Hemodynamic Effects of Basal and Stimulated Release of 
Endogenous Nitric Oxide in Isolated Human Lungs

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Background—We compared the hemodynamic responses to inhibition or stimulation of endothelial nitric oxide (NO) release of isolated explanted lungs from transplantation recipients with pulmonary hypertension and in normotensive unallocated donor lungs.

Methods and Results—Lungs from 10 patients with severe pulmonary hypertension (SPH) and from 16 patients with severe chronic obstructive lung disease (COLD) were studied. Fourteen normotensive lungs were studied as controls. The lungs were perfused at a constant flow. In protocol 1 N\textsuperscript{G}-nitro-L-arginine methyl ester caused a similar rise in baseline pulmonary artery pressure (PAP) that was similar in SPH (+17.1±4.2 mm Hg; n=5), COLD (+15.5±4.8 mm Hg; n=8), and control lungs (+14.5±1.5 mm Hg; n=7). Arterial occlusion demonstrated that most of the changes with N\textsuperscript{G}-nitro-L-arginine methyl ester were precapillary. The response to sodium nitroprusside (10\textsuperscript{-8} to 10\textsuperscript{-4} mol/L) was similar in all groups. In protocol 2, the lungs were preconstricted, and acetylcholine (10\textsuperscript{-9} to 10\textsuperscript{-5} mol/L) caused a lesser fall in PAP in both COLD and SPH lungs compared with control (−41.9±8.6%, −55.7±7.6%, and −73.2±2.5%, respectively; P<0.05), whereas sodium nitroprusside (10\textsuperscript{-5} mol/L) decreased PAP to initial levels in all lungs.

Conclusions—Stimulated release of NO is impaired in arteries of lungs with plexogenic or hypoxemic pulmonary hypertension. In contrast, basal release of NO appears to be maintained. (Circulation. 1999;100:1316-1321.)

Key Words: endothelium-derived factors ■ lung ■ endothelium ■ hypertension ■ vasculature ■ hypoxia

Pulmonary hypertension, both unexplained and secondary to chronic hypoxic lung disease or congenital heart disease, is associated with increased resistance to pulmonary blood flow. Failure of the pulmonary circulation to adapt to increased cardiac output is implicated in the marked rise in perfusion pressure observed during exercise and leading to decreased exercise tolerance in these patients. Structural changes such as narrowing, fibrosis, and thrombosis of pulmonary arteries contribute to this abnormal behavior. Endothelium-derived vasoactive substances such as nitric oxide (NO) and prostacyclin (PGI\textsubscript{2}) inhibit cellular proliferation and may be implicated in vascular remodeling. Basal NO contributes to the maintenance of low vascular tone in a number of species, including humans, and this release is affected by blood flow. Evidence exists supporting impaired release of NO\textsuperscript{8,9} and PGI\textsuperscript{10} in pulmonary hypertension, but contrasting results have been reported in the expression of NO synthase in hypertensive lungs. The current study was carried out on isolated, perfused, ventilated explanted lungs from patients with plexogenic pulmonary hypertension and from patients with chronic hypoxemia secondary to airway disease caused by cystic fibrosis or bronchiectasis. The hemodynamic effects of inhibition of basal and stimulated release of NO were studied, and arterial occlusion maneuvers were performed to describe segmental changes in the pulmonary pressure gradient in disease and locate the site of NO release. Sodium nitroprusside was used to assess the response of the pulmonary vascular bed to endothelium-independent nitrovasodilatation.

Methods

The explanted lungs of patients with end-stage chronic obstructive lung disease (COLD) and severe pulmonary hypertension (SPH) caused by primary (unexplained) pulmonary hypertension or Eisenmenger’s syndrome were obtained at the time of transplantation surgery (Table 1). The control group consisted of lungs obtained from patients who had died from head injuries or cerebrovascular accidents and were unallocated for lung transplantation for lack of a suitable recipient. These patients had clear chest radiographs and satisfactory gas exchange and normal pulmonary hemodynamics (Table 2).
The acquisition of explanted lungs for study in isolation has been previously described in detail. Immediately after the first lung (usually the left) had been excised, the main bronchus was intubated and ventilated with room air. A cannula was inserted into the pulmonary artery, and cold (10°C) extracellular preservation solution was flushed through the pulmonary circulation until the lung was warmed (37°C) under a heated Perspex cover.

Pulmonary venous drainage was free and collected into a heated reservoir at 37°C. Pulmonary artery pressure (PAP) was recorded with a pressure transducer (P50, Spectramed) attached to a side port close to the end of the pulmonary artery cannula. Pressures were referenced to the level of the hilum. The lung was kept moist and warm (37°C) under a heated Perspex cover.

At a constant rate of perfusion, the PAP was measured at end-expiration at atmospheric pressure. Analogue signals from the pressure transducer and Doppler flow probe were digitized at 500 Hz (MP100, Biopac) and stored for off-line analysis (Apple Computer, Inc.).

Arterial occlusion maneuvers were carried out by rapidly diverting the flow away from the lung for 10 seconds and recording pressure and flow curves. A monoexponential curve was fitted to a portion of the pressure decay trace after occlusion. This curve was then extrapolated backward to the instant of occlusion (Figure 1). This allowed the identification of the pressure at the distal end of the large, relatively nonstenosable arterial segment (Pa2). In this way, the total pressure gradient across the pulmonary vascular bed can be divided into 2 segments (arterial segment = PAP − Pa2 + precapillary + venous segment = Pa2 − outflow pressure).

Once the perfusate temperature reached 37°C, a 20-minute equilibration period was allowed. Indomethacin (final concentration 10−5 mol/L, dissolved in a small volume of 3% Na2CO3) was then added to the perfusate to inhibit cyclo-oxygenase activity.
Pulmonary perfusion pressure was increased by the addition of the thromboxane analogue U46619 (11α, 9α-epoxymethano-9α, 11β-dideoxy-prostaglandin F2α). A final concentration in the reservoir of $10^{-9}$ to $10^{-7}$ mol/L was used to achieve a rise in PAP of $\approx 12$ to 15 mm Hg. After a stable plateau of pressure was attained, cumulative doses of acetylcholine (ACh, final concentrations in the reservoir $10^{-9}$ to $10^{-7}$ mol/L) were added to the perfusate. A single dose of sodium nitroprusside (SNP; $10^{-6}$ mol/L) was subsequently added to the reservoir to fully vasodilate the lungs.

After the experiments the lungs were inflated, fixed in formaldehyde at pressure of 20 mm Hg, and examined both macroscopically and by light microscopy. About 9 to 10 sections were taken from the upper, lower, and lower lobes, and separate sections were taken from the pulmonary artery and vein, which allowed a qualitative histopathologic description. Staining was done with hematoxylin and eosin and with Verhoeff-van Gieson.

All reagents were obtained from Sigma Chemical Company and were dissolved in 2 mL Krebs-Henseleit solution, except for indomethacin.

### Statistical Analysis

Results are expressed as mean±SEM. The changes in PAP after L-NAME were analyzed by multiple linear regression by use of initial PAP, diagnosis, and their interaction as independent variables. The response to ACh was analyzed as the percentage fall in PAP induced by the vasoconstrictor and the baseline PAP. The half effective dose (ED50) for SNP was determined by interpolation of computer-fitted relaxation curves. Statistical comparisons were carried out by Student's t test or ANOVA with Scheffe’s test for multiple comparisons. Significance was set at a value of $P<0.05$.

### Results

The pathological findings of the pulmonary vessels differed greatly in the 2 disease groups. The COLD lungs all exhibited medial hypertrophy and/or intimal thickening. Besides these findings, the SPH lungs had atheromatous changes in the large arteries and intimal fibrosis in the distal vessels, and, in all but 1 lung, plexiform lesions were observed (Table 1).

The PAP values were higher in lungs from COLD and SPH compared with controls (COLD 12.6±4.5, SPH 57.8±4.9, and control 6.8±0.7 mm Hg; $P<0.01$, Scheffe’s test). At baseline the lungs appeared to have no added baseline tone beyond the baseline PAP did not affect the response to the inhibitor. SNP (10$^{-4}$ mol/L) restored baseline PAP values in all the groups. There was no significant shift of the dose-response curves between the different groups after administration of SNP (10$^{-4}$ to 10$^{-7}$ mol/L; ED50 for COLD 49.3±19.2 μmol/L;
SPH 23.2±11.2 μmol/L, and control 28.1±16.5 μmol/L; Figure 3).

Analysis of the occlusion curves showed that approximately one third of the total pressure drop was found in the proximal arterial segment in the control lungs, whereas this segment accounted for ~40% in COLD lungs but only ~20% of the total pressure gradient in SPH. The main effect of L-NAME (10⁻⁵ mol/L) was to increase the distal segmental pressure (precapillary + venous) gradient all 3 groups. In COLD and control lungs there was also a small increase in the proximal arterial gradient, but there was a reduction in SPH lungs (Figure 4).

Protocol 2: Effects of ACh on Constricted Human Lungs

U46619 (10⁻⁹ to 10⁻¹⁰ mol/L) caused a stable increase in PAP in all the lungs (COLD 13.4±4.3 mm Hg, SPH 14.6±5.3 mm Hg vs control 12.4±2.4 mm Hg, P=0.8, Scheffé’s test), although the dose of U46619 necessary to increase PAP by this amount was 10 to 100 times lower in the SPH and COLD lungs than in donor lungs.

ACh (10⁻⁹ to 10⁻⁵ mol/L) caused a dose-dependent decrease in PAP (COLD, n=8, P=0.004; SPH, n=5, P=0.001; control, n=7, P<0.001, Scheffé’s test, Figure 5). The maximal vasorelaxation with ACh was less in both SPH and COLD lungs compared with control lungs (Figure 5, respectively, -55.7±7.6% and -41.9±8.6% compared with -73.2±2.5%; P=0.02 and 0.01; unpaired t test). In all groups, subsequent addition of SNP (10⁻⁵ mol/L) restored PAP to initial levels (P=0.9 compared with baseline values, paired t test).

Discussion

The main findings of this study are that whereas inhibition of basal NO synthesis caused an equivalent rise in PAP in both normotensive and hypertensive lungs, stimulation of NO
release by ACh after preconstriction caused a smaller decrease in PAP in hypertensive lungs compared with control. In contrast, exogenous NO restored PAP to initial levels in all lungs. The responses in the diseased lungs were uniform despite the disparity in underlying cause, histopathologic findings, airway function, and baseline PAP.

The anatomic and mechanical changes occurring in disease may alter the responsiveness to NO-induced vasorelaxation. The hypertrophied muscular layer may be less responsive to NO. On the other hand, if one assumes the hypertensive vascular beds to consist of narrowed and/or stiff vessels, then a similar change in the radius of the vessels should lead to larger absolute changes in pressure in hypertensive lungs. Likewise, if the hypertensive vasculature consisted of areas of occluded vessels along with areas of normal vessels, then inhibition of a similar release of NO should lead to increases in PAP proportional to the initial values unless the normal vessels had an increased distensibility. The decrease in PAP of the proximal arterial segment after L-NAME in the SPH lungs suggests that such an increase in distensibility is possible.

The efficient response to U46619 in the COLD and SPH vessels as well as the conserved response to nitroprusside indicates that an altered responsiveness of the vascular smooth muscle is unlikely. Furthermore, the lack of correlation between initial PAP and the change in PAP after NO synthesis inhibition suggests that initial PAP did not affect the responsiveness of the lungs. The failure of SNP to reduce PAP to below initial levels in the diseased lungs indicates that structural changes rather than vasocostriction are the likely cause of the elevated values of PAP.13,14 The extensive fibrosclerotic changes observed in SPH lungs may explain the lack of effect of L-NAME on the proximal arterial segment.

Both reduced and increased expression of protein and mRNA for NO synthase has been reported in lungs from patients with pulmonary hypertension,11,12 suggesting that there is a heterogeneity of NO release. However, measurement of NO synthase activity may not reflect NO production and does not give information regarding the effect on pulmonary hemodynamics. We have previously shown that direct measurement of NO output (VNO) in the expired air of isolated lungs is related to pulmonary vascular resistance.15 Furthermore, we showed that VNO was not reduced in patients with primary pulmonary hypertension,16 although sources of VNO other than the pulmonary vasculature could have affected the results. The findings in this study suggest that resistance pulmonary arteries in diseased lungs are able to maintain endothelial release of NO perhaps in those vessels in which structural abnormalities are limited. This release of NO appears to be hemodynamically important.

The finding that pharmacologically stimulated NO-mediated vasodilation is impaired in SPH and COLD is consistent with an earlier study of isolated rings of human conduit elastic pulmonary arteries from explanted lungs.8,9 This decreased response was not due to an impairment in signal transduction because the response to other agents such as calcium ionophore and ADP, which act on different receptors, were similarly altered.17 Furthermore, there was no evidence of substrate deficiency because l-arginine failed to restore normal responses.

The difference in the histopathologic changes in COLD and SPH lungs was striking. The only common factor that linked the diseased lungs was the consistent severe long-term hypoxemia. In piglets exposed to long-term hypobaric hypoxia, basal accumulation of cGMP was unchanged compared with normoxic controls, but the increase of cGMP with ACh was impaired.18 Further, the cGMP accumulation after nitroprusside was unchanged in the hypoxic animals. Long-term hypoxia may impair the phosphorylation of the particulate NO synthase associated with translocation of the enzyme from membrane to cytosol.19 Other mechanisms such as loss of sulfhydryl groups from cell membranes of pulmonary artery endothelium have been shown to cause a marked reduction of NO synthase activity20 and may be affected in chronic pulmonary disease states.

In conclusion, inhibition of NO synthesis caused a similar increase in pulmonary artery pressure in human lungs with COLD or SPH. In contrast, the response to stimulated release of NO with ACh was reduced. Although it is unlikely that reduced NO synthesis is primarily responsible for the raised pulmonary vascular resistance in these conditions, reduced NO release may be of importance in the response of the vascular bed to shear stress and hypoxia.

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References


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