

Pathogenesis and treatment of neonatal and postnatal pulmonary hypertension

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Recent developments in basic vascular biology have provided new insights into mechanisms contributing to pulmonary hypertension and novel approaches to its treatment. Although it has long been recognized that normal vascular endothelium produces endogenous vasodilators, identification of the so-called *endothelium-derived relaxing factor* as a gas, nitric oxide, led to extensive studies in several experimental models of pulmonary hypertension and in various clinical settings. Endogenous nitric oxide production modulates vascular tone in the fetal and postnatal lung, and contributes to the normal decline in pulmonary vascular resistance at birth. Exogenous (inhaled) nitric oxide therapy causes potent and selective pulmonary vasodilation in neonates with severe pulmonary hypertension (ie, persistent pulmonary hypertension of the newborn), as well as in patients with pulmonary hypertension due to congenital heart disease, acute hypoxemic respiratory failure, primary or unexplained pulmonary hypertension, and other diseases. The safety and efficacy of inhaled nitric oxide therapy is currently being examined in multicenter trials. Other endothelium-derived products, such as the potent vasoconstrictor peptide endothelin, may contribute to abnormal vascular tone, reactivity, and structure in the pulmonary circulation. Basic studies of mechanisms to altered vessel growth and remodeling may lead to further therapeutic strategies for chronic pulmonary hypertension.

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Pulmonary hypertension causes significant morbidity and mortality in a wide variety of pediatric cardiac and pulmonary diseases. Therapy has been somewhat limited by our lack of insight into basic mechanisms that regulate vascular tone, reactivity, and growth in the developing pulmonary circulation. Advances in vascular biology over the past decade, however, have provided exciting new information with important implications for our clinical approach to pulmonary hypertension. Recent studies have demonstrated the critical roles of vascular endothelium and its products in governing vascular tone in the perinatal and postnatal lung, as well as vessel growth and remodeling. In pediatrics, understanding pulmonary hypertension, whether related to persistent pulmonary hypertension of the newborn (PPHN), sepsis, congenital heart disease, or chronic lung disease, begins with understanding normal developmental changes *in utero*, at birth,

and during the early postnatal period. For example, in PPHN, mechanisms of altered pulmonary vascular reactivity appear to be at least partly due to changes in endothelial cell behavior, as well as the effects of chronic intrauterine stimuli on smooth muscle function. In congenital heart disease, altered intrauterine vascular remodeling and the failure of decline in pulmonary vascular resistance contribute to late neonatal and infant morbidity and mortality. Finally, several papers demonstrate that in premature infants, sustained elevations of pulmonary vascular resistance are associated with high morbidity and mortality in severe respiratory distress syndrome (RDS). This limited review selectively surveys literature from this past year, and focuses on the theme that vascular endothelium and its products, especially nitric oxide and endothelin, contribute significantly to the development of pulmonary hypertension and its treatment.

Abbreviations

ARDS—adult respiratory distress syndrome; **ECMO**—extracorporeal membrane oxygenation; **ET-1**—endothelin-1; **HFOV**—high-frequency oscillatory ventilation; **PPHN**—persistent pulmonary hypertension of the newborn; **PVR**—pulmonary vascular resistance; **RDS**—respiratory distress syndrome.

Endothelium-derived nitric oxide

Formation and function

Furchgott and Zawadzki [1] first demonstrated that the vasodilator response to certain stimuli, such as acetylcholine, is dependent on the presence of an intact endothelium and the release of a potent but short-lived product called *endothelium-derived relaxing factor* (Fig. 1), a potent endogenous vasodilator that was recently identified as the gas nitric oxide or a nitric oxide-containing compound [2,3]. Formed during the conversion of L-arginine to L-citrulline by nitric oxide synthase, nitric oxide rapidly diffuses to smooth muscle cells, stimulating soluble guanylate cyclase, increasing cyclic GMP content, and causing vasodilation [4•,5•]. On entering the circulation, nitric oxide binds avidly to hemoglobin, leading to its inactivation in blood and limiting its effects in neighboring cells. Although this review focuses on nitric oxide in pulmonary circulation, nitric oxide synthase is present in many different cell types, and nitric oxide plays diverse roles in intra- or intercellular signaling throughout biology [4•]. For example, nitric oxide contributes to potentiation of long-term memory, nonadrenergic, noncholinergic neural responses, macrophage killing, intestinal inflammation, cardiovascular collapse in sepsis, and other physiologic and pathophysiologic activity.

At least 3 different types of nitric oxide synthase have been described, including 2 "constitutive" types: type I (neuronal and respiratory airway epithelium) and type III (endothelial cells). Type II is an "inducible" enzyme (vascular smooth muscle, endothelial, and macrophages). An important clinical example of the pathophysiologic role for endogenous nitric oxide is septic shock. Characterized by systemic hypotension that is often refractory to cardiotoxic therapy, septic shock is associated with excessive nitric oxide production due to the rapid induction of type II nitric oxide synthase in vascular smooth muscle by endotoxin-stimulated cytokine activity [4•]. Basic mechanisms that regulate nitric oxide synthase activity remain under active investigation but include adequate availability of substrate (L-arginine) and cofactors (eg, tetrahydrobiopterin), and the effects of oxygen tension, shear stress, pressure, and growth factors (Fig. 1).

Physiologic role in regulating pulmonary vascular tone

At birth, pulmonary circulation undergoes a dramatic fall in pulmonary vascular resistance (PVR), as blood flow increases nearly eightfold, and pulmonary artery pressure steadily declines. Physical and chemical stim-

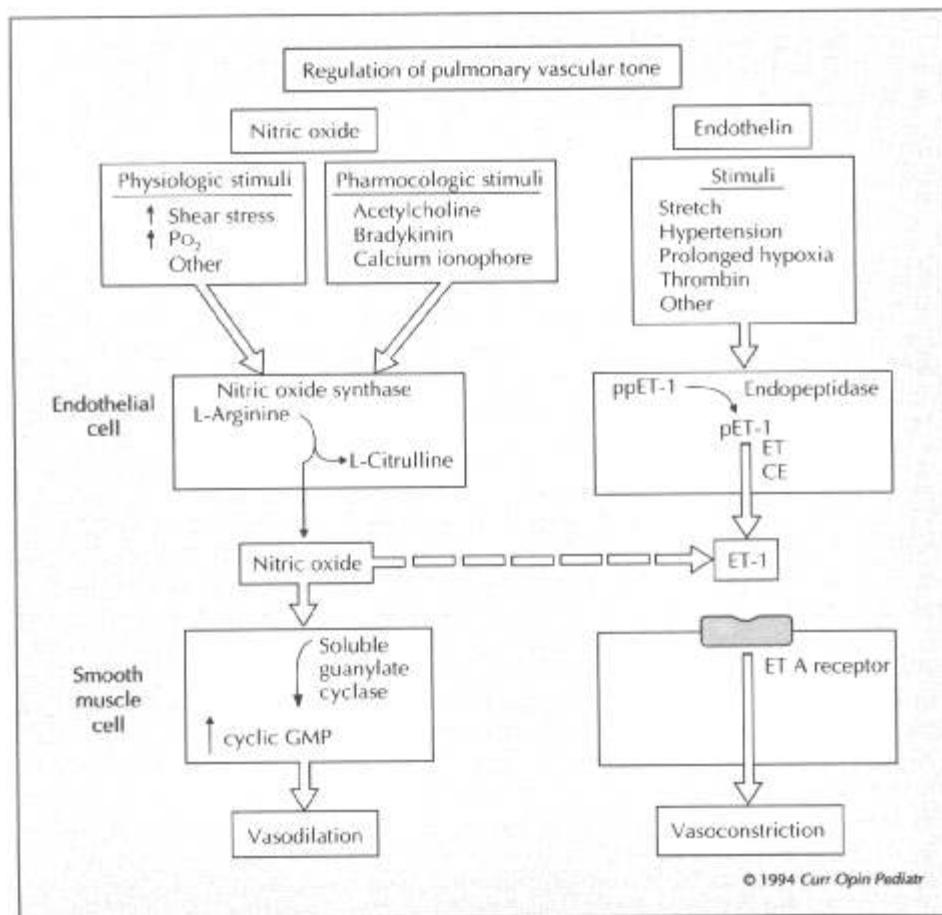


Fig. 1. Role of the endothelium-derived products in regulation of pulmonary vascular tone. Mechanisms leading to the release of nitric oxide, *left side*, and endothelin-1 (ET-1), *right side*, are shown. ET CE—endothelin converting enzyme; pET-1—proendothelin-1; ppET-1—preproendothelin-1.

uli, such as low oxygen tension in the fluid-filled fetal lung, increased vasoconstrictor products (eg, leukotrienes or endothelin), and decreased activity of vasodilator substances (eg, prostacyclin or nitric oxide), may contribute to high fetal PVR. Mechanisms that contribute to normal postnatal adaptation at birth include establishment of an air-liquid interface, rhythmic distension, increased oxygen, and altered production of vasoactive mediators. Multiple homeostatic mechanisms most likely have overlapping effects on decreasing PVR at birth, because no single stimulus appears to be the sole "mediator" of the transition. Recent studies suggest, however, that endogenous nitric oxide activity plays an important role in determining vascular tone in the perinatal lung. Experimental studies suggest that endogenous nitric oxide 1) modulates basal tone in the normal fetal lung [6], 2) can be released by pharmacologic (ie, acetylcholine) and physiologic (eg, shear stress, increased PO₂, ventilation) stimuli [6,7,8,9], 3) contributes to the normal decline in PVR at birth [6,7,9], 4) maintains low PVR and modulates vasoreactivity in the normal postnatal lung [8,10], and 5) may increase with maturation [11].

In addition, direct administration of nitric oxide by inhalation of nitric oxide causes potent pulmonary vasodilation of the normal near-term ovine fetal pulmonary circulation [12]. Inhaled nitric oxide (5 to 20 ppm) increased fetal pulmonary blood flow to levels achieved with 100% oxygen during ventilation, demonstrating the striking responsiveness of the developing lung circulation to nitric oxide and providing a rationale for doses used in subsequent clinical studies. Frostell *et al.* [13] had previously shown potent and selective pulmonary vasodilation during acute pulmonary hypertension in juvenile sheep. Although higher doses of inhaled nitric oxide were studied, the majority of decline in pulmonary vascular resistance occurred at 20 ppm or less. Roberts *et al.* [14] reported the potent and selective pulmonary vasodilator effects of inhaled nitric oxide in newborn lambs with hypoxia and severe respiratory acidosis, providing further experimental support for the inhaled nitric oxide therapy in the sick newborn. Later studies reported similar effects of inhaled nitric oxide in septic piglets [15] and newborn lambs with chronic pulmonary hypertension after intrauterine ligation of the ductus arteriosus [16].

Thus, experimental studies have shown that endogenous nitric oxide modulates pulmonary vasoreactivity and contributes to postnatal adaptation in the early perinatal period. In the postnatal lung, inhibition of endogenous nitric oxide activity augments the pulmonary hypertensive response to acute hypoxia and other constrictor stimuli [10]. In an anesthetized adult rabbit model of acute unilateral alveolar hypoxia, blockade of endogenous nitric oxide increases blood flow from the hypoxic lung to the normoxic lung [17]. These findings suggest that endogenous nitric oxide opposes acute hypoxic pulmonary vasoconstriction and other hypertensive stimuli, attenuating the severity of pulmonary

hypertension [10,17]. Whether chronic hypoxia impairs endogenous nitric oxide activity is controversial. Although past studies suggest loss of nitric oxide activity in the lung in adult rats after chronic normobaric hypoxia [18], a more recent study suggests that nitric oxide activity persisted or was increased in chronic hypobaric hypoxia [19]. In summary, these findings support the speculation that impaired endothelial nitric oxide production augments pulmonary hypertension or vasoreactivity in acute or chronic clinical settings, causing heightened vasoreactivity and more severe pulmonary vascular disease.

Therapeutic role of inhaled nitric oxide in pulmonary hypertension

With their experience in studying the use of inhaled nitric oxide for the clinical measurement of lung diffusion capacity, Pepke-Zaba *et al.* [20] were the first to recognize the potential application of nitric oxide as an inhalational therapy for pulmonary hypertension. Unlike systemic infusions of prostacyclin, brief exposure to inhaled nitric oxide acutely lowered PVR in adults with primary pulmonary hypertension without adverse effects on systemic hemodynamics. Subsequent animal and clinical studies in PPHN, congenital heart disease, and other diseases further illustrated selective pulmonary vasodilation with inhaled nitric oxide. In a landmark study, Zapol *et al.* [21] and Rossaint *et al.* [22] first demonstrated the ability of inhaled nitric oxide not only to lower pulmonary artery pressure, but also to improve ventilation-perfusion matching and oxygenation in patients with parenchymal lung disease and pulmonary hypertension.

Persistent pulmonary hypertension of the newborn

Persistent pulmonary hypertension of the newborn represents the failure of the pulmonary circulation to achieve or sustain the normal decline in PVR at birth. PPHN is a clinical syndrome associated with various cardiopulmonary diseases, including meconium aspiration, asphyxia, group B streptococcal sepsis, congenital diaphragmatic hernia, and respiratory distress syndrome; it can also be idiopathic. Pathophysiologic features of PPHN include high PVR with abnormal vasoreactivity, predominantly right-to-left shunting across the ductus arteriosus or foramen ovale, and severe hypoxemia. High pulmonary vascular resistance may be caused by several mechanisms, such as active vasoconstriction, remodeling, decreased arterial density and number, or combinations of the above. Although mechanisms leading to severe neonatal pulmonary hypertension are variable, it has been hypothesized that decreased release of endogenous nitric oxide and other abnormalities in endothelial function may play a critical role in the development of PPHN [6].

In 1992, two reports described the acute effects of inhaled nitric oxide in severe PPHN. Roberts *et al.*

[23•] reported that inhaled nitric oxide at 80 ppm improved pre- and postductal oxygenation without changing PaCO_2 or systemic blood pressure in five of six neonates with severe PPHN. Inhaled nitric oxide was discontinued after brief exposure, and five of six patients subsequently required extracorporeal membrane oxygenation (ECMO). Kinsella *et al.* [24•] studied the effects of inhaled nitric oxide in nine neonates with PPHN who had qualified for ECMO. With lower doses (10 to 20 ppm), each patient acutely improved oxygenation and echocardiographic signs of pulmonary hypertension without systemic hypotension. Three patients treated briefly required ECMO after nitric oxide was discontinued. However, in six neonates treated with inhaled nitric oxide (at 6 ppm) for 24 hours showed sustained improvement in oxygenation throughout the study period and recovered without the need for ECMO.

Based on this initial experience, Kinsella *et al.* [25•] studied the response to this treatment strategy (20 ppm for 4 hours, then 6 ppm for prolonged therapy) in nine consecutive neonates with severe PPHN who met ECMO criteria despite aggressive therapy. Prior to entry, patients in this study were ventilated with either conventional pressure-limited ventilators or high-frequency oscillatory ventilation (HFOV), depending on which approach provided optimal gas exchange. Eight of nine neonates with PPHN, including patients with meconium aspiration, congenital diaphragmatic hernia, and sepsis, improved oxygenation and subsequently recovered without ECMO (Fig. 2). None of the patients developed high methemoglobin levels. Systemic hypotension and poor cardiac performance with sepsis were identified as important risk factors for treatment failure and the need for ECMO. In some cases, inhaled nitric oxide during HFOV improved oxygenation to a greater extent than HFOV alone or conventional ventilation with inhaled nitric oxide. Based on this experience and recent data on the efficacy of HFOV in PPHN [26•], the authors concluded that inhaled nitric oxide may provide an effective adjuvant therapy for treating severe PPHN, but that responsiveness to nitric oxide may depend on specific diseases associated with PPHN (eg, congenital diaphragmatic hernia, meconium aspiration, and diffuse parenchymal disease) and optimized ventilator strategies [27•]. Subsequent editorials also underscored the need for multicenter, controlled clinical studies of inhaled nitric oxide prior to its routine application in the treatment of severe PPHN [28•,29•].

Respiratory distress syndrome with prematurity

In addition to high PVR in term or near-term neonates with cardiorespiratory failure and PPHN, recent clinical reports show that pulmonary hypertension is also found in premature newborns with severe RDS [30–33]. Although surfactant deficiency and dysfunction are the central pathophysiologic abnormalities of RDS,

several reports suggest that sustained elevation of PVR appear to be associated with severe disease. For example, fatal cases of RDS have been associated with echocardiographic signs of severe pulmonary hypertension [30]. Greater differences in arterial oxygenation from pre- and postductal blood samples further suggest the presence of significant right-to-left shunting across the ductus arteriosus in severe hyaline membrane disease [32]. Finally, surfactant administration lowers pulmonary artery pressure from systemic arterial levels, suggesting that in some cases, improved oxygenation after surfactant therapy may be partially due to decreased right-to-left shunting [31]. Inhaled nitric oxide therapy has been reported to lower PVR in the premature newborn, demonstrating marked responsiveness to inhaled nitric oxide in the immature lung [34]. It is not known whether altered endogenous nitric oxide activity contributes to the pathophysiology of RDS and whether exogenous (*ie*, inhaled) nitric oxide will be an effective therapy in premature neonates who fail to respond to exogenous surfactant. In light of the potential for oxidant-related injury with nitric oxide therapy and its potential for increasing bleeding time, extra precautions must be taken prior to recommending its use in the premature neonate.

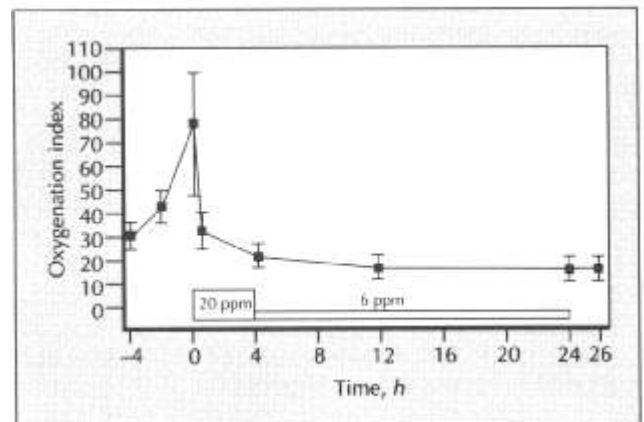


Fig. 2. Improved oxygenation during treatment of neonatal pulmonary hypertension with low-dose nitric oxide. The oxygenation index is mean airway pressure times forced inspiratory oxygen (FiO_2) times 100 divided by arterial PO_2 . (From Kinsella *et al.* [25•]; with permission.)

Congenital heart disease

Inhaled nitric oxide also selectively lowers pulmonary vascular resistance in children with congenital heart disease [35]. Inhaled nitric oxide has been used in patients with congenital heart disease to assess pulmonary vascular reactivity during cardiac catheterization [35], to study basic mechanisms of altered pulmonary vascular tone in postoperative patients after cardiopulmonary bypass [36•], and to treat patients with pulmonary hypertension during the postoperative period. Wessel *et al.* [36•] compared the effects of acetylcholine, the endothelium-dependent vasodilator, with those of inhaled nitric oxide, the endothelium-

independent agonist, in children with congenital heart disease after cardiopulmonary bypass. The pulmonary vasodilator response to acetylcholine was diminished after bypass, whereas direct smooth muscle stimulation with inhaled nitric oxide effectively lowered PVR (Fig. 3). These findings suggest that impaired endothelial function contributes to postoperative pulmonary hypertension after cardiopulmonary bypass and suggest the potential efficacy of inhaled nitric oxide in the management of pulmonary hypertension in this clinical setting. Celermayer *et al.* [37••] further reported that sodium nitroprusside, a pharmacologic nitric oxide donor, caused more potent pulmonary vasodilation than did acetylcholine in children with congenital heart disease and pulmonary vascular disease. Interestingly, pulmonary vasodilation to acetylcholine was not selectively impaired in patients with high pulmonary blood flow and early pulmonary vascular injury. Thus, decreased endogenous nitric oxide activity occurs with progressive pulmonary vascular disease in children with congenital heart disease and may be acutely decreased after cardiopulmonary bypass.

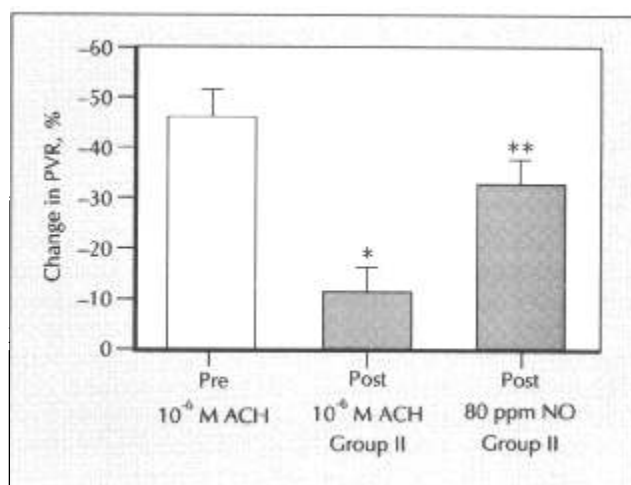


Fig. 3. Changes in pulmonary vascular resistance (PVR) in patients with congenital heart disease before and after cardiac surgery and cardiopulmonary bypass. As shown, the pulmonary vasodilator response to acetylcholine (an endothelium-dependent agent) is markedly attenuated after surgery and bypass, whereas inhaled nitric oxide (NO) (an endothelium-independent stimulus) evokes more potent fall in PVR in the early postoperative period. The *single asterisk* indicates significantly different from preoperative acetylcholine and the *double asterisks* indicate significantly different from postoperative acetylcholine. ACH—acetylcholine; Pre—preoperative; Post—postoperative. (From Wessel *et al.* [36••]; with permission.)

Adult respiratory distress syndrome

The clinical study of inhaled nitric oxide in adults with severe adult respiratory distress syndrome (ARDS) by Rossaint *et al.* [22••] demonstrated not only that inhaled nitric oxide was a selective pulmonary vasodilator, but also that it could improve oxygenation in the presence of severe ventilation-perfusion mismatch. Pulmonary hypertension is a well-recognized feature of severe respiratory failure, but although past studies showed that

pharmacologic vasodilators (eg, prostacyclin or sodium nitroprusside) lowered PVR, their use was severely limited due to marked hypoxemia caused by worsening ventilation-perfusion abnormalities. In contrast, inhaled nitric oxide appeared to redistribute pulmonary blood flow to better ventilated regions of the lung, increasing oxygenation in many patients [21••,22••]. Thus, unlike most vasodilators, inhaled nitric oxide has the unique ability not only to selectively lower pulmonary artery pressure without affecting systemic pressure, but also to enhance ventilation-perfusion matching in the presence of parenchymal lung disease. This clinical response was generally sustained with low-dose chronic nitric oxide therapy [22••]. Because the study group was small and most of the patients were concomitantly treated with venovenous cardiac bypass, these promising results await further study before the optimal role of inhaled nitric oxide in ARDS management can be determined.

Subsequent work has demonstrated that very low doses of inhaled nitric oxide (less than 1 to 10 ppm) are effective in improving oxygenation and lowering pulmonary artery pressure in some patients with severe ARDS [38••]. The dose required to increase PO_2 to 50% of the maximum response was lower than that needed to achieve a similar response with pulmonary artery pressure. Although low-dose nitric oxide therapy has been successful in the management of PPHN, I have observed (Unpublished data) that the acute response of older children with severe hypoxemic respiratory failure (eg, ARDS and bronchopulmonary dysplasia with respiratory syncytial viral pneumonitis) is initially greater with higher nitric oxide doses (10 to 20 ppm) than was reported by Gerslach *et al.* [38••]. With prolonged therapy, however, inhaled nitric oxide is often as effective at lower doses (1 to 5 ppm) as at higher doses.

Other diseases

Improved gas exchange during inhaled nitric oxide treatment was also reported in adults with chronic obstructive lung disease [39•], as well as in selected cases of postoperative pulmonary hypertension associated with congenital heart disease, after heart and lung transplantation, and in a young infant with persistent pulmonary hypertension or unexplained pulmonary hypertension after the failure of several pharmacologic agents [40•]. It should be emphasized that in all of these studies, some patients have little response to inhaled nitric oxide; whether this reflects severe structural remodeling of small pulmonary arteries, functional impairment of the soluble guanylate cyclase system in hypertensive smooth muscle cells, or other mechanisms requires further study.

Safety of nitric oxide therapy

Despite current enthusiasm for the potential therapeutic role of inhaled nitric oxide, many concerns exist

regarding potential toxicities, such as lung injury, decreased surfactant production or activity, and down-regulation of endogenous nitric oxide and potential impairment of central nervous system vasoreactivity [41•,42••]. Low doses of inhaled nitric oxide may provide effective responses in various clinical settings, and in some cases, clinical improvement can be sustained without tachyphylaxis at these doses. Whether low doses of inhaled nitric oxide can be safely used for prolonged therapy requires further study. The long-term response to nitric oxide and its potential role in treating chronic pulmonary hypertension with or without severe lung disease are unknown.

In addition to regulation of vascular tone, endothelial-derived nitric oxide decreases neutrophil adherence [43••] and platelet aggregation and influences smooth muscle cell growth and proliferation [44••]. If decreased responsiveness to acetylcholine reflects impaired nitric oxide release, one may further speculate that this functional abnormality also contributes to structural changes as well, because thrombosis *in situ* and vascular remodeling are common histologic findings in patients with chronic pulmonary hypertension. For example, *in vitro* studies with human umbilical vein endothelial cells suggest that impaired nitric oxide release may increase production of potent mitogens, such as endothelin-1 (ET-1) and platelet-derived growth factor [44••]. Whether chronic therapy with nitric oxide donors or other strategies to enhance cyclic GMP content in the pulmonary circulation will decrease vascular remodeling remains speculative. At the present time, two multicenter trials are in progress to address the safety and efficacy of nitric oxide in PPHN.

Potential role of endothelin in pulmonary hypertension

In addition to nitric oxide, vascular endothelium releases several vasoactive products, including the potent constrictor peptide ET-1 (Fig. 1). ET-1 is part of a family of peptides, related to snake venom toxin, that can cause progressive and sustained vasoconstriction [45,46••]. Along with its direct hypertensive effects, subthreshold concentrations of ET-1 augment vasoconstrictor responses to other stimuli, such as norepinephrine and a thromboxane analogue. Despite its potent hemodynamic properties, little is known about its physiologic or pathophysiologic roles. High circulating levels have been reported in several clinical vascular diseases, however, including renal failure, systemic hypertension, strokes, coronary artery disease, and scleroderma. Recent experimental and clinical studies have implicated ET-1 in pulmonary hypertension as well. Increased ET-1 gene expression or protein content has been identified in several adult rat models of chronic pulmonary hypertension, including

chronic hypoxia, as well as in a genetic strain of rats and after monocrotaline treatment. For example, an endothelin receptor antagonist attenuates the severity of cardiopulmonary changes in monocrotaline-induced chronic pulmonary hypertension [47••].

Clinically, high circulating levels of immunoreactive ET-1 were first reported by Stewart *et al.* [48] in adults with primary and secondary pulmonary hypertension. This group subsequently reported increased ET-1 protein and gene in patients with pulmonary hypertension by immunostaining and *in situ* hybridization [49••]. More recently, Rosenberg *et al.* [50•] reported high circulating levels in PPHN, with the highest levels found in neonates who required ECMO. Allen *et al.* [51•] also found high circulating immunoreactive ET-1 levels in pediatric patients with pulmonary hypertension; they also found that basal levels correlated with the degree of pulmonary vasoconstrictor response to acute hypoxia. Until ET-1 antagonists become available for clinical studies, the actual clinical significance of ET-1 in clinical pulmonary hypertension will remain uncertain.

Pulmonary vascular remodeling in infants with congenital heart disease

Past studies have demonstrated the early presence of pulmonary vascular remodeling in young infants with certain types of congenital heart disease. Several recent clinical papers have reinforced the concept that early hypertensive structural changes in the lung circulation contribute to the clinical course and outcome of children with transposition of the great vessels with intact septum [52•], truncus arteriosus [53•], total anomalous pulmonary venous return [54•], scimitar syndrome [55•], and pulmonary veno-occlusive disease [56•]. These papers illustrate how intrauterine [57•] and early postnatal events can rapidly alter normal postnatal adaptation and influence subsequent growth and remodeling of the pulmonary circulation.

Conclusions

During this past year, direct links between basic studies of vascular endothelial cell function and clinical disease led to the rapid application of inhaled nitric oxide to the management of pediatric pulmonary hypertension. Further insight into other basic mechanisms in vascular biology, including the regulation of vascular tone, growth, and remodeling, as well as the response to injury, is certain to lead to other novel approaches toward the treatment of acute and chronic pulmonary hypertension.

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