0031-3998/05/5705-0021R
PEDIATRIC RESEARCH
Vol. 57, No. 5, Pt 2, 2005
Printed in U.S.A.

Developmental Expression of Vasoactive and Growth Factors in Human Lung. Role in Pulmonary Vascular Resistance Adaptation at Birth

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ABSTRACT

The factors that mediate the postnatal fall in pulmonary vascular resistance, which is crucial for normal gas exchange, are not fully understood. The endothelium has been implicated in this phenomenon, through the release of vasorelaxant factors such as nitric oxide (NO). Human pulmonary expression of endothelial NO synthase increases up to 31 wk of gestation, together with vascular endothelial growth factor (VEGF), and both factors potently mediate pulmonary angiogenesis and vasorelaxation. During the perinatal period, when pulmonary vasodilatation is maximal, endothelial NO synthase and VEGF are weakly expressed. This raises the involvement of vasorelaxant factors other than NO at birth. One candidate is endothelial-derived hyperpolarizing factor, which induces smooth muscle cell hyperpolarization by activating K_{ATP} channels. The marked vasorelaxation induced by activation of these channels in newborn animals, and their strong perinatal expression in the human lung, suggest their involvement during this phase. Another candidate is endothelin (ET)-1, together with its receptors ET-A and ET-B. ET-A receptors are located exclusively on smooth muscle cells and mediate vasoconstriction, whereas ET-B receptors mediate vasoconstriction when located on smooth muscle cells and vasodilatation when located on endothelial cells. ET-B receptors, which are strongly expressed in the human fetal lung both at the end of gestation and after birth, may be involved in perinatal pulmonary vasodilatation. Thus, in human fetal lung, K_{ATP} channels and ET-B receptors could be important in mediating the perinatal pulmonary vasodilatation crucial for adapting the pulmonary circulation to extrauterine life. **(*Pediatr Res* 57: 21R–25R, 2005)**

Abbreviations

BMP, bone morphogenic protein
EDHF, endothelium-derived hyperpolarizing factor
eNOS, endothelial nitric oxide synthase
ET-1, endothelin-1
ET-A, type A endothelin receptor
ET-B, type B endothelin receptor
FGF, fibroblast growth factor
K_{ATP}, ATP-sensitive potassium channel
NO, nitric oxide
PPHN, persistent pulmonary hypertension of the neonate
TGF, transforming growth factor
VEGF, vascular endothelial growth factor

Fetal pulmonary circulation is characterized by high vascular resistance: <10% of ventricular output enters the lungs (1). The underlying mechanisms likely involve physical factors such as the lack of ventilation and low oxygen tension. An imbalance between vasorelaxant and vasoconstrictor mediators (2–4) (Fig. 1) is also likely. These mediators, derived from the endothelium, have either vasorelaxant effects (NO, prostacyclin, and EDHF) or vasoconstrictive effects (*e.g.* ET-1) (5–7). VEGF, crucial for endothelial growth and angiogenesis, also exerts a potent vasorelaxant effect by interacting with the NO pathway (8). Although these endothelial vasoactive factors and growth factors appear to be involved in regulating pulmonary vascular tone during fetal life and after birth, human data are scarce and conflicting data have been obtained in animal models.

THE FETAL PULMONARY CIRCULATION

Fetal lung development is classically classified into five stages (9), namely embryonic (up to 7 wk), pseudoglandular (7–16 wk), canalicular (16–24 wk), saccular (24–35 wk), and alveolar (>35 wk).

Received August 9, 2004; accepted January 13, 2005.
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This work was supported by Société Française de Cardiologie (Hélène de Marsan Grant).

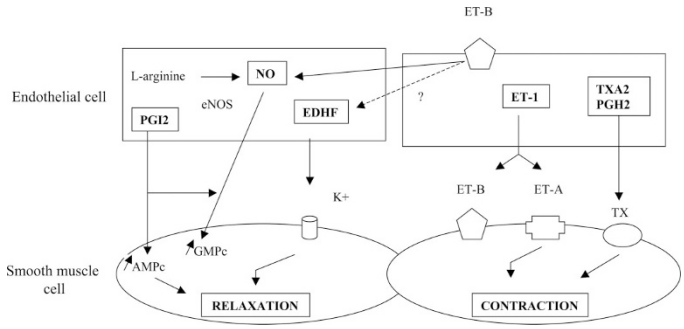

Figure 1. Diagram showing the different pathways involved in neonatal pulmonary vascular tone.

The lungs originate from the foregut endoderm. These endodermal buds branch and differentiate within the surrounding mesoderm, giving rise to the airways, blood vessels, and alveoli. Cross-talk between epithelial and mesenchymal cells is crucial for lung differentiation. Factors involved in this cross-talk include TGF-β, BMP-4, IGF, FGF, and fibronectin (9,10). The lung vasculature develops through two processes, namely vasculogenesis, in which new blood vessels form *in situ* from angioblasts, and angiogenesis, in which new vessels sprout from existing ones (11). Vessel development begins at the outset of lung development, requires epithelial-mesenchymal cross-talk, and is inextricably linked to airway development (11–13). In the fetus, the bulk of right ventricular output is diverted away from the lungs through the patent *ductus arteriosus* to the aorta (6), as gas exchange occurs in the placenta. The involvement of endothelial factors in this high fetal pulmonary vascular resistance has been widely studied in animals (6,7), but their ontogeny is poorly documented in the human fetal lung.

In fetal lambs, ET-1 appears to be involved in pulmonary vasoconstriction (14,15). In this model, pulmonary ET-1 levels fall markedly before birth, yet pulmonary vascular resistance remains high. This points to the involvement of factors other than ET-1. In addition, the role of ET-1 depends on which of its receptors is activated (16–19). ET-A activation induces vasoconstriction, whereas ET-B activation induces vasoconstriction when the receptors are located on smooth muscle cells and vasodilatation when they are located on endothelial cells. In fetal lambs, ET-B receptors are strongly expressed in the perinatal period and their blockade attenuates O_2-induced vasodilatation (15,18,19), whereas in newborn piglets ET-B receptor activation induces pulmonary vasodilatation (20). These data strongly support a vasodilatory role of ET-B receptors in the perinatal period. As regards the human fetal lung, we have found that the expression of both ET-1 and ET-A is stable throughout gestation, whereas ET-B expression increases in mid-term and remains high until birth (21). ET-B receptors may thus have an important role in perinatal vasodilation. Prostaglandin I_2 is synthesized primarily in vascular endothelial cells and exerts its vasodilatory action by activating adenyl cyclase through receptor G protein-coupled mechanisms. PGI_2 production increases gradually throughout gestation, but its inhibition does not markedly change resting pulmonary vascular resistance (6). In addition, the ductus arteriosus is particularly sensitive to PGE_2 at mid-term and less so at the end of gestation, suggesting only a modest effect on vascular tone in the perinatal period (22). This increased sensitivity during fetal life could explain the high incidence of patent ductus arteriosus in preterm infants.

NO is synthesized by endothelial cells after eNOS activation. In fetal lambs, inhibition of NO synthesis increases resting pulmonary vascular resistance, strongly pointing to NO involvement in maintaining low pulmonary vascular tone in basal conditions (7,23,24). In this model, eNOS expression peaks three-quarters of the way through gestation and falls before birth, whereas it peaks at birth in piglets and after birth in rats (24–27). Differences in the chronology of lung parenchymal and vascular development between species may account for these differences in eNOS expression kinetics (28–30). The temporal relationship between airway and pulmonary vasculature development is firmly established in animals (24) and was recently confirmed in humans (9,12). In human fetal lung, the concomitance of the increase in eNOS expression and the onset of alveolarization points to an important role of eNOS in airway maturation (31). Indeed, in addition to its vasorelaxant effect, the NO pathway is involved in both angiogenesis and lung development (31–33). However, eNOS-null mice have abnormal lung development but a functional pulmonary circulation in adulthood, suggesting that eNOS is not vital for angiogenesis, at least in this model (33,34). No data are available on human eNOS ontogeny. We recently observed a gradual increase in eNOS expression in the human fetal lung until 31 wk of gestation, followed by a sharp decrease close to birth (21). As in other animals, peak eNOS expression matches the onset of alveolarization in humans. This, together with very weak eNOS expression at birth, suggests a role in lung maturation rather than in neonatal vasodilatation. It is noteworthy in this respect that prenatal glucocorticoid administration, while activating lung maturation, also increases pulmonary eNOS expression in fetal lambs (35) through an unknown mechanism.

VEGF potently induces endothelial cell growth *in vitro* and angiogenesis *in vivo* (36–39). VEGF expression is stimulated by TGF-β, hypoxia, and shear stress (39,40). Recent studies indicate that VEGF acts as a paracrine mediator of angiogenesis in the developing lung (36,41,42), whereas abundant growth of intra-acinar capillaries coincides with alveolar development in lambs and humans (12,30). VEGF is also involved in epithelial cell proliferation in human fetal lung, highlighting its role as both an autocrine and a paracrine growth factor (41,43,44). VEGF is detected in endothelial and bronchial epithelial cells of the fetal and mature human lung, but only in bronchial epithelial cells of term infants, indicating a paracrine role after birth (41). Although the role of VEGF in lung development appears to be crucial, little is known of its human ontogeny. In the above-mentioned study, we found that VEGF expression followed the same pattern as eNOS, with an increase between the canalicular and saccular stages, then a sharp decrease at the alveolar stage (21). A number of studies have described parallel effects of NO and VEGF, which both have vasodilative and angiogenic properties (31–33). Their peak expression in the human lung at 31 wk of gestation, just

before the alveolar stage, suggests their possible involvement in alveolar development. This is in keeping with the fact that premature infants born after this time point do not usually require mechanical ventilation (45,46).

THE NEONATAL CIRCULATION

The mechanisms underlying the fall in pulmonary vascular resistance and the immediate increase in pulmonary blood flow at birth are unclear. The decrease in pulmonary vascular resistance is tightly regulated by an interplay between metabolic and mechanical factors triggered by the ventilatory and circulatory changes that occur at birth, including closure of the ductus arteriosus through decreased sensitivity to prostaglandins (22). The initial rapid vasodilatation is at least partly stimulated mechanically by lung inflation and by the increase in oxygen tension, but endothelial factors also play a critical role.

Animal studies. Physical expansion of the fetal lamb lung, with no concurrent change in oxygen tension, induces a modest decrease in pulmonary vascular resistance, partly due to prostaglandin synthesis (6,47–49). Ventilation of near-term lambs with air or oxygen induces marked pulmonary vasodilatation, possibly through the release of NO, prostaglandins, bradykinin, or EDHF. Prostaglandin inhibition attenuates the fall in pulmonary vascular resistance induced by lung inflation but not that induced by oxygenation, suggesting only minor involvement of prostaglandins in neonatal pulmonary vasodilatation (6). NO appears to be more important, as eNOS inhibition in lambs attenuates the increase in pulmonary blood flow at birth (21,47). In newborn piglets, however, NO-dependent vasodilatation in response to acetylcholine is totally absent at birth and only appears at 3 d of age, even though eNOS expression increases after 1 d of age, suggesting a dysregulation of NO pathway rather than absence of eNOS (20,50,51). Vasorelaxant factors other than NO, such as prostacyclins, bradykinin, and K+ channel openers, might therefore be involved during the first hours of life in this model. Inhibition of these factors in fetal lambs reduces the pulmonary blood flow induced by lung expansion but not that induced by oxygenation (48). Bradykinin blockade, on the other hand, has no effect on the fall in pulmonary vascular resistance (52). Hyperpolarization of smooth muscle cells through potassium channel activation is therefore likely to be involved. Various types of K+ channels have been described in vascular smooth muscle, including voltage-activated K+ channels (Kv), Ca2+-activated K+ channels (Kca), pH-sensitive K+ channels (TASK), and ATP-sensitive K+ channels (K_{ATP}) (53). The respective roles of these K+ channels is controversial, although oxygen-induced pulmonary vasodilatation is markedly inhibited by blocking K_{CA} and K_{ATP} channels in near-term fetal lambs (54). K+ channel expression is controversial in experimental pulmonary hypertension of newborn animals, and appears to be strongly dependent on the model used. Indeed, Kv and Kca expression is reduced in the rat model of nitrofen-induced congenital diaphragmatic hernia (55), Kv expression is not modified in shunted lambs, and K_{Ca} expression fell in some studies and rose in others (56–58). The role of K_{ATP} channels in neonatal pulmonary vasodilation is poorly documented. K_{ATP} channel openers induce strong vasorelaxation in both newborn lambs and piglets, and this effect is partly inhibited by the endothelium, as we and others have shown (20,59,60). This effect was initially attributed partly due to a lack of NO activity (61), although one alternative is an excess of ET-1. Indeed, plasma levels and pulmonary expression of ET-1 are high in the newborn piglet then fall to adult levels after 3 d (20,61). Furthermore, we have found that the vascular relaxation induced by K_{ATP} openers is potentiated by specific ET-A receptor blockade (20,60), an effect that had previously only been reported in cerebral arteries and the heart (62,63). ET-1, strongly expressed in both normal and hypertensive lungs, might therefore inhibit neonatal pulmonary vasodilation *via* its ET-A receptors.

Human studies. Published data on the ontogeny of vasoactive factors and growth factors in the human lung are scarce. We have shown that eNOS and VEGF, both involved in pulmonary angiogenesis and vasorelaxation, are weakly expressed in the perinatal period (21). These data suggest the involvement of vasorelaxant factors other than NO in human neonatal pulmonary vasodilatation. Potassium channels are good candidates, but pharmacological and functional studies of human fetal lungs are difficult, for obvious reasons. In a search for indirect evidence, we examined K_{ATP} channel subunit Kir 6.1 expression in lung tissues from aborted human fetuses. Interestingly, the subunit was strongly expressed on vascular smooth muscle cells in both near-term fetuses and newborns (Fig. 2) (64). These are the first data pointing to a crucial role of K_{ATP} channels in human pulmonary vascular adaptation at birth.

THERAPEUTIC IMPLICATIONS

PPHN is a severe disorder, for which current treatments are disappointing. Inhaled NO is a selective pulmonary vasodilator with a short half-life. necessitating continuous administration (65,66). Inhaled NO has improved the prognosis of PPHN, but some patients are resistant and others remain dependent (67). Continuous prostacyclin infusion attenuates the clinical symptoms and reduces pulmonary pressure but has troublesome systemic effects (68). Bosentan, a drug antagonizing the effects of ET-1, appears to be as effective as prostacyclin in adults and is also better tolerated (69). However, bosentan is a nonselec-

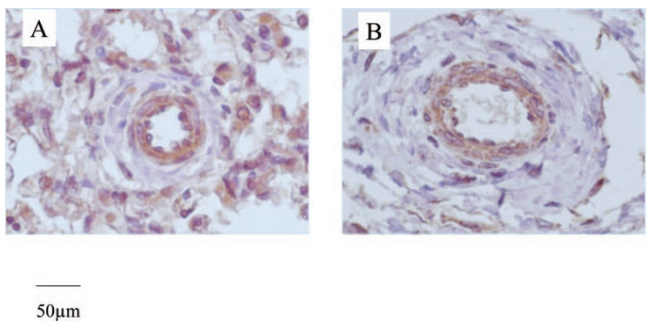

Figure 2. Immunostaining for Kir 6.1 in lungs of fetuses at 31 wk (*A*) and neonate (*B*). Kir 6.1 subunit of K_{ATP} channels is expressed by smooth muscle cells and epithelial cells. Magnification is ×250.

tive endothelin antagonist blocking both ET-A and ET-B receptors (70). It has been suggested that ET-B receptors, which are strongly expressed in the human neonatal lung, might have a vasorelaxant effect (18,19). The use of selective ET-A blockers might therefore be preferable in PPHN. The phosphodiesterase V inhibitor sildenafil, which acts by promoting cGMP accumulation in smooth muscle cells, facilitates NO weaning of patients with PPHN and is also effective on adult pulmonary hypertension (71,72). Trials of this drug are now underway in infants. Finally, K_{ATP} channel openers, currently used in coronary heart disease (73), have never been tested in pulmonary hypertension. Their enhanced vasorelaxant effects in experimental models of PPHN call for clinical trials in human PPHN (59,60).

SUMMARY

The mechanisms underlying pulmonary vasodilatation at birth are poorly understood in humans. Expression of eNOS and VEGF peaks just before the alveolar stage of lung development, indicating a key role of these two mediators in pulmonary maturation. In the perinatal period, when pulmonary vasodilatation is maximal, eNOS and VEGF expression is very low and ET-1 and ET-1 receptor expression is very high. This suggests that mediators other than NO participate in the marked pulmonary vasodilatation occurring in human newborns. ET-B receptors, strongly expressed in the human lung from mid-gestation until birth, might have potent vasodilatory effects in the newborn. K_{ATP} channels mediate vasorelaxation in newborn animals and are strongly expressed throughout human lung development, but their role in the adaptation of pulmonary vascular resistance at birth remains to be established.

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