Nitric Oxide in the Pulmonary Vasculature
Matthew P. Coggins, Kenneth D. Bloch

Abstract—Homeostasis in the pulmonary vasculature is maintained by the actions of vasoactive compounds, including nitric oxide (NO). NO is critical for normal development of the pulmonary vasculature and continues to mediate normal vasoregulation in adulthood. Loss of NO bioavailability is one component of the endothelial dysfunction and vascular pathology found in pulmonary hypertension (PH). A broad research effort continues to expand our understanding of the control of NO production and NO signaling and has generated novel theories on the importance of pulmonary NO production in the control of the systemic vasculature. This understanding has led to exciting developments in our ability to treat PH, including inhaled NO and phosphodiesterase inhibitors, and to several promising directions for future therapies using nitric oxide-donor compounds, stimulators of soluble guanylate cyclase, progenitor cells expressing NOS, and NOS gene manipulation.

Key Words: nitric oxide pulmonary vasculature pulmonary hypertension treatment

The low resting tone of the pulmonary circulation is established at birth and is modulated by the balance of vasoconstrictors (endothelin-1, thromboxane A2, and serotonin) and vasodilators (prostacyclin and NO) produced by the pulmonary endothelium (reviewed in1). Vasoconstriction of the pulmonary vasculature in response to acute hypoxia is the clearest divergence from the systemic vasculature, which is characterized by hypoxic vasodilation in the periphery. The acute hypoxic response in the pulmonary bed is thought to be critical for matching ventilation with perfusion. Disruption of the balance of pulmonary vasodilators and vasoconstrictors, for example by environmental stress (eg, prolonged hypoxia) or endothelial dysfunction, can lead to the pulmonary vasoconstriction, vascular smooth muscle cell (VSMC) proliferation, and in situ thrombosis that characterize pulmonary hypertension (PH).

NO is a free radical gas that diffuses from its site of production in the endothelial cell to its target, soluble guanylate cyclase (sGC), in the VSMC. In this classical NO signaling pathway, activation of sGC enhances cyclic guanosine monophosphate (cGMP) production, which in turn mediates vasodilatation. Alternative NO signaling pathways involve the oxidation of NO to nitrite1 or reactions of NO with protein thiols to form S-nitrosothiols (SNOs),4 NO derivatives that can function as vasodilators or as posttranslational modifiers of protein function. Intriguing new theories are founded on the interaction of nitrite and SNOs with hemoglobin (Hb): by exploiting the allosteric nature of Hb within red blood cells (RBCs), the NO signal is transported to the periphery, where its vasodilator potential enables selective delivery of oxygenated blood to hypoxic tissue.
The importance of NO signaling in pulmonary vascular homeostasis and disease has been established by the use of genetically-modified mice (reviewed in5) and models of pulmonary injury and hypertension. The mechanisms regulating NO signaling have been extensively studied revealing an elegant interplay of multiple cell signaling pathways and protein–protein interactions. Moreover, understanding these pathways and interactions provides opportunities to manipulate NO signaling in the treatment of pulmonary vascular diseases, particularly PH.

This review will focus on the mechanisms regulating NO production, the classical and alternative pathways through which NO exerts its effects, the role of NO in pulmonary vascular development and pulmonary vascular physiology, the beneficial and potentially harmful effects of NO on the pulmonary vasculature, and current and emerging treatment options for pulmonary vascular disease related to NO signaling.

**Regulation of NO Production**

The supply of NO is tightly regulated at the level of its synthesis through the oxidation of L-arginine by NO synthases (NOSs), a family of 3 isoenzymes with overlapping patterns of expression. The NOS isoforms are distinguished not only by their sites of expression, but also by the regulation of their activities. NOS3 (endothelial NOS) is expressed in vascular endothelial cells throughout the body (reviewed in6) and is the predominant source of NO production in the pulmonary circulation.7 NOS1 (neuronal NOS) and NOS2 (inducible NOS) are both expressed in airway epithelium and at low levels in the adult in VSMCs. The expression of NOS2 is induced in virtually all pulmonary cells by exposure to endotoxin or inflammatory cytokines.

The elucidation of the crystal structures of the NOS domains and related enzymes has lent a framework for understanding not only the catalytic behavior of the enzyme, but also its varied regulatory interactions (reviewed in8–10). NOS is active only as a homodimer: each subunit contains a reductase domain and an oxygenase domain, separated by a calmodulin-binding sequence. The oxygenase domain is the active site of NO synthesis and has binding sites for heme and L-arginine, as well as the cofactor tetrahydrobiopterin (BH4).

Electron transfer from the reductase domain leads to the activation of molecular oxygen (O2) bound to heme iron. In a 2-step oxygenation of the guanidino-nitrogen of L-arginine, NO and L-citrulline are produced.

NOS dimer assembly is dependent on heme binding and is further stabilized by zinc9 and BH411. In addition, BH4 has a direct role in catalysis by stabilizing the favorable spin state of the Fe(II)-O2 heme intermediate.9 As described in more detail below, this stabilization of the active site of the enzyme prevents “uncoupling” of the enzyme and formation of reactive oxygen species (ROS).

The fine control of NOS3 activity and its ability to respond to agonists and physiological stimuli depend on transcriptional regulation, posttranslational modification, protein–protein interaction, and intracellular localization (reviewed in6,12,13; see also supplemental materials, available online at http://atvb.ahajournals.org).

**Regulation of NO Responsiveness**

Nitric Oxide Signaling via cGMP—The Classical Pathway

The best-described target for NO is sGC, a heterodimer composed of 1α and 1β subunit with a heme group bound by the apposed N-terminal regions of the subunits (reviewed in14). The predominant sGC isoform in the cardiovascular is α1β1. A second sGC isoform, α2β1, is present at low levels in the cardiovascular system and is more abundant in the placenta and the brain. Reaction of NO with the sGC heme (in the ferrous state) induces a conformational change at the catalytic site leading to a several hundred–fold increase in production of cGMP from GTP (reviewed in15). In VSMCs, the effects of cGMP are mediated through activation of its effector proteins—cGMP-dependent protein kinase (PKG; reviewed in16), cGMP-gated ion channels, and cGMP-regulated phosphodiesterases (PDEs) (see figure). cGMP relaxes VSMCs via several mechanisms including hyperpolarization of the cell membrane and inhibition of agonist-induced calcium influx. In addition, cGMP decreases myofilament calcium sensitivity by activating myosin light chain (MLC) phosphatase (MLCP) via PKG-mediated phosphory-
lation. PKG also phosphorylates the GTPase RhoA, inactivating it and preventing the activation of Rho kinase and Rho kinase-dependent inhibition of MLCP.17

The cGMP signal is limited primarily by degradation of the cyclic nucleotide by PDEs (reviewed in18). Several PDEs degrade both cAMP and cGMP, whereas others, such as PDE5, are specific to cGMP. PDE5 is abundant in the pulmonary vasculature in adult mammals.19 PDE5 requires cGMP binding for full activation, and phosphorylation of PDE5 by PKG may serve to stabilize this interaction.

cGMP-Independent Nitric Oxide Signaling—The Alternative Pathway

S-nitrosylation can modulate the function of a broad range of proteins, with a high degree of substrate specificity (reviewed in4,20). For example, in bovine aortic endothelial cells, NOS3 itself is S-nitrosylated at rest.21 Stimulation by vascular endothelial growth factor (VEGF) induces denitrosylation and activation of the enzyme, followed by re-nitrosylation on return to the resting state. In addition to the NO radical, the source of the NO group for S-nitrosylation may be nitrite, other SNOs or NO oxidation products, or metal-NO complexes. In addition to modifying protein function, SNOs also exert NO-like vasodilatory effects (reviewed in22). These stable compounds enable transport of the NO signal at greater distances than would be achieved by simple diffusion of NO gas. A similar paradigm applies for nitrite, an oxidative product of NO that has a longer half-life than NO in tissues and in plasma. The oxidation of plasma NO to nitrite is likely catalyzed by ceruloplasmin in a rapid reaction that competes with the scavenging of NO by Hb.23 Nitrite can then regenerate NO under low pH conditions or in the presence of a reducing enzyme.4 The emerging role of both SNOs and nitrite in pulmonary and respiratory physiology is presented in more detail below.

Role of NO in the Pulmonary Vascular Transition at Birth

The fetal pulmonary circulation is a high-resistance system, enabling blood flow from the placenta to be diverted via the foramen ovale and ductus arteriosus to the systemic circulation. At birth, there is an acute drop in pulmonary vascular resistance facilitating gas exchange in the lungs and closure of the foramen ovale. In animal models, NOS3 protein expression and enzyme activity have been shown to increase in late gestation.24–26 The peak of pulmonary NOS3 expression in the fetal lamb precedes the development of a pulmonary vasodilator response to acetylcholine or increased oxygen tension.26,27 Although the gestational timing of peak NOS3 expression varies slightly among species, it appears to correlate with the timing of accelerated pulmonary angiogenesis and peripheral vessel formation.26 A critical role for NOS3 in pulmonary vascular development is further supported by the observations of Han and colleagues,28 who reported that, although NOS3-deficient mice are viable and have normal litter sizes, pups are frequently cyanotic at birth with a high rate of neonatal loss (40% within 1 hour of birth). A defect in pulmonary angiogenesis in developing NOS3−/− mice is suggested by a decreased density of distal arterial branches and areas of pruning of arterioles with nearly absent capillary perfusion.28 Striking abnormalities in pulmonary vascular development were evident as early as gestational day 13, the time when pulmonary NOS3 gene expression is beginning to increase in the wild-type mouse embryo, and the abnormalities were duplicated by exposing wild-type embryos to the NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME) during mid- to late gestation. The morphological abnormalities are reminiscent of the syndrome of alveolar capillary dysplasia seen in human infants with persistent pulmonary hypertension of the newborn.29

Like NOS3, pulmonary sGC expression undergoes dramatic changes during the transition from the fetal lung to the adult lung. sGC is abundant in VSMCs of the pulmonary resistance vessels of fetal and perinatal animals,30,31 with peak activity in the perinatal period suggesting a role in the acute drop in pulmonary vascular resistance (PVR) after birth. sGC expression and activity decrease in the adult.30,32 Developmental changes in NOS3 and sGC expression in the pulmonary veins also contribute to the regulation of PVR, as highlighted in a review by Gao and Raj.33 Taken together, a larger picture begins to emerge integrating multiple components of the NO/cGMP signal transduction system in pulmonary vascular development in general, and the pulmonary vascular changes associated with the transition from fetal to postnatal physiology.

Role of NO in Pulmonary Vascular Physiology

Early studies of the role of NO in the pulmonary vasculature were based on the use of NOS inhibitors that do not distinguish between NOS isoforms. Our understanding of the contributions of individual NOS isoforms in pulmonary vascular biology has been greatly facilitated by the development of genetically-modified mice deficient in each NOS isoform. For example, deletion of the NOS3 gene in adult mice causes systemic hypertension34 and mild PH without evidence of pulmonary vascular remodeling.35 Fagan and colleagues evaluated the importance of each NOS isoform in the maintenance of pulmonary vasomotor tone: using isolated-perfused lungs from mice deficient in each NOS isoform, these investigators reported that NOS3 has the greatest role in regulating resting tone in the pulmonary circulation.36 Under basal conditions, NOS2 and NOS1 have little or no role in the maintenance of low pulmonary tone. After prolonged hypoxia, however, NOS1 expression is increased in the pulmonary and systemic vascular smooth muscle,7,37 potentially contributing to the impaired endothelium-dependent vasodilation associated with prolonged hypoxia.

Prolonged exposure to low oxygen concentrations induces PH and pulmonary vascular remodeling in animal models and in human beings residing at high altitudes. The clinical applicability of these findings, though, has been questioned because chronic hypoxia is not a common cause of PH in patients.38 Oxygen is required for NOS activity, and NO production is decreased under low oxygen tension. However, the response to hypoxia in vivo is complex, and conflicting data from multiple different models has caused uncertainty as to the importance of NO signaling in modulating the pulmonary vascular response to hypoxia (reviewed in39–41). Hypoxic pulmonary vasoconstriction (HPV) is augmented in NOS3−/− mice.36,42 Several groups reported that prolonged
hypoxia induced greater PH and pulmonary vascular remodelling in NOS3−/− than in wild-type mice, but another group reported the converse.45

In intact mouse and rat models of chronic hypoxia (1 to 3 weeks exposure), pulmonary NOS3 expression has generally been found to increase.7,46,47 However, in a study of pulmonary arteries from rats exposed to 1 week of hypoxia, decreased NO production correlated with tighter association of NOS3 with caveolin-1, decreased calmodulin and hsp90 binding, and decreased Ser177 phosphorylation.48 These results suggest that early hypoxic PH involves protein–protein interactions and posttranslational modifications that favor the inactive state of NOS3.

Attention has also focused on the endogenous NOS inhibitor asymmetrical dimethylarginine (ADMA) (reviewed in49). In a rat model of chronic hypoxic PH (10% inspired O2 for 1 week), an increase in ADMA levels and a decrease in activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme responsible for the degradation of ADMA, correlated with increased pulmonary artery pressure (PAP).50 The role of ADMA in PH of other etiologies has also been investigated, and patients with idiopathic PH were found to have higher plasma ADMA levels than healthy controls.51 A critical role for DDAH in the regulation of pulmonary vascular tone was recently highlighted by Leiper and colleagues, who studied mice heterozygous for a null DDAH1 mutation.52 They observed that DDAH1+/− mice had decreased DDAH activity and increased ADMA levels, associated with an increase in RV systolic pressure (RVSP) and thickened walls of pulmonary arterioles.

In the absence of L-arginine or BH4, NOS3 may become “uncoupled”. Electron transfer across the dimer interface of the enzyme is interrupted, and molecular oxygen becomes the electron recipient, resulting in the generation of superoxide with a concomitant decrease in NO production (reviewed in53). A model of BH4 deficiency is the hph-1 mouse, which contains a homozygous mutation in the gene for GTP cyclohydrolase-I (GTPCH-I), the enzyme that catalyzes the rate-limiting step in BH4 biosynthesis.54 Khoo and colleagues studied mice with varying pulmonary BH4 levels—wild-type, hph-1, and hph-1 mice carrying a transgene directing GTPCH-I expression in endothelium—before and after prolonged exposure to low O2 concentrations.55 Pulmonary BH4 levels correlated inversely with RVSP, and hph-1 mice had decreased pulmonary NOS activity, RV hypertrophy (RVH), and histological evidence of pulmonary vascular remodelling at baseline. Restoration of BH4 levels in hph-1 mice using the GTPCH-I transgene normalized RVSP and attenuated RVH. The pulmonary hypertensive response to chronic hypoxia was exaggerated in hph-1 mice and was essentially abolished in GTPCH-I–overexpressing mice. It is not yet known whether BH4-deficient states represent a significant cause of PH in humans. However, these findings suggest that BH4 metabolism may represent an important target for future pharmaceutical approaches to the treatment of PH.

The role of NO, particularly that produced by the inducible NOS isoform, NOS2, in the pathogenesis of pulmonary injury and pulmonary vascular dysfunction is discussed in the supplemental materials, available online.

The Lung as the Source of Systemic NO

Because of its very short half-life in biological fluids, NO was not thought to be capable of affecting vascular tone remote from its site of production. One of the primary scavengers of free NO is Hb: reaction with the heme iron to form nitrate or iron-nitrosyl adducts reduces circulating NO levels below those required to induce VSMC relaxation. S-nitrosothiols, introduced above, were first described as stable NO derivatives that retain vasodilator properties. Rather than being degraded by Hb, SNOs can undergo a transnitrosylation reaction using the heme iron to form SNO on a conserved cysteine residue of the β subunit of Hb (SNO-Hb) (reviewed in52,56). This reaction is favored in the R (oxygenated) state of hemoglobin, whereas inactivation of NO by binding to heme iron (Hb[FeNO]) predominates in the T (deoxygogenated) state.57 Furthermore, once SNO-Hb is formed, subsequent oxygen desaturation of the Hb molecule with an R to T conformational change results in release of SNO to acceptor thiols.22 It is hypothesized that, through these reactions, red blood cells (RBCs) deliver NO to the periphery in parallel with oxygen delivery.22 In this model, release of SNO from RBCs under low oxygen saturations is responsible for the hypoxic vasodilation of systemic vascular beds, augmenting microvascular blood flow and oxygen delivery. The depleted SNO-Hb appears to be replenished both by transfer of NO from the heme group of Hb[FeNO] on reoxygenation58 and by generation of NO from NOS3 in well-oxygenated pulmonary capillaries and venules.

In the pulmonary arteries, the hypoxic vasodilating effect of RBCs should be in opposition to baseline hypoxic vasoconstriction, thus reducing PVR and potentially impairing ventilation/perfusion (V/Q) matching. In an animal model, McMahon and colleagues demonstrated that enhancing the SNO-Hb content of RBCs caused a reduction in PVR and an increase in arterial pO2, suggesting that the vasodilator role of RBCs tempers hypoxic vasoconstriction (HPV) and even improves V/Q matching.

NO produced in the lung may also exert systemic effects via nitrite, as recently reviewed by Kim-Shapiro and colleagues.2 When infused at near physiological concentrations into the peripheral vasculature, nitrite induces vasodilation.60 Deoxygenated Hb possesses a nitrite reductase activity, which is most efficient when the Hb tetramer is in the R state. Hence, the peak rate for the enzyme is near the p50 of the oxygen-Hb dissociation curve. The highest concentration of nitrite in the vasculature is found in the cytoplasmic fraction of erythrocytes.61 It is hypothesized that nitrite in the plasma and within RBCs is reduced to NO in the periphery, forming Hb[FeNO], subsequently leading to release of NO from the RBC to direct microvascular vasodilation. As noted above, the NO oxidase activity of ceruloplasmin has recently been described, and may represent an important source of nitrite formation.23 Whether this activity of ceruloplasmin is responsive to changes in oxygen concentration has not been defined, though ceruloplasmin gene expression is upregulated by hypoxia in the mouse liver and in hepatoma cell lines.62 The lung is likely not the primary source of nitrite in the blood, and it may accumulate throughout the circulation because of the effects of NO oxidases, in addition to dietary intake.
In view of its pulmonary and systemic effects, NO has been proposed as a “third gas” that is transported by the respiratory system.58 NO exploits the allosteric properties of hemoglobin to enhance O₂ and CO₂ exchange, directing microvascular distribution of flow into hypoxic tissue and away from oxygenated tissue. To a degree, particularly in the SNO-Hb model, the lung functions as the source of vasodilatory NO for the peripheral circulation.

**NO-Based Therapies of Pulmonary Vascular Diseases**

Perhaps the most exciting implication of the research into the roles of NO in the pulmonary vasculature has been the translation of these findings into new therapeutic agents (see Figure). For at least 2 of these agents, inhaled NO (iNO) and PDE5 inhibitors, efficacy has been demonstrated in clinical settings leading to approval by the US Food and Drug Administration (FDA). For the remainder, preclinical studies and in some cases small clinical trials have revealed promising beneficial effects for the treatment of PH or respiratory failure. These novel therapeutic agents have led to new insights into the role of NO in the pulmonary vasculature and have raised important new questions.

**Nitric Oxide Equivalents**

**Inhaled Nitrile Oxide**

Agents that augment NO concentrations in the pulmonary vasculature have been used to treat a variety of pulmonary disorders including PH, acute lung injury (ALI), and the acute respiratory distress syndrome (ARDS). Inhaled NO, delivered by continuous inhalation between 5 to 80 ppm, is perhaps the most widely used agent (reviewed in63,64). NO that reaches the bloodstream is rapidly inactivated by Hb, limiting its vasodilator effects to the pulmonary vascular bed. This pulmonary vascular selectivity allows a decrease in PVR and RV afterload without the attendant fall in systemic vascular resistance associated with most other pulmonary vasodilators. Even within the lung, Hb-mediated NO inactivation is so efficient that NO selectively vasodilates well-ventilated lung units and can reverse V/Q mismatching associated with lung injury improving systemic oxygenation. By contrast, intravenous vasodilators dilate the entire pulmonary vascular bed and can worsen V/Q mismatching and hypoxemia.

In 1999, the FDA approved iNO therapy for term and near-term infants with hypoxic respiratory failure and PH. The primary benefits in randomized trials were improved oxygenation and a decreased need for extracorporeal membrane oxygenation (ECMO), with no significant reduction in mortality (reviewed in65). Although the FDA indication requires evidence of PH, it does not appear to be required for a response to iNO.55 The use of iNO for preterm infants with hypoxic respiratory failure has also been explored. The etiologies of hypoxia and PH in the preterm neonate are different from those in the term infant, as are the options for treatment, and there has been controversy regarding the benefit of iNO in this group (reviewed in66,67). In premature infants, bronchopulmonary dysplasia (BPD) is a frequent cause of respiratory failure and chronic lung disease.68 Clinical trials of iNO have shown a reduction in the risk of BPD and death in premature infants: in 2 trials this benefit was seen,69,70 This benefit was seen in all infants studied whereas in 2 others71,72 it was seen only in those with birth weights over 1000 g. There was also variation among these trials in the timing and duration of therapy. More investigation is required to identify the subgroup of preterm infants that is most likely to benefit from treatment with iNO.

In the treatment of PH in adults, a 30% decrease in PVR during a 10-minute inhalation of iNO at 10 ppm has been validated as a predictor of patients who would benefit from chronic calcium channel blockers.73,74 iNO has been used in the management of acute PH and right ventricular dysfunction complicating right ventricular myocardial infarction,75 implantation of a left ventricular assist device,76 other cardiac surgery,77 and lung transplantation,78 as well as acute chest syndrome79 and vasoocclusive crises80 in sickle cell disease. However, further study is required to determine the full utility of iNO in all of these settings (reviewed in63,64). A common use of iNO is for treatment of hypoxemia associated with ALI or ARDS. Several studies have shown a significant improvement in hypoxemia in ARDS patients treated with iNO between 1 and 80 ppm.63,81–84 Unfortunately, no trial has shown that iNO improves mortality or shortens the duration of mechanical ventilation, findings that are likely attributable to the high mortality of ARDS patients from multisystem organ failure, as well as the diverse underlying pathologies seen in these critically ill patients.

The risks of iNO therapy, including methemoglobinemia, and the appropriate monitoring of its effect have been reviewed elsewhere.64,65 Although iNO given to patients with chronic PH from varied causes has been shown to decrease PVR and improve exercise tolerance,66,87 chronic outpatient therapy with iNO has been limited by expense, mode of delivery, the relatively short duration of action, and the need for close monitoring.

**Ethyl Nitrate**

The inhaled agent ethyl nitrate (ENO₂) is designed to mimic the effect of NO by formation of S-nitrosothiols. This agent appears to have a lower tendency than iNO to generate toxic NO species on reaction with O₂, and a lower risk of methemoglobinemia than that seen in earlier studies using inhaled ethyl nitrite (ENO).88 ENO₂ was shown to lower pulmonary artery pressure and PVR selectively in a model of hypoxia-induced PH, without significant systemic effect.89 Although ENO₂ alone has a potency 100-fold lower than iNO, the addition of glutathione dramatically increased its activity, presumably by enhancing the formation of SNOs.

**Inhaled Nitrite**

By analogy with the known nitrite reductase action of deoxyhemoglobin, inhaled nitrite would be expected to undergo conversion to NO in the presence of deoxyhemoglobin, thus providing a hypoxia-specific vasodilatory effect. Hunter and colleagues compared the hemodynamic effects of inhaled nitrite in animal models of hypoxic and normoxic PH.90 They found that nitrite induced greater pulmonary vasodilation in the presence of hypoxia than under normoxic conditions. In support of its possible therapeutic application, the duration of the reduction in PA pressure after discontinuing nitrite administration was significantly longer than that for iNO.
PDE-Dependent Mechanism
Inhibitors of PDE5 (eg, sildenafil) augment NO/cGMP-mediated vascular relaxation by preventing breakdown of cGMP to GMP, an effect that is dependent on adequate sGC stimulation by NO. Sildenafil was shown to cause a dose-dependent reduction in PVR in an animal model of acute PH without a significant change in systemic vascular resistance, and this effect has also been demonstrated in patients with idiopathic PH. A number of studies have suggested that long-term use of sildenafil in patients with PH is beneficial. The largest of these was a randomized, double-blind, placebo-controlled trial of 278 patients with idiopathic PH or PH attributable to connective tissue disease or systemic to pulmonary shunting treated with sildenafil 20 to 80 mg 3 times daily. Patients had significant improvements in (PAP), WHO functional class, and the distance walked in 6 minutes. Two hundred twenty-two patients followed for 1 year had a sustained improvement in the 6-minute walk distance. In 2005, sildenafil was approved for the treatment of PAH at a dose of 20 mg 3 times daily.

NOS3-Dependent Mechanisms
3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase Inhibitors
As the multiple pathways involved in the regulation of NOS3 have become recognized, attempts have been made to manipulate them for therapeutic effect. The 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitor, simvastatin, was shown to attenuate hypoxic PH and pulmonary vascular remodeling without a change in NOS3 expression. A subsequent study with rats showed that treatment with fluvastatin attenuated the increase in RVSP and RVH, as well as the pulmonary vascular remodeling, induced by prolonged hypoxia. The proposed mechanisms by which HMG-CoA reductase inhibitors attenuate pulmonary vascular remodeling involves NOS3 activation by shifting the binding of NOS3 from its inhibitor caveolin-1 to hsp90, resulting in increased NO synthesis.

NOS Gene Manipulation
Adenoviral gene transfer of NOS3 to rat lungs by inhalation has produced sustained improvement of pulmonary hemodynamics in models of hypoxic PH and in NOS3-deleted animals, without affecting systemic blood pressure. Transfer of the NOS2 gene via inhalation protected rats from hypoxic PH and pulmonary vascular remodeling to a greater extent than did NOS3 gene transfer. The NO pathway may be more amenable to this approach than pathways that depend on intracellular localization of the gene product because diffusion of NO from the airway epithelium to VSMC occurs at a physiologically-relevant rate.

PH research has benefited from the growing interest in the ability of bone marrow–derived progenitor cells to repair endothelial injury (reviewed in99). Infusion of bone marrow endothelial-like progenitor cells (ELPCs) in rats prevents experimental PH after vascular injury and halts the progression of established PH. Bone marrow–derived progenitor cells transduced with the NOS3 gene have been used to treat established PH in 2 different animal protocols, leading to near normalization of RVSP and improvement in pulmonary microvascular perfusion, as well as improved survival. Engraftment of progenitor cells in the pulmonary microvasculature in PH may improve the microvascular remodeling associated with PH either by direct endothelial repair or by release of paracrine signals to stimulate local repair mechanisms. The combination of progenitor cell therapy with supplementation of the NO pathway is a promising approach that appears to focus therapy to the vascular target. Research in viral and cell vectors is improving the tolerability and safety of these techniques, the specificity of gene delivery, and the regulation of gene expression (reviewed in102).

L-Arginine
Attempts at stimulating the endogenous NOS machinery by providing excess L-arginine substrate have had limited success. Short-term infusion or oral supplementation of L-arginine in patients with PH of varied etiologies caused a reduction in PVR. Of note, treatment of normal subjects with L-arginine did not alter pulmonary hemodynamics and was associated with modest reduction in systemic blood pressure. Treating rats with L-arginine before and during exposure to either hypoxia or monocrotaline prevented the development of PH and pulmonary vascular remodeling. Functional L-arginine deficiency in PH may be attributable to elevated ADMA levels, as described above.

sGC Inhibitors and Activators
A variety of agonists and antagonists have been used to explore the interaction of NO and sGC. One early agent was the quinoxalin derivative 1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one (ODQ), an inhibitor of sGC with limited specificity. ODQ competes with NO for binding at the heme group of sGC, irreversibly inhibiting the enzyme by oxidizing the heme iron.

Classical sGC activators, such as organic nitrates, act through their conversion to NO. A new class of drugs has been developed that activate sGC independently of NO. YC-1, an indazole derivative, was the first of these compounds. Its activation of sGC depends on the presence of the reduced heme group (ie, it requires the enzyme to be NO-responsive) and results in “NO sensitization”, an increase in cGMP production for a given NO signal. This sensitization, which has been demonstrated for both NO and CO, is explained by the stabilization of the active conformation of sGC. The sGC sensitizer, BAY 41-2272, holds promise as a therapeutic approach to PH of multiple etiologies. An analog of YC-1, BAY 41-2272 is a more potent NO sensitizer. BAY 41-2272 reduced PAP and PVR in sheep with PH induced by a thromboxane analogue, as well as systemic arterial pressure and vascular resistance. L-NAME abolished the effects of BAY 41-2272 on the systemic vasculature, whereas the pulmonary effects were not diminished. When coadministered with iNO, BAY 41-2272 augmented and prolonged the vasodilator response of iNO.

A second class of compounds, known as sGC-activators, appears to increase the activity of the enzyme in the NO-unresponsive
oxidized state, independent of heme.\textsuperscript{111} The observation that BAY 58-2667 is a more potent stimulator when combined with the inhibitor ODQ suggests that oxidation of the heme group may cause a conformational change in sGC that enhances binding to or activation of the enzyme by BAY 58-2667.\textsuperscript{112}

Both BAY 41-2272 and BAY 58-2667 decrease acute hypoxic vasconstriction in mice.\textsuperscript{113} In both chronic hypoxia-induced PH in mice and monocrotaline-induced PH in rats, oral administration of either agent reduced RVSP. Most impressively, both agents reversed RVH and pulmonary vascular remodeling in both PH models. In NOS3\textsuperscript{+/-} mice subjected to hypoxia, neither agent caused a reduction in RVH, raising the possibility that the antiremodeling effects of these drugs are dependent on endogenous NO production. The clinical appeal of these novel agents is that they obviate the limitations of insufficient NO signal produced by NO donors and, in the case of BAY 58-2667, impaired responsiveness of sGC attributable to oxidative stress. In addition to their therapeutic potential, these agents also offer new tools to investigate the biology of NO-sGC signaling.

**Conclusion**

NO signaling pathways have a vital role in pulmonary vascular physiology and pathophysiology. The apparent participation of NO in the respiratory cycle introduces another fascinating vasoregulatory function for NO produced in the lung, linking it with distant vascular beds. Numerous experimental findings have been translated into current and potential therapies for PH and respiratory failure, while at the same time raising new questions and opening new directions for investigating NO biology.

**Sources of Funding**

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