibitors, indicating that oxygenation may cause pulmonary vasodilatation by activating BK channels via cGMP-PKG pathway (137, 506).

Kv channels seem to play a smaller role than BK channels in fetal pulmonary vessels. The resting membrane potential in ovine fetal PASMCs is more depolarized than in the adult (36 vs. 49 mV). It is less sensitive to Kv blockers than that of the adult. Kv2.1 channel protein and mRNA expression in distal pulmonary vasculature increase developmentally in fetal, newborn, and adult sheep (140, 484). The maturational changes in pulmonary vasoconstriction in response to acute hypoxia, which is of physiological importance for optimizing ventilation-perfusion ratio, is in part related to the increased Kv channel activity. Acute hypoxia has been shown to induce a more rapid and greater change in Ca$^{2+}$ in magnitude in PASMC of adult sheep compared with late gestation fetal sheep.
(140). K⁺ currents in adult ovine PASMCs that are sensitive to Kᵵ channel blocker are partially inhibited by acute hypoxia (484). It is not clear whether or not a maturational increase in Kᵵ channel activity is species specific. In piglets, the Kᵵ inhibitor 4-AP causes a greater pulmonary vascular contraction in newborn versus older animals (131).

Infusion of Kₐtp activator into the fetal ovine pulmonary artery produces dose-dependent increases in blood flow. The effect is prevented by glibenclamide (a Kₐtp channel blocker), indicating that Kₐtp channels are present in the fetal pulmonary vasculature (113, 136). Infusion of glibenclamide alone in most studies has had no effect on basal tension of fetal pulmonary vessels or on shear stress-induced pulmonary vasodilatation (113, 136, 506, 551, 577). Electrophysiological study also shows that the resting membrane potential of fetal ovine PASMCs is not affected by glibenclamide (437).

In summary, there is sufficient accumulated evidence to support a prominent role for BK channel activity in the resting membrane potential of fetal PASMCs and in pulmonary vasodilatation due to hyperpolarization caused by ventilation, oxygenation, and shear stress at birth. The relative importance of K⁺ channel types regarding the resting membrane potential and hypoxic response of PASMCs shifts from BK to Kᵵ channels in an age-dependent manner.

2. Calcium channels

The cytosol Ca²⁺ ([Ca²⁺]ᵢ) concentration of pulmonary vascular SMCs is tightly controlled. The primary pathways for Ca²⁺ influx across the plasma membrane are voltage-gated L-type Ca²⁺ channels and nonselective cation channels (NSCC). [Ca²⁺]ᵢ can also come from the sarco(endo)plasmic reticulum (SR) Ca²⁺ stores through IP₃ and ryanodine-sensitive receptors. Cytosol Ca²⁺ is extruded into the extracellular space via the plasma membrane Ca²⁺-ATPase and the Na⁺/Ca²⁺ exchanger and sequestered by the SR Ca²⁺-ATPase (SERCA) into the SR. Depletion of the SR Ca²⁺ stores leads to extracellular Ca²⁺ entry through Ca²⁺ release-activated Ca²⁺ channels (CRAC), also known as capacitative Ca²⁺ entry (CCE) or store-operated Ca²⁺ entry. Studies suggest that CRAC may be composed of canonical transient receptor potential (TRPC) proteins and activated by stromal interacting molecule 1 (STIM1). STIM1 and five of seven known TRPC isoforms (TRPC1 > TRPC6 > TRPC4 > TRPC3 > TRPC5) are expressed in rat pulmonary arteries. STIM1, TRPC1, TRPC6, and TRPC4 were greater in distal than proximal PASMCs. Studies conducted under conditions of extracellular Ca²⁺-free medium, when Ca²⁺ stores in SR are depleted, and L-type voltage-operated Ca²⁺ channel are blocked, demonstrate that hypoxia-induced [Ca²⁺]ᵢ elevation through SOCC is greater in distal than in proximal PASMCs, indicating an important role for SOCE in HPV (54, 74, 89, 359, 373, 537).

In fetal ovine PASMCs, membrane depolarization and increase in [Ca²⁺]ᵢ induced by hypoxia are inhibited by verapamil [an inhibitor of voltage-operated calcium channels (VOCC)] and attenuated by ryanodine, suggesting that extracellular calcium influx through VOCC channels and intracellular calcium release from ryanodine-sensitive calcium stores contribute to hypoxia-evoked [Ca²⁺]ᵢ elevation in fetal pulmonary vascular SMCs (141, 142).

The basal cytosolic and SR Ca²⁺ levels of term fetal ovine PASMCs are comparable with those in adult ovine PASMCs. Ca²⁺ efflux pathways from the cytosol and basal as well as capacitative Ca²⁺ entry are also matured before birth. The elevation in [Ca²⁺]ᵢ evoked by 5-hydroxytryptamine (5-HT) occurs in very few fetal compared with adult PASMCs; in contrast, phenylephrine elevated [Ca²⁺]ᵢ to a similar degree in fetal and adult PASMCs. Such differences in Ca²⁺ signaling may be due to maturational changes in receptor and receptor coupling. The elevation in [Ca²⁺]ᵢ evoked by caffeine is greater in fetal PASMCs while [Ca²⁺]ᵢ elevation by purinergic ATP is greater in adult cells, indicating that IP₃-sensitive Ca²⁺ stores are mature and ryanodine-sensitive Ca²⁺ stores can be upregulated in fetal PASMCs (215).

Pulmonary vasodilatation in term fetal lambs and reduction in [Ca²⁺]ᵢ of fetal PASMCs induced by oxygenation are attenuated by ryanodine. Since these effects are also inhibited by iberiotoxin, oxygenation may increase spontaneous sparking release of Ca²⁺ through ryanodine-sensitive Ca²⁺ stores, which leads to activation of BK channels, thus leading to membrane hyperpolarization and vasodilatation. Indeed, the frequency of spontaneous transient outward currents, indicative of intracellular calcium spark activation of BK channels, is increased by oxygenation in fetal PASMCs (463). Ryanodine increases [Ca²⁺]ᵢ in distal PASMCs from term fetal but not from newborn (<2 day) and juvenile (4–6 wk old) rabbits. The effect is prevented by BK channel blocker. Fetal rabbit PASMCs exhibit spontaneous transient outward currents and calcium sparks (462). These observations suggest that a close relationship exists between ryanodine-sensitive Ca²⁺ stores and BK channel activity.

G. ROS

ROS including superoxide and H₂O₂ cause pulmonary vasoconstriction in adult animals. In particular, ROS derived from mitochondria and NADPH oxidases (Nox) may serve as a sensor to mediate hypoxic pulmonary vasoconstriction through inhibition of potassium channels and elevation of [Ca²⁺]ᵢ (31, 25, 483, 602, 605, 616). It should be noted that a change in detection of ROS, specifically superoxide and H₂O₂, may not be equal to a
change of its production. For instance, in the presence of NO, the rate constant of the reaction with superoxide to form peroxynitrite is such that superoxide is likely to be removed before it can reach the detector (192).

In near-term fetal lambs, an increase in oxygen tension dilates while a decrease in oxygen tension constricts pulmonary vessels (364, 408). In human fetuses of late gestation, maternal hyperoxegenation significantly increases pulmonary blood flow (480). If ROS acts as the key mediator for hypoxic pulmonary vasoconstriction in the fetus as in the adult, the above studies would suggest an active role for ROS in modulating fetal pulmonary vasoactivity. In addition to promoting vasocostriction, ROS may also exert their effects by inhibiting the production of vasodilators such as EDNO. In pulmonary arterial endothelial cells from late-gestation fetal lambs, eNOS promoter activity is decreased by H2O2 through c-Jun (341). The inhibition of ET-1 on eNOS activity observed in fetal ovine pulmonary arterial endothelial cells may also be via a mechanism with H2O2 being involved (608).

Intracellular ROS levels are maintained by enzymatic and nonenzymatic antioxidant defenses within a physiological range to prevent their harmful effects. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase, and catalase. Nonenzymatic antioxidants include ascorbic acid, α-tocopherol, glutathione, carotenoids, flavonoids, and others (192, 581). The activity of antioxidant enzymes in the lung has been found to increase during late gestation in a number of species including rat, rabbit, hamster, guinea pig, sheep, and humans (37, 118, 125, 126, 190, 191, 246, 391, 492, 598). The developmental regulation of specific antioxidant enzymes appears to be species specific. In fetal ovine lung, the activity of SOD, catalase, and GPx increases between 0.8 gestation to term (598). In human lung throughout gestation, maternal hyperoxegenation significantly increases H2O2 production. For instance, in the presence of NO, the rate constant of the reaction with superoxide to form peroxynitrite. In rat pulmonary arteries preconstricted with thromboxane A2 analog, exogenous peroxynitrite causes a lesser contraction of juvenile and adult vessels than in newborn vessels. This may be due to the “priming” effect of peroxynitrite that results in lower production of 8-isoprostane in the juvenile and adult vessels (59).

Involvement of ROS in cell proliferation has been reported in various cell types (53, 473, 557). Proliferation of fetal ovine pulmonary vascular SMCs can be stimulated by exogenous ROS such as H2O2 and endogenous ROS, when their levels are elevated by either inhibition of antioxidant enzymes or by ET-1 (612). In contrast, fetal PASMCs proliferation is inhibited when endogenous ROS is reduced with a combined superoxide dismutase/catalase mimic. When the amount of endogenous ROS was not completely eliminated, there was an increase in fetal PASMC proliferation and in levels of the cell-cycle regulatory protein p21, suggesting that ROS levels determine the fate of fetal PASMCs proliferation and, when below a threshold level, trigger apoptosis (607, 609).

H. ROK

ROK is a serine/threonine protein kinase with a molecular mass of ~160 kDa. It is present in two isoforms, types I and II. They are encoded by different genes and are expressed ubiquitously. Within the cells, ROKs are distributed in the cytoplasm but are translocated to the peripheral membrane on RhoA activation. Currently, more than 15 ROK substrates have been identified. The phosphorylation of these proteins by ROKs is related to a variety of functions such as contraction, migration, proliferation, apoptosis/survival, gene expression, and differentiation (534). ROK activity may be preferentially augmented in a number of vascular diseases such as hypertension, ath-
erosclerosis, and pulmonary hypertension (371, 534). In the lung, increased ROK expression and/or activity are associated with chronic hypoxia-induced pulmonary hypertension (222, 273, 312, 419). ROK inhibitors have been found to be effective in preventing and reversing chronic hypoxia-induced pulmonary hypertension in animal models and seem to have some therapeutic benefit in the treatment of pulmonary hypertension in humans (169, 222, 273, 418–420, 632).

The fetal pulmonary circulation is uniquely characterized by its inability to exhibit sustained vasodilatation. Its dilatation response is often transient despite continued exposure to dilator stimuli (4, 5, 287). This may in part be due to its high intrinsic myogenic tone (58, 315, 446, 550, 552, 576). In chronically catheterized late-gestation fetal lambs, intrapulmonary infusion of ROK inhibitors causes a sustained pulmonary vasodilatation and converts the transient pulmonary vasodilatation caused by ductus compression to a sustained one. These findings suggest that high ROK activity opposes pulmonary vasodilatation in utero, which may contribute to maintenance of high PVR in the normal fetal lung (448, 576).

ROK-mediated phosphorylation of MYPT1, the regulatory subunit of myosin light-chain phosphatase (MLCP), is recognized as a critical mechanism in the regulation of contractility of vascular SMCs. When stimulated with various constrictors, the activated regulation of contractility of vascular SMCs. When (MLCP), is recognized as a critical mechanism in the regulatory subunit of myosin light-chain phosphatase fetal lung (448, 576).

HIF-1α contributes to maintenance of high PVR in the normal poses pulmonary vasodilatation in utero, which may contribute to the unique myogenic tone and myogenic response of the fetal pulmonary vasculature, which include the sensors for myogenic response, membrane channels affecting cytosolic Ca²⁺ concentration, and factors affecting calcium sensitivity of myofilaments (157, 185, 521).

I. Hypoxia and Hypoxic Vasoconstriction

The fetal lung develops in a relatively hypoxic environment in utero that appears to be essential for the proper development of the lung and for pulmonary vasculogenesis (198, 499–501). Effects of hypoxia are largely mediated by HIF-1 (36, 38, 39, 65). HIF-1α-deficient mice die in utero and exhibit severe vascularization defects, malformation of the neural fold, and cardiovascular defects (297, 337, 502). HIF-2α−/− mouse embryos die as a consequence of inadequate blood vessel fusion and remodeling, impaired fetal lung maturation, and a very slow heart rate because of insufficient catecholamine production (452, 569). HIF-1β−/− embryos die displaying defects in blood vessel formation, defective angiogenesis of the yolk sac and branchial arches, stunted development, and embryo wasting (380).

Under normoxic conditions (∼5% O₂), the α-subunits of HIF-1 are hydroxylated and degraded by proteosomes. Under hypoxic conditions (≤5% O₂), the α-subunits are stabilized via dimerization with a HIF-β or aryl hydrocarbon receptor nuclear translocator (ARNT), then translocated to the nuclei, where they can initiate transcription of target genes (432, 515, 516). Hypoxia-induced stimulation of gene transcription is mainly by HIF-1α and HIF-2α (321). HIF-2α subunits are structurally similar to those of HIF-1α in their DNA binding and dimerization domains but differ in their transactivation domains. In mammalian cells, HIF-1α is ubiquitously expressed and HIF-2α is predominantly expressed in the lung, endothelium, and carotid body (269, 321). In lung, HIF-1α is primarily expressed in the branching epithelium. HIF-2α and ARNT proteins are localized to the developing epithelium as well as mesenchymal structures in the parenchyma, most likely vascular in nature (219, 478).

HIF-1 modulates the expression of an array of mitogenic factors that have broad functional implications. VEGF, a key promoter for blood vessel formation, is regulated by HIF-1 (327, 592). HIF-1α−/− mice die at midgestation in association with defects in VEGF expression and vasculogenesis (502). In mouse lung explants, pulmonary vascular development was greater when cultured at 3% oxygen compared with 20% oxygen. The effect of oxygen is inhibited by antisense oligonucleotides against either HIF-1α or VEGF, indicating that hypoxia promotes vascular formation via HIF-1α-VEGF (585). In vascular development stimulated by hypoxia, in addition to VEGF, several other mitogens whose expression is regulated by HIF-1 may also be involved such as TGF-β1, PDGF-BB, bFGF, and/or their receptors. Their precise roles in lung vascular development are being studied (214, 432, 515, 516).

The development and maturation of the pulmonary vasculature is accompanied by increased vessel reactivity towards oxygen. In humans, maternal hyperoxygenation
significantly increases pulmonary blood flow in near-term fetuses (31–36 wk) but has no effect in midterm fetuses (480). In fetal sheep, a change in blood oxygen tension has no or very little effect on pulmonary vasoreactivity in fetuses at \( \sim 0.6 – 0.7 \) gestation. In contrast, an increase in oxygen tension markedly dilates while a decrease in oxygen tension strikingly constricts pulmonary vessels of the near-term fetus (364, 407). These observations suggest that the fetal pulmonary circulation acquires the ability to respond to changes in oxygen tension with advancing gestation (407, 480).

The increase in hypoxic pulmonary vasoconstriction (HPV) during the latter part of gestation has functional significance for the proper distribution of cardiac output among different organs. In the fetus, gas exchange occurs in the placenta. Pulmonary blood flow is low and primarily supports the growth and metabolic functions of the lung (350). In ovine fetuses, the number of resistance pulmonary vessels increases by 10-fold per unit of lung weight in the latter third of gestation, yet pulmonary blood flow only increases from 3.5 to 7% of total cardiac output (407, 500). In humans, the proportion of total fetal cardiac output that goes to the lung increases by 60% from 20 to 38 wk of gestation (481). If fetal pulmonary vessels do not demonstrate hypoxic vasoconstriction, there would be a striking redistribution of cardiac output from other organs to the lung (407, 500). With the onset of breathing and oxygenation, hypoxic vasoconstriction in the pulmonary circulation is reversed and the pulmonary circulation relaxes. After birth, hypoxic vasoconstriction is still physiologically very important as it diverts blood flow from less well aerated areas to better-aerated areas of the lung so as to optimize the ventilation-perfusion ratio (1).

Hypoxic constriction is unique to pulmonary vessels, in particular resistance pulmonary vessels, since systemic vessels relax in response to hypoxia (473–475, 604). HPV was first reported by von Euler and Liljestrand in 1946 (593). Despite years of study, the precise mechanisms underlying this phenomenon remain unresolved. It is generally recognized that HPV is a response intrinsic to pulmonary vascular SMCs and that the endothelium plays an important role to augment HPV (1, 2, 25, 64). There are currently two leading hypotheses for HPV. One hypothesis postulated by Archer et al. is that hypoxia decreases the production of mitochondrial ROS including \( \text{H}_2\text{O}_2 \) which shifts the cytosolic redox balance to a more reduced state and causes an inhibition of redox-sensitive voltage-gated potassium channel. This is followed by membrane depolarization and vasoconstriction. (25, 28, 394). An alternate hypothesis postulated by Schumacker et al. is that an increased production of mitochondrial ROS induced by hypoxia triggers the release of \( \text{Ca}^{2+} \) from SR and \( \text{Ca}^{2+} \) entry via capacitative calcium entry channels and/or voltage-dependent calcium, resulting in vasoconstriction (604, 605). Hypoxia may also increase ROS generation from NADPH oxidase, probably through the mitochondrial ROS-PKC signaling axis (385, 483). In addition to these two hypotheses, some alternative mechanisms have recently been proposed. Evans (167) proposed that inhibition of mitochondrial oxidative phosphorylation by hypoxia leads to a rise in cellular AMP/ATP ratio and consequent activation of AMP-activated protein kinase (AMPK), which leads to cADPR-dependent \( \text{Ca}^{2+} \) release from the SR resulting in vasoconstriction. Other studies suggest that an increase in release of zinc from nitrosylation of metallothionein (a metal-binding, zinc-storing protein) (68) and an increase in \( \text{Na}^+ \) influx following the activation of membrane-bound transient receptor potential channel 6 (TRPC6) channels (615) may be involved in HPV.

In summary, the relative hypoxic environment of the fetus is critical for lung development and pulmonary vasculogenesis (297, 337, 502, 585). HPV acquired with advancing gestation is important for the proper distribution of cardiac output among different organs in fetal life, and postnatally, HPV serves to optimize ventilation-perfusion ratio during localized alveolar hypoxia (350, 407, 480). Several mechanisms contribute to the high vasomotor tone in the fetal pulmonary circulation, which include the high extraluminal pressures around the pulmonary vasculature resulting from the fluid-filled lung, thick vessel walls due to greater vascular smooth muscle mass, high vascular contractility caused in part by high PAF and ROK activity, and the inhibition of the EDNO-cGMP-PKG and the PGL2-cAMP signaling pathways (180, 331, 476). Increased sensitivity of the pulmonary vasculature to changes in oxygen tension also facilitates its function as a gas exchanging organ (56, 201, 364, 407, 408, 477, 480).

### IV. PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

PPHN is a syndrome defined by severe hypoxemia and persistent pulmonary hypertension resulting from failure of PVR to decrease adequately at birth. It is characterized by persistently high PVR, elevated vascular reactivity, smooth muscle remodeling, and impaired angiogenesis (3, 143, 212, 545, 568). PPHN can be idiopathic or secondary to a variety of conditions including intrapartum asphyxia, infection, pulmonary hypoplasia, congenital heart disease, or drug therapy. It occurs in \( \sim 1.9 \) per 1,000 live births with a high risk of mortality (216, 545, 568).

#### A. Anatomical Abnormalities and Vascular Remodeling

Vascular remodeling is a prominent feature in PPHN characterized by increased smooth muscle proliferation.
and muscularization of the normally muscle-free peripheral arteries. The intima is also infiltrated with fibroblasts, and the adventitia shows increased production of extracellular matrix (ECM), with deposition of collagen and elastin. These changes result in thickening of the blood vessel wall, increased PVR, and reduced compliance (244, 271, 336, 347, 638) (Fig. 4). While SMC proliferation has drawn great attention, substantial evidence also indicates that the adventitial remodeling is equally important in the development of pulmonary hypertension (336, 347, 638). Studies indicate that under pathological insults such as hypoxia, increased activity of elastases may degrade ECM, release growth factors, induce matrix metalloproteinases (MMPs), and thus promote vascular remodeling (638). Increased elastase activity has been found to be an early event associated with pulmonary hypertension in both the newborn and the adult. Furthermore, treatment with inhibitors of elastases inhibits SMC proliferation and alleviates medial thickening of the distal pulmonary arteries (336, 638). The adventitial remodeling is regulated by MMPs, which are neutral proteinases involved in the breakdown of ECM and by the tissue inhibitors of metalloproteinases (TIMPs). In developing lung, collagen is primarily broken down by MMP-2 (196, 503). In newborn mice, chronic hypoxia leads to thick-walled pulmonary arteries and impaired alveolarization, accompanied by decreased MMP-2 and increased TIMP-2. MMP2−/− mice in normoxia have thick-walled pulmonary arteries, impaired alveolarization, and increased perivascular collagen and elastin. These results suggest that reduced MMP-2 activity, partially due to increased TIMP-2 activity, may contribute to abnormal pulmonary arterial remodel-

![FIG. 4. Diagram showing postnatal structural changes and the effect of chronic hypoxia on pulmonary arteries based on studies with fetal and newborn piglets (225, 242, 243, 258, 259, 324, 325). Fetal pulmonary arteries have thick walls and a narrow lumen. The smooth muscle cells (SMCs) are rounded, densely packed, and appear immature and synthetic in type, and contractile organelles do not predominate. After birth, the overlap of adjacent SMCs rapidly decreases, and a reorganization of the cytoskeleton occurs within 1 wk of life so that the SMCs become thinner and spread around a larger lumen. Later, the medial thickness of the vessel wall decreases further to mature levels at ~6 mo of age with reduced cell packing density and increased myofilament density. The shape of SMCs is elongated with a stable cytoskeleton. Postnatal exposure to chronic hypoxia prevents these postnatal adaptational changes and promotes SMC proliferation, leading to the thickened vessel wall and the narrowed vessel lumen. [From Gao and Raj (204) with permission.]

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ing (23). It is of interest to note that in contrast to the decreased MMP-2 activity observed in neonatal pulmonary hypertension models, MMP-2 activity is increased in adult pulmonary hypertension (194, 358). Such a difference may be explained by differences in intrinsic properties of neonatal versus adult pulmonary arteries. Neonatal pulmonary arteries have thick vessel walls that become thinner with maturation. A decrease in MMP activity in hypoxia would prevent this process and lead to persistent fetal-like, thicker-walled arteries (Fig. 4). Conversely, adult pulmonary arteries are thin-walled. An increase in MMP activity may promote ECM break down and thus facilitate the migration of SMC precursors and vascular remodeling (23, 547).

Many factors promote vascular remodeling in PPHN such as an increase in pulmonary blood flow in utero; hypoxia; hyperoxia; various mediators including ET-1, PAF, ROS, PDGF, TGF-β, and other growth factors; as well as increased RhoA/ROK activity. On the other hand, some mediators may inhibit vascular remodeling such as NO/cGMP pathway and VEGF. Thus stimuli suppressing these inhibitory signaling mechanisms may also contribute to pulmonary vascular remodeling (76, 244, 271, 311, 547).

1. ET-1

ET-1 level is elevated in the blood of newborn infants with persistent pulmonary hypertension (3, 493). In animal models of PPHN, in mice, rats, and piglets, ET-1 has also been implicated in the increased pulmonary vascular remodeling, probably primarily mediated by ET₁ receptors (20, 304, 307, 308, 510, 639).

PDGF, a potent SMC mitogen, is implicated in the vascular remodeling associated with pulmonary hypertension (544). In a neonatal rat model of PPHN obtained after chronic exposure to 60% O₂, the elevation in ET-1 levels, increased SMC proliferation and medial thickening of pulmonary resistance arteries were prevented by an ET-1 receptor blocker, indicating a causal relationship between ET-1 and vascular remodeling (307, 308). In this model, the expression of the β-receptor for PDGF was upregulated, which was prevented by an ET-1 receptor blocker. PDGF-BB, a major ligand for PDGF-β receptors, is found to be a potent inducer of hyperplasia and DNA synthesis in cultured PASMCs from infant rats. Moreover, the vascular remodeling observed in this model was markedly attenuated by a truncated soluble PDGF-β receptor intervention. These results provide evidence for PDGF to be a key downstream mediator of vascular remodeling induced by ET-1 in neonatal lung (304). Similar results were also obtained in an ovine PPHN model created by patent ductal arteriosus ligation. In this lamb model PDGF α- and β-receptor proteins are increased. The development of muscular thickening of small pulmonary arteries and right ventricular hypertrophy is reduced by treatment with NX1975, an aptamer that selectively inhibits PDGF-B (46). Vascular wall remodeling in pulmonary hypertension may reflect an increased proliferation but also reduced apoptosis of smooth muscle. In the neonatal rat model of PPHN obtained after chronic exposure to 60% O₂, the expression of proapoptotic Bax in pulmonary arteries was decreased while that of antiapoptotic Bcl-xL was increased, which was accompanied by increased numbers of TUNEL-positive cells in the walls of pulmonary arteries. Hence, ET-1 may mediate remodeling of neonatal rat pulmonary arteries by inhibiting SMC apoptosis (303).

TGF-β is involved in the regulation of cellular proliferation, differentiation, and other functions (86). It is dynamically regulated during late lung development in mouse and human (15, 451). Genetic defects in TGF-β receptors are implicated in the etiology of PAH in childhood (239). Since TGF-β synthesis and phosphorylation of its downstream effector Smad3 are stimulated by ET-1 via ET₁ receptors in the lung (299), it is possible that ET-1 may influence vascular remodeling through the TGF-β pathway (91, 119, 454). In newborn mice, chronic hypoxia leads to PASMC proliferation and altered extracellular matrix protein deposition, accompanied by increases in active TGF-β and phosphorylated Smad2. These effects are abrogated by inducing dominant-negative mutation of the TGF-β type II receptor (21, 22).

It is of interest to note that in vitro ET-1 alone is less potent as a mitogen for PVSMS than in the presence of other growth factors or serum, indicating a coordinating or synchronizing action for this peptide in vivo (301, 311, 640). Vascular remodeling represents an altered balance between proliferation and apoptosis, resulting in abnormal cell accumulation. Studies suggest that the inhibitory effect of ET-1 on apoptosis of SMCs may be more important than its moderate mitogenic effect on SMCs in hypoxic pulmonary vascular remodeling (303, 339, 531, 625). ET-1 attenuates Paclitaxel- and serum deprivation-induced PASMC apoptosis in newborn rats. In pulmonary vessels of rat pups exposed from birth to 60% O₂ for 7 days, the expression of proapoptotic Bax is decreased while antiapoptotic Bcl-xL is increased. When treated with SB217242, an ET receptor antagonist, the numbers of TUNEL-positive cells are increased. These observations suggest that ET-1 mediates remodeling of neonatal rat pulmonary arteries by inhibiting SMC apoptosis (303). There is evidence indicating that the anti-apoptosis effect of ET-1 in vascular SMCs is mediated by the prosurvival phosphoinositide 3-kinase (PI3K)/AKT and p38 mitogen-activated protein kinase (303, 531) (Fig. 5).
2. PAF

PAF contributes to the development of chronic pulmonary hypertension induced by chronic hypoxia in adult rats (412). In fetal lambs exposed to chronic high-altitude hypoxia, the percentage medial wall thickness was significantly increased in arteries at all levels, and this was associated with a threefold increase in PAF synthesis in these pulmonary arteries. Hypoxic pulmonary arteries show greater PAF receptor expression and increased PAF receptor binding. Furthermore, PASMCs obtained from chronically hypoxic fetal lambs demonstrate greater proliferation rates. These findings suggest that in chronic hypoxia in utero increased PAF-R protein expression and increased PAF binding contribute to pulmonary vascular remodeling and may predispose the fetuses to persistent pulmonary hypertension after birth (75). A recent study shows that PAF may promote fetal pulmonary vascular SMC proliferation through transactivation of EGFR, via activation of matrix metalloproteinases and heparin-binding EGF-like growth factor, resulting in p38 and ERK activation. EGFR transactivation has been found to be essential for the mitogenic effect of PAF in PVSMCs (274, 646). PAF-induced proliferation of term fetal lamb PASMCs and PVSVMCs is accompanied by increased NF-κB protein expression. PAF may stimulate proliferation of fetal Smooth muscle by activating MAPK that in turn activates NF-κB and sequentially activates the nuclear cell-cycle regulators CDK2 and CDK4 and retinoblastoma protein. Phosphorylated retinoblastoma induces gene expression and cell growth (274).

3. ROS

The vascular remodeling associated with the development of PPHN in the lamb ductal ligation model is associated with increased production of superoxide originating from elevated expression of p67phox, a subunit of the NADPH oxidase complex (79, 97). Chronic hypoxia causes a significant increase in superoxide production in intrapulmonary arteries, which was obliterated in NADPH oxidase (gp91phox) knockout (KO) mice, suggesting that NADPH oxidase was the major source of ROS. Pathological changes associated with chronic hypoxia-induced medial wall thickening of small pulmonary arteries and right heart hypertrophy were completely abolished in NADPH oxidase (gp91phox) KO mice (368). In newborn ovine PASMCs, inhibition of Rac1, an important subunit of the NADPH oxidase complex, inhibited ROS generation by inhibiting geranylgeranyl transferase, which is critical for Rac1 in the formation NADPH oxidase complex. Also, inhibition of Rac1 by gene transfer of dominant negative Rac1 reduced ROS production and led to decreased cell proliferation and cell cycle arrest at G2/M (450).

A role for xanthine oxidase (XO) is implicated in a rat PPHN model. Newborn rats exposed to hypoxia for 14 days from birth led to pulmonary vascular remodeling and right ventricular hypertrophy, accompanied by increased serum and lung XO activity and increased vascular XO-derived superoxide production, vascular nitrotyrosine formation, and reduced relaxation of pulmonary arteries to ACh and sodium nitroprusside (305).

4. RhoA-ROK signaling

Pulmonary artery endothelial cells taken from piglets with chronic hypoxia-induced pulmonary hypertension display a stable abnormal phenotype manifested by increased formation of stress fibers and increased permeability under normoxic conditions. This may result from sustained activation of RhoA that leads to sustained inhibition of Rac1 and its downstream effector PAK-1 (622). In mice, the deficiency of the Na+/H+ exchanger isoform 1 (NHE1) gene has been found to prevent hypoxia-induced pulmonary hypertensive and vascular remodeling (636). ROK (ROK1 and ROK2) expression and activity in pulmonary arteries are reduced in these mice, suggesting that NHE1 may promote pulmonary vascular remodeling via ROK (636). Pulmonary SMC growth requires an alkaline shift in intracellular pH in which the activation of NHE is involved (465, 466). A wide range of mitogenic factors can activate NHE such as PDGF, which is released by endothelial cells exposed to hypoxia (311, 348). In pulmonary SMCs, hypoxic induction of NHE1 expression seems to be mediated by HIF-1 (533).
5. **NO-cGMP-PKG**

In eNOS-deficient mice, pulmonary arterial muscularity was greater (396), which is evidence of hypertensive remodeling (528). Exposure to mild hypoxia in the neonatal period led to a failure of capillary and alveolar growth in eNOS$^{-/-}$ mice, which was not seen in normal mice, suggesting that EDNO preserves normal distal lung growth during hypoxic stress (47). NO donor spermine NONOate has been found to attenuate serum-induced proliferation of fetal ovine PASMCs resulting in a G0/G1 cell cycle arrest. The effect of the NO donor on cell proliferation may be mediated by inhibition of the mitogenic action of superoxide or through peroxynitrite, since spermine NONOate has been found to decrease superoxide levels and increase peroxynitrite production in PASMCs (610). Major targets for NO-mediated inhibition of cell cycle progression at the G1/S transition may include the downregulation of cyclin D and cyclin A expression, the upregulation of the expression of both the inhibitory protein p21Cip1/Waf1 and the transcription factor E2F, and the dephosphorylation of retinoblastoma protein. NO may also inhibit cell proliferation and differentiation through interference with MAPK signaling in both a cGMP-dependent and -independent manner (588).

Chronic hypoxia triggers pulmonary vascular remodeling, which is associated with modulation of vascular SMC phenotype from a contractile, differentiated state to a synthetic, dedifferentiated state. In ovine fetal PVSMCs, hypoxia-induced reduction in PKG protein expression strongly correlated with the repressed expression of SMC phenotype markers, myosin heavy chain, calponin, and vimentin, indicating that hypoxia induces phenotype modulation of SMC to a dedifferentiated state. Overexpression of PKG in SMCs reversed the effect of hypoxia on the expression of these phenotype marker proteins. These results suggest that decreased PKG activity may be involved in the maintenance of the dedifferentiated phenotype in PVSMCs in hypoxia (645). Myocardin and E-26-like protein 1 (Elk-1) are possible downstream effectors of PKG. Exposure to hypoxia of fetal PVSMCs for 24 h decreased the promoter activity of multiple SMC marker genes, downregulated protein and mRNA expression of myocardin, and upregulated mRNA expression of Elk-1. Inhibition of PKG by siRNA transfection downregulated the expression of myocardin and upregulated that of Elk-1. It is postulated that PKG induces displacement of myocardin from serum response factor and upregulates myocardin expression, thus activating transcription of SMC genes (647) (Fig. 6).

6. **VEGF**

VEGF plays a vital role in lung vascular growth in the embryo, by maintaining normal endothelial cell function and modulating vascular structure during late fetal life. In an ovine model of PPHN created by ductal ligation, the surge in lung VEGF expression during late gestation was reduced by 78%. In fetal lambs treated with EYE001, an aptamer that specifically inhibits VEGF-165, pulmonary artery pressure was elevated with associated right ventricular hypertrophy and increased wall thickness of small pulmonary arteries. EYE001 treatment also reduced lung eNOS protein content and impaired EDNO-mediated pulmonary vasodilation. These results suggest that hypertension downregulates VEGF expression in the developing lung and that impaired VEGF signaling may contribute to the pathogenesis of PPHN (221, 352).

**B. High Vasomotor Tone and Abnormal Dilator Responses**

1. **NO-cGMP pathway**

Decreased eNOS expression, reduced release of EDNO, and impaired pulmonary vasodilatation have all been documented in PPHN in human infants and in a number of animal models (3, 176, 181, 424, 528, 529, 590). EDNO production can be reduced by an endogenous inhibitor of eNOS, asymmetric dimethylarginine (ADMA) (582). Urinary ADMA levels in infants with PPHN are markedly higher than in healthy control infants at days 1
and 3 postpartum and fall to levels similar to controls at day 5 (455, 456). ADMA is metabolized to citrulline by dimethyl-arginine dimethylaminohydrolase (DDAH). In the newborn pig with pulmonary hypertension, one study suggests that decreased DDAH type 2 may be responsible for the elevated ADMA levels (33). In pulmonary arterial endothelial cells, eNOS uncoupled by ADMA has been found to be translocated from the plasma membrane to the mitochondria, which results in increased mitochondrial-derived ROS and decreased generation of ATP. The decrease in ATP may further lead to a reduction in the chaperone activity of Hsp90 and decrease in its interaction with eNOS (555). Studies also show that Hsp90 significantly augments NO production and inhibits superoxide generation from eNOS. In a lamb model of pulmonary hypertension caused by increased pulmonary blood flow (shunt), Hsp90-eNOS interaction is decreased, associated with decrease in NO generation and increase in eNOS-dependent generation of superoxide (554).

In PPHN, the downstream enzymes of EDNO signaling may also be impaired (203, 232, 233, 580). In fetal lambs with surgically created left congenital diaphragmatic hernia, a major cause of severe PPHN, relaxation of pulmonary arteries to the NO donor sodium nitroprusside is impaired (567). In fetal lambs with PPHN following prenatal ligation of the ductus arteriosus, cGMP content, sGC protein, and activity in pulmonary arteries are significantly lowered (78, 580). In a lamb model of pulmonary hypertension created by an aorto-pulmonary shunt that mimics congenital heart disease with increased pulmonary blood flow, both sGC expression and cGMP levels are increased; however, PDE5 expression and activity are also increased, and the upregulated PDE5 may contribute to the impairment of endothelium-dependent pulmonary vasodilatation (80). An increased expression and/or activity of PKG have been documented in pulmonary hypertension in both the adult and the newborn (233, 249, 619). PKG is the primary enzyme that mediates cGMP actions. In fetal lambs exposed to chronic high-altitude hypoxia, PKG-dependent relaxation of pulmonary arteries was attenuated due to decreased PKG specific activity (203).

2. Prostanoids

In pulmonary arteries of newborn pigs, prolonged hypoxia reduces the production of PGIL but does not affect that of TxA2. This leads to an increased ratio of TxA2 to PGIL that favors vasoconstriction (178). TxA2 is a potent constrictor prostanoid. Neonatal pulmonary arterial myocytes show increased sensitivity and reactivity to the TxA2 mimic after prolonged hypoxia. Hypoxia increases the abundance and affinity of TxA2 receptors in neonatal resistance pulmonary arteries, which would contribute to the increased pulmonary arterial pressure observed in PPHN (253, 254). Activation of TxA2 receptors, probably by lipid peroxidation products such as 8-isoprostanone, is also thought to contribute to pulmonary hypertension in newborn rats caused by 60% oxygen via a COX-independent pathway (302).

cAMP is the primary mediator for relaxation induced by vasodilator prostaglandins. In newborn lambs, elevation in intracellular cAMP as well as relaxation of pulmonary arteries to PGE2 were markedly potentiated by EDNO, resulting from inhibition of degradation of cAMP by the cGMP-inhibitory phosphodiesterase PDE3 (207). These results suggest that the combined use of vasodilator prostanoids with EDNO or PDE3 inhibitors may be a useful alternative in the treatment of PPHN. Indeed, intravenous milrinone, a PDE3 inhibitor, significantly shortened the onset and prolonged the duration and degree of pulmonary vasodilatation produced by PGIL in newborn lambs with pulmonary hypertension (482).

3. ET-1

Many studies on the effectiveness of ET-1 receptor antagonists in the treatment of both adult pulmonary arterial hypertension and PPHN indicate a key role for ET-1 in the development of pulmonary hypertension (72, 94, 467). In patients with PPHN, plasma levels of ET-1 are elevated and have been shown to correlate with disease severity, and they decline with clinical improvement (122, 164, 493). In fetal lambs, prolonged ETA receptor blockade attenuates chronic pulmonary hypertension (293), while prolonged ETB receptor blockade causes pulmonary hypertension (292). A genetic rat model of ETB receptor deficiency, after 3 wk of severe hypoxia, develops exaggerated pulmonary hypertension characterized by elevated pulmonary arterial pressure, diminished cardiac output, and increased total PVR. Although mRNA for preproET-1 in the lung is not different from that in control rats, mRNA for ET-converting enzyme-1 in the lung and plasma ET-1 levels are greater than in controls (293). These findings suggest that the activation of ETA receptors promotes the development of PPHN while activation of ETB receptors protects against PPHN. In rats with ETB-receptor deficiency, their elevated ET-1 levels may in part result from increased activity of ET-converting enzyme-1 and decreased clearance of ET-1 (115).

In the ductal ligation model of PPHN in fetal lambs, preproET-1 is increased and ETB receptor gene expression is decreased in the lung. Since ET may cause pulmonary vasodilatation by stimulating EDNO release via ETB receptor on the endothelial cells, the decreased ETB receptor expression combined with increased ET-1 production may shift ET-1 action more towards vasoconstriction (289). Circulating ET levels and ETB binding in the entire pulmonary vasculature were high in newborn piglets exposed to chronic hypoxia (427). Although it is believed that the ETA receptor is the principal subtype for ET-1-
induced pulmonary vasoconstriction, ETB receptors may also play a significant role in mediating ET-1-induced constriction of intrapulmonary conduit and resistance arteries (376, 389). The vasoconstrictor actions of the ETB receptor may become more pronounced in the pathological setting of pulmonary hypertension (160). This would explain the findings that dual blockade is necessary to maximize the inhibition of ET-1-induced pulmonary vasoconstriction in humans (115, 376, 389).

Elevated ET-1 levels in PPHN may in part result from decreased production of EDNO and vasodilator prostaglandins, as these vasodilators can negatively regulate ET-1 production by inhibition of preproET-1 transcription (115, 122, 164, 291). Elevated ET-1 levels may in turn decrease the production of EDNO through downregulation of the expression of eNOS and thus may further potentiate ET-1-mediated pulmonary vasoconstriction (10, 608). Data from a study with fetal ovine pulmonary endothelial cells cocultured with arterial SMCs suggest that ET-1 may downregulate eNOS expression by SMC-derived H2O2 production stimulated by ET-1 (608). PKC has been implicated in mediating ET action in the vascular system (50, 92, 386). In chronically hypoxic piglets, inhibition of PKC with chelerythrine has no effect on baseline pulmonary artery pressure in normoxic piglets but lowers pulmonary vascular tone in chronically hypoxic piglets, suggesting that PKC activation was induced by chronic hypoxic exposure (66).

4. ROS

Increased production of ROS has been documented in various models of PPHN (97, 179, 182, 218, 305, 611). In the ovine PPHN model created by ligation of the ductus arteriosus (97) or by a shunt between the ascending aorta and main pulmonary artery (218), superoxide levels in pulmonary vessels were increased. The expression of NADPH oxidase subunits p67phox in the pulmonary arteries or Rac1 and p47phox in the lung were increased (97). Three days of hypoxic exposure in piglets increased p67phox expression and ROS production from NADPH oxidase. Acetylcholine-induced relaxation was converted to contraction in pulmonary arteries from these animals (179). Multiple isoforms of NADPH oxidase are present in the lung. Increased ROS production and pulmonary vascular remodeling associated with development of pulmonary hypertension in adult mice are mainly related to increased NOX4 activity (398). The specific isoform involved in development of PPHN remains to be determined. Increased production of superoxide derived from XO is implicated in a neonatal rat model of PPHN. These rats had increased serum and lung XO activity, increased vascular XO-derived superoxide production, increased vascular nitrotyrosine formation, and reduced relaxation of pulmonary arteries to ACh and sodium nitroprusside (305).

In the ovine shunt model of PPHN, increased superoxide production is reduced by an inhibitor of NO synthase, indicating that uncoupled eNOS is the source. Indeed, dihydrobipterin levels in the lung were increased, which would have uncoupled eNOS and reduced bioavailability of endogenous NO and contributed to the attenuated pulmonary vasodilatation to an endogenous nitrovasodilator (218, 346, 433). Superoxide reacts readily with NO to form peroxynitrite. In the ovine ductal ligation model of PPHN, peroxynitrite has been shown to reduce eNOS binding to Hsp90 through tyrosine nitration, which leads to further eNOS uncoupling, increased superoxide production, and decreased NO bioavailability (335). In a recent study, treatment with cell-permeable SOD has been shown to restore eNOS expression and function in resistance pulmonary arteries from neonatal lambs with persistent pulmonary hypertension (170).

Elevated H2O2 levels have also been measured in pulmonary arteries from the ovine fetal ductal ligation model of PPHN, which may impair NO-mediated vasodilatation through downregulation of sGC, as overnight exposure of fetal PASMCs to H2O2 decreased sGC expression and NO-dependent cGMP generation (614). H2O2 can also decrease eNOS promoter activity, eNOS expression, and NO production in fetal pulmonary arterial endothelial cells (608).

In the aortopulmonary shunt model of PPHN in lambs, activities of SOD (SOD1, SOD2, and SOD3) and catalase measured 8 wk after birth were not different from controls except that the activity of catalase was less at 2 wk and that of SOD2 was greater at 4 wk. Superoxide levels in pulmonary vessels were higher at 2 and 4 but not at 8 wk, while H2O2 levels were higher at 2 but not 4 and 8 wk (522). In the ductal ligation model of PPHN, H2O2 levels at birth were elevated in pulmonary arteries. The expression and activities of catalase and glutathione peroxidase in the lung and pulmonary vessels of ligated lambs were not different from those of controls. These results indicate that ROS production may increase at certain periods during the development of PPHN. Furthermore, the changes in activity of antioxidant enzymes seem to occur only at certain stages of the development of PPHN (522).

5. K+ channels

In the ductal ligation model of PPHN in fetal lambs, BK mRNA content in distal lung homogenates and PASMCs is reduced (138, 367), and the contribution of BK channels to whole cell current is diminished in PASMCs (436). In contrast, in a neonatal ovine model of pulmonary hypertension created by an aortopulmonary shunt, lung mRNA and protein expression of BK channels was in-
increased (139). The low expression of BK channels in the ductal ligation model of PPHN has been attributed to the low pulmonary blood flow and oxygen tension, while the high expression of BK channels in the aortopulmonary shunt model is attributed to the higher blood flows and oxygen tension (138, 139).

Kv channel activity in the lung has been found to be increased (436) or decreased (177) in PPHN. In PASMCs of lambs with PPHN, inhibition of Kv channels with 4-AP caused a larger depolarization than the inhibition of BK channels with iberiotoxin, which is in contrast to what was observed in PASMCs from control animals, indicating an increased Kv channel activity and decreased BK channel activity in PPHN (436). In resistance pulmonary arteries of piglets at an early stage of pulmonary hypertension, Kv channel activity seems to be downregulated as indicated by the reduced constriction elicited by either 4-AP or correloid accompanied by more polarized membrane potential and decreased K$_{1.2}$. The abundances of K$_{1.5}$ and K$_{2.1}$ are unaltered (177). Unaltered K$_{2.1}$ expression was also noted in the ovine model of PPHN (138, 139).

Bone morphogenetic proteins (BMPs) have been implicated in the pathogenesis of familial pulmonary arterial hypertension. Transgenic mice with a SMC-targeted mutation in the type 2 receptor for BMPs (BMPR2) show increased pulmonary artery pressure and decreased mRNA and protein expression of K$_{1.5}$ in the lung. Human PASMCs treated with recombinant BMP2 increased K$_{1.5}$ protein and macroscopic Kv current density. These studies suggest that BMP regulates Kv channel expression and that loss of this signaling pathway in PASMCs through a mutation in BMPR2 is sufficient to cause pulmonary artery constriction (634). In models of chronic lung disease in newborn lambs and rats, reduced expression/activity of O$_2$-sensitive K$_v$ channels in distal pulmonary arteries was associated with a blunted hypoxic pulmonary vasoconstrictor response. Constriction of distal pulmonary arteries to the K$_{1.2}$-specific inhibitor was attenuated along with decreased K$_{1.5}$ and K$_{2.1}$ mRNA and K$^+$ current. Intrapulmonary gene transfer of K$_{1.5}$ increased O$_2$-sensitive K$^+$ current in PASMCs and restored hypoxic pulmonary vasoconstriction (489). Perinatal hypoxia in mice can have long-term effects into adulthood as evidenced by increased BK and Kv channel activity and higher BK $\alpha$-subunit and K$_{1.5}$ $\alpha$-subunit protein expression in pulmonary arteries of adult mice exposed to perinatal hypoxia (384).

6. Ca$^{2+}$ channels

In piglets with chronic hypoxia-induced pulmonary hypertension, there is an exaggerated pulmonary vasodilator response to nifedipine. Small pulmonary arteries from these piglets exhibit accentuated Ca$^{2+}$-dependent contraction, and the Ca$^{2+}$ channel current is higher in their PASMCs. The Ca$^{2+}$ channel pore forming subunit $\alpha_{1C}$ protein is also upregulated (255). In fetal lamb ductal ligation model of PPHN, the capacitative calcium entry in PASMCs is increased. Protein expression of transient receptor potential channel TRP6 protein expression is increased in hypertensive cells compared with controls. Transient receptor potential channel gene expression is downregulated (487).

7. ROK

An increase in ROK activity is implicated in pulmonary hypertension in adults and the newborn (3, 312, 313, 434, 534). In isolated ovine pulmonary arteries from near-term fetuses exposed in utero to chronic high-altitude hypoxia, the expression of type II ROK and activity of ROKs are increased. Phosphorylation of Thr-696 and Thr-850 of the regulatory subunit MYPT1 of myosin light-chain phosphatase caused by ET-1 via ROK is also upregulated. Inhibition of the phosphorylation of MYPT1 by 8-BrcGMP via PKG as well as PKG-dependent relaxation of pulmonary arteries is attenuated. These results suggest that increased ROK activity following chronic hypoxia may contribute to the diminished pulmonary vasodilatation mediated by PKG (203) (Fig. 7). On the other hand, pulmonary veins from these lambs had normal relaxation responses to 8-BrcGMP. This is because there is a balanced reduction in the expression and activity of both ROK and PKG resulting in unchanged dilator response to 8-BrcGMP (202). In neonatal rat models of severe pulmonary hypertension that are refractory to NO treatment, ROK inhibitors completely normalize PVR (392). In a recent study with neonatal rats, ROK inhibitors (Y-27632 or fasudil), while being effective in preventing the development of pulmonary hypertension and vascular remodeling, augmented chronic hypoxia-induced somatic growth restriction. Whether or not the retarded somatic growth results from inhibition of ROKs or is due to the nonspecific effects of these inhibitors remains to be determined (649).

V. CONCLUSION

The regulation of the pulmonary circulation in the fetus and newborn is under many interacting and redundant signaling pathways. In the fetus, these systems work well under a physiological “fetal” level of hypoxia, resulting in a high level of vasomotor tone that maintains the fetal type of circulation and also promotes normal vascular growth. However, an increase in the degree of hypoxia results in abnormal signaling and vascular remodeling. Similarly, increased oxygenation in the fetus by artificial means may transiently increase blood flow in the fetus, but compensatory mechanisms come into play rapidly to restore the high vascular resistance in utero. At birth,
mainly under the influence of increased oxygenation and increased shear stress, the rapid fall in resistance occurs that enables the newly born to survive.

Much progress has been made over the last two decades in our understanding of the mechanisms underlying the regulation of the fetal and newborn pulmonary circulation, in particular, regarding the roles of NO-cGMP pathway, PGI₂-cAMP pathway, and ET-1 (3, 152, 241, 549). NO and PGI₂ pathways are closely regulated by oxygen tension and shear stress (19, 180, 256, 277, 281, 476). It is now well recognized that the suppression of NO and PGI₂ actions as a result of the low oxygen tension and low pulmonary blood flow is critically involved in the maintenance of the high PVR in the fetal lung. In contrast, the increased NO and PGI₂ activities stimulated by oxygenation and shear stress play a pivotal role in the postnatal decrease in PVR (180, 256, 476, 499). Animal studies of the fetus and the newborn reveal that the effects of ET-1 receptor blockers on pulmonary vascular tone are rather moderate (369, 519, 579). However, chronic inhibition of ET₆ receptor decreases while chronic inhibition of ET₇ receptor increases pulmonary artery pressure (292, 293), suggesting that ET-1 may act mainly through its effect on SMC proliferation (50, 303).

The progress made in our understanding of the regulation of the perinatal pulmonary circulation has helped in the development of new treatments for PPHN, such as NO inhalation (540), PGI₂ inhalation, inhibition of cGMP degradation by PDE5 with sildenafil (270), inhibition of cAMP degradation with milrinone (207), and inhibition of ET-1 with bosentan (55, 494). Studies in recent years on ROK signaling in pulmonary vasoconstriction have led to the discovery that ROK inhibitors are very effective agents in treating PPHN in various animal models and could potentially be used in humans (2, 392). However, the potential side effects of ROK inhibitors remain to be understood. For example, ROK inhibitors (Y-27632 and fasudil) have been found to augment chronic hypoxia-induced somatic growth restriction (649).

Despite the progress being made in understanding the regulation of the perinatal pulmonary circulation, much is left unknown. In particular, our knowledge about the regulation of the development of the pulmonary circulation at the genetic and molecular levels is scanty. Although the roles of various vasoactive agents in the regulation of pulmonary vascular remodeling and vasocontractility are better understood, their intracellular mechanisms under physiological and pathophysiological conditions remain to be elucidated in more depth. The cross-talk among the multiple signaling pathways involved in the different cell types in the pulmonary vasculature needs to be better understood.

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