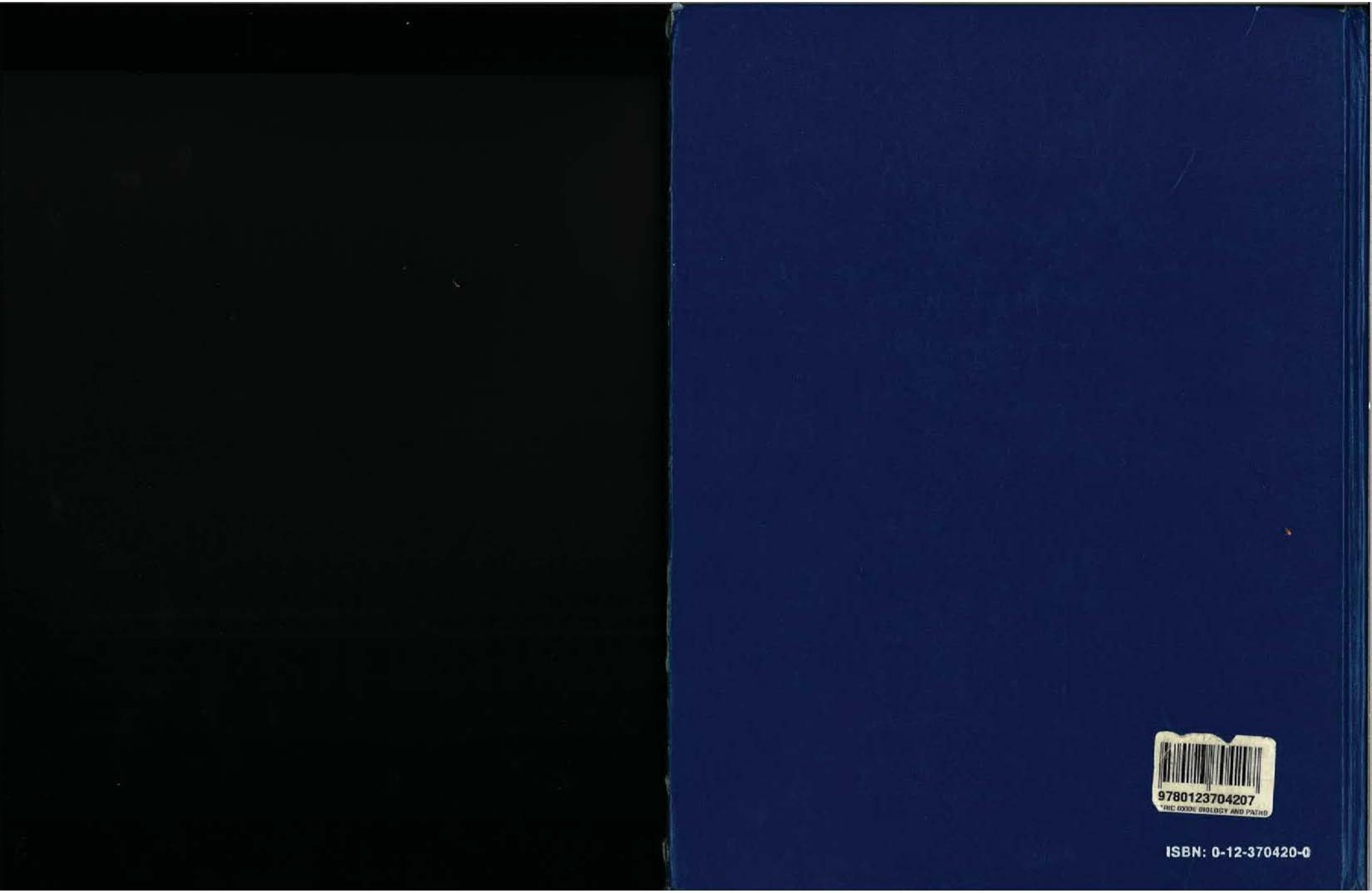
NITRIC OXIDE

BIOLOGY AND PATHOBIOLOGY

EDITED BY

LOUIS J. IGNARRO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Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N. Engl. J. Med.* **323,** 22–27.

Petros, A., Bennett, D., and Vallance, P. (1991). Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* **338,** 1557–1558.

Petros, A., Lamb, G., Leone, A., Moncada, S., Bennett, D., and Vallance, P. (1994). Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc. Res.* **28,** 34–39.

Riezebos, J., Watts, I. S., and Vallance, P. (1994). Endothelin receptors mediating functional responses in human small arteries and veins. *Br. J. Pharmacol.* **111,** 609–615.

Simon, D. I., Stamler, J. S., Loh, E., Loscalzo, J., Francis, S. A., and Creager, M. A. (1995). Effect of nitric oxide synthase inhibition on bleeding time in humans. *J. Cardiovasc. Pharmacol.* **26,** 339–342.

Stamler, J. S., Loh, E., Roddy, M. A., Currie, K. E., and Creager, M. A. (1994). Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation* **89,** 2035–2040.

Steinberg, H. O., Brechtel, G., Johnson, A., Fineberg, N., and Baron, A. (1994). Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* **94,** 1172–1179.

Taddei, S., Virdis, A., Mattei, P., Ghiadoni, L., Sudano, I., and Salvetti, A. (1996). Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation* **94,** 1298–1303.

Vallance, P., and Charles, I. (1998). Nitric oxide in sepsis: Of mice and men. *Sepsis* **1,** 93–100.

Vallance, P., Collier, J., and Moncada, S. (1989a). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **2,** 997–1000.

Vallance, P., Collier, J., and Moncada, S. (1989b). Nitric oxide synthesized from L-arginine mediates endothelium dependent dilation in human veins *in vivo*. *Cardiovasc. Res.* **23,** 1053–1057.

Vallance, P., Benjamin, N., and Collier, J. (1992a). The effect of endothelium-derived nitric oxide on *ex vivo* whole blood platelet aggregation in man. *Euro. J. Clin. Pharmacol.* **42,** 37–41.

Vallance, P., Leone, A., Calver, A., Collier, J., and Moncada, S. (1992b). Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* **339,** 572–575.

Vallance, P., Patton, S., Bhagat, K., MacAllister, R., Radomski, M., Moncada, S., and Malinski, T. (1995). Direct measurement of nitric oxide in human beings. *Lancet* **346,** 153–154.

White, R. P., Deane, C., Vallance, P., and Markus, H. S. (1998). Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. *Stroke* **29,** 467–472.

Williams, D. J., Moosavi, A. H., and Imms, F. J. (1995). Nitric oxide contributes to local heat induced vasodilation in man. *J. Physiol.* **483,** 126–127.

Williams, D. J., Vallance, P., Neild, G. H., Spencer, J. A., and Imms, F. J. (1997). Nitric oxide-mediated vasodilation in human pregnancy. *Am. J. Physiol.* **272,** H748–H752.

CHAPTER 56

Clinical Therapy with Inhaled Nitric Oxide in Respiratory Diseases

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MANY INSIGHTS INTO THE MECHANISMS OF ACTION OF NITRIC OXIDE (NO) HAVE BEEN RAPIDLY APPLIED TO TREAT PATIENTS. SINCE THE REPORTED APPLICATIONS OF INHALED NO IN THE LABORATORY (FROSTELL *et al.*, 1991) AND IN ADULT PATIENTS WITH PRIMARY PULMONARY HYPERTENSION (PEPKE-ZABA *et al.*, 1991), HUNDREDS OF STUDIES HAVE BEEN CONDUCTED TO DETERMINE THE CLINICAL APPLICABILITY OF INHALED NO. IN SELECTED GROUPS OF SEVERELY ILL AND HYPOXIC CHILDREN AND ADULTS, INHALED NO IMPROVES ARTERIAL OXYGENATION AND SELECTIVELY REDUCES PULMONARY ARTERIAL HYPERTENSION (PAH). NO INHALATION THERAPY, IN COMBINATION WITH CONVENTIONAL (NEONATAL INHALED NITRIC OXIDE STUDY GROUP, 1997A; ROBERTS *et al.*, 1997) OR HIGH-FREQUENCY OSCILLATORY VENTILATION (KINSELLA *et al.*, 1997), CAN SIGNIFICANTLY IMPROVE ARTERIAL OXYGENATION AND REDUCE THE NEED FOR EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO), AN EXPENSIVE AND INVASIVE SUPPORT PROCEDURE IN NEWBORN PATIENTS WITH HYPOXIC RESPIRATORY FAILURE. HOWEVER, IT REMAINS UNCERTAIN WHETHER NO INHALATION IMPROVES SURVIVAL RATES IN ADULTS WITH SEVERE ACUTE LUNG INJURY.

NEW APPLICATIONS FOR NO INHALATION HAVE BEEN DISCOVERED. STUDIES INDICATE THAT INHALED NO MAY DECREASE ISCHEMIA–REPERFUSION INJURY (BACHA *et al.*, 1996) AND MAY BE USEFUL TO TREAT THROMBOTIC DISORDERS (ADRIE *et al.*, 1996; NONG *et al.*, 1997). BY INCREASING THE O_2 AFFINITY OF SICKLE CELL HEMOGLOBIN, INHALED NO MAY PREVENT OR TREAT SICKLE CELL CRISIS. THIS CHAPTER WILL REVIEW THESE DIVERSE CLINICAL APPLICATIONS FOR INHALED NO THERAPY.

Background

Nitric oxide (NO) is a small, easily diffusible molecule that can be administered simply by inhalation. Because avid binding of NO to hemoglobin limits the action of NO in the systemic circulation, inhaled NO produces selective pulmonary vasodilation, a long-sought action that should be useful in the treatment of many lung diseases. Many studies have concluded convincingly that inhaled NO is a selective pulmonary vasodilator, in a variety of both animal models and clinical conditions.

The administration of inhaled NO often improves systemic oxygenation during acute lung injury. Commonly used intravenously administered vasodilators diffusely release hypoxic pulmonary vasoconstriction within the lungs and can worsen oxygenation. Inhaled nitric oxide, by being delivered to areas of the lungs that are best ventilated and then by being rapidly bound to hemoglobin and inactivated in

the circulation, can selectively vasodilate ventilated lung regions. Regions of the lungs that are not ventilated are not exposed to inhaled NO. Oxygenation improves via a reduction in relative blood flow to nonventilated regions.

Borland and co-workers administered inhaled NO to patients and volunteers to determine the diffusing capacity of the lung (Borland and Higenbottam, 1989). They found that a single breath of nitric oxide could be administered safely. Because NO is also an atmospheric pollutant (Alberts, 1994), human toxicity studies (von Nieding *et al.,* 1975) and exposure recommendations (Centers for Disease Control, 1988) had been previously reported and provided the foundation for initial clinical studies. Today, substantiated indications for inhaled nitric oxide (Table I) include hypoxic respiratory failure of the newborn (Clark, 1999; Hoffman *et al.,* 1997; Kinsella *et al.,* 1997; Neonatal Inhaled Nitric Oxide Study Group, 1997a; Roberts *et al.,* 1997) and the assessment of pulmonary vascular reactivity in patients with pulmonary hypertension (Fishman *et al.,* 1998). Inhaled NO has also been used in the treatment of acute respiratory distress syndrome (ARDS), lung and cardiac transplants, congenital and acquired heart disease, and chronic pulmonary hypertension, and it has been used to produce desirable direct effects on blood elements, specifically for the treatment of acute chest syndrome in sickle cell disease.

Neonatal Respiratory Failure

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome characterized by sustained pulmonary hypertension and severe hypoxemia, resulting in cyanosis that is unresponsive to oxygen therapy. Persistent pulmonary hypertension of the newborn may be due to a variety of etiologies (Roberts, 1993). Diagnostic confirmation of PPHN includes echocardiographic observation of a right-to-left shunt through the ductus arteriosus or foramen ovale, due to increased pulmonary vascular resistance (PVR), in the absence of congenital heart disease. Conventional treatment strategies include breathing high inspired O_2 concentrations (FiO_2), mechanical ventilation with high-frequency oscillatory ventilation, hyperventilation and infusion of bicarbonate to produce alkalosis, inhalation treatments with bovine surfactant, and intravenous vasodilator therapy. Where available, extracorporeal membrane oxygenation is used to treat severe hypoxemia. The anticoagulation and cannulation of large vessels required for ECMO, however, is associated with hemorrhagic complications. In adults receiving ECMO, bleeding complications have been reported to occur in 88% of patients (Anderson *et al.,* 1993). Bleeding at a rate of over 1.3 liters per day is common (Pesenti *et al.,* 1988). In neonates, intracranial hemorrhage occurs at a rate of approximately 15% of patients receiving ECMO [ECMO registry of the Extracorporeal Life Support Organization (ELSO), 1996].

Table I Clinical Indications for Inhaled Nitric Oxide

Substantiated
Hypoxic respiratory failure and persistent pulmonary hypertension of the newborn (PPHN)
Assessment of pulmonary vascular reactivity in patients with pulmonary hypertension
Investigational
Acute respiratory distress syndrome (ARDS)
Lung and cardiac transplantation
Congenital and acquired heart disease
Chronic pulmonary hypertension
Ischemia–reperfusion injury
Antiplatelet effects
Acute chest syndrome in sickle cell disease
Bronchodilation

In 1992, Roberts *et al.* and Kinsella *et al.* reported that 80 parts per million (ppm) (Roberts *et al.,* 1992) or 6–20 ppm (Kinsella *et al.,* 1992) inhaled NO improved oxygenation in newborns with hypoxic respiratory failure and PPHN. Several controlled, randomized multicenter trials of the effects of inhaled NO in near-term and term hypoxic newborn patients were reported in 1997 (Fig. 1) (Hoffman *et al.,* 1997; Kinsella *et al.,* 1997; Neonatal Inhaled Nitric Oxide Study Group, 1997a,b; Roberts *et al.,* 1997). In the majority of patients with PPHN and hypoxic respiratory failure, NO improved oxygenation and decreased the requirement for ECMO. The decisions to initiate ECMO were made by the clinical team on the basis of center-specific ECMO entry criteria and without knowledge of assignment of the patient to the treatment group or placebo.

It is worthwhile to review several important studies. The Neonatal Inhaled Nitric Oxide Study (NINOS) group of investigators studied 230 infants of at least 34 weeks gestational age with hypoxemic respiratory failure of various etiologies (Neonatal Inhaled Nitric Oxide Study Group, 1997a). They were randomized to receive 100% oxygen or oxygen plus inhaled NO. The use of ECMO was reduced significantly from 54% in the control group to 39% in the NO group. The study did not find a change in mortality that was statistically significant. Roberts and co-workers (1997) randomized 58 infants with severe hypoxemia and PPHN to receive nitrogen or 80 ppm NO.

Oxygenation doubled in 53% of the children receiving inhaled NO versus 7% of controls. This initial effect was sustained in 75% of infants with initial improvement and resulted in a significant reduction in ECMO use from 71% in control patients to 40% in newborns receiving inhaled NO. Kinsella *et al.* (1997) reported that NO inhalation and high-frequency oscillatory ventilation were an effective combination that may increase the rate of responsiveness to inhaled NO. As opposed to studies in adults, in which the ventilatory strategies called for a reduction in lung recruitment (see later), the use of high frequency oscillation in combination with inhaled NO is important for maintaining alveoli open and possibly reducing barotrauma. In follow-up studies of children who received inhaled NO treatment for PPHN, neurodevelopmental scores and growth rates, as well as the frequency of airway disease and need for supplemental O_2, were comparable to conventionally ventilated or ECMO-treated patients (Rosenberg *et al.,* 1997). In summary, inhaled NO improves oxygenation in many newborns and significantly reduces the need for ECMO (Neonatal Inhaled Nitric Oxide Study Group, 1997a; Roberts *et al.,* 1997). These findings have more recently been confirmed in a third multicenter randomized trial (Clark *et al.,* 2000).

The use of inhaled NO to treat PPHN deserves special emphasis. Persistent pulmonary hypertension of the newborn is a relatively uniform disease characterized by severe pulmonary vasoconstriction. By relieving pulmonary vasoconstriction, inhaled NO directly treats one of the major pathological derangements of the disease. Consequently, the efficacy of NO in PPHN has been substantiated in multicenter, randomized, placebo-controlled trials.

Inhaled NO reduces the necessity for ECMO in newborns with PPHN and infants with hypoxic respiratory failure of various etiologies. This improvement in management will justify its continued use, because of the increased expense of ECMO and its morbidity secondary to its invasive nature and the necessity for systemic anticoagulation. Nevertheless, ECMO remains a necessary lifesaving therapy because inhaled NO is not effective in all patients. Such patients should be treated in a facility providing the possibility of ECMO therapy.

Respiratory distress syndrome (RDS), or hyaline membrane disease of the premature newborn, is characterized by a deficiency or dysfunction of surfactant and is often associated with acute PAH (Kinsella *et al.,* 1994). After promising preliminary studies of inhaled NO in the premature newborn with RDS (Abman *et al.,* 1993; Peliowski *et al.,* 1995), Skimming and associates (1997) studied the effect of inhaled NO at 5 and 20 ppm in preterm neonates (without systemic hypotension or congenital malformations and mechanically ventilated at $FiO_2 > 0.5$). They demonstrated that arterial oxygenation improved and that systemic arterial blood pressure was unaffected during a 15-min NO inhalation trial. The conclusions of this study were extremely limited, however, since pulmonary artery pressure was not measured, and only 7% of the initially evaluated premature infants were included in the study. There is concern that an inhibitory effect of NO on platelet aggregation might contribute to the occurrence of intracranial hemorrhages in premature infants (Meurs *et al.,* 1997). An increased rate of bleeding complications has been noted in term infants (Neonatal Inhaled Nitric Oxide Study Group, 1997b), but insufficient data have been published to confirm the safety of inhaled NO therapy in premature newborns. In neonatal lung diseases characterized by abnormal lung development, such as congenital diaphragmatic hernia (Neonatal Inhaled Nitric Oxide Study Group, 1997b), inhaled NO appears to be less effective or without benefit.

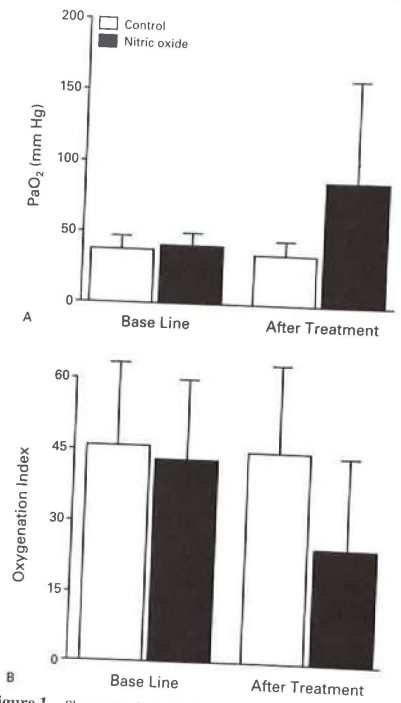

Figure 1 Short-term effects of inhaled NO on systemic oxygenation in infants with severe hypoxemia and persistent pulmonary hypertension of the newborn. Treatment with nitric oxide inhalation (80 ppm at FiO_2 0.9 for 20 min, $n = 30$), but not with placebo inhalation (control, nitrogen at FiO_2 0.9, $n = 28$), after randomized assignment significantly ($p < 0.001$) improved postductal PaO_2 (A) and oxygenation index (B) compared with baseline. Values are means ± SD. Reprinted with permission from Roberts *et al.* (1997).

Acute Respiratory Distress Syndrome

Pulmonary Vascular Resistance

Rossaint *et al.* (1993) demonstrated that inhaled NO produced selective pulmonary vasodilation in patients with severe ARDS (Fig. 2). This was later confirmed by larger studies (Dellinger *et al.,* 1998; Manktelow *et al.,* 1997). In some patients, NO-induced pulmonary vasodilation was sufficient to improve right ventricular performance (Rossaint *et al.,* 1995a). In children with ARDS, inhaled NO (20 ppm) decreased mean pulmonary artery pressure (MPAP) by 25% and increased cardiac index by 14% (Abman *et al.,* 1994). Inhaled NO also effectively relieved the pulmonary hypertension associated with the use of permissive hypercapnia in patients with ARDS (Puybasset *et al.,* 1994).

Inhaled NO (40 ppm) decreased pulmonary capillary pressure (Benzing and Geiger, 1994) and pulmonary trans-

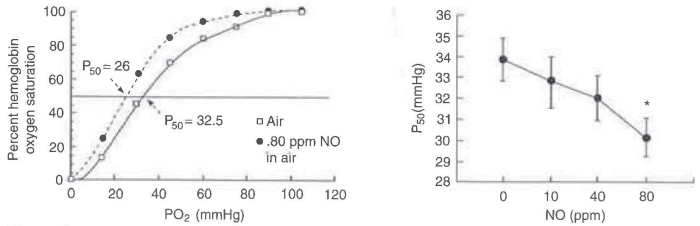

Figure 6 Effects of NO on oxygen affinity of red blood cells. Exposure to NO (80 ppm for 15 min) shifted to the left the oxygen dissociation curve of hemoglobin S erythrocytes (left). The effect of NO exposure on P50 of hemoglobin S erythrocytes was dose dependent (right). Values are means ± SE. Reprinted with permission from Head *et al.* (1997).

Nitrotyrosine residues were detected in the airway specimens of two infants requiring prolonged ventilation with NO in this study. The relative contribution of NO inhalation and endogenous NO formation to nitrotyrosine formation in the lung is unclear, however, because nitrotyrosine formation has been demonstrated in acutely injured lungs without the exogenous administration of NO (Haddad *et al.,* 1994; Kooy *et al.,* 1995). Studies of survivors of ARDS treated with inhaled NO reported no obvious differences in pulmonary function compared with ARDS patients not treated with NO (Luhr *et al.,* 1998). The doses of NO in current clinical use are less than that received with cigarette exposure and are nearly within the range encountered while breathing the air of many urban centers (Lee *et al.,* 1997).

Methemoglobinemia

Blood methemoglobin concentrations have been regularly monitored in clinical trials of inhaled NO in adults and neonates (Dellinger *et al.,* 1998; Kinsella *et al.,* 1997; Neonatal Inhaled Nitric Oxide Study Group, 1997a,b; Roberts *et al.,* 1997). The incidence of methemoglobinemia has been low. Its occurrence is more common in neonates and with high inhaled doses, but it is usually well tolerated. There have been no reports of sequelae to methemoglobinemia in randomized studies. Methemoglobinemia is easily treated by reducing the dose of NO. Single reported cases of more severe methemoglobinemia during NO therapy have occurred in the setting of high doses (Hess *et al.,* 1997). Chemical therapies, such as methylene blue and ascorbic acid, are available, but they should not be necessary if methemoglobin levels are monitored.

Inhibition of Platelet Function

Inhaled NO inhibits platelet function. Increased bleeding times and decreased platelet aggregation have been reported in experimental animals and patients (George *et al.,* 1998; Högman *et al.,* 1993b, 1994; Samama *et al.,* 1995). In randomized studies of adults and term and nearly full-term infants, however, an increased incidence of clinical bleeding has not been substantiated (Dellinger *et al.,* 1998; Kinsella *et al.,* 1997; Neonatal Inhaled Nitric Oxide Study Group, 1997a,b; Roberts *et al.,* 1997). Indeed, platelet inhibition could be therapeutic, rather than detrimental. Nevertheless, a cautious approach to the possibility of worsened bleeding during inhaled NO therapy remains prudent, especially in premature infants who have a high incidence of intracranial hemorrhages (Meurs *et al.,* 1997).

Adverse Hemodynamic Effects

Inhaled NO may also have adverse hemodynamic effects. Inhalation of NO may vasodilate the pulmonary circulation and increase blood flow entering the left ventricle. In patients with preexisting severe left ventricular dysfunction, an increased left ventricular end-diastolic pressure (Hayward *et al.,* 1996; Loh *et al.,* 1994; Semigran *et al.,* 1994) and pulmonary edema (Bocchi *et al.,* 1994) during NO breathing have been reported. This increase may be due to small increases in left ventricular volume associated with improved right ventricular function that, in turn, produced exaggerated increases in pulmonary capillary wedge pressure when the left ventricle is poorly compliant. Monitoring of left ventricular function may be indicated when inhaled NO is administered to patients with severe left ventricular dysfunction.

Rebound hypoxemia and pulmonary hypertension may occur after the sudden discontinuation of NO (Bigatello *et al.,* 1994; Lavoie *et al.,* 1996; Rossaint *et al.,* 1993). It has been suggested that the downregulation of endogenous NO synthesis by NO inhalation is responsible for rebound PAH (Ma *et al.,* 1996; Rengasamy and Johns, 1993). Data obtained in rats with hypoxic pulmonary hypertension, however, suggest that inhibition of endogenous NO synthesis play a minor role in rebound PAH: no changes of lung endothelial nitric oxide synthase (NOS) protein levels, NOS activity, endothelium-dependent and -independent vasodilation were reported after 3 weeks inhaling 20 ppm NO. Lung guanylate cyclase activity was transiently decreased after 1 week of NO inhalation, but guanylate cyclase activity was normal after 3 weeks of NO inhalation (Frank *et al.,* 1998).

Rebound hypoxemia and pulmonary hypertension can be anticipated, and attenuated by increasing the FiO_2, and, perhaps, through the administration of phosphodiesterase inhibitors (Hess *et al.,* 1997). The administration of a type V phosphodiesterase inhibitor, dipyridamole, has been reported to prevent rebound PAH following discontinuation of inhaled NO (al-Alaiyan *et al.,* 1996; Ivy *et al.,* 1998; Ziegler *et al.,* 1998).

Summary

Inhaled NO offers a novel therapy for the treatment of pulmonary hypertensive diseases and the symptomatic relief of hypoxemia. The use of inhaled NO reduces the necessity for ECMO in newborns and infants with acute hypoxemic respiratory failure. Proper indications, contraindications, dosing criteria, and implications of the toxic actions of NO remain to be fully delineated. Randomized clinical studies of patients with carefully defined specific acute disease states characterized by pulmonary hypertension or hypoxemia (e.g., pulmonary embolism, severe PAH, postpneumonectomy pulmonary edema, acute rejection following lung transplantation) and of premature newborns with respiratory failure remain to be completed. If such trials are carefully designed and conducted, we may define additional groups of patients that may benefit from, or may be harmed by, inhaled NO. Chronic ambulatory inhaled NO therapy may someday prove valuable for patients with pulmonary hypertension. The use of inhaled NO continues to be a unique and fascinating approach to studying and treating diseases as diverse as acute rejection of the transplanted lung and sickle cell crisis.

Acknowledgments

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References

Abman, S. H., Kinsella, J. P., Schaffer, M. S., and Wilkening, R. B. (1993). Inhaled nitric oxide in the management of a premature newborn with severe respiratory distress and pulmonary hypertension. *Pediatrics* **92,** 606–609.

Abman, S. H., Griebel, J. L., Parker, D. K., Schmidt, J. M., Swanton, D., and Kinsella, J. P. (1994). Acute effects of inhaled nitric oxide in children with severe hypoxemic respiratory failure. *J. Pediatr.* **124,** 881–888.

Adatia, I., Lillehei, C., Arnold, J. H., Thompson, J. E., Palazzo, R., Fackler, J. C., and Wessel, D. L. (1994). Inhaled nitric oxide in the treatment of postoperative graft dysfunction after lung transplantation. *Ann. Thorac. Surg.* **57,** 1311–1318.

Adatia, I., Perry, S., Landzberg, M., Moore, P., Thompson, J. E., and Wessel, D. L. (1995). Inhaled nitric oxide and hemodynamic evaluation of patients with pulmonary hypertension before transplantation. *J. Am. Coll. Cardiol.* **25,** 1656–1664.

Adrie, C., Bloch, K. D., Moreno, P. R., Hurford, W. E., Guerrero, J. L., Holt, R., Zapol, W. M., Gold, H. K., and Semigran, M. J. (1996). Inhaled nitric oxide increases coronary artery patency after thrombolysis. *Circulation* **94,** 1919–1926.

al-Alaiyan, S., al-Omran, A., and Dyer, D. (1996). The use of phosphodiesterase inhibitor (dipyridamole) to wean from inhaled nitric oxide. *Intensive Care Med.* **22,** 1093–1095.

Alberts, W. M. (1994). Indoor air pollution: NO, NO_2, CO, and CO_2. *J. Allergy Clin. Immunol.* **94,** 524–526.

Allman, K., Young, J., Carapiet, D., Stevens, J., Ostman-Smith, I., and Archer, L. (1996). Effects of oxygen and nitric oxide in oxygen on pulmonary arterial pressures of children with congenital cardiac defects. *Pediatr. Cardiol.* **17,** 246–250.

Amato, M. B. P., Barbas, C. S. V., Medeiros, D. M., Magaldi, R. B., Schettino, G. P., Lorenzi-Filho, G., Kairalla, R. A., Deheinzelin, D., Munoz, C., Oliveira, R., Takagaki, T. Y., and Carvalho, C. R. R. (1998). Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N. Engl. J. Med.* **338,** 347–354.

Anderson III, H., Steimle, C., Shapiro, M., Delius, R., Chapman, R., Hirschl, R., and Bartlett, R. (1993). Extracorporeal life support for adult cardiorespiratory failure. *Surgery* **114,** 161–173.

Argenziano, M., Choudhri, A. F., Moazami, N., Rose, E. A., Smith, C. R., Levin, H. R., Smerling, A. J., and Oz, M. C. (1998). Randomized, double-blind trial of inhaled nitric oxide in LVAD recipients with pulmonary hypertension. *Ann. Thorac. Surg.* **65,** 340–345.

Auler, J. O., Jr., Carmona, M., Bocchi, E., Bacal, F., Fiorelli, A., Stolf, N., and Jatene, A. (1996). Low doses of inhaled nitric oxide in heart transplant recipients. *J. Heart Lung Transplant.* **15,** 443–450.

Bacha, E. A., Herve, P., Murakami, S., Chapelier, A., Mazmanian, G.-M., Montpreville, V. d., Detruit, H., Libert, J.-M., and Darteville, P. (1996). Lasting beneficial effect of short-term inhaled nitric oxide on graft function after lung transplantation. Paris-Sud University Lung Transplantation Group. *J. Thorac. Cardiovasc. Surg.* **112,** 590–598.

Barabrà, J. A., Roger, N., Roca, J., Rovira, I., Higenbottam, T. W., and Rodriguez-Roisin, R. (1996). Worsening of pulmonary gas exchange with nitric oxide inhalation in chronic obstructive pulmonary disease. *Lancet* **347,** 436–440.

Benzing, A., and Geiger, K. (1994). Inhaled nitric oxide lowers pulmonary capillary pressure and changes longitudinal distribution of pulmonary vascular resistance in patients with acute lung injury. *Acta Anaesthesiol. Scand.* **38,** 640–645.

Benzing, A., Bräutigam, P., Geiger, K., Loop, T., Beyer, U., and Moser, E. (1995). Inhaled nitric oxide reduces pulmonary transvascular albumin flux in patients with acute lung injury. *Anesthesiology* **83,** 1153–1161.

Berner, M., Berghetti, M., Spahr-Schopfer, I., Oberhansli, I., and Friedli, B. (1996). Inhaled nitric oxide to test the vasodilator capacity of the pulmonary vascular bed in children with long-standing pulmonary hypertension and congenital heart disease. *Am. J. Cardiol.* **77,** 532–535.

Bigatello, L. M., Hurford, W. E., Kacmarek, R. M., Roberts, J. D., Jr., and Zapol, W. M. (1994). Prolonged inhalation of low concentrations of nitric oxide in patients with severe adult respiratory distress syndrome. Effects on pulmonary hemodynamics and oxygenation. *Anesthesiology* **80,** 761–770.

Bocchi, E. A., Bacal, F., Auler Junior, J. O., Carmone, M. J., Bellotti, G., and Pileggi, F. (1994). Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure. *Am. J. Cardiol.* **74,** 70–72.

Borland, C. D., and Higenbottam, T. W. (1989). A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur. Respir. J.* **2,** 56–63.

Centers for Disease Control (1988). Recommendations for occupational safety and health standard. *MM WR* **37,** 21.

Channick, R. N. (1999). Chronic use of inhaled nitric oxide for pulmonary hypertension. *Respir. Care* **44,** 212–221.

Channick, R. N., Newhart, J. W., Johnson, F. W., Williams, P. J., Auger, W. R., Fedullo, P. F., and Moser, K. M. (1996). Pulsed delivery of inhaled nitric oxide to patients with primary pulmonary hypertension: An ambulatory delivery system and initial clinical tests. *Chest* **109,** 1545–1549.

CHAPTER 58

Nitric Oxide and Persistent Pulmonary Hypertension in the Newborn

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THE TRANSITION OF THE PULMONARY CIRCULATION FROM A HIGH RESISTANCE–LOW FLOW CIRCULATION IN THE FETUS TO A LOW RESISTANCE–HIGH FLOW CIRCULATION IN THE NEWBORN IS A COMPLEX PROCESS THAT INVOLVES THE SIMULTANEOUS DOWNREGULATION OF VASOCONSTRICTOR MEDIATORS AND UPREGULATION OF VASODILATORY MEDIATORS SUCH AS NITRIC OXIDE (NO). IN THE VAST MAJORITY OF INFANTS, THIS PROCESS OCCURS SPONTANEOUSLY AND QUICKLY AND DOES NOT REQUIRE ANY INTERVENTION. OCCASIONALLY, HOWEVER, THE TRANSITION MAY BE DIFFICULT OR ABNORMAL, AND REQUIRE PROMPT AND EFFECTIVE RESUSCITATION TO ENSURE A SUCCESSFUL ADAPTATION TO THE EXTRAUTERINE ENVIRONMENT.

ONE DISEASE THAT LEADS TO COMPLICATIONS DURING TRANSITION IS PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN (PPHN) WHICH IS CHARACTERIZED BY FAILURE OF THE PULMONARY VASCULAR RESISTANCE (PVR) TO DECREASE AFTER BIRTH, RESULTING IN HYPOXIA AND SEVERE DISTRESS. UNDERSTANDING THE PHYSIOLOGY OF THE PULMONARY CIRCULATION DURING THE TRANSITION FROM FETUS TO NEWBORN HAS HELPED NEONATOLOGISTS DEVELOP NEW TECHNIQUES FOR THE MANAGEMENT OF THIS SERIOUS CONDITION ASSOCIATED WITH HIGH MORTALITY AND MORBIDITY. FOR EXAMPLE, BECAUSE INFANTS WITH PPHN EXHIBIT REDUCED NO SYNTHESIS, INHALED NO IS NOW WIDELY USED TO INCREASE PULMONARY BLOOD FLOW AND CORRECT HYPOXIA IN THESE NEONATES. OTHER METHODS TO INCREASE cGMP, SUCH AS INHIBITION OF cGMP SPECIFIC TYPE 5 PHOSPHODIESTERASE ISOFORM (PDE5), EITHER ALONE OR IN COMBINATION WITH NO, ARE BEING INVESTIGATED AS NOVEL THERAPEUTIC MODALITIES FOR TREATING THIS CONDITION. IT IS ANTICIPATED THAT THIS COMBINATION THERAPY WILL DECREASE THE CONCENTRATION OF NO REQUIRED FOR A THERAPEUTIC EFFECT; THUS ATTENUATING POTENTIALLY DELETERIOUS EFFECTS RESULTING FROM THE FREE RADICAL ACTIONS OF NO.

THIS CHAPTER WILL STRESS THE ROLE OF NO IN (1) THE NORMAL FETAL CIRCULATION, (2) THE TRANSITION OF THE CIRCULATION AT BIRTH, AND (3) THE ETIOLOGY AND TREATMENT OF PPHN IN NEWBORNS. IT IS IMPORTANT TO NOTE THAT ALTHOUGH THEY WILL BE DISCUSSED ONLY BRIEFLY, MULTIPLE FACTORS IN ADDITION TO NO CONTRIBUTE TO BOTH THE NORMAL TRANSITION AND THE PATHOPHYSIOLOGY OF PPHN.

Events that Initiate Transition

The stimuli that seem to be most important in decreasing PVR at birth are the rhythmic ventilation of the lungs with a gas and the increase in oxygen tension in the lungs. Each of these stimuli by itself will decrease PVR and increase pulmonary blood flow, but the largest effects are seen when the two events occur simultaneously (Teitel *et al.,* 1990). Studying the role of oxygenation independent of ventilation during transition is technically challenging. Chronically instrumented near-term fetal lambs have been studied while the ewe breathes oxygen in a hyperbaric chamber at three atmospheres. During hyperbaric oxygenation, fetal pulmonary vascular resistance decreases and pulmonary blood flow increases to levels comparable to after birth (Fig. 4).

Pulmonary endothelial cells play a central role in the pulmonary vascular transition through the production and release of numerous mediators that act on the subjacent smooth muscle cell layer. A complete discussion of their products is outside the scope of this chapter. However, the main endothelial products currently believed to be responsible for the pulmonary vasodilation at transition include arachidonic acid metabolites and nitric oxide. Prostacyclin (PGI_2) is the arachidonic acid metabolite most studied in the transition of the pulmonary circulation at birth. Prostacyclin may be important in pulmonary vasodilation following rhythmic distention of the lung, but does not appear to mediate the pulmonary vascular response to oxygenation in the fetus (Morin *et al.,* 1988). Despite a large body of research, the importance of prostacyclin in the transition at birth remains unclear.

Changes in NO–cGMP Signaling at Birth

Within 24 hours after birth, PA pressure decreases to approximately 50% of mean systemic arterial pressure (Fig. 6), and slowly declines to adult values within the next 2–6 weeks (Rudolph, 1985). The immediate increase in oxygen tension at birth alters the status of the smooth muscle and endothelial cells of the pulmonary vasculature (Fig. 7). One immediate effect of the increase in oxygen is reversal of the metabolic blockade of potassium channels in the smooth muscle cell layer leading to potassium efflux and a return of the membrane potential to the more polarized state typical in the adult pulmonary circulation. This closes voltage-dependent calcium channels leading to a decrease in cytosolic calcium, relaxation of pulmonary vessels, and a decrease in PVR.

After birth, eNOS abundance increases and peaks at 2–3 days. Subsequently, eNOS decreases in most vessels by 6 days and is nearly absent in the distal pulmonary arteries of adult animals (Halbower *et al.,* 1994; Hislop *et al.,* 1995). Despite the postnatal decrease of eNOS in small arteries, basal and stimulated production of NO from larger pulmonary arteries continues to increase after birth (Abman *et al.,* 1991; Steinhorn *et al.,* 1993).

The decrease in PVR is further augmented by a rapid increase in the oxygen-mediated availability of NO through a variety of mechanisms. Inhibition of nitric oxide synthase blocks the pulmonary vascular response of the near-term fetal lamb to hyperbaric oxygenation (Fig. 4). Oxygen directly increases basal and acetylcholine-stimulated cGMP production in the pulmonary vasculature (Shaul *et al.,* 1992). Acute changes in oxygen tension do not produce similar changes in mesenteric arteries, suggesting that this dramatic effect of oxygen is due to a direct and specific effect on NO production by fetal pulmonary arteries (Shaul and Wells, 1994). The acute oxygen modulation of pulmonary endothelial NO production does not appear to be a result of production of a local receptor agonist, or changes in availability of oxygen or L-arginine as substrates for NOS. Oxygen may, however, directly effect NOS by altering pulmonary endothelial cell calcium homeostasis.

Oxygen also causes a rapid increase in red blood cell adenosine triphosphate (ATP). ATP or its metabolite adenosine cause pulmonary vasodilation in the fetus, a response that is blocked by inhibition of NOS (Konduri *et al.,* 1992; Steinhorn *et al.,* 1994b). ATP may stimulate endothelial NO production either by binding to purinergic receptors or directly to NOS. Plasma ATP levels increase in the pulmonary arteries of fetal lambs during ventilation with oxygen, and the decrease in PVR that accompanies ventilation with oxygen is abolished by blockade of adenosine and ATP receptors (Konduri *et al.,* 1993). Thus, increased synthesis and release of ATP may cause pulmonary vasodilation in response to birth-related stimuli in the ovine fetus.

The peptide bradykinin stimulates endothelial NO production, and is a potent pulmonary vasodilator in the fetus. Ventilating fetal lambs with oxygen, or exposing the fetus to hyperbaric oxygen, increases the blood concentrations of bradykinin (Heymann *et al.,* 1969). However, blockade of bradykinin receptors does not block the pulmonary vasodilation to oxygen (Banerjee *et al.,* 1994), and it is unknown whether a direct interaction between bradykinin and NO plays a role in the development of the response to oxygen.

Closure of the ductus arteriosus at birth and the decrease in PVR lead to a large increase in pulmonary blood flow. This increase in pulmonary blood flow increases shear stress in the pulmonary vasculature and activates signaling cascades in endothelial cells which produce and/or potentiate pulmonary vasodilation via increased synthesis and release of NO. An increase in shear stress increases eNOS mRNA and protein expression in lung tissue (Black *et al.,* 1997). Therefore, whenever a stimulus initiates an increase in pulmonary blood flow during transition, it increases shear stress and creates a positive feedback loop in which increased NO synthesis increases pulmonary blood flow still further. At least a portion of the pulmonary vasodilation that results from shear stress may be attributed to NO-mediated activation of K^+-channels (Kv) in smooth muscle cells (Storme *et al.,* 1999).

The pattern of expression of soluble guanylate cyclase is similar to eNOS, with the highest levels of expression and activity noted in the first days following birth. Furthermore, within 1 hour following birth, PDE5 activity, protein, and mRNA dramatically decrease in newborn lamb and mouse lungs (Hanson *et al.,* 1998b). The low level of PDE5 activity following birth would be expected to enhance the effect of NO on pulmonary vascular smooth muscle by increasing cGMP levels in a manner that correlates well with the decrease in PVR early in transition.

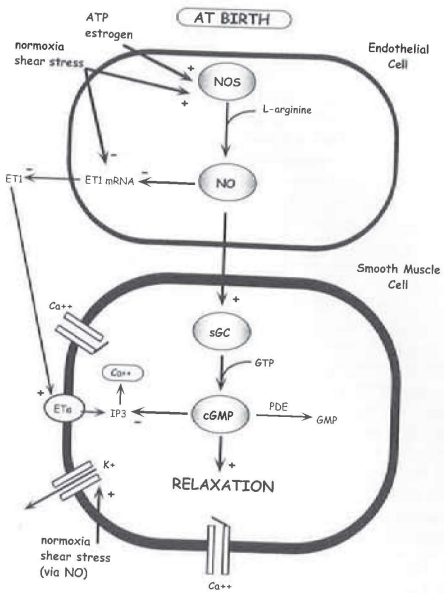

Figure 7 Diagram of the factors that favor low pulmonary vascular resistance at birth. The size and density of letters and lines reflect relative concentrations and activity.

Summary of Developmental Changes Leading to Transition

Early in fetal life PVR is high due to relatively few pulmonary capillaries, high vascular smooth muscle tone induced by hypoxia and endothelin, and a poorly developed NO–cGMP signaling pathway (Fig. 3). As lung development progresses toward term, the NO–cGMP pathway matures such that, at birth, it assumes a major role in the transition of the pulmonary circulation to a high flow/low pressure circuit (Fig. 7). Abnormalities in fetal lung development can interrupt maturation of the NO–cGMP pathway at several sites and interfere with this transition. The lack of a properly functioning NO–cGMP pathway for inhibition of pulmonary vascular tone at birth is a major contributing factor to the development of PPHN. The causes of PPHN, animal models for investigating this disease, and therapeutic regimens based on manipulation of the NO–cGMP pathway are discussed in the following sections.

Persistent Pulmonary Hypertension

Definition

The expected decrease in pulmonary vascular resistance and increase in pulmonary blood flow described earlier do not always occur normally during the transition to extrauterine life. Persistent pulmonary hypertension of the newborn (PPHN) is the result. This syndrome complicates more than 1 in 1000 live births and up to 10% of admissions to intensive care nurseries. There are no known genetic factors involved in its pathogenesis. Although it can occur in premature infants, PPHN is classically a disorder of the term infant.

PPHN is characterized by pulmonary hypertension causing right to left extrapulmonary shunting of blood and hypoxemia (Fig. 1C). It is a clinical syndrome rather than a specific disease, and is associated with a wide array of cardiac and respiratory disorders. PPHN may result when vasoconstriction of structurally normal pulmonary vessels

occurs in response to acute asphyxia, or alveolar hypoxia due to parenchymal disorders such as hyaline membrane disease or meconium aspiration syndrome. However PPHN can occur idiopathically in the absence of underlying parenchymal disease. It is believed that in these cases, the syndrome is the result of an abnormal remodeled vasculature that develops *in utero* in response to prolonged fetal stress, hypoxia, and pulmonary hypertension. PPHN causes substantial morbidity and mortality in otherwise normal term infants. Clinically, once recovery occurs, neurologic sequelae are a concern. Although there are theoretical concerns that an early insult may "imprint" the vasculature unfavorably, clinical outcome studies do not currently indicate that pulmonary hypertension recurs later in life.

Presently, over 1000 infants per year with PPHN require transport to specialized centers that provide extended heart lung bypass, known as extracorporeal membrane oxygenation or ECMO. Although ECMO is life-saving, it is expensive, invasive, and carries a substantial risk of morbidity. By design, cardiopulmonary bypass will decrease pulmonary blood flow and pressure and therefore may indirectly benefit the pulmonary vasculature. However it is not specific therapy designed to reverse PPHN. Therefore, understanding the role of mediators such as nitric oxide in the abnormal transition is important. This understanding should lead to development of safer, more effective therapies for PPHN and ultimately its prevention.

Human Studies

It is difficult to measure endogenous nitric oxide production in the newborn infant, and direct assay of NO production by pulmonary endothelial cells is not currently feasible. Therefore, the few studies in human infants have all have been based on more global measures of nitric oxide production. Urinary nitrites and nitrates are lower in infants with PPHN than in healthy term infants (Dollberg *et al.,* 1995). Plasma cGMP concentrations are also low, and increase rapidly in response to inhaled NO (Christou *et al.,* 1997). More recently, eNOS expression was found to be absent in umbilical vein endothelial cells cultured from four out of six infants who subsequently developed PPHN (Villaneuva *et al.,* 1998). Taken together, these studies provide indirect evidence for a deficiency of endogenous NO production in infants with PPHN. However, it is impossible to determine whether the absence of eNOS is the cause of PPHN, or whether PPHN leads to a loss of eNOS.

Effects of NO Synthase Inhibition or Disruption

Does a decrease in endogenous NOS activity produce clinical PPHN? Acute or chronic infusions of the nonspecific NO synthase inhibitor, L-NA, in fetal lambs produce physiological abnormalities consistent with PPHN following delivery (Abman *et al.,* 1990; Fineman *et al.,* 1994). Pulmonary arterial pressure and pulmonary vascular resistance are increased relative to control lambs, and hypoxemia results due to shunting of deoxygenated blood across the foramen ovale. These lambs are fully responsive to inhaled nitric oxide and nitric oxide donor agents, and following birth their pulmonary hypertension can be completely reversed with L-arginine. These findings suggest that inhibition or decreased activity of NOS could cause acute PPHN in some infants. Of particular interest is that persistent pulmonary hypertension develops in these lambs *without* associated pulmonary vascular remodeling. Therefore, reduction of endogenous NO production alone is probably not sufficient to produce the full physiological and anatomic picture of PPHN.

Mice with targeted disruption of endothelial or neuronal NOS offer an alternative experimental approach to infusions of pharmacological inhibitors, although measurements of pulmonary hemodynamics are technically challenging in adult animals, and currently not feasible in the newborn. Disruption of endothelial or neuronal NOS expression does not increase perinatal mortality. Adult eNOS $-/-$ mice have at most a modest increase in pulmonary vascular resistance in a baseline unstressed state, but exhibit a striking exaggerated physiological response and increase in muscularization in peripheral arterioles in response to even mild hypoxia (Fagan *et al.,* 1999; Steudel *et al.,* 1998). Interestingly, even a 50% reduction of eNOS protein results in augmentation of the pulmonary vascular sensitivity to hypoxia.

Effect of PPHN on NO–cGMP Signaling

Most studies have addressed the converse of the previous question: Is PPHN associated with alterations in NOS expression and/or activity? Similar to the problem in evaluating the human studies, it must be kept in mind that it is difficult to differentiate whether alterations in specific enzymes are responsible for producing PPHN, or occur in response to it. Still, important insights into pathophysiology and therapeutic options can be gained from the study of animal models of PPHN.

Prenatal Development of Pulmonary Vascular Abnormalities

Newborns who die due to idiopathic persistent pulmonary hypertension of the newborn display an increase in pulmonary arterial medial smooth muscle and extension of muscle to normally nonmuscular pulmonary arteries (Haworth and Reid, 1976; Murphy *et al.,* 1981). The muscle cells are frequently surrounded by heavy elastic laminae, suggesting they formed several weeks before death. Further, these anatomic changes are observed in infants dying in the first 24 hours of life, which strongly suggests that an altered intrauterine environment may produce structural changes in the pulmonary circulation of the fetus.

To study the antenatal development of PPHN, intrauterine models of persistent pulmonary hypertension have been developed in the fetal lamb. The relatively large size of the fetal lamb makes it suitable for surgical intervention and physiological study as a fetus and immediate newborn. Furthermore, the ewes tolerate uterine surgical intervention well, and are relatively resistant to premature labor.

Ductal Constriction or Ligation In the normal fetus, diversion of right ventricular output away from the lungs across the ductus arteriosus may be an important mechanism that protects against remodeling of pulmonary resistance arteries. PPHN is more common in postterm newborns, which may be due to intrauterine constriction of the fetal ductus arteriosus. A rare cause of PPHN in infants is prenatal constriction of the ductus arteriosus due to maternal ingestion of prostaglandin synthesis inhibitors. The most common model currently in use for the study of PPHN is the ductal ligation model.

Following surgical constriction of the ductus arteriosus, pulmonary blood flow acutely increases. However, within 2 hours pulmonary blood flow decreases back to baseline while pulmonary vascular resistance remains high. Fetal lambs born 7 to 14 days following ductal constriction or ligation have persistent pulmonary hypertension (Abman *et al.,* 1989; Morin, 1989), with all the physiological hallmarks of the human syndrome, including pulmonary arterial pressure equal to aortic pressure and hypoxemia unresponsive to ventilation with 100% oxygen. Structural alterations also occur, including extension of smooth muscle into the normally nonmuscular distal arteries and the formation of periadventitial fibrosis surrounding the intraacinar arteries (Wild *et al.,* 1989) (Fig. 8). These changes are identical to those observed in human infants.

Activity, message, and protein content of endothelial nitric oxide synthase are decreased by approximately 50% in lung extracts of ligated compared to control fetal lambs (Black *et al.,* 1998a; Shaul *et al.,* 1997; Villamor *et al.,* 1997). Preliminary studies indicate that neuronal NOS expression in the peripheral arterioles is also decreased (Tzao *et al.,* 1999). The pulmonary vasculature of ductal ligation lambs dilates in response to nitric oxide inhalation, but high concentrations are required to decrease pulmonary arterial pressure and pulmonary vascular resistance to near normal levels. This may be due in part to increased production of potent competing vasoconstrictors such as endothelin-1. However, this response pattern to exogenous NO can also be explained by other alterations in the nitric oxide–cGMP pathway as described later.

Relaxations to atrial natriuretic peptide and cGMP analogs are similar in control and hypertensive lambs, indicating that the remodeled pulmonary vessels relax normally when cGMP concentrations increase sufficiently. However, in addition to decreased endothelial NO synthase content and activity, soluble guanylyl cyclase content and activity are decreased. Pulmonary arteries isolated from PPHN lambs have diminished relaxations and cGMP accumulation in response to sodium nitroprusside and nitric oxide gas compared to controls (Steinhorn *et al.,* 1995). Protein contents for both the α and β subunits of soluble guanylate cyclase are decreased (Black *et al.,* 1998a; Tzao *et al.,* 1998), and immunostaining localizes the decrease in sGC expression to all levels of the arterial tree, with the most striking changes in the smallest resistance arterioles. Cyclic GMP specific phosphodiesterase activity appears to be elevated in PPHN lambs, by a mechanism that may involve posttranslational modification by phosphorylation. Increased phosphodiesterase activity would further depress the already decreased cGMP concentrations in response to endogenous and exogenous NO.

Abnormal Lung Growth Congenital diaphragmatic hernia (CDH) occurs when the diaphragmatic leaflets fail to fuse early in gestation, allowing the bowel to migrate into the chest cavity. Pulmonary hypoplasia occurs to a variable degree, probably in proportion to the volume and duration of intestinal herniation. In lung development, the vessels develop in parallel with the conducting airways. As a result, the pulmonary vascular bed in CDH is reduced in proportion to the degree of pulmonary hypoplasia, and abnormal muscularization of arterioles occurs as described earlier (Bohn *et al.,* 1987). Even with advanced support techniques such as ECMO, mortality has remained nearly 50%.

CDH can be induced in approximately 50% of rat fetuses after maternal ingestion of the herbicide nitrofen early in gestation. In this model, eNOS mRNA and protein abundance are decreased in the lung ipsilateral to the hernia compared to lungs from unaffected littermates. Congenital diaphragmatic hernia can also be surgically produced in the second trimester in the fetal lamb. Physiological and anatomic findings are similar to that seen in severely affected human infants. In this model, NO synthase content and functional activity are not altered in large pulmonary arteries (Karamanoukian *et al.,* 1995). However, NO synthase activity is abnormal in pulmonary veins, with the most striking abnormalities observed in veins isolated from the smaller lung ipsilateral to the hernia (Irish *et al.,* 1998). Relaxations to NO donor agents are normal in both pulmonary arteries and veins, indicating that at least prior to birth the vascular smooth muscle responds normally to NO. However, as described later, even when there is an initial dramatic improvement in oxygenation, infants with CDH are less likely to sustain a response to inhaled NO.

Postnatal Development of Pulmonary Vascular Abnormalities

Hypoxic Vasoconstriction The development of acute vasoconstriction and chronic hypertension in response to hypoxia is a key feature that distinguishes the pulmonary circulation from the systemic circulation. In newborn and adult animals, acute hypoxia produces a prompt rise in pulmonary artery pressure and pulmonary vascular resistance. However, hypoxic vasoconstriction appears to be attenuated in the early newborn period, and increases strikingly with postnatal age (Fike and Hansen, 1987). This attenuation correlates well with the rapid alterations in NO–cGMP signaling that occur at birth and favor sustained increased cGMP concentrations and pulmonary vasodilation. The site of hypoxic vasoconstriction also varies with postnatal age. In contrast

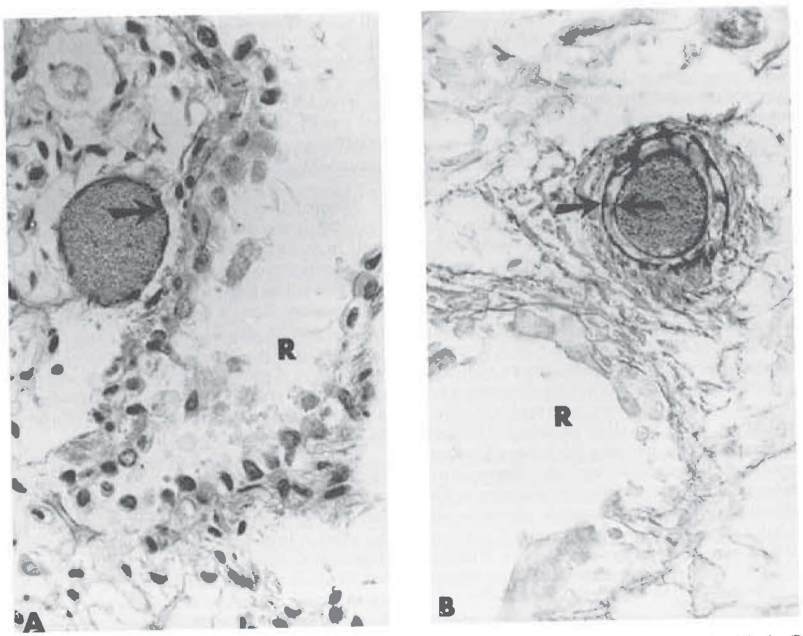

Figure 8 Example of pulmonary arterioles from normal near-term fetal lambs (A) and from lambs with PPHN following ductal ligation (B). In PPHN, the arterioles have a muscularized arterial wall, double elastic lamina (arrows), and adventitial proliferation. From Wild *et al.* (1989), with permission.

to adult lungs, in which the pulmonary arteries are the major site of hypoxic vasoconstriction, both pulmonary arterial and small diameter venous pressures increase following acute hypoxia in newborn lambs and piglets (Fike and Kaplowitz, 1992; Raj and Chen, 1986).

The role of NO synthase in modulating acute hypoxic pulmonary vasoconstriction remains controversial. Nitric oxide synthase inhibitors potentiate hypoxic vasoconstriction in newborn piglets and lambs (Gordon and Tod, 1993), suggesting that endogenous NO production may attenuate hypoxic vasoconstriction. However other studies demonstrate that decreased oxygen tension inhibits NO synthase expression and activity in fetal lambs (North *et al.*, 1996; Shaul *et al.*, 1992), and exhaled NO and NO metabolites fall during acute hypoxia in perfused lungs isolated from newborn piglets (Nelin *et al.*, 1996). Studies examining the newborn response to hypoxia following knockout of the NOS gene would be helpful, but have not been done because of the technical challenges.

Chronic Hypoxia Prolonged hypoxia following birth is an important cause of PPHN, and a widely used model for its study. Neonatal pulmonary vascular smooth muscle cells respond to hypoxia with a more vigorous proliferative response than adult cells, leading rapidly to structural changes. The vasculature of newborn piglets exposed to 72 hours of hypoxia from the moment of birth retains a fetal shape and spacial relationship, and the vascular smooth muscle cells retain a fetal phenotype (Haworth, 1988). In addition to retaining the fetal potential for smooth muscle cell proliferation, there is enhanced accumulation of extracellular matrix connective tissue components.

Important functional changes in NO-mediated relaxations accompany these structural changes. Endothelial nitric oxide synthase expression and activity are decreased in newborn animals exposed to chronic hypoxia (Fike *et al.*, 1998; Hislop *et al.*, 1997; Orton *et al.*, 1988). This is in contrast to the multiple studies indicating that in adult rats, prolonged hypoxia *enhances* expression of NOS (Lecras *et al.*, 1996; Shaul *et al.*, 1995), and that this upregulation occurs precisely at the onset of vascular remodeling (Xue and Johns, 1996). Further investigation will help delineate whether these opposite findings represent important development differences in NO synthase function, or whether chronic hypoxia produces alterations in the nitric oxide target enzyme pathways in the vascular smooth muscle cell which decrease sensitivity to endogenous and exogenous NO.

Alterations in smooth muscle response to NO may occur following chronic hypoxia. Relaxations and cGMP accumulation in response to both endogenous and exogenous NO are blunted following 7 days of hypoxia in adult rats, but responses to atrial natriuretic peptide (ANP) remain equivalent to controls (Crawley *et al.*, 1992). Rodman (1992), found that relaxations to endogenous NO, exogenous NO, and cGMP were all blunted in pulmonary arteries isolated from rats exposed to 35 days of hypoxia. Taken as a whole, these data indicate that in chronic hypoxia, defects in relaxation may develop over time, first at the level of the endothelium, followed by soluble guanylyl cyclase, followed by alterations in smooth muscle response to cGMP. Even briefer periods of hypoxia disrupt responses to exogenous nitric oxide in newborn piglets, indicating increased sensitivity to hypoxia during the early newborn period (Tulloh *et al.*, 1997).

Congenital Heart Disease Pulmonary hypertension commonly develops in infants with congenital heart lesions that are associated with increased pulmonary blood flow, such as truncus arteriosus or atrioventricular canal. If the heart lesion is not corrected, vascular changes of medial and intimal thickening occur, which ultimately lead to luminal obliteration. The obvious differences in pulmonary hypertension induced by a chronic increase in pulmonary blood flow from that induced by hypoxia has led to the development of specific animal models. As described earlier, increases in shear stress stimulate endothelial cells to produce several modulators of vascular tone, including NO. Large aortopulmonary shunts have been successfully placed in the late gestation ovine fetus, producing a model with the greatest similarity to children with congenital heart disease (Reddy *et al.*, 1995). At 1 month of age, the ratio of pulmonary to systemic blood flow is approximately 2 to 1; and while pulmonary vascular resistance is low after birth, it rises to near systemic values by 4 to 6 weeks of age. Morphological changes occur at this time, characterized by extension of muscle into small peripheral arteries, medial hypertrophy of small muscular arteries, and an increase in the total number of vessels (Reddy *et al.*, 1995).

High pulmonary blood flow from these shunts produces complex functional alterations in NO-mediated vasodilation. While endothelium-dependent vasodilation is decreased in 4 week old lambs, pulmonary vascular constriction to blockade of nitric oxide synthase is enhanced and plasma concentrations of cGMP are high (Reddy *et al.*, 1996). Expression of eNOS, the α and β subunits of soluble guanylyl cyclase, and Type 5 phosphodiesterase are all increased in lung parenchyma from shunted lambs, and *in situ* hybridization and immunohistochemistry localized the increase in eNOS to the endothelium of small and large pulmonary arteries (Black *et al.*, 1998b). These changes in NO–cGMP signaling are an interesting contrast to the changes observed in the ductal ligation model, indicating that pressure and flow may induce different abnormalities in endothelium–smooth muscle signaling.

Pulmonary hypertension can be dramatically exacerbated following cardiopulmonary bypass even in very young infants. Microemboli, neutrophil activation and sequestration, interruption of normal pulmonary blood flow, excessive thromboxane production, hypoxic vasoconstriction, and platelet adhesion all occur, and may disrupt endothelial function. If the endothelium is producing large amounts of NO prior to bypass, it is easy to envision that its disruption following bypass could shift the balance toward vasoconstrictors such as endothelin.

Clinical Importance of the NO–cGMP Pathway in PPHN

RESULTS OF CLINICAL TRIALS

To restore the normal transition in infants with PPHN, a vasodilator selective for the pulmonary circulation is needed. Nitric oxide is a gas, which allows it to be delivered directly to the lung. Further, the systemic circulation is protected because NO is rapidly inactivated by its combination with hemoglobin, forming nitrosohemoglobin and subsequently methemoglobin. Initial studies in animal models and human infants showed that NO inhaled at doses between 5 and 80 ppm improved systemic oxygenation in newborns with persistent pulmonary hypertension without decreasing systemic blood pressure. The animal studies further showed that inhalation of 80 ppm NO for 24 hours did not increase lung injury.

The clinical applications of inhaled NO have been studied in a wide range of populations and disease states, but to date the results are most compelling in the hypoxic newborn with PPHN. In two multicenter, randomized, placebo-controlled studies of term infants with PPHN (Table I), inhaled NO significantly improved systemic oxygenation and decreased the need for ECMO by approximately 30% (Neonatal Inhaled Nitric Oxide Study Group, 1997a; Roberts *et al.*, 1997). Although they report similar results (Fig. 9), the two studies used different concentrations of NO and enrolled quite different populations of infants. For example, although both studies enrolled infants with hypoxemia, documentation of pulmonary hypertension was only required for entry in the Roberts *et al.* (1997) trial. The similar outcomes in the two different patient populations may indicate that the clinical response to NO is not completely determined by the underlying disease state. A third large multicenter trial of NO inhalation studied patients earlier in the course of PPHN, and found a reduction in ECMO use similar to the two previous studies (Davidson *et al.*, 1998). However, *no* study has shown that NO reduces the incidence of death, neurologic sequelae, or chronic lung disease, findings that have been attributed to the availability and efficacy of ECMO. Although inhaled NO is an extraordinary advance in the therapeutic approach to PPHN, these studies clearly demonstrate that NO is not universally effective.

Table I Characteristics of Multicenter, Randomized, Placebo-Controlled Clinical Trials of Inhaled NO for PPHN

Authors	*N*	Entry criteria	Initial NO dose	Other therapies	Outcome measure
NINOS (1997a)	235	OI > 25 × 2, ≥34 weeks, ≤14 days old	20 ppm, may increase to 80 ppm	HFV permitted (55%), surfactant permitted (72%)	Death, ECMO
Roberts *et al.* (1997)	58	PPHN, ≥37 weeks, PaO_2 < 55	80 ppm	No HFV, surfactant permitted	Oxygenation (PaO_2 > 55)
Davidson *et al.* (1998)	155	PPHN, ≤72 hours old, >37 weeks, PaO_2 > 40 < 100	5, 20, or 80 ppm	No HFV, no surfactant	Death, ECMO, adverse sequelae

OI, oxygenation index; HFV, high frequency ventilation.

The correct clinical dose of inhaled NO is controversial. Animal studies, which allow for direct measurement of hemodynamics, indicate that pulmonary vascular resistance decreases in a dose-dependent fashion. However, in clinical studies, oxygenation and clinical efficacy were not different whether NO was inhaled at doses of 5, 20, or 80 ppm (Davidson *et al.,* 1998). Potential toxicities of NO are important in considering the ideal NO dose. NO is clinically delivered in combination with high concentrations of oxygen. This may favor the oxidation of NO to nitrogen dioxide, which even in very low concentrations can acutely injure the distal airways and alveoli, and disrupt the vascular endothelium. Furthermore, there may be increased production of superoxide due to inflammatory lung disease and high inspired oxygen concentrations. When NO comes into contact with superoxide, peroxynitrites are formed that may damage surfactant associated proteins, inhibit surfactant function, and cause cell damage. Another concern is that nitrosylhemoglobins formed when NO combines with hemoglobin are oxidized to methemoglobin. The balance of methemoglobin will depend on its rate of production, and the rate of elimination by methemoglobin reductase in the erythrocyte, an enzyme which has reduced activity in the newborn period. Significant increases in methemoglobin (> 7% of total hemoglobin) occur commonly during delivery of doses of 80 ppm to newborns. Finally, nitric oxide increases cGMP concentrations in platelets as well as in vascular smooth muscle, which can inhibit platelet aggregation and adhesion. NO inhalation increases bleeding time in healthy adult humans by 30%, and initial clinical trials indicate that premature infants treated with inhaled NO may have a very high incidence of intracranial hemorrhage.

Congenital Diaphragmatic Hernia: A Unique Subset of Patients

Because of its unique pathophysiology, all of the previously mentioned clinical studies using inhaled NO specifically excluded infants with congenital diaphragmatic hernia. A multicenter trial enrolling only infants with CDH showed a significant *increase* in ECMO use following NO treatment, indicating that nitric oxide affects the hypoplastic lung adversely (Neonatal Inhaled Nitric Oxide Study Group, 1997b). The reason for this adverse effect is not clear. In some cases it may be due to poor lung recruitment due to surfactant deficiency. It is also likely that the hypoplastic pulmonary vasculature allows for sustained postnatal pulmonary hypertension when pulmonary blood flow increases, subsequently leading to rapid vascular remodeling and decreased vascular smooth muscle responsiveness to NO. Infants with hypoplastic lungs who are initially refractory to inhaled NO do respond after a period of ECMO support (Karamanoukian *et al.,* 1994). This could result from improved lung recruitment due to restored surfactant synthesis, or possibly due to protection and recovery of the pulmonary vasculature during ECMO support.

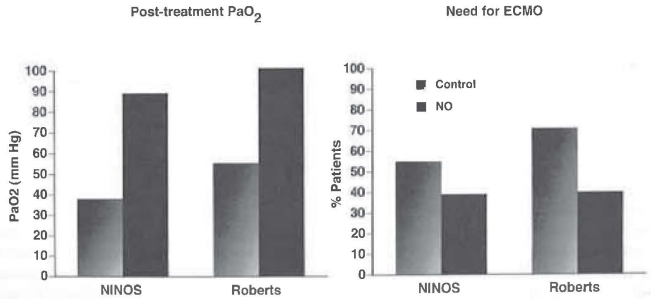

Figure 9 Comparison of results of two major clinical trials (NINOS, 1997a; Roberts *et al.,* 1997) of NO inhalation in infants with PPHN.

Clinical Problems Encountered Using NO

Inhaled NO is clearly not universally effective. In some cases, this may be due to lack of delivery to the target site. It is widely presumed that when NO is delivered as an inhaled gas, its small molecular weight allows it to simply diffuse through the pulmonary interstitium and vascular adventitia into the vascular smooth muscle cell. As discussed earlier, in reality, nitric oxide does not readily cross the adventitia of pulmonary vessels (Steinhorn *et al.,* 1994a). The clinical implication is that nitric oxide must be delivered to peripheral lung units to be effective, which can be difficult when parenchymal lung disease is present.

The clinical response to NO is more heterogenous than can be explained by lack of effective delivery to the lung periphery. Even a dramatic initial response to NO is often transient. To further complicate clinical use of NO, life-threatening rebound pulmonary hypertension may occur when NO is discontinued after only a few hours of inhalation (Miller *et al.,* 1995). Rebound pulmonary hypertension occurs even if the initial response to inhaled NO was modest, and may leave the patient in worse condition than prior to initiation of NO. It is attractive to theorize that decreased expression of endothelial NOS in response to exogenous NO is responsible for this response. NO donor agents acutely alter NOS activity, but do not alter eNOS expression in pulmonary artery endothelial cell cultures (Sheehy *et al.,* 1998). Chronic administration of NO downregulates soluble guanylyl cyclase activity in pulmonary vascular smooth muscle cells, indicating that the vascular smooth muscle cell may also alter its response to NO during prolonged exposures.

Enhancement of NO Effect

Because NO must be delivered to peripheral lung units, clinical strategies designed to improve lung recruitment become critical components of successful therapy. Studies delivering inhaled nitric oxide in combination with high frequency oscillatory ventilation or exogenous surfactant indicate that these strategies may enhance the clinical efficacy of nitric oxide. Ventilation with oxygen-carrying perfluorochemicals is an exciting new experimental strategy that may improve lung recruitment in the face of severe surfactant deficiency or inactivation. Preliminary data indicate that the pharmacokinetics of inhaled nitric oxide are similar whether it is delivered to the perflurocarbon-filled or the conventionally gas-ventilated lung. The combination of these two therapies may therefore represent an additional way to deliver nitric oxide in the face of severe parenchymal lung disease.

Lung recruitment does not provide the whole answer to this clinical problem. It is important to note that the clinical efficacy of NO in the NINOS trial, which allowed use of lung recruitment strategies such as surfactant and high frequency ventilation, was not different than the Roberts *et al.* (1997) trial which restricted access to these therapies. After vascular injury, the adventitia, as well as the vascular smooth muscle cell, are sites of cellular proliferation in both animal models and human infants with PPHN. New reports indicate that the adventitia is metabolically active and produces superoxide. Furthermore, exogenous NO may induce superoxide formation in endothelial and other cells (Munzel *et al.,* 1995; Sheehy *et al.,* 1998). The relative intracellular activities of superoxide and superoxide dismutase (SOD) provide a potential pathway for modulating the effects of nitric oxide in the lung (Fig. 10). By reducing NO clearance by superoxide, SOD significantly enhances responses to nitric oxide *in vitro* (Cherry *et al.,* 1990). A newly developed recombinant human Cu,Zn-SOD is being tested in infants at high risk for lung injury. Experimental studies indicate that SOD in conjunction with nitric oxide may decrease oxidative lung injury and enhance the physiological effects of inhaled nitric oxide (Davis, 1998).

Similar to the animal models described earlier, some patients may not sustain a response to nitric oxide due to

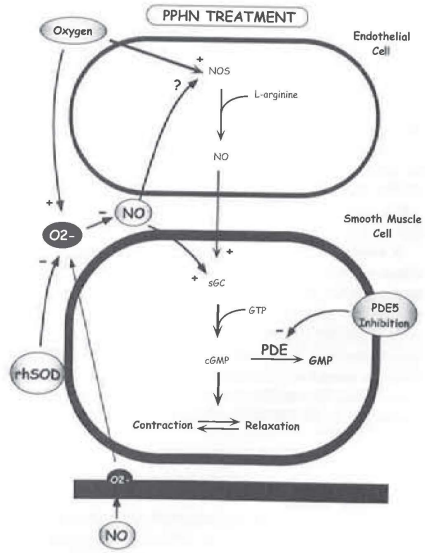

Figure 10 Diagram of potential points of the NO–cGMP signaling pathway that may allow for therapeutic intervention and enhancement of effect.

abnormalities of vascular development or function. This may be due to altered content or activity of soluble guanylyl cyclase or the cGMP phosphodiesterase isoenzyme. If so, inhibition of cGMP phosphodiesterase activity may provide an avenue for increasing efficacy of inhaled nitric oxide in human infants with PPHN by increasing cGMP concentrations (Fig. 9). Dipyridamole, which has been used for many years in humans, has significant inhibitory activity against cGMP phosphodiesterase. Dipyridamole augments the pulmonary vascular response to inhaled NO in adult patients with pulmonary hypertension following cardiopulmonary bypass (Fullerton *et al.,* 1997), and in some pediatric patients with pulmonary hypertension (Ziegler *et al.,* 1998). Dipyridamole may also prevent rebound pulmonary hypertension when inhaled NO is withdrawn (Ivy *et al.,* 1998b), and thereby allow for safer transport of infants who fail to respond to inhaled NO. There is little experience to date with its use in human infants. Dipyridamole selectively dilates the pulmonary circulation in the ovine fetus by augmenting the response to endogenous NO production (Ziegler *et al.,* 1995). However, dipyridamole inhibits other phosphodiesterase isoforms involved in cAMP metabolism, and its use must be approached with caution in the newborn period. In newborn lambs with PPHN, dipyridamole decreased pulmonary vascular resistance, but produced marked systemic hypotension at the same time (Dukarm *et al.,* 1998). Although the use of lower dipyridamole doses avoided systemic hypotension, they did not enhance the effect of inhaled NO.

Experimental pharmacological inhibitors such as zaprinast (M&B 22,948) and E4021 have more recently been developed for the Type 5 phosphodiesterase, and may be more potent and selective than dipyridamole. When zaprinast is administered in combination with a threshold dose of inhaled NO (6 ppm) in the ductal ligation lamb model, the drop in pulmonary vascular resistance and increase in oxygenation are quadrupled compared to nitric oxide alone (Thusu *et al.,* 1995). The combination of zaprinast and NO also significantly increases the duration of vasodilation to inhaled NO (Ichinose *et al.,* 1995).

If selective enough, phosphodiesterase inhibition may be effective even *without* the use of exogenous NO. For example, E4021, an experimental agent, is 100 times as potent as zaprinast for PDE5 (Saeki *et al.,* 1995), and has minimal to no inhibitory activity for other PDE isoenzymes. In newborn lambs with PPHN, increasing doses of E4021 result in selective pulmonary vasodilation without systemic hypotension. Using this strategy, a pulmonary vascular response equivalent to 50–100 ppm inhaled NO is possible by using E4021 alone.

Summary

Persistent pulmonary hypertension of the newborn is a syndrome that results from stresses on the pulmonary vasculature during critical developmental periods before or just after birth. Alterations in NO production and NO–cGMP signaling are clearly associated with PPHN, although it is not as clear whether they directly produce PPHN. Inhaled NO is now widely used to increase pulmonary blood flow and correct hypoxia in newborns with PPHN, but the clinical response is often absent or not sustained. Other methods to increase cGMP concentrations, such as inhibition of superoxide or cGMP-specific phosphodiesterase (PDE5), may enhance the response to inhaled NO and allow more infants to respond, to respond to lower concentrations of NO, and to sustain their response.

References

Abman, S. H., and Accurso, F. J. (1991). Sustained fetal pulmonary vasodilation with prolonged natriuretic factor and GMP infusions. *Am. J. Physiol.* **260,** H183–H192.

Abman, S. H., Shanley, P. F., and Accurso, F. J. (1989). Failure of postnatal adaptation of the pulmonary circulation after chronic intrauterine pulmonary hypertension in fetal lambs. *J. Clin. Invest.* **83,** 1849–1858.

Abman, S. H., Chatfield, B. A., Hall, S. L., and McMurtry, I. F. (1990). Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. *Am. J. Physiol.* **259,** H1921–H1927.

Abman, S. H., Chatfield, B. A., Rodman, D. M., Hall, S. L., and McMurtry, I. F. (1991). Maturational changes in endothelium-derived relaxing factor activity of ovine pulmonary arteries *in vitro*. *Am. J. Physiol.* **260,** L280–L285.

Banerjee, A., Roman, C., and Heymann, M. A. (1994). Bradykinin receptor blockade does not affect oxygen mediated pulmonary vasodilation in fetal lambs. *Pediatr. Res.* **36,** 474–480.

Black, S. M., Johengen, M. J., Ma, Z. D., Bristow, J., and Soifer, S. J. (1997). Ventilation and oxygenation induce endothelial nitric oxide synthase gene expression in the lungs of fetal lambs. *J. Clin. Invest.* **100,** 1448–1458.

Black, S. M., Johengen, M. J., and Soifer, S. J. (1998a). Coordinated regulation of genes of the nitric oxide and endothelin pathways during the development of pulmonary hypertension in fetal lambs. *Pediatr. Res.* **44,** 821–830.

Black, S. M., Fineman, J. R., Steinhorn, R. H., Bristow, J., and Soifer, S. (1998b). Altered molecular expression of nitric oxide synthase in a lamb model of increased pulmonary blood flow. *Am. J. Physiol.* **275,** H1643–H1651.

Bloch, K. D., Filippov, G., Sanchez, L. S., Nakane, M., and deluMonte, S. M. (1997). Pulmonary soluble guanylate cyclase, a nitric oxide receptor, is increased during the perinatal period. *Am. J. Physiol.* **272,** L400–L406.

Bohn, D., Tamura, M., Perrin, D., Barker, G., and Rabinovitch, M. (1987). Ventilatory predictors of pulmonary hypoplasia in congenital diaphragmatic hernia, confirmed by morphologic assessment. *J. Pediatr.* **111,** 423–431.

Cherry, P. D., Omar, H. A., Farrell, K. A., Stuart, J. S., and Wolin, M. S. (1990). Superoxide anion inhibits cGMP-associated bovine pulmonary arterial relaxation. *Am. J. Physiol.* **259,** H1056–H1062.

Christou, H., Adatia, I., VanMarter, L. J., Kane, J. W., Thompson, J. E., Stark, A. R., Wessel, D. L., and Kourembanas, S. (1997). Effect of inhaled nitric oxide on endothelin-1 and cyclic guanosine 5′-monophosphate plasma concentrations in newborn infants with persistent pulmonary hypertension. *J. Pediatr.* **130,** 603–611.

Crawley, D. E., Zhao, L., Giembycz, M. A., Liu, S., Barnes, P. J., Winter, R. J. D., and Evans, T. W. (1992). Chronic hypoxia impairs soluble guanylyl cyclase-mediated pulmonary arterial relaxation in the rat. *Am. J. Physiol.* **263,** L325–L332.

Cummings, J. J. (1997). Nitric oxide decreases lung liquid production in fetal lambs. *Am. J. Physiol.* **83,** 1538–1544.

D'Angelis, C., Nickerson, P. A., Steinhorn, R. H., and Morin III, F. C. (1998). Heterogeneous distribution of soluble guanylate cyclase in the pulmonary vasculature of the fetal lamb. *Anat. Rec.* **250,** 62–69.