

REDUCED EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN THE LUNGS OF PATIENTS WITH PULMONARY HYPERTENSION

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Abstract *Background.* Pulmonary hypertension is characterized by abnormal thickening of the pulmonary arteries and increased pulmonary vascular resistance. Nitric oxide is a potent endothelium-derived vasorelaxant substance and an inhibitor of smooth-muscle-cell growth. Nitric oxide is produced in various cell types by the action of an enzyme, nitric oxide synthase. We compared the expression of endothelial nitric oxide synthase in the lungs of control subjects with that in the lungs of patients with pulmonary hypertension.

Methods. We investigated the expression of endothelial nitric oxide synthase by histochemical and immunohistochemical analysis, in situ hybridization, and Northern blot analysis in the lungs of 22 patients with plexogenic pulmonary arteriopathy (arteriopathy of grades 4 through 6), 24 patients with secondary pulmonary hypertension (arteriopathy of grades 1 through 3), and 23 control subjects.

Results. In the lungs of the control subjects, nitric oxide

synthase was expressed at a high level in the vascular endothelium of all types of vessels and in the pulmonary epithelium. In contrast, little or no expression of the enzyme was found in the vascular endothelium of pulmonary arteries with severe histologic abnormalities (i.e., plexiform lesions) in patients with pulmonary hypertension. The intensity of the enzyme immunoreactivity correlated inversely with the severity of histologic changes. There was an inverse correlation between the arterial expression of the enzyme and total pulmonary resistance in patients with plexogenic pulmonary arteriopathy ($r = -0.766$, $P = 0.004$).

Conclusions. Pulmonary hypertension is associated with diminished expression of endothelial nitric oxide synthase. It is possible that decreased expression of nitric oxide synthase may contribute to pulmonary vasoconstriction and to the excessive growth of the tunica media observed in this disease. (N Engl J Med 1995;333:214-21.)

PULMONARY hypertension is generally characterized by increased thickening of the walls of pulmonary arteries, narrowing of the pulmonary-artery lumen, increased pulmonary vascular resistance, and right-sided heart failure.¹⁻³ Clinically, patients have increasing dyspnea, cyanosis, precordial discomfort, anginal pain, and cardiomegaly.¹⁻³ Histologically, pulmonary arteries with such resistance, particularly those less than 100 μm in diameter, show various degrees of intimal thickening and muscular hypertrophy.^{1,4,5} Pulmonary hypertension can be either idiopathic (primary) or due to other disease conditions. A number of humoral factors have been implicated in the pathogenesis of pulmonary hypertension, but there is no evidence that any contribute directly to the disorder.

Recently, attention has been given to the endothelium as an important mediator of pulmonary hypertension by virtue of its ability to produce factors that regulate blood flow and vascular tone.⁶⁻⁸ One of the most important factors so produced is the endothelium-derived relaxing factor nitric oxide,^{9,10} which is produced from the guanidino nitrogen of L-arginine by the enzyme nitric oxide synthase.¹⁰ There are three isoforms of this enzyme. Two, expressed in neurons and endothelial cells, are calcium-dependent,^{11,12} whereas a third is calcium-independent and is expressed by macrophages and other cells after induction with cytokines.¹³ In addition to its vasodilative effects, nitric oxide acts as a bronchodilator, neurotransmitter, an-

ticoagulant, antiproliferative, and antimicrobial substance.¹⁰ Several studies have shown that nitric oxide plays an important part in the physiology of the lung,¹⁴ particularly in maintaining low pressure in the normal pulmonary circuit.¹⁵ Continuous inhalation of nitric oxide protects against the development of pulmonary hypertension in chronically hypoxic rats,¹⁶ and chronic deprivation of the substance in utero produces pulmonary hypertension in newborn lambs.¹⁷ Inhalation of nitric oxide reduces pulmonary vascular resistance in patients with pulmonary hypertension.^{18,19}

On the basis of these considerations, we asked whether the endothelial expression of nitric oxide synthase might be abnormal in the pulmonary vasculature of patients with pulmonary hypertension. To date, there are only a few reports concerning the expression of nitric oxide synthase in human lungs,^{20,21} none of which has dealt with the expression of the constitutive endothelial isoform. In this study we provide evidence of abundant expression of endothelial nitric oxide synthase in normal lungs. We also show that the expression of this enzyme is diminished in the endothelium of pulmonary arteries of patients with pulmonary hypertension and that this diminution correlates inversely with the total pulmonary resistance in patients with plexogenic pulmonary arteriopathy.

METHODS

Study Patients

The study patients were divided into three groups on the basis of clinical and histologic characteristics. Group 1 consisted of 22 patients with plexogenic pulmonary arteriopathy (Heath and Edwards grades 4 through 6).⁴ Eighteen of these had a clinical diagnosis of primary pulmonary hypertension,²² two had cirrhosis, one had postpartum pulmonary hypertension, and one had systemic lupus erythematosus. Group 2 consisted of 24 patients with pulmonary hyperten-

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sion due to various disease conditions (Heath and Edwards grades 1 through 3).⁴ Group 3 consisted of 10 patients with nonspecific pneumonitis. Also included in group 3 were 13 normal lungs that were donor organs not used for transplantation. The characteristics of the study patients are shown in Table 1. Specimens of lung tissue were collected during open-lung biopsy, at transplantation, or at autopsy. Multiple pieces of tissue were fixed in either paraformaldehyde or formalin for routine histologic diagnosis, immunohistochemical analysis, and in situ hybridization. For the Northern blot analysis, fresh samples were snap-frozen in liquid nitrogen.

Immunohistochemical Analysis

Paraffin and cryostat sections of tissue were immunostained with antiserum to human endothelial nitric oxide synthase produced in our laboratory with a modification of the avidin–biotin–peroxidase method.²³ The specificity of the antiserum used was determined by Western blotting and by comparison with a commercial antiserum (Transduction Laboratories, Lexington, Ky.). In the immunohistochemical analysis, tissue sections were made permeable with Triton X-100, incubated in hydrogen peroxide to block endogenous peroxidase activity, and incubated first with normal serum for 30 minutes and then with the primary antiserum for 16 hours at 4°C. Sections were then incubated with biotinylated IgG and stained with an immunoperoxidase technique according to the manufacturer’s instructions (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, Calif.). The samples of primary antiserum were incubated with their respective antigens (1 µg per milliliter of solution) before incubation with tissue sections, or sections were incubated with the normal serum instead of the primary antiserum samples and used as negative controls. Antiserum to the endothelial-cell marker von Willebrand factor (factor VIII) and antiserum to endothelin-1 were also used. Extra sections from all patients were stained with hematoxylin and eosin and with Verhoeff–van Gieson stains for the histologic diagnosis of pulmonary hypertension. The intensity of immunostaining was graded semiquantitatively as described elsewhere.²⁴

In Situ Hybridization

Cryostat sections of paraformaldehyde-fixed tissues were placed on RNase-free glass slides and hybridized with an RNA probe labeled

with sulfur-35 to detect endothelial nitric oxide synthase,¹² according to a previously described method.²⁴ In brief, tissue sections were made permeable with Triton X-100 and proteinase K. To reduce background noise, they were treated with acetic anhydride and triethanolamine and with a further solution of *N*-ethylmaleimide and iodoacetamide. After overnight incubation with the radiolabeled probe at 42°C, unhybridized RNA probes were removed with RNase A and high-stringency washes with 2× to 0.1× saline sodium citrate (SSC; 1× SSC is 0.15 mol of sodium chloride and 0.015 mol of sodium citrate per liter, pH 7) at 22 to 55°C. The sections were then processed for autoradiography. Negative control experiments included lung sections hybridized with the sense probe or with the hybridization buffer in the absence of the labeled antisense probe. Extra sets of sections were immunostained with the endothelial nitric oxide synthase antiserum before they were processed for autoradiography for colocalization of the mature protein and messenger RNA (mRNA) on the same sections. Another set of sections was hybridized with the RNA probe for human endothelin-1 as a positive control.²⁴

The experiments using immunohistochemical analysis and in situ hybridization were further complemented by Northern blot analysis²⁵ and histochemical staining for NADPH diaphorase.²⁶

Statistical Analysis

Data are presented as means ±SE. Differences between groups were assessed by analysis of variance, with Bonferroni’s correction for multiple comparisons, with a commercial program (Statview). The correlation between immunohistochemical grades and total pulmonary resistance or the severity of the lesion was assessed with ordinary least-squares linear regression techniques.²⁷

RESULTS

Histologic analyses of consecutive sections from the patients with plexogenic pulmonary arteriopathy (group 1), after staining with hematoxylin and eosin and the Verhoeff–van Gieson stain, revealed pulmonary arteries with morphologic changes of grades 4 through 6 on the Heath and Edwards scale.⁴ The parenchyma

Table 1. Characteristics of the Study Patients.*

CHARACTERISTIC	GROUP 1 (PLEXOGENIC PULMONARY ARTERIOPATHY)	GROUP 2 (SECONDARY PULMONARY HYPERTENSION)					GROUP 3 (CONTROL)	
		CONGENITAL HEART DEFECT	IDIOPATHIC PULMONARY FIBROSIS	COPD	CONGESTIVE HEART FAILURE	BRONCHIECTASIS	NORMAL LUNGS	PNEUMONITIS
Sex (M/F)	12/10	2/6	3/2	3/2	3/1	1/1	7/6	5/5
Age (yr)	38±4	40±5	49±5	60±6	62±4	48±2	30±4	43±5
Hemoglobin (g/liter)	143±5	164±21	126±7	113±15	120±13	130±21	115±8	—
Pulmonary hemodynamics								
PaO ₂ (mm Hg)	70±4	62±11	75±7	73±16	65±3	55±8	—	—
Mean pulmonary arterial pressure (mm Hg)	67±3	76±13	50±6	37±1	70±1	58±8	—	—
Pulmonary-capillary wedge pressure (mm Hg)	11±2	8±3	17±3	NA	NA	10	—	—
Cardiac output (liters/min)	3±0.3	4±1	4±0.5	NA	3±0.2	3	—	—
Pulmonary vascular resistance (Wood units)	17±2	16±4	9±3	NA	17	16	—	—
Pulmonary function								
Total pulmonary resistance (Wood units)	21±1	20±8	12±3	NA	22±1	20	—	—
FEV ₁ (% of predicted value)	72±6	72±10	39±12	23±4	98	36±18	—	—
FVC (% of predicted value)	72±5	75±10	42±9	44±2	109	41±6	—	—
DLCO (% of predicted value)	70±9	47	25±7	19±1	64	43±17	—	—
Immunohistochemical grade, small muscular pulmonary arteries	0.2±0.1	0.9±0.2	0.4±0.1	0.5±0.2	0.3±0.2	0.8±0.5	3.5±0.1	3.3±0.1

*Plus–minus values are means ±SE. COPD denotes chronic obstructive pulmonary disease, PaO₂ arterial oxygen tension, NA not available, FEV₁ forced expiratory volume in one second, FVC forced vital capacity, and DLCO single-breath diffusing capacity for carbon monoxide.

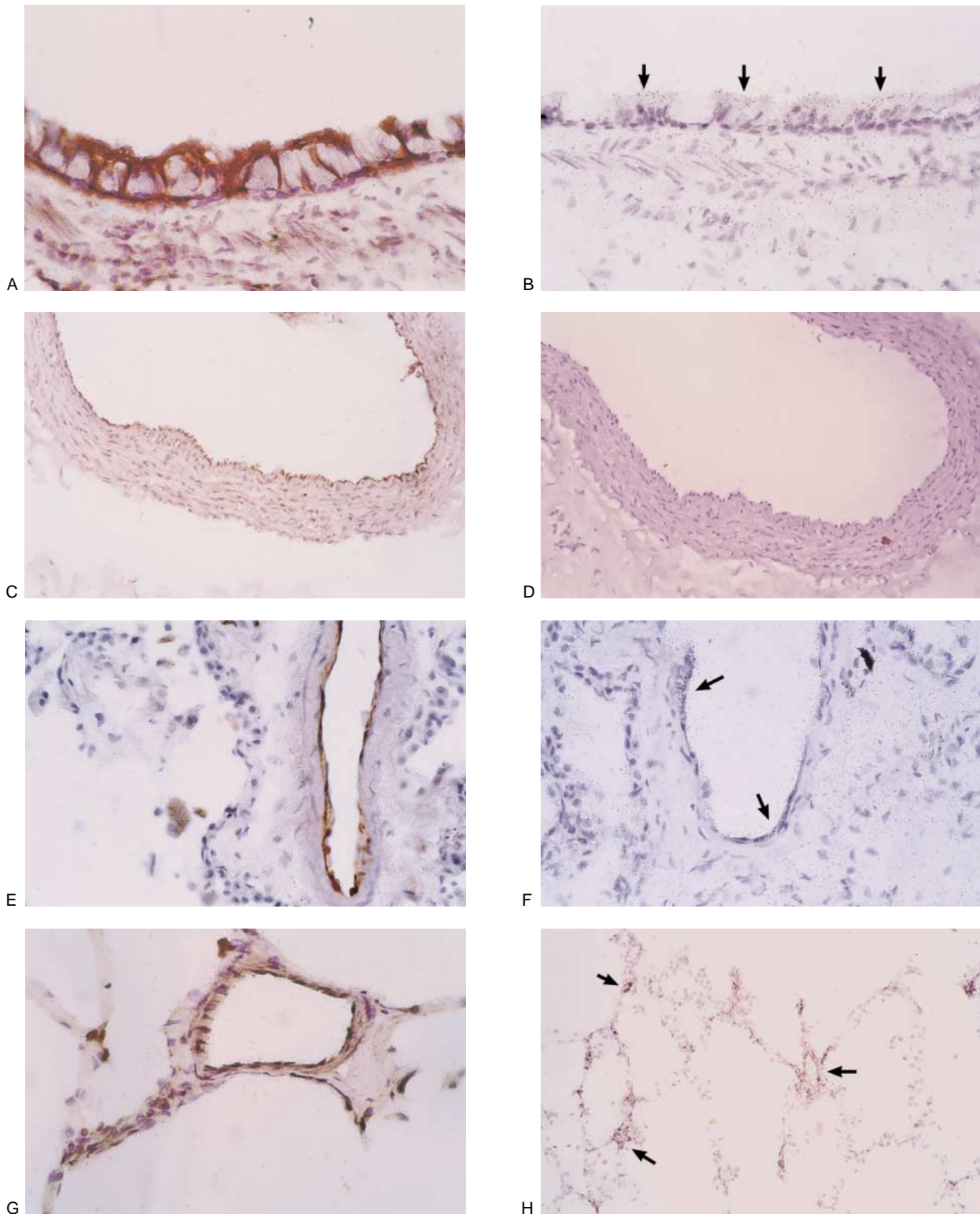


Figure 1. Endothelial Nitric Oxide Synthase–Like Immunoreactivity and mRNA in Normal Lung Tissue.

Immunoreactivity to nitric oxide synthase is shown in the bronchial epithelium (Panel A, $\times 400$) and in the vascular endothelium of the elastic (Panel C, $\times 200$) and the medium-sized muscular (Panel E, $\times 400$) and small muscular (Panel G, $\times 400$) pulmonary arteries of normal lungs. Panel D shows a section consecutive to that shown in Panel C, first hybridized with the sense RNA probe and then immunostained with the mixture of antiserum and antigen to serve as a negative control for in situ hybridization and the immunohistochemical analysis ($\times 200$). The expression of endothelial nitric oxide synthase mRNA is shown in the bronchial epithelium (Panel B, $\times 400$) and in the vascular endothelium of the medium-sized (Panel F, $\times 400$) and small (Panel H, $\times 200$) muscular pulmonary arteries in normal lungs.

Arrows indicate hybridization signals in the bronchial epithelium (Panel B) and the vascular endothelium (Panels F and H).

showed no apparent morphologic abnormalities. Specimens from patients with secondary pulmonary hypertension showed pulmonary arteries with muscular hypertrophy and intimal fibrosis (grades 1 through 3 on the same scale), with various underlying parenchymal changes, such as interstitial fibrosis, edema, or hemorrhage.

Immunohistochemical analyses revealed strong immunostaining for endothelial nitric oxide synthase in the pulmonary vascular endothelium and the pulmonary epithelium of the control lungs (group 3) (Fig. 1). Dense, diffuse immunostaining was observed in the endothelial cells of pulmonary arteries of all sizes (Fig. 1C, 1E, and 1G). The endothelial cells of pulmonary veins, bronchial arteries, and microvessels were also stained. The intensity of the immunoreaction was similar to that of the immunoreaction to von Willebrand factor. Strong immunoreactivity was observed in the airway epithelium, excluding goblet cells (Fig. 1A). Moderate-to-weak immunoreactivity was occasionally seen in the serous glands and nerve fibers around vessels and airways. In comparison, the lungs of patients with plexogenic pulmonary arteriopathy (group 1) had little or no endothelial nitric oxide synthase in the pulmonary arteries with severe morphologic abnormalities (Fig. 2A and 2C). In the same specimens, pulmonary arteries with few histologic changes or none showed weak-to-moderate immunostaining for the enzyme. The immunoreactivity to endothelial nitric oxide synthase remained strong in the pulmonary epithelium (Fig. 2A). In the patients with secondary pulmonary hypertension (group 2), there was weak immunoreactivity to endothelial nitric oxide in the endothelium of pulmonary arteries with medial thickening and intimal proliferation (Fig. 2D). In the patients with pulmonary hypertension due to pulmonary fibrosis, moderately diffuse staining was also seen in proliferative type II pneumocytes (Fig. 2E). In groups 1 and 2, the immunostaining in the endothelium of pulmonary veins remained relatively unchanged. Consecutive sections immunostained with the endothelin-1 antiserum showed strong expression of the peptide at sites where endothelial nitric oxide was absent or weak (data not shown). Immunostaining of heart and kidney sections from normal subjects and patients with pulmonary hypertension showed a strong immunoreaction within the vascular endothelium, as well as in the tubular epithelium of kidney sections from both diseased patients and normal subjects. No staining was seen in sections immunostained with the mixture of antiserum and antigen or with the nonimmune serum (Fig. 1D).

In situ hybridization revealed the expression of endothelial nitric oxide synthase mRNA at sites similar to those at which immunoreactivity was present. Strong hybridization signals were seen in the endothelium of pulmonary arteries and the pulmonary epithelium of control lungs (group 3) (Fig. 1B, 1F, and 1H). In contrast, only scattered hybridization signals or none were seen in pulmonary arteries with severe arteriopathy (group 1) (Fig. 2B). As was noted in the immunohisto-

chemical analyses, pulmonary arteries with mild morphologic abnormalities in groups 1 and 2 showed moderate hybridization signals in the vascular endothelium. In general, in the patients with pulmonary hypertension, small and medium-sized pulmonary arteries had the fewest hybridization signals. The intensity of hybridization signals in the airway epithelium remained unchanged in all groups (Fig. 2F). These observations were further confirmed in the study of lung sections that were hybridized with the radiolabeled probe and then immunostained with antiserum to endothelial nitric oxide synthase (Fig. 2F). No hybridization signals were seen in sections hybridized with the sense RNA probe (Fig. 1D). Northern blot analysis showed stronger hybridization signals for the enzyme in normal lungs than in the lungs of patients with secondary pulmonary hypertension. The lung samples from patients with plexogenic pulmonary arteriopathy showed very weak signals only after a long exposure.

Histochemical staining for NADPH revealed the localization of nitric oxide synthase-like immunoreactivity in the pulmonary epithelium, vascular endothelium, and nerve fibers. In general, strong staining was seen in the pulmonary epithelium, vascular endothelium, and nerve fibers of normal lungs (Fig. 2G). In contrast, there was no staining in the endothelium of any of the pulmonary arteries with severe morphologic changes (Fig. 2H).

Semiquantitative analyses of the immunohistochemical data revealed a significant difference in the arterial expression of endothelial nitric oxide synthase among the study patients. There was significantly more immunoreactivity to endothelial nitric oxide synthase in the endothelium of elastic and muscular pulmonary arteries of all sizes in the control lungs (group 3) than in the lungs of the patients with plexogenic pulmonary arteriopathy (group 1) or secondary pulmonary hypertension (group 2) ($P < 0.001$) (Fig. 3). Small and medium-sized pulmonary arteries of patients in group 2 showed a greater mean (\pm SE) degree of immunostaining (immunohistochemical grades, 0.6 ± 0.1 and 1.0 ± 0.1 , respectively) than those of patients in group 1 (0.2 ± 0.07 and 0.6 ± 0.1 ; $P = 0.009$ and $P = 0.014$). Linear regression analysis indicated significant inverse correlations between the immunohistochemical grade and the severity of the lesion in all patients with pulmonary hypertension ($r = -0.787$; 95 percent confidence interval, -0.858 to -0.685 ; $P < 0.001$) (Fig. 4) and between arterial expression of nitric oxide synthase and total pulmonary resistance in patients with plexogenic pulmonary arteriopathy (group 1; $r = -0.766$; 95 percent confidence interval, -0.95 to -0.307 ; $P = 0.004$).

DISCUSSION

In our study, we observed prominent expression of endothelial nitric oxide synthase in the endothelium of pulmonary vessels and the airway epithelium of normal lungs. In contrast, in patients with plexogenic pulmonary arteriopathy or secondary pulmonary hypertension the expression of the enzyme in the endothelium

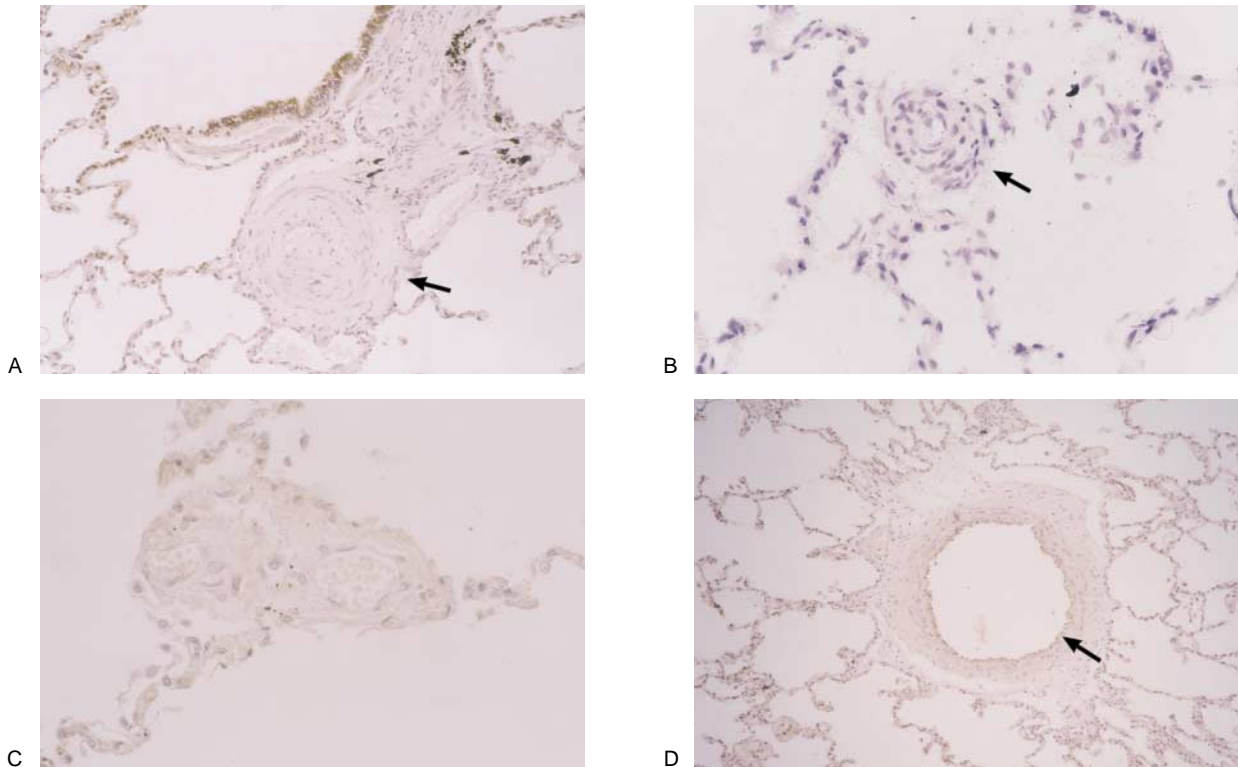
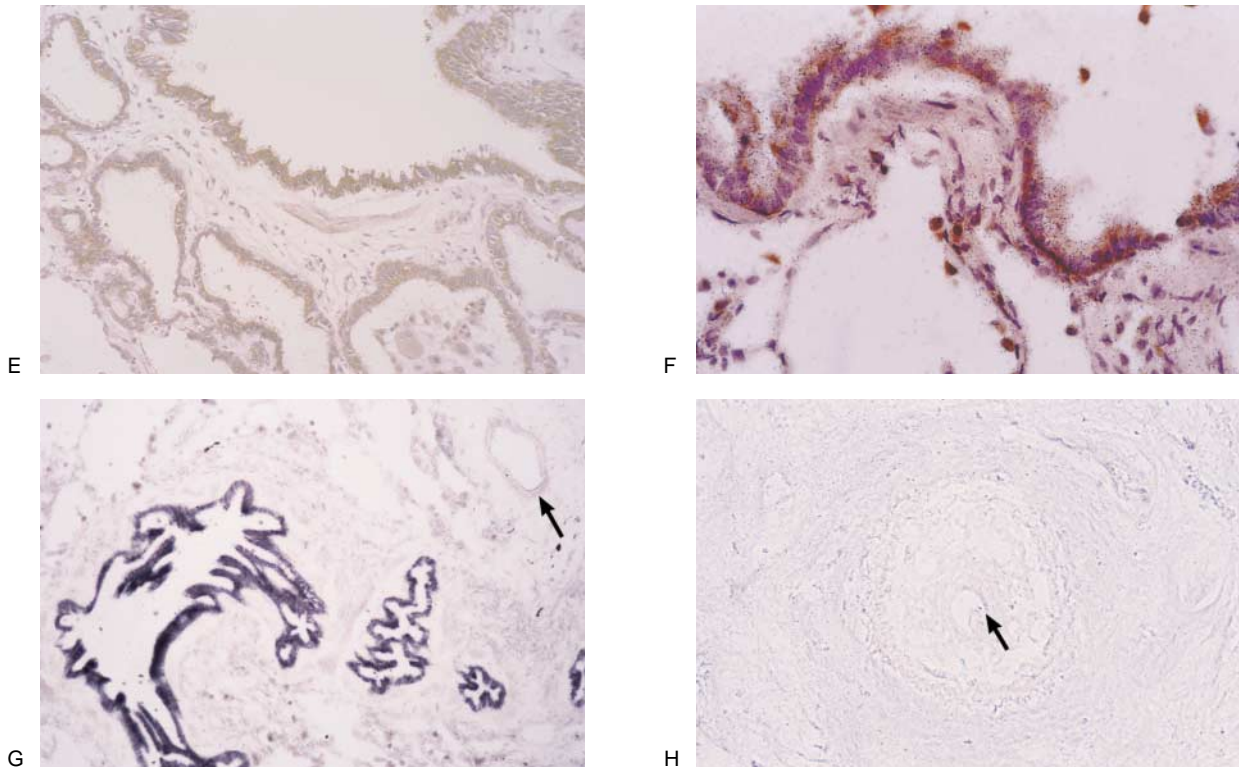


Figure 2. Endothelial Nitric Oxide Synthase-Like Immunoreactivity and mRNA in the Lungs of Patients with Pulmonary Hypertension. Panels A, B, and C show sections of lung from patients in group 1, demonstrating strong immunoreactivity to nitric oxide synthase in the bronchiolar epithelium but no staining in the adjacent pulmonary artery (arrow, Panel A) with severe intimal fibrosis and muscular hypertrophy ($\times 200$). Panel B shows the absence of hybridization signals in a small pulmonary artery with morphologic changes similar to those in Panel A (the arrow indicates the presence of a few scattered hybridization signals over the occluded vessel; $\times 400$). Panel C shows the absence of immunostaining with endothelial nitric oxide synthase in a plexiform lesion of a small pulmonary artery ($\times 400$). Panels D and E, showing sections from patients in group 2, demonstrate weak-to-moderate immunoreactivity in the vascular endothelium of a medium-sized pulmonary artery with muscular hypertrophy (Panel D; the arrow shows a weak brown color in the endothelium; $\times 200$) and in the alveolar epithelium of a patient with pulmonary hypertension due to idiopathic pulmonary fibrosis (Panel E; $\times 200$). Coexisting endothelial nitric oxide synthase-like immunoreactivity and mRNA are shown in the bronchiolar epithelium of a patient with secondary pulmonary hypertension (Panel F, $\times 400$). Strong staining for NADPH diaphorase was seen in the pulmonary epithelium, nerve fibers, and vascular endothelium (arrow) of normal lungs (Panel G, $\times 100$). No NADPH activity was detected in pulmonary arteries with severe morphologic changes (intimal fibrosis and muscular hypertrophy; the arrow indicates narrowing of the lumen) (Panel H, phase-contrast micrograph, $\times 200$).

of pulmonary arteries with abnormal wall morphology was substantially reduced. We also observed an inverse correlation between the arterial expression of endothelial nitric oxide synthase and total pulmonary resistance in patients with plexogenic pulmonary arteriopathy. By virtue of its biologic activities, nitric oxide is likely to play an important part in pulmonary pathophysiology.¹⁴ Indeed, endothelial nitric oxide synthase has been implicated in the maintenance of low pressure in the normal pulmonary vascular bed.¹⁵ Conceivably, constitutive production of nitric oxide by this enzyme is important for the regulation of blood flow and homeostasis and for the maintenance of normal vascular-wall structure.

Nitric oxide has vasodilative effects on pulmonary vessels, and it inhibits the thrombogenicity and proliferation of vascular smooth-muscle cells.^{10,14} Several studies have shown that nitric oxide and L-arginine can

reduce pulmonary pressure in patients with pulmonary hypertension.^{28,29} Dinh-Xuan et al.¹⁵ showed that in patients with chronic obstructive lung disease there was a positive correlation between intimal thickening of blood vessels and the impaired release of nitric oxide. More recently, the same group found that in chronic hypoxia, pretreatment with an excess concentration of L-arginine does not reverse the effects of *N*^ω-monomethyl-L-arginine, a specific inhibitor of nitric oxide synthase, indicating that uptake of L-arginine into the cell is not affected.³⁰ Muscular pulmonary arteries 100 μ m in diameter, those just before the capillary bed, contribute most to the resistance to flow in pulmonary hypertension.³¹ Indeed, they are the most likely arteries to have severe morphologic abnormalities in patients with pulmonary hypertension.^{4,5} Immunohistochemical analysis and in situ hybridization clearly demonstrate abundant expression of nitric oxide synthase in the vascular en-



dothelium of pulmonary arteries, veins, and bronchial vessels of normal lungs, as compared with very sparse signals for this enzyme in the pulmonary arteries of patients with pulmonary hypertension. These observations are confirmed by histochemical staining for NADPH diaphorase and by Northern blot analysis. Our data also showed a significant inverse correlation between the expression of endothelial nitric oxide synthase and the severity of morphologic changes in the study patients. Vessels with arteriopathy of grades 1 through 3 on the Heath and Edwards scale often had weak-to-moderate staining, whereas those with arteriopathy of grades 4 through 6 often showed no staining. Indeed, the small and medium-sized pulmonary arteries of patients in group 1 had significantly less immunoreactivity than those of patients in group 2. Furthermore, we found a significant inverse correlation between the arterial expression of nitric oxide synthase and total pulmonary resistance that appeared only in patients with plexogenic pulmonary arteriopathy. These findings suggest that in patients with pulmonary hypertension, impaired constitutive expression of the enzyme by the vascular endothelium of pulmonary arteries may play a part in either the initiation or the progression of pulmonary hypertension. Indeed, from the present study it is difficult to determine whether the reduction in the expression of this enzyme is a cause or an effect of pulmonary hypertension.

Imbalances in the expression of endothelium-derived vasoactive substances are thought to contribute to the pathogenesis of pulmonary hypertension.²⁹ Christman

et al.³² reported an imbalance between the excretion of thromboxane and that of prostacyclin metabolites in patients with pulmonary hypertension. We have elsewhere demonstrated increased expression of the vasoconstrictor peptide endothelin-1 in the endothelium of pulmonary arteries with severe arteriopathy in patients with pulmonary hypertension.²⁴ In the present study, when colocalization experiments were performed on consecutive sections of tissue, endothelial nitric oxide synthase and endothelin-1 appeared to have inverse patterns of expression (data not shown). Whereas endothelin-1 was strongly expressed by the diseased vessels,²⁴ endothelial nitric oxide synthase predominated in the normal vascular bed. Both the pharmacologic and the pathological features of pulmonary hypertension indicate that the initial reduction of pulmonary arterial blood flow results from vasoconstriction. Shear stress and alteration in flow are known to modulate the production of endothelin-1 and nitric oxide by endothelial cells.^{33,34} Although we have not determined the mechanisms of reduced expression of endothelial nitric oxide synthase in pulmonary hypertension, it is reasonable to postulate that in the normal lung, shear stress and flow maintain high constitutive expression of the enzyme in the pulmonary vasculature. Taken together, these considerations suggest that down-regulation of the endothelium-derived relaxing and antiproliferative factors (e.g., nitric oxide) and up-regulation of the endothelium-derived vasoconstrictor and mitogenic factors (e.g., endothelin-1) contribute to pulmonary hypertension.

The finding that airway and alveolar epithelial cells

express endothelial nitric oxide synthase is interesting. It is known that pulmonary epithelial cells produce factors that cause relaxation of smooth-muscle cells.^{35,36} Nitric oxide, recently shown to be a product of epithelial cells,^{20,21,37} has potent bronchodilatory effects and regulates ciliary beat.¹⁴ All previous reports have demonstrated the expression of inducible nitric oxide synthase by either histochemical staining for NADPH or immunostaining with antiserum to inducible nitric oxide synthase.^{20,37} Here, we have demonstrated by immunohistochemical analysis that pulmonary epithelium *in vivo* produces endothelial nitric oxide synthase and by *in situ* hybridization that it expresses the respective mRNA. The constitutive expression of endothelial nitric oxide synthase in the pulmonary epithelium and in the pulmonary endothelium may have similar effects, regulating smooth-muscle tone and homeostasis of the airway.

In conclusion, the current findings demonstrate that in the normal lung there is high basal expression of endothelial nitric oxide synthase in the endothelium of pulmonary vessels. In contrast, under pathologic conditions such as primary or secondary pulmonary hypertension, the expression of this enzyme in the endo-

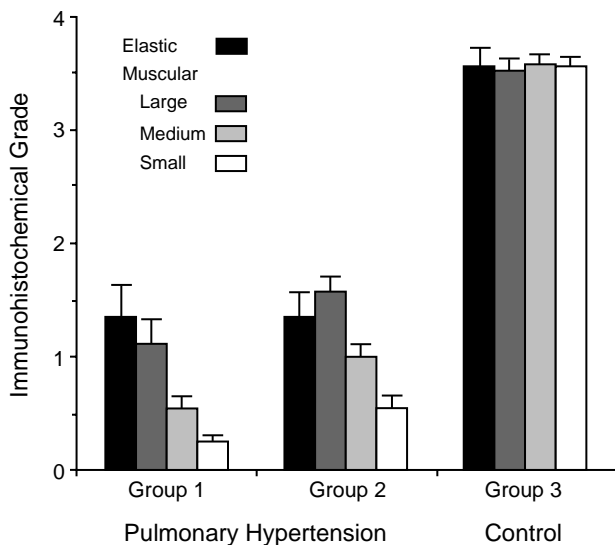


Figure 3. Mean (+SE) Endothelial Nitric Oxide Synthase-Like Immunoreactivity in the Vascular Endothelium of Pulmonary Arteries in the Two Groups of Patients with Pulmonary Hypertension and the Control Group.

The patients with pulmonary hypertension were subdivided according to morphologic and clinical criteria into the group with plexogenic pulmonary arteriopathy (group 1) and the group with secondary pulmonary hypertension (group 2). Endothelial nitric oxide synthase-like immunoreactivity was assessed in the endothelium of elastic pulmonary arteries ($\geq 500 \mu\text{m}$ in diameter) and large (300 to $500 \mu\text{m}$), medium-sized (>100 to $<300 \mu\text{m}$), and small ($\leq 100 \mu\text{m}$) muscular pulmonary arteries. The level of immunostaining was significantly higher in the control group (group 3) than in the patients with pulmonary hypertension ($P < 0.001$ for all artery types). When group 2 was compared with group 1, $P = 0.014$ for medium-sized pulmonary arteries and $P = 0.009$ for small pulmonary arteries. Immunohistochemical grades were determined semiquantitatively, as described elsewhere.²⁴

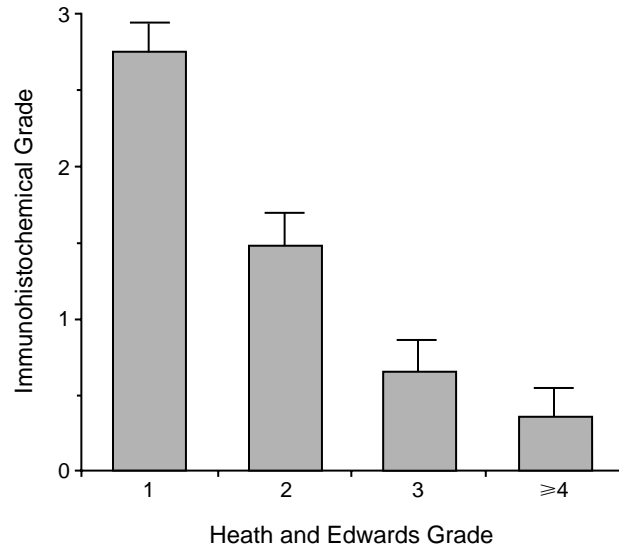


Figure 4. Relation between Immunoreactivity to Endothelial Nitric Oxide Synthase and the Severity of Morphologic Changes in the Pulmonary Arteries of Patients with Pulmonary Hypertension.

Mean (+SE) endothelial nitric oxide synthase-like immunoreactivity was measured semiquantitatively by immunohistochemical methods²⁴ in the vascular endothelium of pulmonary arteries, and histologic changes in the arteries were graded on the Heath and Edwards scale⁴ in patients with pulmonary hypertension. Arteries with Heath and Edwards grades of 1 or 2 had significantly higher immunoreactivity ($P < 0.001$) than those with grades of 3 or above.

thelium of pulmonary arteries with severe morphologic abnormalities is reduced. The reduced expression of this enzyme correlates inversely with increased vascular resistance. Therapy with analogues of endothelial nitric oxide synthase may provide a new, safe, more widely applicable and comprehensive therapeutic approach to the prevention of pulmonary hypertension.

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