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## Inhaled Nitric Oxide

### Basic Biology and Clinical Applications

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A REMARKABLY exciting field of research has developed since nitric oxide (NO) was identified in 1987 as a key endothelium-derived relaxing factor (EDRF).<sup>1,2</sup> The awarding of the 1998 Nobel prize in physiology or medicine to three seminal researchers in the field of NO biology provided the most recent evidence for the emerging prominence of this area of study.<sup>3</sup> The understanding of the roles of NO in the cardiovascular, immune, and nervous systems; the isolation and localization of NO synthases (NOS); the manipulation of the genes for NOS, including their cloning and selective transfer or knock-out; and the therapeutic use of inhaled NO gas have revolutionized many fields of physiologic research and are influencing clinical therapy.

Many insights into the mechanisms of action of NO have been gained. Since the reported applications of inhaled NO in the laboratory<sup>4</sup> and in adult patients with primary pulmonary hypertension in 1991,<sup>5</sup> hundreds of studies have been conducted to determine the clinical

applicability of inhaled NO. In subgroups of severely ill and hypoxic children and adults, inhaled NO improves arterial oxygenation and selectively decreases pulmonary arterial hypertension (PAH). NO inhalation therapy, in combination with conventional<sup>6,7</sup> or high-frequency oscillatory ventilation,<sup>8</sup> can reduce the need for extracorporeal membrane oxygenation (ECMO), an expensive and invasive procedure in newborn patients with hypoxic respiratory failure.<sup>6,7</sup> However, it remains uncertain whether NO inhalation improves survival rates in adults or children with severe lung injury.

New applications for NO inhalation have been discovered. Recent studies indicate that inhaled NO may decrease intestinal ischemia-reperfusion injury<sup>9</sup> and may be useful to treat thrombotic disorders.<sup>10,11</sup> By increasing the oxygen affinity of sickle cell hemoglobin,<sup>12</sup> inhaled NO may prevent or treat sickle cell crisis. This article reviews the relevant physiologic effects, therapeutic uses, side-effects, and toxicity of NO inhalation. The first portion of this article concentrates on the chemistry, biochemistry, toxicology, and biology of NO; the second portion summarizes the results of NO inhalation studies to date in experimental settings and the results of clinical studies in newborns, children, and adults.

### Chemistry, Biochemistry, and Toxicology of Nitric Oxide

Nitric oxide is a colorless, almost odorless gas that is slightly soluble in water (2 or 3 mM).<sup>13</sup> Environmental NO arises from combustion processes (e.g., fossil fuel combustion and tobacco smoke) and lightning.<sup>14</sup> Atmospheric concentrations of NO usually range between 10 and 500 parts per billion (ppb), but can exceed 1.5 parts per million (ppm) in areas of heavy traffic.<sup>15</sup> Concentrations of NO produced in the hot cone of a glowing cigarette can reach 1,000 ppm in a 40-ml puff.<sup>16</sup> The Occupational Safety and Health Administration has set 8-h time-weighted average exposure limits in the work-

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place at 25 ppm for NO breathing and at 5 ppm for nitrogen dioxide (NO<sub>2</sub>).<sup>17</sup> Commercially, NO is manufactured from the reaction of sulfur dioxide with nitric acid, from the reaction of sodium nitrite and sulfuric acid, or by the oxidation of ammonia over a platinum catalyst at a high temperature (> 500°C).<sup>18</sup> In an anaerobic environment (*i.e.*, in highly purified nitrogen), NO can be stored for several years.

#### *Reaction of Nitric Oxide with Oxygen*

In the gaseous phase, NO reacts with molecular oxygen to form NO<sub>2</sub>. The conversion rate of NO to NO<sub>2</sub> can be described by the relation

$$-d[\text{NO}]/dt = k \cdot [\text{NO}]_2 \cdot [\text{O}_2]^{19,20}$$

where  $k$  is the rate constant for conversion of NO to NO<sub>2</sub>. The rate constant has been reported to be between  $0.79 \times 10^{-9}$  to  $2.26 \times 10^{-9} \cdot \text{ppm}^{-2} \cdot \text{min}^{-1}$ , dependent on experimental conditions.<sup>19</sup> Approximately half of a 10,000-ppm NO mixture in air is converted into NO<sub>2</sub> within 24 s, whereas 50% of a 10-ppm NO mixture in air is converted into NO<sub>2</sub> within 7 h at 20°C.<sup>21</sup> In aqueous solution, NO<sub>2</sub> decomposes to give equal amounts of nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>).<sup>13</sup>

The pathologic effects of NO<sub>2</sub> inhalation have been studied in various animal species. High levels of inhaled NO<sub>2</sub> (> 10 ppm) induce pulmonary edema, alveolar hemorrhage, changes in the surface tension activities of surfactant, hyperplasia of type 2 alveolar epithelial cells, intrapulmonary accumulation of fibrin, neutrophils, and macrophages, and death.<sup>22,23</sup> Lower inhaled NO<sub>2</sub> concentrations (< 2 ppm) can alter surfactant function, produce alveolar cell hyperplasia, and alter the epithelium of the terminal bronchioles.<sup>24</sup> Inhalation of 2 ppm NO<sub>2</sub> in humans increases alveolar permeability<sup>25</sup> and airway reactivity.<sup>26-28</sup> Inhalation of 0.5-1.5 ppm NO<sub>2</sub> for 9 weeks caused focal degeneration of pulmonary interstitial cells, with mild emphysematous changes, in rats.<sup>29</sup>

#### *Reaction of Nitric Oxide with Superoxide*

Nitric oxide and superoxide (O<sub>2</sub><sup>-</sup>) readily react to form peroxynitrite (OONO<sup>-</sup>) at nearly a diffusion-limited rate.<sup>13</sup> During physiologic conditions, O<sub>2</sub><sup>-</sup> is scavenged by endogenous O<sub>2</sub><sup>-</sup> scavengers (*e.g.*, superoxide dismutase) and formation of OONO<sup>-</sup> is minimal. During pathologic conditions, such as in the presence of increased concentrations of O<sub>2</sub><sup>-</sup> or after O<sub>2</sub><sup>-</sup> scavengers are exhausted, significant concentrations of OONO<sup>-</sup> may be produced.<sup>30</sup> Peroxynitrite directly causes oxidation, peroxidation, and nitration of biologically impor-

tant molecules (*e.g.*, lipids, proteins, DNA; for review articles see Szabo *et al.*<sup>31,32</sup>). The cytotoxic effects of OONO<sup>-</sup> provide protective functions if they are directed by inflammatory cells against invading microorganisms or tumor cells.

An important example of a reaction caused by OONO<sup>-</sup> is the nitration of tyrosine. Tyrosine nitration inhibits tyrosine phosphorylation, alters the dynamics of assembly and disassembly of cytoskeletal proteins, and inhibits tyrosine hydroxylase, thereby reducing dopamine production by neurons and inhibiting cytoskeletal movements of endothelial cells.<sup>31</sup> Nitrotyrosine has been detected in lung tissue sections from patients with lung injury,<sup>33,34</sup> in atherosclerotic lesions,<sup>35,36</sup> and in lungs after ischemia-reperfusion injury.<sup>37</sup>

Exposure of surfactant to high concentrations of OONO<sup>-</sup> *in vitro* reduced its minimum surface tension.<sup>38</sup> Peroxynitrite exposure impaired pulmonary surfactant function, because of peroxidation of surfactant lipids, and decreased the ability of the major hydrophilic surfactant, protein A, to aggregate lipids and act synergistically with other surfactant proteins to reduce the minimum surface tension.<sup>39,40</sup> These changes of surfactant protein A were associated with nitrotyrosine formation.<sup>39</sup> A mixture of surfactant proteins B and C exposed to OONO<sup>-</sup> was incapable of reducing phospholipid minimum surface tension during dynamic compression.<sup>41</sup>

Peroxynitrite can cause cell apoptosis by DNA strand breakage, activation of poly-adenosine-diphosphate-ribosyltransferase and by inhibition of mitochondrial respiratory enzymes.<sup>31,32</sup> Peroxynitrite rapidly reacts with carbon dioxide to form an adduct that participates in nitration and oxidation reactions.<sup>42</sup> Interestingly, in a model of thrombin or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced vascular injury of the rat mesenteric endothelium and in an ischemia-reperfusion model of the rat heart, infusion of OONO<sup>-</sup> significantly reduced neutrophil adhesion to the endothelium and expression of adhesion molecules, suggesting that OONO<sup>-</sup> exerts inhibitory effects on neutrophil adhesion in inflammatory processes.<sup>43</sup>

In summary, OONO<sup>-</sup> is more cytotoxic than NO in a variety of experimental systems,<sup>32</sup> and the balance of NO, O<sub>2</sub><sup>-</sup>, and O<sub>2</sub><sup>-</sup>-OONO<sup>-</sup> scavenging systems determines whether biologically relevant OONO<sup>-</sup> concentrations will occur in tissues.<sup>30</sup>

#### *Reaction of Nitric Oxide with Heme Proteins and Metals*

Nitric oxide binds to intracellular iron and heme-containing proteins. Examples of heme proteins that are

directly affected by NO are oxyhemoglobin, soluble guanylate cyclase (sGC), cyclooxygenase, and cytochrome p450. Guanylate cyclase is stimulated by NO; cyclooxygenase is stimulated by low NO concentrations<sup>44</sup> and inhibited by high NO concentrations.<sup>45</sup> The cytochrome p450 system is inhibited by NO.<sup>46,47</sup> The ratio of rates of uptake and release of NO for ferrous ( $\text{Fe}^{2+}$ ) hemoglobin is  $10^5$ - $10^6$  times larger than for oxygen.<sup>48</sup> Different from other iron-heme ligands, such as carbon monoxide or oxygen, NO can bind with the ferric ( $\text{Fe}^{3+}$ ) and  $\text{Fe}^{2+}$  oxidation state of hemoglobin. The vasodilating effects of NO *in vivo* are limited by its rapid reaction with oxyhemoglobin or oxymyoglobin to form nitrosylhemoglobin or nitrosylmyoglobin. Methemoglobin ( $\text{Fe}^{3+}$  hemoglobin) is produced when the heme iron is oxidized from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  and  $\text{NO}_3^-$  is released.<sup>49</sup> Most of the methemoglobin is reduced back to  $\text{Fe}^{2+}$  hemoglobin by NADH-cytochrome b<sub>5</sub>/cytochrome b<sub>5</sub> methemoglobin reductase within erythrocytes. In addition, reduced glutathione reduces methemoglobin.<sup>50</sup>

#### *Reaction of Nitric Oxide with Thiols*

Nitric oxide can nitrosate thiol groups to form S-nitrosothiols. Common, naturally occurring S-nitrosothiols include S-nitrosocysteine, S-nitrosohomocysteine, and S-nitrosoglutathione.<sup>51</sup> S-nitrosothiols have similar platelet-inhibitory and vasorelaxant activities to NO, which are mediated through guanylate cyclase activation,<sup>52</sup> but which differ in other important physiologic characteristics from gaseous NO.<sup>53-55</sup>

In addition to binding to the iron-heme center of hemoglobin, NO participates in transnitrosation reactions with the sulfhydryl group of hemoglobin to form S-nitrosohemoglobin. Such reactions may serve as important steps in the uptake and distribution of NO in the systemic circulation. *In vivo* analysis reveals that arterial blood samples from normal rats contained larger concentrations of S-nitrosohemoglobin than did venous samples,<sup>56</sup> suggesting that S-nitrosylation is regulated by hemoglobin oxygenation and changes with erythrocyte transit through the lungs. Stamler *et al.*<sup>57</sup> demonstrated that hemoglobin cysteines (Cys $\beta$ 93) participate in the binding and release of NO. When deoxygenated hemoglobin with a high oxygen affinity enters the pulmonary circulation, the affinity of the hemoglobin thiol groups for NO is high and NO uptake occurs. In the peripheral circulation, where oxygenated hemoglobin with a low oxygen affinity releases oxygen to tissues at a low partial pressure of oxygen ( $\text{PO}_2$ ), release of NO is enhanced.

Such localized release of NO permits vasodilation and increased oxygen delivery to occur in tissues with reduced  $\text{PO}_2$ .<sup>57</sup>

#### *Effect of Nitric Oxide on DNA*

Nitric oxide can alter DNA by the formation of mutagenic nitrosamines,<sup>58,59</sup> by direct modification and strand breakage of DNA from the formation of radical nitrogen oxide species (e.g.,  $^-\text{OONO}$ ),<sup>60-62</sup> and by inhibition of enzyme systems that are necessary to repair DNA lesions.<sup>63-65</sup> NO deaminates desoxynucleosides and desoxynucleotides in mammalian cell preparations and in aerobic solutions of nucleic acids<sup>66</sup> and causes dose-dependent DNA strand breakage.<sup>67</sup> In contrast, NO can abate DNA damage caused by xanthine oxidase and  $\text{H}_2\text{O}_2$ .<sup>68-70</sup>

Tumoricidal and tumor-promoting effects of NO have been reported.<sup>65</sup> Nitric oxide derived from the inducible NOS (iNOS) of macrophages, Kupffer cells, natural killer cells, and endothelial cells produces tumoricidal effects against many types of tumors,<sup>71-79</sup> reduces the viability of several tumor cell lines,<sup>80</sup> and inhibits angiogenesis, tumor growth, and metastasis.<sup>81</sup> Transfection of iNOS into metastatic melanoma cells reduces their potential for metastasis.<sup>82</sup> NO inhibits tumor cell adhesion<sup>83</sup> and decreases the metastatic activity of colon cancer cells.<sup>84</sup> In other studies, NO mediates tumor growth through NO-mediated control of angiogenesis and of growth factors.<sup>65,85,86</sup> Wink *et al.*<sup>65</sup> recently concluded that the role of NO in carcinogenesis is multidimensional. Tissues that are exposed for prolonged durations to high NO concentrations in combination with long-term inflammation and production of reactive oxygen species may accumulate mutations caused by the direct or indirect effects of NO. As a tumor develops, NO produced from iNOS can kill tumor cells through cytostatic and cytotoxic activity. As the tumor progresses, NO may inhibit or support angiogenesis, may limit leukocyte infiltration, and may limit metastasis or kill tumor cells through the induction of apoptosis.<sup>65</sup>

#### *Effect of Nitric Oxide on Lipids*

Nitric oxide has contrasting effects on lipids, particularly on the oxidation of low-density lipoproteins in the pathogenesis of atherosclerotic lesions (for review see Rubbo *et al.*<sup>87</sup>). NO inhibits lipid peroxidation by inhibiting radical chain propagation reactions *via* radical-radical reaction with lipid peroxy and alkoxy groups.<sup>87,88</sup> As a ligand to iron (and other transition metals), NO modulates the prooxidant effects of iron

and thereby limits the formation of hydroxyl radicals and iron-dependent electron-transfer reactions.<sup>87</sup> NO inhibits cell and <sup>-</sup>OONO-mediated lipoprotein oxidation in macrophage and endothelial cell systems.<sup>89</sup> However, NO-induced <sup>-</sup>OONO formation can oxidize low-density lipoproteins to potentially atherogenic species.<sup>90,91</sup> The antioxidant *versus* prooxidant outcome of these reactions appears to depend on the relative concentration of the various reactive molecules.<sup>88,92</sup>

#### *Endogenous Nitric Oxide Synthesis*

Nitric oxide synthase catalyzes a partially tetrahydrobiopterin-dependent five-electron oxidation of the terminal guanidino nitrogen of L-arginine.<sup>93</sup> The reaction stoichiometrically consumes oxygen and nicotinamide adenine dinucleotide hydrogen phosphate (NADPH), requires the cofactors flavin adenine dinucleotide, flavin mononucleotide, and calmodulin, and produces L-citrulline and NO. NOS does not produce detectable levels of NO unless superoxide dismutase is present.<sup>94</sup> During conditions of L-arginine depletion, NOS generates O<sub>2</sub><sup>-</sup>.<sup>95,96</sup> NOS is homologous to the cytochrome p450 reductase enzyme containing iron-protoporphyrin IX.<sup>97,98</sup>

Three NOS isoforms have been identified and classified based on the tissue in which they were first identified, the regulation of their activity, and their substrate-inhibitor profile. Constitutive neuronal NOS (nNOS, NOS1) initially was discovered in nerve tissue. Inducible NOS (iNOS, NOS2), a cytokine-inducible isoform, is expressed in a variety of inflammatory cells. Constitutive endothelial NOS (eNOS, NOS3) was originally described in vascular endothelial cells. More recent studies have shown that expression of the constitutive NOS isoforms (nNOS and eNOS) is regulated,<sup>99-102</sup> and that the inducible isoform (iNOS2) is constitutively present without previous stimulation.<sup>103</sup> NOS isoforms are expressed in many different cell types and intracellular organelles, and most cells are able to synthesize NO.<sup>104,105</sup> Altered NOS expression and endogenous NO synthesis have been reported in a large variety of ischemic, traumatic, neoplastic, inflammatory, and infectious diseases.<sup>106-115</sup> In addition to enzymatic generation of NO by NOS isoforms, nonenzymatic formation of NO *in vivo* during reduced and acidotic conditions (*e.g.*, organ ischemia) has been reported<sup>116</sup> and can contribute to NO production during pathologic conditions.

**Neuronal NOS.** Neuronal NOS fulfills a myriad of disparate functions in a wide variety of tissues. In the peripheral nervous system, NO acts as a neurotransmitter, regulating smooth muscle relaxation in the gastroin-

testinal, urogenital, and respiratory tracts *via* nonadrenergic noncholinergic nerves containing nNOS.<sup>114</sup> Neuronal NOS expression is also present in vasodilator nerves that innervate large cerebral vessels.<sup>117</sup> In the central nervous system, NO is essential in neuronal plasticity to modulate information storage in the brain<sup>118</sup> and has effects on brain development, memory function, behavior, and pain perception.<sup>114</sup> In human skeletal muscle, nNOS modulates contractile force, myocyte development, myofiber differentiation, and myotube innervation.<sup>114</sup> Other nNOS expression sites include cardiac nerve terminals that regulate the release of catecholamines in the heart,<sup>119</sup> and the retina, where nNOS is involved in NO production in photoreceptors and bipolar cells.<sup>120</sup>

**Inducible NOS.** Inflammatory cells (*e.g.*, macrophages and granulocytes), among many other cell types, express iNOS in response to a variety of infectious and inflammatory stimuli. Inducible NOS produces effects that are beneficial and critical for survival during important bacterial and parasitic infections (*e.g.*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*) and in the response to inflammation (*e.g.*, decrease of neutrophil adhesion in endotoxemia, increase of wound closure, and neovascularization of wounds), as shown in murine models of congenital iNOS deficiency.<sup>115</sup> In contrast, increased iNOS expression has been associated with the worsening of other infectious diseases (*e.g.*, influenza pneumonitis) and inflammatory states (*e.g.*, endotoxin-induced hypotension, autoimmune vasculitis).<sup>115</sup> Enhanced expression of iNOS and increased vascular NO synthesis and release have been associated with systemic arterial vasodilation and the "low-tone state" in sepsis.<sup>121</sup>

**Endothelial NOS.** The 1998 Nobel prize in physiology or medicine was awarded to three researchers who discovered endothelium-derived relaxing factor and demonstrated that NO, generated from eNOS in vascular endothelial cells, is endothelium-derived relaxing factor.<sup>1,2,122</sup> Endothelial NOS activity is increased by acetylcholine, bradykinin, and other mediators that increase intracellular calcium concentrations.<sup>122</sup> Endothelial NOS activity modulates systemic<sup>122</sup> and pulmonary vascular tone<sup>123</sup> and plays important roles in lung development and disease. Endothelial NOS expression in the fetal lung changes with lung maturation.<sup>124,125</sup> NO production and eNOS expression by endothelial cells is increased by vascular shear stress.<sup>126,127</sup> For example, pulmonary vascular eNOS expression is reduced in patients with chronic pulmonary hypertension.<sup>128</sup> Congenital absence of eNOS in mice results in pulmonary hypertension and

increased right ventricular remodeling if the mouse is stressed by long-term hypoxic breathing.<sup>129</sup> Endothelial NOS can be reversibly inhibited by NO<sup>130</sup> and eNOS expression can be upregulated by cyclic guanosine monophosphate (cGMP).<sup>102</sup> In cardiac endothelial cells, eNOS activity inhibits contractile tone and proliferation of the underlying vascular smooth muscle cells, reduces platelet aggregation and monocyte adhesion, promotes diastolic relaxation, and decreases the oxygen consumption rate of cardiac muscle.<sup>119</sup> Endothelial NOS is constitutively expressed in cardiac myocytes, where its activity opposes the inotropic action of catecholamines after muscarinic cholinergic or  $\beta$ -adrenergic receptor stimulation.<sup>119</sup>

#### Smooth Muscle Relaxation by Inhaled NO

Soluble guanylate cyclase (sGC) mediates many of the biologic effects of NO and is responsible for conversion of guanosine-5'-triphosphate to cGMP (fig. 1). Cyclic 3':5' GMP is an important second messenger in a variety of cell types and is found in the cytosol of almost all mammalian cells. A variety of nitrovasodilators (e.g., nitroglycerin, sodium nitroprusside) stimulate cGMP synthesis, which, in turn, is responsible for the smooth muscle relaxation mediated by these drugs.<sup>131</sup> The common mechanism of action of these drugs is attributed to the release of NO.<sup>132</sup>

Soluble guanylate cyclase is a heme-containing protein composed of an  $\alpha$  and  $\beta$  subunit. The heme moiety in sGC is essential for activation of the enzyme. The presence of heme results in a 100-fold increase of enzyme activity after stimulation with NO, whereas basal enzyme activity is low without heme and does not change with the addition of NO.<sup>133</sup> Effects of cGMP on vascular tone, cardiac function, and intestinal water and ion transport by protein kinase-dependent and -independent mechanisms have been reviewed in detail by others.<sup>134,135</sup>

The physiologic action of cGMP is limited by its hydrolysis to GMP by a family of cyclic nucleotide phosphodiesterases. Of the seven known phosphodiesterase isozymes, phosphodiesterases 1 and 5 hydrolyze cGMP. Phosphodiesterase 1 catalyzes cyclic adenosine monophosphate and cGMP hydrolysis and is found in high concentrations in the brain, the heart, the lung, and the testis. Phosphodiesterase 5 is cGMP specific and has been found in lung tissue, platelets, vascular smooth muscle, and the kidney. It has a high affinity for cGMP and can be inhibited by the selective phosphodiesterase 5 inhibitors zaprinast, sildenafil, and dipyridamole. Inhibition of phosphodiesterase 5 enhances endothelium-

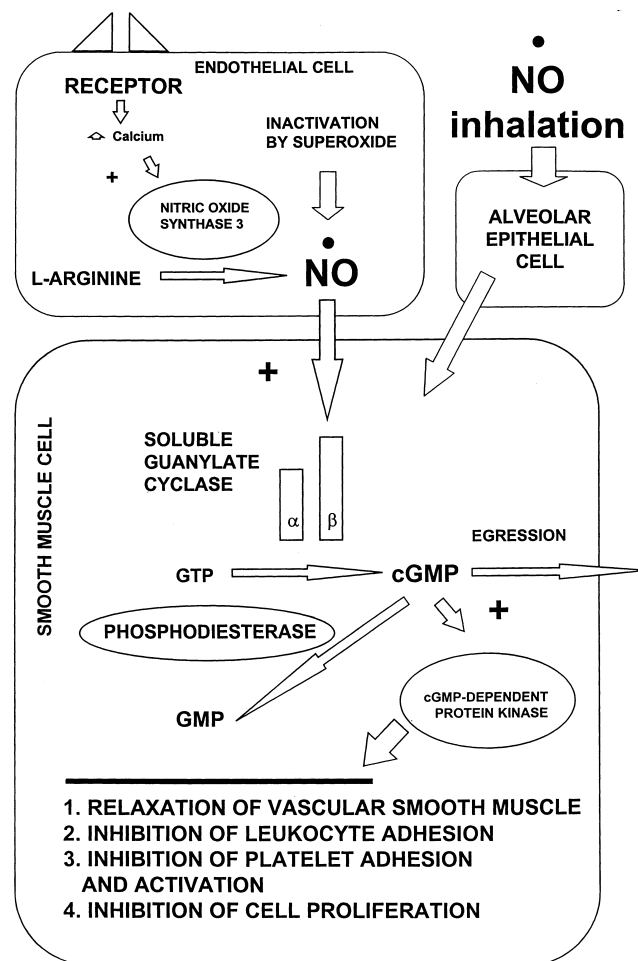


Fig. 1. Nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signal transduction pathway. NO, formed by endothelial cells (left) or administered by inhalation (right), diffuses to vascular smooth muscle cells (lower). NO activates soluble guanylate cyclase, which in turn catalyzes the production of cGMP. Through cGMP-dependent protein kinase mediated effects, increased intracellular concentration of cGMP relaxes smooth muscle and inhibits leukocyte adhesion, platelet adhesion, and cellular proliferation. The action of cGMP is limited by phosphodiesterases, which convert cGMP to GMP.

dependent vasorelaxation, reduces pulmonary vascular tone, and enhances the hypotensive effects of nitrovasodilators.<sup>136,137</sup>

## Physiology of Inhaled Nitric Oxide Therapy

### Selective Pulmonary Vasodilation

**Alveolar Hypoxia.** The ability of NO to selectively dilate the pulmonary vasculature was evaluated in an awake lamb model of alveolar hypoxia. Alveolar hypoxia

## NITRIC OXIDE INHALATION

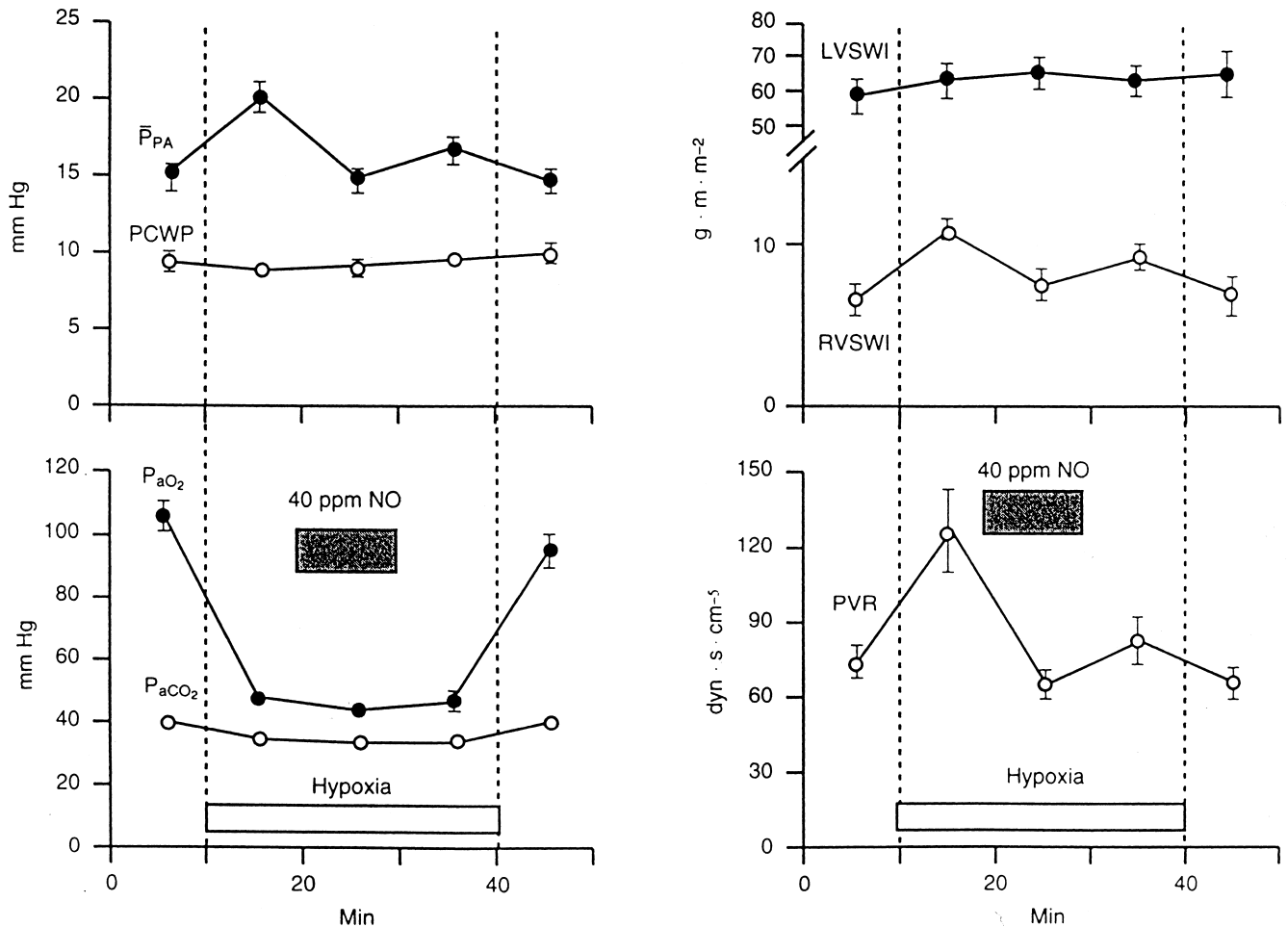


Fig. 2. Physiologic effects of inhaled NO (40 ppm) during hypoxia in nine healthy volunteers. Note the decrease of mean pulmonary artery pressure and pulmonary vascular resistance achieved by NO inhalation. Values are mean  $\pm$  SE. LVSWI = left ventricular stroke work index;  $P_{aO_2}$  = arterial partial pressure of oxygen,  $P_{aCO_2}$  = arterial partial pressure of carbon dioxide; PCWP = pulmonary capillary wedge pressure; PPA = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; RVSWI = right ventricular stroke work index. Reprinted with permission from Frostell *et al.*<sup>139</sup>

produces reversible pulmonary vasoconstriction mediated by an unknown mechanism. During normoxia, inhalation of 80 ppm NO did not alter the normally low mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR). With alveolar hypoxia (inspired fraction of oxygen [ $F_{I_{O_2}}$ ], 0.1), pulmonary vasoconstriction increased MPAP from 17 mmHg to 28 mmHg. With NO inhalation (40 ppm), MPAP decreased to 20 mmHg and further to 18 mmHg (80 ppm NO) within 3 min of NO breathing.<sup>4</sup> Cardiac output and systemic arterial pressure were not affected by NO inhalation. These results were confirmed in mechanically ventilated sheep,<sup>138</sup> in awake healthy volunteers breathing low oxygen concentrations at ambient pressure<sup>139</sup>

(fig. 2), and in volunteers at high altitude (at 4,559 m, hypobaric hypoxia).<sup>140</sup>

#### Pulmonary Selectivity and Vascular Sites of Vasodilation

The pulmonary selectivity of inhaled NO and its rapid inactivation by hemoglobin was first evaluated in an isolated perfused rabbit lung model. The effluent of the perfused lung was conducted to an isolated, pharmacologically precontracted segment of aorta. The pulmonary vasculature was then precontracted with U46619, a thromboxane analog. When the perfusate was a hemoglobin-free aqueous buffer, inhalation of NO first decreased MPAP and subsequently the tone of the sequen-

tially perfused aorta. When erythrocytes were added to the perfusate, inhaled NO still caused pulmonary vasodilation, but its vasodilatory effect on the effluent-perfused aorta was abolished,<sup>141</sup> suggesting that inhaled NO was inactivated by contact with hemoglobin. The pulmonary vascular selectivity of NO inhalation (*i.e.*, pulmonary vasodilation in the absence of systemic arterial vasodilation) has been confirmed in numerous subsequent studies.<sup>142-147</sup>

The longitudinal effects of inhaled NO within the pulmonary vasculature are important because increases of arterial or venous vascular tone differentially influence hydrostatic fluid exchange within the lung. Pulmonary venoconstriction increases pulmonary capillary pressure and promotes edema formation.<sup>148,149</sup> If venous and arteriolar constriction both contribute to increased PVR, a vasodilator selectively acting on arterial tone may worsen edema formation by increasing mean pulmonary vascular surface area and pressure. Lindeborg *et al.*<sup>150</sup> reported that inhalation of 5, 20, and 80 ppm NO decreased arterial, microvascular, and venous resistances to the same extent in an isolated rabbit lung model. Shirai *et al.*<sup>151</sup> used an X-ray television system to visualize the *in vivo* effects of NO inhalation on the internal diameter of pulmonary arteries and veins in a feline model. Inhaling 5-40 ppm NO caused a dose-dependent increase of the diameter of small arteries and veins during normoxic conditions. After induction of hypoxic vasoconstriction by lobar anoxia, NO inhalation dilated smaller constricted and larger nonconstricted arteries, as well as veins. These results suggest that the pulmonary vasodilator response to inhaled NO is similar in pulmonary arteries and veins.

#### *Selective Vasodilation of Ventilated Areas*

The intrapulmonary distribution of blood flow and ventilation (ventilation-perfusion [ $\dot{V}/\dot{Q}$ ] distribution) is a major determinant of transpulmonary oxygenation effectiveness, and the resulting partial pressure of oxygen in arterial blood ( $P_{aO_2}$ ). In a normal, healthy lung, ventilated areas are well perfused. The shunt from the right to the left side of the circulation is mainly extrapulmonary (*e.g.*, bronchial veins) and is less than 5-8% of cardiac output.<sup>152</sup> Local alveolar hypoxia constricts the vascular bed adjacent to hypoxic regions and redistributes blood flow to lung regions with better ventilation and a higher intraalveolar  $PO_2$ . It has been proposed that inhaled NO amplifies this mechanism by increasing blood flow through well-ventilated lung areas. Pison *et al.*<sup>138</sup> studied the effects of inhaled NO on distribution in an ovine

model of acute hypoxia. Because they studied generalized alveolar hypoxia, no improvement of  $P_{aO_2}$  during NO inhalation was expected. However, they demonstrated increased blood flow to better ventilated (but still hypoxic) lung areas and a stable  $P_{aO_2}$  during NO inhalation.

The effects of NO inhalation on gas exchange have been assessed using lung injury models that induce mismatch. The mismatch induced by oleic acid injury in sheep was significantly improved by inhalation of 40 ppm NO<sup>153</sup> and was augmented by the simultaneous use of continuous positive airway pressure to open collapsed alveoli.<sup>154</sup> Hopkins *et al.*<sup>155</sup> studied the effects of inhaled NO on gas exchange in dogs by selectively creating areas of shunt or areas with a low ratio. NO (80 ppm) decreased blood flow to shunting regions. In areas with a mismatch, NO produced an inconsistent response. When the PVR of the partially obstructed airway regions was decreased by NO inhalation, inequality was increased because blood flow to the relatively poorly ventilated areas was increased by vasodilation. When NO did not reach the lung regions distal to the partial obstruction, and thus did not reduce local PVR, matching was improved.

#### *Bronchodilator Action*

Nitric oxide synthase inhibitors suppress the bronchodilator actions of nonadrenergic noncholinergic-mediated bronchodilation, suggesting that endogenous NO synthesis is involved in the control of bronchial tone.<sup>156,157</sup> The expression of various NOS isoforms in peripheral nonadrenergic noncholinergic nerve endings<sup>158</sup> and in human bronchial epithelium<sup>159</sup> supports this finding. Dupuy *et al.*<sup>160</sup> demonstrated that inhaled NO decreased airway resistance after bronchoconstriction with methacholine in guinea pigs, later confirmed in various experimental models using rabbits,<sup>161,162</sup> dogs,<sup>163-165</sup> and pigs.<sup>166,167</sup> In human volunteers, however, inhaled NO only reduced airway resistance minimally after a methacholine challenge.<sup>168</sup> Large airways appeared to be preferentially dilated by inhaled NO.<sup>169</sup>

#### *Pulmonary Surfactant*

Surfactant synthesized by type 2 alveolar epithelial cells affects lung mechanics by reducing surface tension, modifies pulmonary gas exchange, and has antimicrobial functions.<sup>40</sup> Isolated type 2 alveolar epithelial cells exposed to NO (generated by the NO donor drugs S-nitroso-N-penicillamine, spermine NONOate, or 3-morpholino-sydnominine) in the presence of superoxide

dismutase reduced their surfactant synthesis by approximately 60%.<sup>170</sup> Exposure of surfactant to NO *ex vivo* was not associated with changes of surface activity.<sup>38</sup> *In vivo*, a combination of high inspired oxygen concentrations and high inspired NO concentrations (100 ppm) inhaled by newborn piglets for 48 h significantly decreased the minimum surface tension of surfactant recovered by bronchoalveolar lavage.<sup>38</sup>

In lambs, high inhaled NO concentrations (80–200 ppm) resulted in abnormal surface activities and inhibition of surfactant protein A lipid aggregation.<sup>171</sup> In addition, NO (from NO donor drugs) can decrease surfactant protein A gene expression by distal respiratory epithelial cells.<sup>172</sup> In contrast, in an experimental model of acute lung injury, combining exogenous surfactant therapy with inhaled NO improved ventilation–perfusion matching and arterial oxygenation.<sup>173</sup> Other interactions of NO and surfactant were recently summarized by Hallman and Bry.<sup>40</sup>

#### *Metabolic Fate of Inhaled Nitric Oxide*

Nitric oxide is inactivated by reaction with its biologic target molecules. The half-life of NO *in vivo* is only a few seconds. The main metabolic pathways are the binding of NO to O<sub>2</sub><sup>-</sup> and to the heme iron of hemoglobin with the subsequent release of NO<sub>3</sub><sup>-</sup>. The binding and release of NO to thiols presents another important metabolic pathway. Approximately 90% of NO is absorbed during a steady state inhalation. Almost 70% of the inhaled gas appears within 48 h as NO<sub>3</sub><sup>-</sup> in the urine.<sup>174</sup> The remaining 30% of inhaled NO is recovered as NO<sub>2</sub><sup>-</sup> in the oral cavity through secretion from salivary glands. NO<sub>2</sub><sup>-</sup> is also partly converted to nitrogen gas in the stomach and some NO<sub>2</sub><sup>-</sup> in the intestine is reduced to ammonia, reabsorbed, and converted to urea.<sup>175,176</sup>

### **NO Inhalation in Experimental Acute Lung Injury and Pulmonary Artery Hypertension**

#### *Models of Persistent Pulmonary Hypertension of the Newborn and Respiratory Distress Syndrome*

Hypoxia in the preterm and term newborn is usually characterized by severe PAH, extrapulmonary right-to-left shunting, hypoxemia, and acidosis. Roberts *et al.*<sup>177</sup> studied the effects of inhaled NO in hypoxic and acidotic term newborn lambs delivered by cesarean section. Hypoxia associated with hypercapnia doubled PVR. In this model, inhaling 20 ppm NO during hypoxia completely abolished pulmonary vasoconstriction, despite the pres-

ence of a marked respiratory acidosis. Similar results were obtained in hypoxic, mechanically ventilated late-gestation ovine fetuses.<sup>178</sup> In an experimental model of persistent pulmonary hypertension of the newborn (PPHN) in lambs (induced by ductal ligation), inhaled NO decreased PVR and markedly increased survival rates.<sup>179,180</sup>

The responsiveness of the premature lung to inhaled NO depends on gestational age and the maturity of the pulmonary vasculature. In the immature lung of the ovine fetus at 0.78 of term, initiation of mechanical ventilation caused maximal pulmonary vasodilation and the addition of NO (20 ppm) or 100% oxygen did not increase vasodilation further. If surfactant was administered and mechanical ventilation at 0.78 term continued with 100% oxygen for 2 h, PVR continued to increase and the initial beneficial effect of mechanical ventilation on PVR and oxygenation decreased. With initiation of NO inhalation 2 h after commencing mechanical ventilation, PVR was again decreased and oxygenation improved.<sup>181</sup> At 0.86 of term, initiation of mechanical ventilation caused pulmonary vasodilation, which was further increased by NO inhalation but not by 100% oxygen. Near term (0.96), NO inhalation and 100% oxygen administration both further increased the pulmonary vasodilation caused by mechanical ventilation.<sup>182</sup>

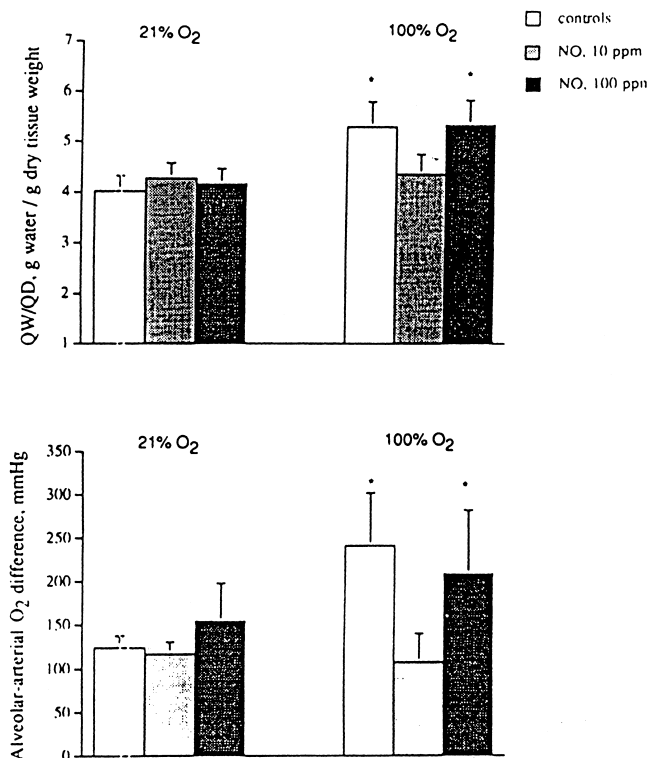
#### *Models of Acute Pulmonary Artery Hypertension and Lung Injury in Adult Animals*

Selective pulmonary vasodilation during NO inhalation has been shown in numerous animal models: after pharmacologic precontraction of the pulmonary vasculature with U46619, a synthetic thromboxane analog<sup>4</sup>; after a heparin–protamine reaction that induces thromboxane-mediated pulmonary vasoconstriction<sup>183</sup>; after pulmonary oleic acid instillation, which induces endothelial and alveolar edema, cell necrosis, and PAH<sup>184,185</sup>; and after bilateral lung lavage, which depletes surfactant.<sup>186</sup> Inhaled NO is also an effective pulmonary vasodilator in endotoxin-induced PAH<sup>187–189</sup> and after smoke inhalation injury.<sup>190</sup>

#### *Lung Injury Induced by Neutrophil-derived Oxidants and by Molecular Oxygen*

Reactive oxygen species (*e.g.*, H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>) promote lung injury in various clinical settings.<sup>191–194</sup> The use of high inspired concentrations of oxygen is sometimes necessary during the treatment of acute lung injury, but these high oxygen concentrations may cause or worsen lung injury.<sup>195,196</sup> Thus, the effects of NO inhalation





**Fig. 3.** Effects of 100% oxygen exposure with or without inhalation of nitric oxide (NO; 10 and 100 ppm) on pulmonary edema in rats. (Upper) After 40 h of oxygen exposure, the increase of the wet to dry ratio (QW/QD) was prevented by inhalation of 10 ppm NO, but not by 100 ppm NO. (Lower) The increase of the alveolar-arterial oxygen difference was also prevented by inhalation of 10 ppm NO, but not by 100 ppm NO. Data are mean  $\pm$  SD of six rats in each group. Reprinted with permission from Garat *et al.*<sup>200</sup>

during lung injury caused by neutrophil oxidants or oxygen are of particular interest. Reaction of NO with oxygen or O<sub>2</sub><sup>-</sup> results in NO<sub>2</sub> or <sup>-</sup>OONO formation, which may damage the lung.<sup>33,34,96</sup> In contrast, NO may also protect against the cellular toxicity of H<sub>2</sub>O<sub>2</sub>, alkyl peroxides, and O<sub>2</sub><sup>-</sup>.<sup>197</sup>

Data from isolated lung studies suggest that the net physiologic effects of NO inhalation protects against tissue injury by typical neutrophil oxidants. In buffer-perfused isolated rabbit lungs, NO inhalation decreased H<sub>2</sub>O<sub>2</sub>-induced pulmonary edema, pulmonary vascular permeability,<sup>198</sup> and edema formation after injury with O<sub>2</sub><sup>-</sup> (generated by the reaction of purine with xanthine oxidase).<sup>199</sup> In hyperoxic lung injury, low-dose (10 ppm) but not high-dose (100 ppm) inhaled NO completely prevented pulmonary edema caused by inhalation of 100% oxygen for 40 h (fig. 3).<sup>200</sup> In rats exposed for 60 h to 95% oxygen, pulmonary endothelial permeability,

protein transfer, and type I alveolar epithelial cell injury were attenuated by inhalation of 20 ppm NO.<sup>201</sup> *In vitro*, however, using isolated microvascular endothelial cells and alveolar epithelial cells, simultaneous exposure to a NO donor drug and hyperoxia was associated with earlier cell death, as compared to hyperoxia alone. These *in vitro* findings have been related to an increased production of <sup>-</sup>OONO.<sup>202</sup>

#### *Inhibition of Neutrophil Adhesion by NO Inhalation*

Migration and adherence of neutrophils to the pulmonary vasculature and the local release of their oxidants are believed to be key events in oxidant lung injury.<sup>203,204</sup> The effects of inhaled NO on neutrophil activity and neutrophil-endothelium interactions, in addition to direct effects of their oxidizing products, have been studied extensively. NO inhalation reduced pulmonary neutrophil accumulation after intestinal ischemia-reperfusion injury in rats<sup>205</sup> and after dialysis in pigs.<sup>206</sup> In an isolated rat lung model perfused with a mixture of human neutrophils and either N-formyl-L-methionyl-L-leucyl-L-phenylalanine<sup>207</sup> or interleukin 1,<sup>208</sup> inhalation of 50 ppm NO markedly decreased lung edema formation, neutrophil accumulation and neutrophil migration from the vascular into the alveolar space. Similarly, inhaled NO reduced pulmonary leukocyte sequestration in premature lambs with severe respiratory distress.<sup>209</sup> In an *in vivo* porcine model of *Pseudomonas aeruginosa* sepsis, inhalation of NO (20 ppm) for 5 h after bacterial infusion had significant beneficial effects on pulmonary neutrophil sequestration and neutrophil oxidant activity. Inhaled NO reduced protein and neutrophil sequestration into the alveolar space. Neutrophil oxidant activity (stimulated O<sub>2</sub><sup>-</sup> production) and alveolar structural damage (assessed by electron microscopy) were also reduced in these septic lungs treated with inhaled NO.<sup>210</sup> However, opposing effects also have been reported. Increased oxidant activity (production of O<sub>2</sub><sup>-</sup> and <sup>-</sup>OONO) from intraalveolar neutrophils and increased protein sequestration into the alveolar space after NO inhalation have been observed.<sup>211</sup> In a rat model of intratracheally administered endotoxin, inhaled NO (15 ppm) failed to prevent neutrophil sequestration and activation when inhalation was commenced 8 h after endotoxin challenge.<sup>212</sup> One important difference in these studies was the time point when NO inhalation was begun; early NO inhalation (with respect to bacterial or endotoxin challenge) appeared to be associated with more effective inhibition of neutrophil activation and

sequestration than did NO inhalation begun at a later time.

The effects of inhaled NO on pulmonary neutrophil sequestration may be mediated by modification of adhesion molecule expression and inhibition of the adherence of stimulated neutrophils to the endothelium and their migration through endothelial cell layers. *In vitro* studies report that expression of a variety of endothelial and neutrophil adhesion molecules in response to inflammatory stimuli or ischemia-reperfusion injury is modified by molecular NO, NO donor drugs, or the inhibition of endogenous NO synthesis.<sup>213-217</sup> NO also scavenges O<sub>2</sub><sup>-</sup> released from migrating neutrophils and thereby reduces neutrophil oxidant activity after adherence.<sup>218,219</sup> Recent *in vitro* data showed a dose-dependent effect of NO on neutrophils: exposure of isolated human neutrophils to an environment of 80% oxygen and 20 ppm NO increased cell death by DNA inhibition, whereas 5 ppm NO did not induce significant DNA fragmentation.<sup>220</sup> Whether this apoptotic effect is significant *in vivo* is unknown.

#### *High-altitude Pulmonary Edema*

Severe (hypoxic) pulmonary vasoconstriction and hypertension characterize high-altitude pulmonary edema (HAPE). Scherrer *et al.*<sup>140</sup> hypothesized that inhalation of NO would reduce MPAP and thus the severity of HAPE. NO (40 ppm), inhaled at high altitude (4,559 m), decreased MPAP both in subjects prone to HAPE and those with HAPE, but not in HAPE-resistant control subjects. In subjects with HAPE, perfusion scintigraphy showed that inhaled NO redistributed pulmonary blood flow from edematous to nonedematous lung regions. This was associated with an improved PaO<sub>2</sub>.

#### *Models of Prolonged Hypoxia*

Prolonged exposure to hypoxia induces PAH, pulmonary vascular wall remodeling with neomuscularization, and right ventricular hypertrophy.<sup>221,222</sup> Inhaled NO (10–20 ppm), added while breathing at FiO<sub>2</sub> 0.1 for 2–3 weeks, effectively prevented PAH, pulmonary vascular remodeling, and right ventricular hypertrophy in adult rats,<sup>223,224</sup> in newborn rats,<sup>225</sup> and in wild-type and eNOS-deficient mice.<sup>129</sup> These salutary effects of inhaled NO may be mediated by direct vasodilatory mechanisms in the pulmonary vasculature and antiproliferative effects of NO on smooth muscle cells.<sup>226</sup>

#### *Models of Pulmonary Embolism and Thrombosis*

Because inhaled NO can reduce reactivity and adhesion of circulating blood cells (*e.g.*, leukocytes, thrombocytes), it has been hypothesized that thrombus formation may be decreased by inhaled NO. In a rat model of collagen-induced pulmonary thrombosis, inhalation of 80 ppm NO reduced the MPAP increase associated with collagen injection and inhibited *ex vivo* collagen-induced platelet aggregation. Rats treated with inhaled NO showed fewer platelet thrombi in small pulmonary vessels and a higher residual circulating platelet count.<sup>11</sup> In an *in vivo* porcine model of microsphere-induced pulmonary embolism, inhaled NO (5–80 ppm) reduced the increase of MPAP and increased the end-tidal carbon dioxide concentration. Platelet aggregation was increased with pulmonary embolism in control animals. Inhaled NO decreased the initial and maximum platelet aggregation.<sup>227</sup>

#### *Experimental Models of Lung Transplantation*

A beneficial effect of NO inhalation on ischemia-reperfusion injury, graft function, PAH, and oxygenation after lung transplantation has been reported in various experimental studies.<sup>228-232</sup> The shortage of suitable donor lungs allows only a small percentage of potential recipients to receive a lung transplant.<sup>233,234</sup> It has been suggested to harvest donor lungs from non-heart-beating donors to increase the number of lungs available for transplantation.<sup>235,236</sup> Bacha *et al.* studied whether NO inhalation can improve the function of lungs harvested after cardiac arrest in the donor. In pig and rat models, they treated the donor (after cardiac arrest) and recipient with inhaled NO (30 ppm) and demonstrated a significant improvement of oxygenation and short-term graft survival after transplantation, as well as reduction of PAH and decreased pulmonary neutrophil accumulation.<sup>237,238</sup>

### **Systemic Effects of Nitric Oxide Inhalation**

#### *Bleeding Time*

Nitric oxide stimulates cGMP formation in platelets and, thus, NO inhalation may inhibit platelet function and augment a bleeding tendency in some species. Höglman *et al.*<sup>239</sup> reported that the bleeding time increased after rabbits inhaled NO. They noted that breathing 30 ppm NO for 15 min increased the bleeding time from 51 ± 5 to 72 ± 7 s (mean ± SE), and breathing 300 ppm NO for 15 min increased the bleeding time from 48 ± 12

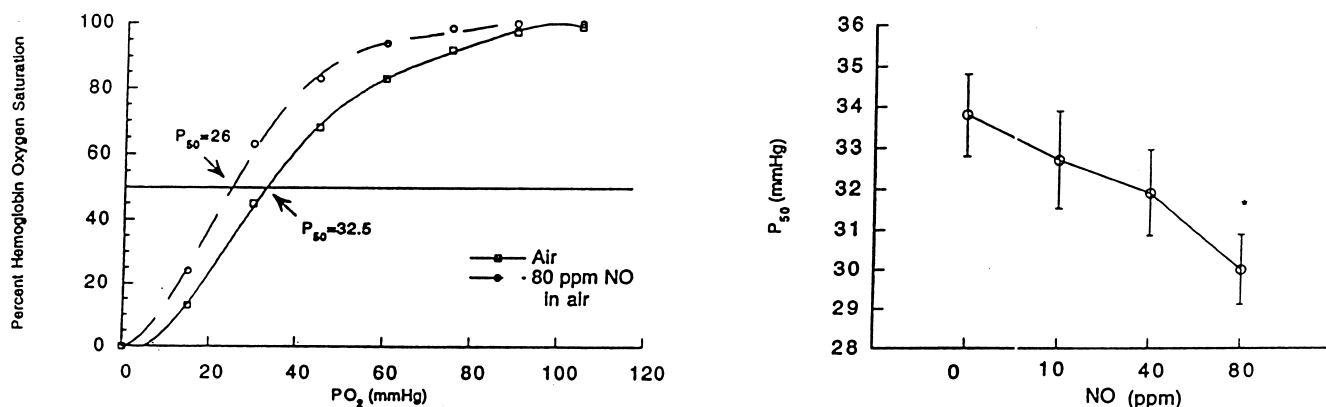


Fig. 4. Effects of nitric oxide (NO) on oxygen affinity of erythrocytes. Exposure to NO (80 ppm for 15 min) shifted the oxygen dissociation curve of hemoglobin S erythrocytes to the left (*left*). The effect of NO exposure on  $P_{50}$  of hemoglobin S erythrocytes was dose-dependent (*right*). Values are mean  $\pm$  SE. Reprinted with permission from Head *et al.*<sup>12</sup> by copyright permission of The American Society for Clinical Investigation.

to  $78 \pm 17$  s. However, bleeding time was not altered in rats breathing 80 ppm NO for 1 h<sup>240</sup> or in dogs breathing 20–200 ppm NO for 45 min.<sup>10</sup> The reason for these differences among species is unknown.

#### Vascular Injury in the Systemic Circulation

Inhaled NO may affect the systemic vasculature and cells circulating within the systemic circulation, possibly by reversibly binding to hemoglobin<sup>57</sup> or other proteins, with subsequent transport and release of NO at distant sites, or by modification of leukocytes and platelets during their transit through the lung. In a rat model of carotid injury, which is associated with migration and proliferation of smooth muscle cells in the arterial intima, inhalation of 80 ppm for 2 weeks decreased the degree of neointimal formation.<sup>240</sup> In a canine model of coronary artery thrombosis and lysis, NO inhalation decreased the cyclic flow variation frequency and increased the duration of periods of coronary artery patency. In the latter model, inhaled NO had no vasodilatory effects on the pharmacologically precontracted coronary artery segment and did not change the bleeding time. Therefore, coronary patency after thrombolysis was increased by NO inhalation, independent of direct vasodilatory activity or increased bleeding time.<sup>10</sup> In contrast to pulmonary vasodilation, these actions of inhaled NO cannot be mediated by any direct effects of gaseous NO on smooth muscle cell relaxation.

Further insights into the mechanisms of the systemic effects of NO inhalation were recently provided by Fox-Robichaud *et al.*<sup>9</sup> In a cat model of intestinal ischemia and reperfusion, inhalation of 80 ppm attenuated the

reduction of perfusion, increase of leukocyte rolling, adhesion and migration, and endothelial dysfunction. Changes in leukocyte activity and vessel size were directly visualized by *in vivo* microscopy and were induced by inhalation of 80 ppm but not 20 ppm NO. These effects were independent of intrapulmonary modification of leukocyte adhesion molecules, suggesting that inhaled NO was bound to transport molecules and was released in the peripheral circulation.<sup>9</sup>

#### Sickle Cell Hemoglobin

Homozygous sickle cell anemia is a genetic disease characterized by severe hemolytic anemia, frequent vasoocclusive events, and a reduced life expectancy. A single amino acid substitution from valine to glutamic acid of the hemoglobin  $\beta$  chain results in hemoglobin S (HbS) formation. At deoxygenation, an erythrocyte containing HbS changes its shape from a biconcave disk to a crescent sickle cell because of intracellular hemoglobin polymerization. Sick cells can occlude the microcirculation.<sup>241–243</sup>

Hemoglobin S has a markedly decreased oxygen affinity, measured as a markedly increased  $P_{50}$  (partial pressure of oxygen at half saturation of hemoglobin), compared with adult hemoglobin. In studies by Head *et al.*,<sup>12</sup> inhalation of 80 ppm NO for 45 min by patients with homozygous sickle cell disease shifted the oxygen dissociation curve of their erythrocytes  $4.6 \pm 2.0$  mmHg to the left, significantly decreasing the  $P_{50}$  (fig. 4). Methemoglobin concentrations did not increase substantially. In five of seven volunteers with sickle cell disease, the effect persisted for at least 60 min after discontinuing

## NITRIC OXIDE INHALATION

**Table 1. Multicenter Trials of Inhaled Nitric Oxide in Patients With PPHN**

Reference	Year	No. of Patients	Inclusion Criteria	Treatment Protocol	Length	Outcome
6	1997	58	PPHN (by echo) Pa <sub>O<sub>2</sub></sub> < 55 mmHg on 2 consecutive measurements	80 ppm at Fi <sub>O<sub>2</sub></sub> 0.9 vs. Fi <sub>O<sub>2</sub></sub> 0.9 (control)	Up to 14 days	Responders: 53% in NO group; 7% in control group Need for ECMO: 40% NO group; 70% control group Survival: 93% in NO and control groups
7	1997	235	Hypoxic respiratory failure, PPHN Requiring mechanical ventilation OI > 25 on 2 consecutive measurements	20 and 80 ppm NO vs. control (Fi <sub>O<sub>2</sub></sub> 1.0)	Up to 14 days	Responders: 51% 20 ppm NO; 15% (control) Need for ECMO: 39% NO group; 55% control group Survival: 86% NO group; 84% control group
249	1997	53	Congenital diaphragmatic hernia (PPHN in 51 of 53 patients) OI > 25 on 2 consecutive measurements	20 and 80 ppm NO vs. control (Fi <sub>O<sub>2</sub></sub> 1.0)	Up to 14 days	Responders: 48% 20 ppm NO; 19% control Need for ECMO: 80% NO group; 54% control group Survival: 52% NO group; 57% control group
8	1997	205	PPHN (by echo) Pa <sub>O<sub>2</sub></sub> < 80 mmHg at Fi <sub>O<sub>2</sub></sub> 1.0	NO (20, 40 ppm) vs. HFOV Crossover and combination	24 h reported	Overall response rate 60% All responders survived 72% of nonresponders treated with ECMO survived Overall survival 86%

PPHN = persistent pulmonary hypertension of the newborn; OI = oxygenation index; NO = nitric oxide; HFOV = high-frequency oscillatory ventilation; ECMO = extracorporeal membrane oxygenation.

NO.<sup>12</sup> In normal volunteers, the P<sub>50</sub> was not affected by breathing NO.<sup>12</sup> Precisely how inhaled NO alters sickle hemoglobin is unknown. One hypothesis is that the Cysβ93 residue of HbS is modified by NO, increasing HbS solubility and decreasing the tendency to polymerize during deoxygenation.<sup>244,245</sup>

### Clinical Studies of Nitric Oxide Inhalation

The first clinical studies of inhaled NO focused on whether the physiologic effects measured in animal models were reproducible in patients. Acute respiratory distress syndrome (ARDS) and persistent PPHN have been the most commonly studied clinical syndromes. The results of large multicenter studies of NO inhalation in the treatment of critically ill newborns and adults recently have been reported. Inhaled NO also has been tested clinically in other conditions, including chronic PAH, chronic obstructive pulmonary disease (COPD), and lung transplantation and heart surgery.

### Respiratory Failure of the Newborn

**PPHN and Hypoxic Respiratory Failure.** Persistent pulmonary hypertension of the newborn is a clinical syndrome characterized by sustained pulmonary hypertension and severe hypoxemia, resulting in cyanosis unresponsive to oxygen therapy. Persistent pulmonary hypertension of the newborn may be caused by a variety of etiologies (e.g., aspiration) or can be idiopathic.<sup>246</sup> Diagnostic confirmation of PPHN includes echocardiographic observation of a right-to-left shunt through the ductus arteriosus or foramen ovale, caused by increased PVR, in the absence of congenital heart disease. Conventional treatment strategies include breathing high inspired concentrations of oxygen, hyperventilation, and infusion of bicarbonate to produce alkalosis, inhalation treatments with bovine surfactant, and intravenous vasodilator therapy. ECMO may be used to treat hypoxemia. However, the anticoagulation and cannulation of large vessels required for ECMO is associated with hemorrhagic complications.

**Table 2. Short-term Effects of Inhaled Nitric Oxide on Systemic Oxygenation in Infants with Severe Hypoxemia and Persistent Pulmonary Hypertension**

	Control*	Nitric Oxide†
Postductal Pa <sub>O</sub> <sub>2</sub> (mmHg)		
Baseline	38 ± 9	41 ± 9
Treatment	40 ± 8	89 ± 70‡

Values are mean ± SD.

\* Nitrogen at F<sub>I</sub>O<sub>2</sub> 0.9, n = 28.

† 80 ppm at F<sub>I</sub>O<sub>2</sub> 0.9 for 20 min, n = 30.

‡ P < 0.001 vs. baseline.

Data reprinted with permission from Roberts *et al.*<sup>6</sup>

In 1992, Roberts *et al.*<sup>247</sup> and Kinsella *et al.*<sup>248</sup> reported that 80 ppm<sup>247</sup> or 6–20 ppm<sup>248</sup> inhaled NO improved oxygenation in patients with PPHN. Several large controlled, randomized multicenter trials of the effects of inhaled NO in near-term and term hypoxic newborn patients were reported in 1997 (tables 1 and 2).<sup>6–8,249,250</sup> In the majority of patients with PPHN and hypoxic respiratory failure (in whom the decision 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), NO improved oxygenation and decreased the requirement for ECMO. Kinsella *et al.* reported that NO inhalation and high-frequency oscillatory ventilation were an effective combination that may increase the rate of responsiveness to inhaled NO.<sup>8</sup> In a 1- to 2-yr follow-up study of children who received inhaled NO treatment for PPHN, neurodevelopment scores, growth rates (growth percentiles for weight, length, and occipitofrontal circumference), the frequency of airway disease, and the need for supplemental oxygen were comparable to conventionally ventilated or ECMO-treated patients.<sup>251</sup> In summary, NO improved oxygenation in many newborns and, although it did not change overall survival, it reduced the need for ECMO (*P* < 0.05).<sup>6,7</sup> Inhaled NO therapy did not appear to impart any benefits, however, to newborns with congenital diaphragmatic hernia.<sup>249</sup>

**Preterm Neonates with Respiratory Distress Syndrome.** Respiratory distress syndrome (RDS), or hyaline membrane disease, of the premature newborn is characterized by deficiency or dysfunction of surfactant and is often associated with acute PAH.<sup>181</sup> After promising preliminary studies of inhaled NO in the premature newborn with RDS,<sup>252,253</sup> Skimming *et al.*<sup>254</sup> studied the effect of inhaled NO at 5 and 20 ppm in preterm neonates (without systemic hypotension or congenital mal-

formations and mechanically ventilated at F<sub>I</sub>O<sub>2</sub> > 0.5). They demonstrated that arterial oxygenation improved and systemic arterial blood pressure was unaffected during a 15 min NO inhalation trial. The conclusions of this study were limited, however, because MPAP was not measured, and only 7% of the initially evaluated premature infants were included in the study.

### *Acute Lung Injury and Acute Respiratory Distress Syndrome*

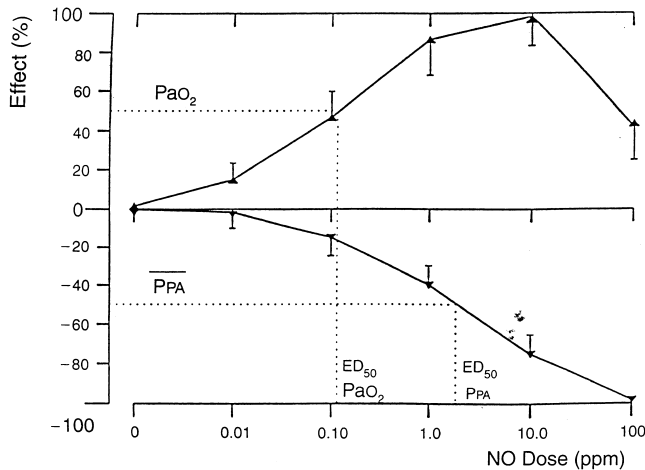
**Selective Pulmonary Vasodilation.** In severe ARDS, PAH augments pulmonary edema and may impede right ventricular function and decrease cardiac output. Ros-saint *et al.*<sup>144</sup> demonstrated in patients with severe ARDS that inhaled NO produced selective pulmonary vasodilation. This was later confirmed by larger studies.<sup>142,147</sup> Occasionally, the NO-induced pulmonary vasodilation has been associated with improved right ventricular performance, as indicated by improvements in right ventricular ejection fraction and decreased right ventricular end-diastolic and end-systolic volumes.<sup>255</sup> In children with ARDS, inhaled NO (20 ppm) decreased MPAP by 25% and increased cardiac index by 14%.<sup>256</sup> Inhaled NO also effectively decreased MPAP associated with the use of permissive hypercapnia in patients with ARDS.<sup>257</sup>

**Pulmonary Capillary Pressure.** Inhaled NO (40 ppm) has been reported to decrease pulmonary capillary pressure<sup>258</sup> and pulmonary transvascular albumin flux,<sup>259</sup> partly caused by its effect on venous PVR in patients with acute lung injury.<sup>258</sup> Such reductions of pulmonary venous and pulmonary capillary pressure should promote resolution of pulmonary edema, an important component of ARDS.

**Arterial Oxygenation.** Severe hypoxemia caused by extensive intrapulmonary right-to-left shunting is characteristic of ARDS. Common current strategies of management include lung recruitment by high levels of positive end-expiratory pressure, prone positioning, and ventilation with a high F<sub>I</sub>O<sub>2</sub>. Therapies that permit lower airway pressures and F<sub>I</sub>O<sub>2</sub> might reduce the risk of barotrauma and oxidant injury to the lung. Inhaling 18 ppm NO for 40 min reduced the shunt fraction by 5% and increased the Pa<sub>O</sub><sub>2</sub>/F<sub>I</sub>O<sub>2</sub> ratio by 30% in patients with ARDS.<sup>144</sup> In a dose-ranging study of ARDS patients breathing NO, the ED<sub>50</sub> (the dose producing 50% of maximal effect) for increasing Pa<sub>O</sub><sub>2</sub> (10–100 ppb) was markedly less than the ED<sub>50</sub> producing pulmonary vasodilation (1–10 ppm, fig. 5).<sup>260</sup>

In a phase 2 multicenter trial, the effects of NO inhalation on oxygenation were studied in 177 patients who

## NITRIC OXIDE INHALATION



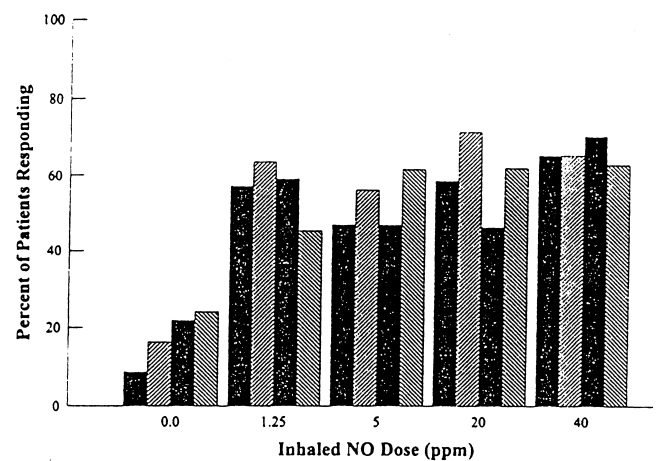
**Fig. 5.** Dose-response of inhaled nitric oxide (NO) for  $\text{Pa}_{\text{O}_2}$  (upper) and mean pulmonary arterial pressure (lower) in 12 patients with acute respiratory distress syndrome. The estimated  $\text{ED}_{50}$  for  $\text{Pa}_{\text{O}_2}$  increase was 110 ppb and the estimated  $\text{ED}_{50}$  for mean pulmonary artery pressure ( $\text{P}_{\text{PA}}$ ) decrease was 1.2 ppm. Values are mean  $\pm$  SD. Reprinted with permission from Gerlach *et al.*<sup>260</sup>

met the criteria of ARDS ( $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2} < 200$  mmHg within the last 72 h, bilateral chest infiltrates, pulmonary capillary wedge pressure (PCWP)  $< 18$  mmHg, positive end-expiratory pressure requirement  $> 8$  cm  $\text{H}_2\text{O}$  and  $\text{Fi}_{\text{O}_2}$  requirement  $> 0.5$ ). Sixty-five percent of the patients who received inhaled NO (pooled results of patients receiving 1.25, 5, 20, 40, 40, or 80 ppm NO) had a significant ( $P = 0.0002$  vs. placebo) improvement in  $\text{Pa}_{\text{O}_2}$  (defined as a 20% increase of  $\text{Pa}_{\text{O}_2}$  after 4 h of therapy). Only 24% of the patients receiving placebo (nitrogen) responded similarly (fig. 6).<sup>142</sup> The improved oxygenation induced by NO allowed physicians to reduce  $\text{Fi}_{\text{O}_2}$  and positive end-expiratory pressure and thereby decreased the oxygenation index ( $\text{Fi}_{\text{O}_2} \times \text{mean airway pressure} \times 100/\text{Pa}_{\text{O}_2}$ ) for the first 4 days of therapy. The MPAP was slightly lower in the inhaled NO group, compared with placebo, for 2 days. Similar transient improvements of  $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$  during inhaled NO therapy in patients with ARDS have been shown in prospective studies conducted by Michael *et al.*<sup>261</sup> and Troncy *et al.*<sup>262</sup> The reasons for such transient effects remain unclear.

**Outcome.** The phase 2 U.S. multicenter study of the effects of inhaled NO on ARDS patients (discussed previously) reported a mortality rate of 30% in NO- (all doses pooled) and placebo-treated patients both.<sup>142</sup> The patients were assigned randomly to receive 1.25, 5, 20, 40, or 80 ppm NO or nitrogen placebo. Subgroups receiving the same dose of NO for the treatment period consisted

of 8–34 patients. Mortality rates in the subgroups were 32% (7 of 22 patients) in the 1.25 ppm group, 24% (8 of 24 patients) in the 5 ppm group, 31% (9 of 29 patients) in the 20 ppm group, 30% (8 of 27 patients) in the 40 ppm group, and 38% (3 of 8 patients) in the 80 ppm group. A prospective randomized study reported by Troncy *et al.*<sup>262</sup> similarly found no significant difference between ARDS patients receiving inhaled NO and controls with regard to 30-day mortality or days of mechanical ventilation. Several hypotheses should be addressed in the interpretation of these data and should be kept in mind for the design of future studies:

1. The beneficial effects of NO inhalation (*e.g.*, improvement of gas exchange) may not alter the overall outcome because the survival of patients with ARDS may not be primarily dependent on gas exchange. The majority of patients dying with ARDS also have severe sepsis or multiple organ failure. The incidence of death primarily because of respiratory failure varies among studies. In a study by Montgomery *et al.* published in 1985, the percentage of ARDS deaths specifically because of hypoxemia and respiratory failure was reported to be 16%.<sup>263</sup> A study of patients treated at the LDS Hospital in Salt Lake City reported that 40% of deaths in ARDS patients were caused by respiratory failure.<sup>264</sup> It is unknown whether this subgroup of patients would benefit from inhaled NO because such severely hypoxemic patients have been excluded from prospective studies.



**Fig. 6.** Percentage of patients with acute respiratory distress syndrome who respond with a  $\text{Pa}_{\text{O}_2}$  increase of 20% or more while receiving 0–40 ppm nitric oxide during a 4-h inhalation period. Bars from left to right within each dose group indicate progressive exposure periods of 30 min, 1 h, 2 h, and 4 h. Reprinted with permission from Dellinger *et al.*<sup>142</sup>

2. The beneficial effects of NO inhalation are offset by its toxic effects. As described previously, NO has several negative effects on biologic molecules and tissues, especially if  $\text{^-OONO}$  is formed. This hypothesis needs further testing in patients, *e.g.*, by analyzing lung specimens for evidence of NO or  $\text{^-OONO}$  toxicity after NO inhalation.
3. NO inhalation has a narrow therapeutic range. Small doses, *e.g.*, 1–5 ppm, could be effective and improve survival, but smaller doses might be not effective, and larger doses could be toxic. The effective dose may change over time and vary among different patients and different disease states.<sup>265</sup> It is necessary to establish improved dosing criteria. Larger patient groups receiving similar doses are necessary to discover statistically robust differences. Currently, a blinded, multicenter phase 3 study is being completed that investigated the effects of 5 ppm inhaled NO, compared with placebo, in patients with ARDS. Such a protocol assumes that the dose–response relation of inhaled NO is similar among ARDS patients and over time. This may not be true.<sup>265</sup>

Performing such trials is difficult and expensive. The incidence of ARDS is relatively low, and the precipitating events are often multifactorial. Usually a large number of centers must participate to recruit sufficient numbers of patients. There may be significant differences in response rates and outcomes among different centers, as reported for PPHN patients by Kinsella *et al.*<sup>8</sup> Different treatment strategies and the experiences of individual institutions and caregivers may provide confounding variables. As a result, conclusive studies evaluating the effects of a drug such as inhaled NO, which at best has a modest effect on the survival of a diverse population of patients with ARDS, may not be economically viable.

#### *Chronic Pulmonary Artery Hypertension*

The pathophysiology of chronic PAH includes a partially reversible increase of MPAP in the early stages of the disease, leading to a nonreactive and irreversibly remodeled pulmonary vasculature after long-standing PAH.<sup>266</sup> Vascular remodeling is characterized by muscularization of previously nonmuscular small resistance arteries, medial hypertrophy of proximal pulmonary arteries, and a reduced number of arteries within the lung. Diagnosis and medical treatment of chronic PAH relies on vasodilator therapy. When medical treatment is no longer possible (*e.g.*, no vasodilator response) or ineffec-

tive (*e.g.*, tachyphylaxis or tolerance), lung transplantation remains as the last option to prolong life.

**Evaluation.** The determination of pulmonary vascular responsiveness is essential for prognosis and long-term treatment. Drugs commonly used to assess pulmonary vasodilatory responses include intravenous prostacyclin ( $\text{PGI}_2$ ), adenosine, and calcium channel blockers.<sup>267</sup> After studying adult patients with inhaled NO and infused prostacyclin, Sitbon *et al.* suggested using inhaled NO as the “gold-standard” to assess pulmonary vasoreactivity<sup>268</sup> because inhaled NO was reported to selectively reduce MPAP in patients with pulmonary hypertension.<sup>5</sup> A recent survey of long-term vasodilator treatment in approximately 800 patients with primary pulmonary hypertension reported that inhaled NO was used as the primary vasodilator to test pulmonary vascular responsiveness by 32% of the participating U.S. tertiary hospitals.<sup>269</sup>

As opposed to commonly used intravenous vasodilators, which can produce systemic hypotension, inhaled NO does not significantly affect systemic vascular resistance. This permits the rapid and safe evaluation of changes of biventricular function during a brief trial of pulmonary vasodilation and, therefore, provides an important diagnostic tool for the decision to begin medical treatment or to plan lung transplantation or combined heart and lung transplantation.<sup>270</sup>

**Treatment.** In chronic PAH, a positive response to a short-term vasodilator trial usually results in long-term drug treatment that may include a wide spectrum of systemic vasodilator drugs (*e.g.*, acetylcholine,  $\alpha$ -adrenergic agonists, direct-acting vasodilators, angiotensin-converting enzyme inhibitors, calcium channel blockers, prostaglandins) and permanent anticoagulation.<sup>267</sup> The effectiveness of current vasodilator treatment often is limited by systemic hypotension. Channick *et al.*<sup>271</sup> tested an ambulatory NO delivery system consisting of an 80-ppm NO tank, a gas-pulsing device, and a nasal cannula in eight PAH patients with a pulmonary artery catheter. They reported that this technique produced effective pulmonary vasodilation without evidence of significant nitrogen dioxide formation. One of the eight patients was discharged from the hospital and treated for 9 months with inhaled NO without any apparent adverse events. Long-term domiciliary NO inhalation as an alternative or a bridge to lung transplantation<sup>272</sup> requires investigation in larger patient groups, after consideration of the beneficial and toxic effects caused by long-term NO inhalation.

### *Obstructive Airway Disease*

Inhaled NO has been tested for use as a pulmonary vasodilator in COPD and as a bronchodilator in COPD and asthma. COPD is characterized by irreversible airway obstruction and is associated with irregular enlargement of alveoli and destruction of alveolar walls after chronic inflammation. Hypoxia produces pulmonary vasoconstriction, resulting in chronic PAH and right ventricular hypertrophy. Important characteristics of asthma include inflamed, hyperreactive airways with reversible bronchoconstriction.

**Bronchodilator Action.** Inhaled NO has been reported to be a bronchodilator in many experimental animal models. Högman *et al.*<sup>169</sup> evaluated inhalation of 80 ppm NO in healthy volunteers, in patients with hyperreactive airways, bronchial asthma, and COPD. Inhaled NO caused mild bronchodilation in patients with asthma but not in patients with COPD. In other studies, however, the bronchodilator action of NO has been reported to be much weaker than commonly used inhaled  $\beta_2$ -adrenergic agonists.<sup>168,273,274</sup>

**Pulmonary Vasodilation.** Hypoxemia in COPD is primarily caused by a mismatch and not by intrapulmonary right-to-left shunting (as in ARDS). Hypoxic vasoconstriction augments blood flow to better ventilated regions and improves oxygenation. Inhaled NO may oppose this physiologically useful mechanism by vasodilating poorly ventilated areas in the obstructed lung, and thus increasing blood flow to these areas, as reported by Hopkins *et al.*<sup>155</sup> Indeed, transcutaneous arterial oxygen tension,<sup>275</sup>  $\text{Pa}_{\text{O}_2}$ , and  $\dot{V}/\dot{Q}$  distribution<sup>276</sup> were worsened by NO inhalation in air-breathing COPD patients. However, when NO was used in combination with modest oxygen enrichment,<sup>277</sup>  $\text{Pa}_{\text{O}_2}$  was improved to a greater extent than with oxygen therapy alone. The combination also more effectively decreased MPAP. Thus, the combined use of supplemental oxygen and inhaled NO (*e.g.*, *via* an ambulatory inhalation device) may offer a valuable therapeutic strategy for improving oxygenation and providing pulmonary vasodilation in selected COPD patients.

### *Lung Transplantation*

Pulmonary artery hypertension frequently occurs in the immediate postoperative period after lung transplantation and has been effectively treated with inhaled NO.<sup>278</sup> Inhaled NO has been reported to be effective in the treatment of post-lung transplant pulmonary dysfunction. In a retrospective study by Date *et al.*,<sup>279</sup> 243 patients undergoing lung transplantation over 6 yr were

analyzed. Thirty-two patients had immediate severe graft dysfunction, as indicated by a  $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$  ratio  $< 150$  mmHg. Comparing patients in whom NO treatment was not available with patients in whom NO treatment was begun after graft dysfunction was diagnosed, inhaled NO reduced MPAP and increased the  $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$  ratio within the first hour of treatment. The requirement for ECMO was similar in both patient groups. The rate of airway complications and hospital mortality (7% in NO group *vs.* 24% in control group) was markedly reduced in patients receiving NO therapy. As in all retrospective studies using historical controls, changes of treatment strategies and increased clinical experience over time must be considered. Although this study suggests that inhaled NO may decrease post-lung transplant organ dysfunction, it should be confirmed in prospective controlled studies.

### *Congenital Heart Disease*

The degree and reversibility of the increased PVR determine the various treatment options and outcome in children with congenital heart disease and PAH.<sup>280</sup> Roberts *et al.*<sup>281</sup> demonstrated that inhaled NO (80 ppm for 10 min) decreased MPAP without causing systemic vasodilation in children between 3 months and 7 yr of age with congenital cardiac lesions (*e.g.*, atrioseptal defect, ventricular septal defect, atrioventricular canal). The ability of inhaled NO to decrease the PVR of children with congenital cardiac defects has been confirmed by others.<sup>282,283</sup> In preoperative patients with severe right-to-left shunting, inhaled NO increased pulmonary blood flow, decreased extrapulmonary shunt flow and improved oxygenation.<sup>284</sup> Inhaled NO, therefore, might provide a therapeutically useful tool for the acute non-surgical treatment of these patients.

### *Cardiac Surgery*

Transient PAH is common after repair of congenital cardiac lesions and has been related to damage to the pulmonary vascular endothelium, probably induced by the use of cardiopulmonary bypass.<sup>285</sup> Inhaled NO has been reported to ameliorate the postoperative PAH of congenital heart disease<sup>286-291</sup> and decrease the need for postoperative ECMO.<sup>292</sup>

Patients with left ventricular valvular disease may have preoperative PAH caused by an increased left atrial pressure with retrograde transmission of pressure into the pulmonary arterial circulation. Pulmonary vascular remodeling occurs as a result of chronic pulmonary venous hypertension and PAH. Pulmonary vascular remodel-



eling and vasoconstriction may persist or slowly decrease with time after valve replacement. Treatment with NO after repair of the valvular disease might relieve the vasoconstrictor component of PAH in these patients.

Fullerton *et al.*<sup>293</sup> reported that 20–40 ppm inhaled NO produced pulmonary vasodilation in patients after aortocoronary bypass. In patients after heart transplantation, inhalation of 20 ppm NO caused significant pulmonary vasodilation but also decreased systemic vascular resistance.<sup>294</sup> The decreased SVR was most likely secondary to an improved cardiac output because systemic arterial pressure and PCWP remained unchanged. Intravenous administration of the phosphodiesterase inhibitor dipyridamole markedly augmented the pulmonary vasodilatory response to inhaled NO in some patients after aortic or mitral valve replacement.<sup>295</sup>

### Effectiveness of Nitric Oxide Inhalation Therapy

#### *Hyporesponsiveness to Inhaled Nitric Oxide*

A considerable number of patients who receive inhaled NO therapy do not respond by either pulmonary vasodilation or improvement of systemic oxygenation. The reported rate of hyporesponders ranges from 30% to 45%, depending on the threshold value chosen to define hyporesponsiveness.<sup>142,147</sup> Several hypotheses have been raised to explain the mechanisms of hyporesponsiveness. Manktelow *et al.*<sup>147</sup> and Krafft *et al.*<sup>296</sup> reported that ARDS patients with sepsis were less likely to respond to inhaled NO (60–70% of septic patients were hyporesponders). The presence of high levels of endogenously produced NO and the opposing pulmonary vasoconstrictor action of catecholamines used for the treatment of septic vasodilation have been suggested as possible reasons for the decreased response to inhaled NO. Holzmann *et al.*<sup>297</sup> evaluated the effects of sepsis on NO responsiveness in an isolated rat lung model. They reported that hyporesponsiveness was associated with decreased pulmonary cGMP release (table 3), suggesting that signal transduction in the NO response pathway is downregulated in sepsis. This was attributed to increased phosphodiesterase activity and therefore increased cGMP breakdown. Bigatello *et al.* reported that hyporesponsive patients with ARDS have a reduced accumulation of plasma cGMP during NO breathing.<sup>298</sup>

Increased vascular production of O<sub>2</sub><sup>-</sup>, as observed in systemic nitrate tolerance, also may contribute to hyporesponsiveness to inhaled NO. Munzel *et al.* reported

**Table 3. Inhaled Nitric Oxide–stimulated Pulmonary cGMP Release in Control and Septic Isolated Perfused Rat Lungs (treated with LPS) before and after Ventilation with 40 ppm Nitric Oxide**

	Control	LPS
Total perfusate cGMP (pmol)		
Before NO	25 ± 5	25 ± 3
NO	190 ± 67*	52 ± 25*†

Values are mean ± SD. Data reprinted with permission from Holzmann *et al.*<sup>297</sup>

NO = nitric oxide; cGMP = cyclic guanosine monophosphate; LPS = lipopolysaccharide.

\*  $P < 0.05$  vs. before NO.

†  $P < 0.05$  vs. control NO.

that long-term nitrate treatment of rabbits resulted in increased O<sub>2</sub><sup>-</sup> production by the aorta and hyporesponsiveness (tolerance) to acute nitroglycerin administration, related to an activated membrane-associated oxidase.<sup>299,300</sup> Increased O<sub>2</sub><sup>-</sup> production in the pulmonary vasculature may have similar effects.

Lastly, Weimann *et al.*<sup>301</sup> recently reported a linkage between ABO blood group distribution and hyporesponsiveness to inhaled NO, demonstrating that ARDS patients with the major blood groups A or O had a larger increase of PaO<sub>2</sub>/FiO<sub>2</sub> in response to NO inhalation than patients with blood groups B or AB. The underlying mechanism of these results is unknown, but they indicate that the pulmonary vascular response to inhaled NO may be determined or modified by genetic factors.

#### *Strategies to Increase Responsiveness*

**Phosphodiesterase Inhibition.** Because cGMP is hydrolyzed by phosphodiesterase, inhibition of phosphodiesterase may increase the effectiveness and duration of the action of inhaled NO. In awake lambs with U46619-induced PAH, intravenous infusion of zaprinast, a phosphodiesterase 5–specific inhibitor, increased the duration of action of inhaled NO and accentuated the NO-induced reduction of PVR. With administration of zaprinast, the half-time of pulmonary vasodilation after discontinuing a 4-min 40-ppm NO inhalation trial was increased from 1 or 2 min to 10–12 min (fig. 7).<sup>302</sup> Potentiation of the effects of NO by another phosphodiesterase inhibitor, dipyridamole, has been reported in the ovine fetal pulmonary circulation.<sup>303,304</sup>

**Inhibition of Vascular Superoxide Production.** No experimental or clinical studies have yet determined whether inhibition of pulmonary vascular O<sub>2</sub><sup>-</sup> produc-

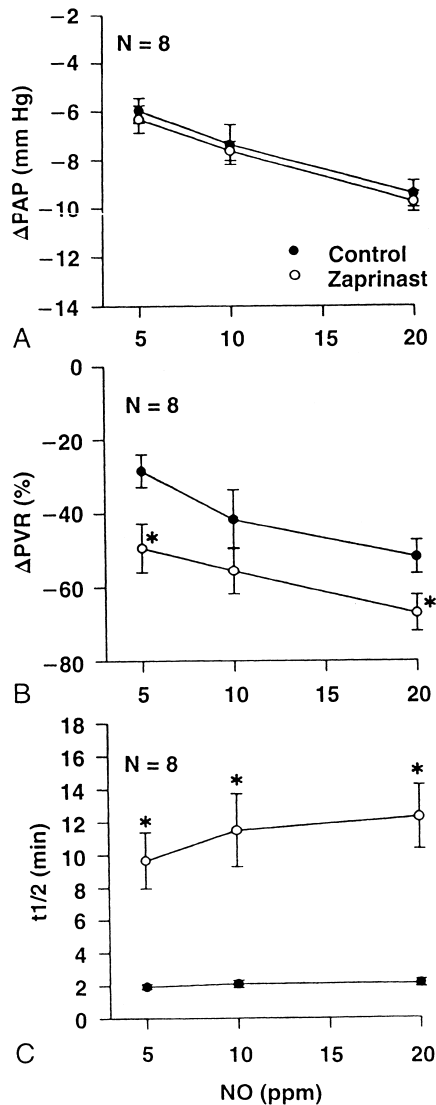


Fig. 7. Influence of continuous intravenous zaprinast infusion ( $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) on the magnitude of peak decreases of mean pulmonary arterial pressure (A), percent changes of pulmonary vascular resistance (B), and half-times of vasodilating effects (C) in response to nitric oxide inhalation during pulmonary arterial hypertension induced by U46619 in awake lambs. Values are mean  $\pm$  SE. \*Significantly different from control value ( $P < 0.05$ ). Reprinted with permission from Ichinose *et al.*<sup>302</sup>

tion may increase responsiveness to inhaled NO. Inhibition of the  $\text{O}_2$ -generating membrane-associated oxidase in rabbit aorta by hydralazine normalized vascular  $\text{O}_2$ -production and restored the vasodilatory activity of nitroglycerin.<sup>299,300</sup>

**Almitrine Infusion.** Almitrine bismesylate acts as an agonist at peripheral arterial chemoreceptors and in-

creases discharge of the carotid sinus nerve.<sup>305</sup> Intravenous administration of low concentrations of almitrine has been reported to restore or enhance hypoxic vasoconstriction in the acutely injured lung. Inequalities and the  $\text{Pa}_{\text{O}_2}$  in ARDS patients usually are improved by almitrine administration, which augments hypoxic vasoconstriction and redistributes pulmonary arterial blood flow toward better ventilated areas with a higher  $\text{PA}_{\text{O}_2}$ . The combination of almitrine infusion and NO inhalation, each having different mechanism, might synergistically reduce mismatch. Almitrine administration enhanced the oxygenation beneficial effects of inhaled NO on oxygenation<sup>306</sup> and allowed a further reduction of  $\text{F}_{\text{I}\text{O}_2}$  in patients with ARDS.<sup>307</sup> The relatively long *in vivo* half-life (mean tissue half-life, 12 h) and possible toxic side effects of almitrine (including a slowly reversible peripheral sensory neuropathy) require careful investigation before this regimen can be recommended for routine clinical use.

**Partial Liquid Ventilation.** Perfluorocarbons are inert liquids that lower surface tension in surfactant-depleted lungs and dissolve large concentrations of respiratory gases. Zobel *et al.*<sup>308</sup> demonstrated that inhaled NO enhanced the effects of perfluorocarbons on pulmonary gas exchange in a piglet model of acute lung injury induced by repeated bilateral lung lavage. An additive effect of inhaled NO and perfluorocarbons on pulmonary gas exchange in acute lung injury has been confirmed by others.<sup>309,310</sup>

## Clinical Side Effects of Nitric Oxide Inhalation

### Left Ventricular Function

The risks of inhaled NO should be carefully considered in patients with markedly impaired left ventricular function (e.g., heart transplant candidates). Inhalation of NO may vasodilate the pulmonary circulation and increase blood flow to the left ventricle, thereby acutely increasing left atrial pressure and PCWP<sup>311-313</sup> and promoting pulmonary edema formation.<sup>314</sup> Cardiac output, left atrial pressure, or PCWP should be monitored if NO is administered to patients with severe left ventricular dysfunction.

### Discontinuation of Nitric Oxide Inhalation

Rebound PAH, an increase of intrapulmonary right-to-left shunting and a decreased  $\text{Pa}_{\text{O}_2}$  after acute NO discontinuation is well-described.<sup>144</sup> Lavoie *et al.*<sup>315</sup> re-

ported four patients with severe acute respiratory failure in whom NO therapy was discontinued abruptly after a decision to discontinue life-extending measures. A sustained decrease of arterial oxygen saturation occurred immediately after NO discontinuation, which was reversed by restarting NO therapy. All four patients died within 24 h after NO discontinuation.

It has been suggested that downregulation of endogenous NO synthesis by NO inhalation is responsible for rebound PAH.<sup>130,316-318</sup> However, recent data obtained in rats with hypoxic pulmonary hypertension suggest that inhibition of endogenous NO synthesis plays a minor role in rebound PAH: no changes of lung eNOS protein levels, NOS activity, endothelium-dependent and -independent vasodilation were reported after 3 weeks inhaling 20 ppm NO. Lung GC activity was transiently decreased after 1 week of NO inhalation, but GC activity was normal after 3 weeks of NO inhalation.<sup>319</sup>

To avoid rebound PAH, a slow stepwise reduction of the inhaled NO concentration with immediate control of any adverse effects (e.g., reduced oxygen saturation or blood gas tensions, increased MPAP<sup>144</sup>) are important to safely wean the patient from inhaled NO. In addition, administration of the phosphodiesterase 5 inhibitor dipyridamole has been reported to prevent rebound PAH in children after cardiac surgery.<sup>320</sup>

#### *Bleeding Time*

Inhaled NO can inhibit platelet function and increased the bleeding time in rabbits. In ARDS patients receiving inhaled NO (3-100 ppm), NO decreased platelet aggregation and agglutination *in vitro*. However, the *in vivo* bleeding time (Ivy bleeding time) was not altered.<sup>321</sup> In neonates, a recent study reported that bleeding time doubled after 30 min of 40-ppm NO inhalation.<sup>322</sup> However, in the multicenter studies in newborns, no difference in the frequency of bleeding events was observed in NO-treated compared with placebo-treated patients.<sup>6-8,249</sup>

### **Toxicity of Nitric Oxide Inhalation**

Nitric oxide inhalation therapy should be instituted after careful consideration of potential acute and long-term toxicity. The major concerns are (1) methemoglobinemia, (2) NO<sub>2</sub> formation, and (3) cellular toxicity. Acute inhaled NO overdose (> 500-1,000 ppm) leads to rapid NO<sub>2</sub> formation, severe methemoglobinemia, pulmonary alveolar edema and hemorrhage, hypoxemia, and death within minutes to hours.<sup>323</sup>

Blood methemoglobin concentrations and inspired NO<sub>2</sub> concentrations have been regularly monitored in clinical trials of inhaled NO in adults and neonates.<sup>6,8,142,249</sup> In the large number of patients studied in these trials (n = 471) receiving inhaled NO therapy at doses ranging from 1.25 to 80 ppm, significant methemoglobinemia or NO<sub>2</sub> formation was uncommon (table 4). If methemoglobin or NO<sub>2</sub> levels increased above predetermined limits, the inhaled NO concentration was decreased. Discontinuation of NO administration because of NO<sub>2</sub> or methemoglobin formation was only necessary in 3 of 471 patients (0.6%).

The most important requirements for safe NO inhalation therapy are (1) continuous analysis of NO and NO<sub>2</sub> concentrations (using chemiluminescence or electrochemical analyzers<sup>324,325</sup>); (2) frequent calibration of the monitoring equipment; (3) frequent analysis of blood methemoglobin levels; (4) the use of certified tanks; and (5) administration of the lowest NO concentration required.

Little is known about the long-term sequelae of NO inhalation in humans. In 12 newborns receiving NO inhalation treatment (< 20 ppm) for up to 4 days, there were no signs of increased lipid peroxidation product, impaired surfactant activity, or changed cytokine profile.<sup>326</sup> However, in two infants requiring prolonged ventilation with NO, nitrotyrosine residues were detected in airway specimens.<sup>326</sup> The relative contribution of NO inhalation and endogenous NO formation to nitrotyrosine formation in the lung is unclear because nitrotyrosine formation has been demonstrated in acutely injured lungs without the exogenous administration of NO.<sup>33,34</sup> Follow-up studies of adult patients 8 months after NO treatment for ARDS showed no obvious differences in pulmonary function compared to ARDS patients not treated with NO.<sup>327</sup>

In summary, reported data of clinical NO toxicity are sparse. Studies appropriately designed to detect long-lasting or irreversible pathologic effects of NO breathing will be necessary to predict the long-term effects of inhaled NO and establish time and dose limits.

### **Alternatives to Nitric Oxide Inhalation Therapy**

#### *Inhaled Prostacyclin*

Prostacyclin is a natural product of the cyclooxygenase pathway and a potent vasodilator. The initial clinical studies of NO inhalation in ARDS patients compared the

**Table 4. Adverse Events in Multicenter Trials of Inhaled Nitric Oxide in Newborn and Adult Patients**

Reference	Year	N	N (NO)	Diagnosis	Time	NO <sub>2</sub>	MethHb	Adverse Events	Therapeutic Consequence
142	1998	177	120	ARDS	Up to 28 days	Mean over study period: 0.1 ppm (1.25 ppm NO); up to 0.35 ppm (40 ppm NO) Max 3-4 ppm in three patients	Mean over study period: 0.8% (1.25 ppm NO); up to 1.4% (40 ppm NO) Max 5-7% in three patients	<ul style="list-style-type: none"> <li>Myopathy/agitation in one patient (1.25 ppm NO)</li> <li>Abnormal liver enzymes in one patient (1.25 ppm NO)</li> <li>Apnea, hemorrhage and coagulation disorder in one patient (40 ppm NO)</li> <li>NO<sub>2</sub> &gt; 3 ppm (one patient)</li> <li>MethHb &gt; 5% (two patients)</li> <li>MethHb increased from 1% to 18% on first day of treatment in one patient</li> </ul>	N/A N/A N/A → NO discontinued → NO discontinued → NO continued because oxygenation was improved; MethHb then decreased → reduction of NO concentration → reduction of NO concentration → reduction of NO concentration
6	1997	58	30	PPHN	Up to 8 days	N/A	Median < 4% Max 18.2% 90% of patients < 10%	<ul style="list-style-type: none"> <li>Elevation of MethHb between 5%–10%</li> <li>NO overdose: one patient 100 ppm for 36 min</li> <li>One patient: 101 ppm for 60 min, MethHb: 6%, NO<sub>2</sub> 5.1 ppm</li> <li>None reported related to NO inhalation</li> <li>No infant required discontinuation of treatment because of NO<sub>2</sub> or MethHb elevations</li> </ul>	→ reduction of NO concentration
7	1997	235	114	HRF	Up to 14 days	Mean peak level: 0.8 ppm	Mean peak level: 2.4% N/A	<ul style="list-style-type: none"> <li>Mean peak level: 2.4%</li> </ul>	
8	1997	205	182	PPHN	24 hours	N/A	Mean (24 h): 1.3% (6 ppm NO) Max: 5.1% (20 ppm NO) N/A	<ul style="list-style-type: none"> <li>None reported related to NO inhalation</li> </ul>	
249	1997	53	25	Congenital diaphragm hernia	Up to 14 days	N/A	N/A	<ul style="list-style-type: none"> <li>None reported related to NO inhalation</li> </ul>	

ARDS = acute respiratory distress syndrome; PPHN = persistent pulmonary hypertension of the newborn; HRF = hypoxic respiratory failure; N/A = not available; MethHb = methemoglobin; N = total number of patients enrolled in study; N (NO) = number of patients who received NO treatment.

pulmonary vasoactive properties of intravenously administered prostacyclin with inhaled NO.<sup>144</sup> Although prostacyclin infusion decreased MPAP to the same degree as inhaled NO, prostacyclin decreased PaO<sub>2</sub>, presumably by reducing hypoxic pulmonary vasoconstriction and caused systemic hypotension. Such adverse effects are commonly observed during intravenous infusion of commonly used vasodilator drugs.

It was hypothesized that the administration of prostacyclin *via* aerosol would limit its hemodynamic effects to the lung. This hypothesis was tested in ARDS patients, and the effectiveness of prostacyclin when used as a short-term inhaled aerosol was compared with inhaled NO.<sup>328</sup> Similar effects, namely decreased MPAP, decreased intrapulmonary right-to-left shunting, and increased PaO<sub>2</sub>/FiO<sub>2</sub> were observed. Systemic vasodilation was not reported with either drug. These effects of inhaled prostacyclin have been confirmed in other studies.<sup>329,330</sup> A disadvantage of aerosolized prostacyclin therapy is that systemic absorption can occur, which makes it difficult to maintain pulmonary vasodilation without producing systemic vasodilation for periods lasting more than a few hours. Larger studies, including randomized trials studying the effectiveness and responsiveness to inhaled prostacyclin for longer time periods, are necessary to support a useful therapeutic role.

#### *Nitric Oxide Donor Drugs*

The use of inhaled NO donor drugs has been proposed as an alternative to NO inhalation. Administration of such drugs, which release a defined amount of NO over a prolonged time period, might permit intermittent NO dosing. Adrie *et al.* compared the pulmonary vascular effects of inhaled sodium 1-(N,N-diethylamino) diazen-1-ium-1,2-diolate (DEA/NO), which spontaneously generates NO, with the inhalation of sodium nitroprusside and NO gas in awake sheep with pharmacologically induced pulmonary hypertension.<sup>331</sup> DEA/NO caused nonselective vasodilation, and sodium nitroprusside was only selective for the pulmonary circulation at low inhaled concentrations, compared with the highly selective effect of NO gas.<sup>331</sup> In a pig model of acute pulmonary hypertension, Brilli *et al.* compared the effects of the aerosolized NO donors ethylputreanine NONOate (EP/NO) and 2-(dimethylamino) ethylputreanine NONOate (DMAEP/NO).<sup>332</sup> They reported that 3-min DMAEP/NO aerosolization caused selective pulmonary vasodilation, which lasted for approximately 30–50 min without effects on systemic arterial pressure or cardiac output.<sup>332</sup> Similarly, tracheal instillation of DMAEP/NO resulted in

prolonged and pulmonary selective vasodilation.<sup>333</sup> EP/NO, aerosolized or instilled, was less effective and the effects were inconsistent.<sup>332,333</sup>

The intravenous infusion of ultra-short-acting NO donor agents might also be an alternative to inhaled NO for producing selective pulmonary vasodilation. In awake, healthy sheep with pulmonary hypertension, the intravenous (systemic) infusion of PROLI/NO (C<sub>5</sub>H<sub>7</sub>N<sub>3</sub>O<sub>4</sub>Na<sub>2</sub> · CH<sub>3</sub>OH), an ultra-short-acting nucleophile/NO adduct that generates NO, produced selective pulmonary vasodilation without affecting the systemic circulation. The selective effect of this intravenous drug was caused by its short half-life *in vivo*, which resulted in complete NO release during transit of the pulmonary circulation and before reaching the systemic arterial circulation.<sup>334</sup> Because such drugs are intravenously administered, oxygenation might be adversely affected in the injured lung through indiscriminant release of hypoxic pulmonary vasoconstriction. Nevertheless, such newly designed NO donor drugs appear promising as a selective pulmonary vasodilator and provide an alternative to NO inhalation.

#### *Inhaled Phosphodiesterase Inhibitors*

The use of inhaled phosphodiesterase inhibitors has been investigated as an alternative or adjunct to NO inhalation. Inhalation of nebulized zaprinast induced selective pulmonary vasodilation and enhanced the effects of inhaled NO in awake lambs.<sup>335</sup> However, at a zaprinast concentration (50 mg/ml) producing a similar decrease of MPAP to 20 ppm NO, significant systemic vasodilation was observed (fig. 8).

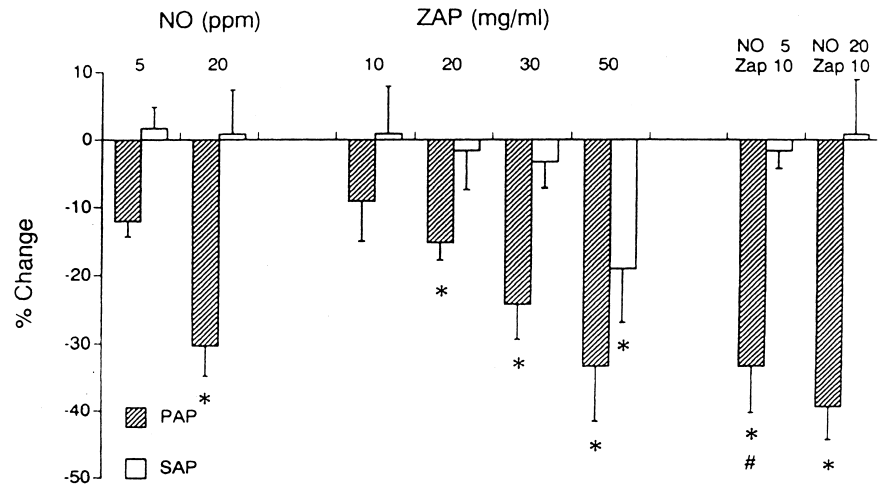
## Summary

Nitric oxide is produced by almost every healthy mammalian tissue. In health, NO has a myriad of functions that are essential for life. In disease, NO has many effects that can be both helpful and deleterious.

Inhaled NO has made a rapid journey from the laboratory bench to the bedside because of its unique selective pulmonary vasodilator activity and its ready availability. It is the first vasodilator described to provide truly selective pulmonary vasodilation. A large number of basic and clinical research studies have made great steps in delineating its physiology, side effects, and clinical efficacy. Nearly simultaneously, the clinical use of inhaled NO has become widespread. During the past 8 yr, inhaled NO

## NITRIC OXIDE INHALATION

**Fig. 8.** Percentage change of mean pulmonary arterial pressure and systemic arterial pressure (SAP) during inhalation of nitric oxide (NO; 5 and 20 ppm), aerosolized zaprinast (ZAP, 10–50 mg/ml), or both, in awake lambs. \*Significantly different from values at pulmonary arterial hypertension ( $P < 0.05$ ). #Significantly different from the value at 5 ppm NO ( $P < 0.05$ ) and from 10 mg/ml zaprinast ( $P < 0.01$ ). Values are mean  $\pm$  SD. Reprinted with permission from Ichinose *et al.*<sup>302</sup>



has been used to treat pulmonary hypertension and hypoxemia in thousands of patients worldwide. Inhaled NO is an effective pulmonary vasodilator in many disease states characterized by pulmonary hypertension. In addition, inhaled NO decreases pulmonary venous admixture in diffuse lung injury, and therefore increases systemic oxygenation in many patients. Most importantly, randomized multicenter studies of NO inhalation have shown that this new therapy significantly reduces the necessity for ECMO in newborns with PPHN or hypoxic respiratory failure. NO inhalation has many additional effects that may be clinically beneficial. Inhaled NO has been reported to decrease pulmonary edema formation and lung injury. In the systemic circulation, NO inhalation inhibits peripheral vascular restenosis in animal models of arterial injury, augments the oxygen binding of sickle erythrocytes, and reduces cyclic coronary occlusion in a model of coronary injury and thrombolysis. However, important questions remain:

1. Does the reduction of pulmonary artery pressure and increased  $\text{Pa}_{\text{O}_2}$  caused by NO inhalation improve clinical outcome for patients with acute lung injury?

In a relatively uniform and well-defined population of patients, newborns with hypoxic respiratory failure, NO inhalation effectively improves oxygenation and significantly reduces the use of ECMO. Avoiding ECMO, a complicated and expensive invasive procedure with limited availability, is an important clinical endpoint and undoubtedly would justify the use of inhaled NO.<sup>6,7</sup> The clinical usefulness of inhaled NO in adults remains unclear. Clinical studies of ARDS in adults are complicated by the diverse nature of the patient population, the precipitating causes of lung

injury, and the common occurrence of sepsis and multiple organ system failure. Current data from multicenter trials suggest that the mortality rate in moderate lung injury is not significantly changed by NO inhalation. Whether this is because of inappropriate study design, the complex nature and spectrum of ARDS, inefficacy of NO, inappropriate dosing, or counterbalancing toxic effects of NO is unknown.

2. If available data suggest that survival is unchanged, should clinicians continue to study NO inhalation? Researchers concentrating on the cellular and subcellular effects of NO properly express concerns that NO inhalation may worsen lung injury and damage important structures. Initial clinical studies, however, suggest that toxicity, if present, is extremely low. The doses of NO now commonly used are less than those received with cigarette exposure and are nearly within the atmospheric background range of many urban areas. Many clinical scientists continue to evaluate inhaled NO and find it useful for short-term symptomatic treatment of hypoxemic respiratory failure and pulmonary vasoconstriction.

The pharmacologic and toxicologic profiles of NO inhalation are incomplete. It is necessary to delineate further (1) proper indications, (2) contraindications, (3) sound dosing criteria, (4) organ disease from cellular and subcellular toxicity, and (5) the causes of NO hyporesponsiveness. Randomized clinical studies of patients with carefully defined specific disease states characterized by pulmonary hypertension or hypoxemia (*e.g.*, pulmonary embolism, severe PAH, postpneumonectomy pulmonary edema, acute rejection after lung transplan-

tation) and in 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. 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. In evaluating this complex field, it is critical that our view does not become colored by a single study or effectiveness in a particular disease state. As with most medical advances, it is the evolution of a wide-ranging body of research that will properly determine the place for NO inhalation therapy in our armamentarium.

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