Use of Inhaled Nitric Oxide and Acetylcholine in the Evaluation of Pulmonary Hypertension and Endothelial Function After Cardiopulmonary Bypass

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Background. Increased pulmonary vascular resistance is common in congenital heart disease and is exacerbated by cardiopulmonary bypass (CPB). We investigated whether CPB is responsible for pulmonary endothelial dysfunction and contributes to postoperative pulmonary hypertension.

Methods and Results. We infused the endothelium-dependent vasodilator acetylcholine (ACH) into the pulmonary circulation of pulmonary hypertensive children with congenital heart disease either before (n=12) or after (n=22) surgical repair on CPB. The dose response to ACH (10^{-9} to 10^{-4} M) was recorded for all hemodynamic variables. Nine additional postoperative patients were studied with ACH followed by inhalation of 80 ppm nitric oxide, an endothelium-independent smooth muscle relaxant. Plasma levels of cyclic GMP (cGMP) were measured before and after ACH and nitric oxide administration. Pulmonary vasodilation with 10^{-4} M ACH was seen in all preoperative patients but was markedly attenuated in postoperative patients. Baseline pulmonary vascular resistance (5.6±1.0 U·m²) fell 46±5% in preoperative patients but declined only 11±4% from baseline (5.8±0.9 U·m²) in postoperative patients (P<.002). However, inhalation of 80 ppm nitric oxide after ACH infusion in postoperative patients lowered pulmonary vascular resistance by 33±4% (P<.0002 compared with postoperative ACH response) with minimal effects on the systemic circulation. This finding suggests that the capacity for smooth muscle relaxation and pulmonary vasodilation was present in postoperative patients but could not be induced by ACH. Plasma levels of cGMP in postoperative patients were unchanged after acetylcholine infusion but rose more than threefold during pulmonary vasodilation with nitric oxide (P<.0001). This finding is consistent with the purported role of cGMP as the second messenger effecting smooth muscle relaxation in this process.

Conclusions. CPB may be responsible for postoperative dysfunction of the pulmonary endothelial cell and may contribute to postoperative pulmonary hypertension in children. Inhaled nitric oxide is a potent pulmonary vasodilator after CPB with minimal systemic circulatory effects. It may have important diagnostic and therapeutic applications in patients with congenital heart disease. (Circulation. 1993;88[part 1]:2128-2138.)

KEY WORDS • congenital heart disease • pediatrics • cyclic GMP

E levated pulmonary vascular resistance (PVR) is common in congenital heart disease1-4 and is especially important after open-heart surgery, where it appears to be exacerbated by cardiopulmonary bypass (CPB).5-12 Attempts to understand and manage post-CPB pulmonary hypertension have met with limited success. However, recent developments in vascular biology have offered new insights into the possible causes and correction of post-CPB pulmonary hypertension.

Acetylcholine (ACH) relaxes preconstricted vascular smooth muscle by binding to muscarinic receptors on the endothelial cell, causing release of a potent vasodilating substance called endothelium-derived relaxing factor (EDRF). The active component of EDRF has recently been identified as nitric oxide.13-16 Nitric oxide is thought to diffuse from the endothelium to adjacent smooth muscle and produce relaxation by activating guanylate cyclase and increasing intracellular cyclic GMP (cGMP).17 This response is dependent on an intact, functioning endothelium.

ACH-induced vasodilation is attenuated or abolished in a variety of illnesses that impair endothelial function, including atherosclerotic diseases,18-22 chronic congestive heart failure,23,24 and systemic hypertension.25 Pulmonary vascular endothelial dysfunction may contribute to pulmonary hypertension since preconstricted pulmonary vascular ring preparations from explanted lungs of patients with pulmonary hypertension fail to vasodilate in response to ACH.26 This loss of physiologic function has an anatomic correlate, with demonstrable changes
in endothelial structure in both laboratory-induced pulmonary hypertension and human disease.27,28

Pulmonary vascular endothelial dysfunction may be a contributing factor in post-CPB pulmonary hypertension.29-30 Structural damage to the pulmonary endothelium is demonstrable after CPB, and the degree of pulmonary hypertension is correlated to the extent of endothelial damage after CPB.31,32 The decreased pulmonary blood flow on CPB may result in postoperative impairment of endothelial function and inability to release nitric oxide. To overcome this, gaseous nitric oxide can be delivered to the pulmonary circulation, by direct inhalation, to produce pulmonary vasodilation. Nitric oxide–induced pulmonary vasodilation of preconstricted vessels has been shown to occur in sheep and humans.33-35 When the endothelium-dependent effect of ACH fails to produce pulmonary vasodilation but vasodilation can be elicited by inhaled nitric oxide, the functional integrity of the smooth muscle can be established in the presence of endothelial dysfunction.

Therefore, we have investigated whether CPB is responsible for a change in the function of either the pulmonary endothelial cell or the underlying smooth muscle cell. We sought to characterize the functional disturbance induced by CPB by measuring both hemodynamic responses and changes in cGMP production during endothelial stimulation (ACH infusion) and direct smooth muscle relaxation (inhalation of nitric oxide).

Methods

Population of Patients

We used three protocols. Twelve preoperative patients (pre-CPB) were studied in the cardiac catheterization laboratory with a single ACH dose. Twenty-two postoperative patients (post-CPB; group 1) were studied immediately after CPB with increasing doses of ACH. Five of the 12 preoperative patients presented for surgery later during the study and were enrolled for postoperative investigation. Nine additional postoperative patients (post-CPB; group 2) were studied immediately after CPB with a single ACH dose followed by nitric oxide.

Preoperative (pre-CPB) patients—ACH only. Patients were selected from children undergoing cardiac cathe-
erative pulmonary resistance with a mean pulmonary artery pressure of ≥25 mm Hg with a transpulmonary pressure gradient ≥15 mm Hg (PVR ≥ 3 U·m⁻²) or increased risk for postoperative pulmonary hypertension based on preoperative pulmonary hypertension and left-to-right ventricular shunts (even though actual postoperative PVR was ≤3 U·m⁻²). Patients were studied in the cardiac intensive care unit within 24 hours of repair on CPB. Diagnoses included closure of ventricular or atrioventricular septal defect (n=14 including 5 with Down syndrome), arterial switch operation and closure of ventricular septal defect (n=4), tetralogy of Fallot repair with nontransannular right ventricular outflow tract reconstruction (n=3), and repair of total anomalous pulmonary venous connection (n=1). Patients ranged in age from 8 days to 8 years (median, 7.0 months) and had a mean body surface area of 0.39±0.4 m². Baseline arterial blood gases were normal with no evidence of hypoventilation. Indwelling catheters were placed intraoperatively in the radial and pulmonary arteries and the left and right atria. The pulmonary arterial catheter included a thermistor, permitting cardiac output determination by thermodilution technique. A second catheter was placed in the right atrium for infusion of ACH. The intraoperative anesthetic and CPB technique, including intravenous pancuronium and fentanyl (50 to 75 μg/kg), has recently been described. Residual anatomic lesions were excluded by analysis of pulmonary artery and atrial pressures and oxygen saturations, inspection of the thermodilution cardiac output curve, physical examination, and postoperative echocardiography.

Postoperative sedation included intravenous morphine and midazolam. Patients were mechanically ventilated with a Siemens 900 C Servo ventilator using the volume preset mode. The fraction of inspired oxygen was 0.40 to 0.50, and ventilatory settings were unchanged throughout the study. All patients were hemodynamically stable on less than 10 μg·kg⁻¹·min⁻¹ doxamine and received no vasodilator therapy. After baseline hemodynamic and blood gas data were obtained, sequential infusion of increasing log molar doses of ACH (10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ M) into the right atrium was begun. Hemodynamic variables (including thermodilution cardiac output) were recorded at the end of each 2-minute infusion. After the final infusion of ACH, repeat hemodynamics, blood gas data, and hemoglobin levels were measured.

All group 1 patients with a mean pulmonary artery pressure ≥25 mm Hg and PVR ≥ 3 U·m⁻² (n=9) were analyzed separately to allow comparison of preoperative and postoperative patients with comparable baseline pulmonary vascular tone.

Postoperative (post-CPB) patients—group 2 (ACH and nitric oxide). We studied nine additional patients with sustained elevation in postoperative pulmonary artery pressure (≥25 mm Hg) and PVR (≥3 U·m⁻²) using a single dose of ACH (10⁻³ M) followed by inhalation of nitric oxide (80 ppm). Diagnoses included closure of ventricular or atrioventricular septal defect (n=4), mitral valvuloplasty or valve replacement (n=3), arterial switch operation and ventricular septal defect closure (n=1), and repair of total anomalous pulmonary venous connection (n=1). Patients ranged in age from 1 day to 11 years (median age, 15 months) and had a mean body surface area of 0.44±0.08 m². Baseline arterial blood gases were normal with no evidence of hypoventilation.

Catheters were placed for monitoring as described above. After baseline measurements, a single infusion of ACH was administered over 2 minutes via the right atrial catheter, and the hemodynamic measurements were repeated at the end of the infusion. After 15 minutes, inhaled nitric oxide was administered for 15 minutes. Hemodynamic changes were monitored continuously and recorded at the beginning and end of each intervention. Methemoglobin levels were measured 15 minutes after nitric oxide administration, with a co-oximeter (CIBA-Corning model 2500) using a multiple wavelength spectrophotometric method.

**cGMP Measurement**

Two milliliters of blood was collected in EDTA from either the left atrial or radial artery catheter at five separate times in group 2 patients: (1) before ACH infusion; (2) at completion of the ACH infusion; (3) 15 minutes after the ACH infusion; (4) after 15 minutes of nitric oxide inhalation; and (5) 15 minutes after the end of the inhalation. Samples were placed on ice and centrifuged for 15 minutes at 4°C as soon as the study was completed. The resulting plasma was stored at −80°C until assayed. Extraction of cGMP was performed by adding 0.5 mL deionized water and 1 mL acetonitrile to 250 μL plasma, vortexing for 20 seconds, and then centrifuging at 1500g for 15 minutes at 4°C. The supernatant was added to trimethylaminopropyl SAX columns (Amprep minicolumns, Amersham Int PLC, UK) that had been conditioned with 2 mL methanol followed by 2 mL deionized water. The columns were washed under vacuum with 3 mL methanol, and the cGMP was eluted with 3 mL methanol in 0.1N HCl. The eluant was taken to dryness by spinning in a vacuum centrifuge. The lyophilate was reconstituted in 2 mL 0.05 mol/L acetate buffer with 0.01% sodium azide. Recovery, estimated by adding a known amount of assay standard, was 99%. cGMP was assayed using the Amersham cGMP [¹²⁵I] assay system (Amersham Int PLC, UK), using a method modified from Steiner et al. The sensitivity of the assay was 0.5 fmol, and the cross-reactivity with other adenosine or guanosine phosphates was less than 0.001. cGMP levels are expressed in pmol/mL.

**Acetylcholine Preparation**

ACH (Miocol) was diluted in 5% dextrose in water to yield five concentrations: 10⁻⁴, 10⁻⁵, 10⁻⁶, and 10⁻⁷ M. ACH was infused into the pulmonary circulation at a rate (in mL/min) equal to the baseline measured pulmonary blood flow (in L/min), so as to achieve final concentrations of ACH in the pulmonary artery of 10⁻³ to 10⁻⁴ M for all patients, independent of the amount of pulmonary blood flow.

**Nitric Oxide Preparation and Delivery**

Nitric oxide was supplied from Scott Specialty Gases (Plumsteadville, Pa) in cylinders containing nitric oxide in nitrogen at a concentration of 800 ppm. The gas was specially prepared and determined, in accordance with the Food and Drug Administration, to be medical-grade quality. The nitric oxide was then blended with gas from a pure nitrogen tank and fed into a low-flow blender at
50 psi. The percentage of nitric oxide distal to the blender thus could be controlled by varying the blended flow from pure nitrogen (no nitric oxide) to all 800 ppm nitric oxide. The resulting mixture of nitric oxide and nitrogen passed to one inlet of a second blender, where it was mixed with 100% oxygen. The fraction of inspired oxygen delivered to the patient could be varied over a wide range of nitric oxide concentrations. The flow distal to the second blender was controlled by a flowmeter. Patients were mechanically ventilated in a volume-preset mode that allowed the nitric oxide/nitrogen/oxygen gas mixture to enter into the low pressure inlet at a rate equal to the minute ventilation of the patient. The expired gas was scavenged through an exhaust system. Nitric oxide and nitrogen dioxide were continuously analyzed from a sampling line at the Y connector attached to the endotracheal tube, using a chemiluminescence device41,42 (model 42A, Thermoenvironmental Instruments, Franklin, Mass) that was calibrated before each use with a calibration gas of known 80±1 ppm nitric oxide in nitrogen (Scott Specialty Gases).

Statistical Analysis
A paired t test was used to test the significance of a change in hemodynamic variables after a single dose of ACH or nitric oxide compared with baseline. Repeated-measures ANOVA was used to test the effect of sequential doses of ACH on hemodynamic parameters. If significance was established, a paired t test was applied to each dose to test significance of change from baseline value, and a correction for comparison of baseline data to several levels of intervention was used (Dunnett’s). Comparison of baseline hemodynamic variables between preoperative and postoperative patients was performed with a t test for unpaired observations. Postoperative (post-CPB) patients—group 1 (ACH only) who had been studied preoperatively were eliminated from analysis of the comparative response (preop versus postop) to ACH. Postoperative patients with high pulmonary artery pressure were compared with other postoperative patients using a two-way ANOVA. Values are expressed as mean±standard error of the mean and P values <.05 were considered statistically significant.

Informed Consent
Informed consent was obtained from the parents of all patients under a protocol approved by the Food and Drug Administration (FDA) and the Clinical Investigation Committee of Children’s Hospital. Inhaled nitric oxide in humans is an investigational drug, and an investigational new drug number was obtained from the FDA for this study.

Results
Response to Acetylcholine
Preoperative (pre-CPB) patients (ACH only). Preoperative patients demonstrated a significant fall in baseline pulmonary artery pressure (42.8±4.1 versus 31.6±3.6 mm Hg, P<.0001) and PVR (5.6±1.0 versus 3.0±0.7 U·m², P<.0005) after a single dose of ACH (10⁻⁶ M). Systemic blood pressure fell (70.1±4.3 versus 57.0±5.2 mm Hg, P<.005), as did systemic vascular resistance (18.0±1.8 versus 14.2±2.2 U·m², P<.03). The rise in pulmonary blood flow was not significant. Cardiac index and left atrial pressure were unchanged, although right atrial pressure fell slightly (7.0±0.5 versus 6.3±0.5 mm Hg, P<.02). Arterial blood gases were unchanged (Table 1). The response of mechanically ventilated patients breathing supplemental oxygen was no different than that of other preoperative patients.

Postoperative (post-CPB) patients—group 1 (ACH only). In 8 of 22 postoperative patients (group 1), the dose-response investigation was continued through the 10⁻³ M infusion of ACH. However, at this dose, mean systemic blood pressure fell by 16±5 mm Hg, pulmonary artery pressure was unchanged, and 4 patients developed signs of transient side effects of ACH with bronchospasm and tachycardia associated with systemic hypotension lasting for 10 to 15 seconds. Thus, 10⁻⁶ M ACH was judged to be the maximal safe and effective dose to elicit pulmonary vasodilation. No additional patients received 10⁻³ M ACH, and data were analyzed from 10⁻⁶ through 10⁻⁴ M only.

In the postoperative patients (group 1), no significant change in pulmonary artery pressure or PVR occurred at any dose of ACH. Although atrial pressures rose slightly over the course of the study, no other hemodynamic or arterial blood gas variable changed to any clinically or statistically significant extent throughout the study (Table 2).

Postoperative (post-CPB) patients—group 1 (ACH only) subgroup with high pulmonary artery pressure. Analysis of the subgroup of postoperative patients with high pulmonary artery pressure again demonstrated no significant change in pulmonary artery pressure at any dose of ACH. At the maximal ACH dose (10⁻⁴ M), a small fall in pulmonary artery pressure (30.8±1.4 versus 28.9±2.1 mm Hg, P=NS) associated with a small rise in left atrial pressure with unchanged cardiac index produced a decrease in PVR (5.4±0.6 versus 4.2±0.5 U·m², P<.02) (Table 3).

Postoperative (post-CPB) patients—group 2 (ACH and nitric oxide). In the postoperative patients (group 2), pulmonary artery pressure fell somewhat (32.9±3.0 versus 30.0±2.7 mm Hg, P<.005), and PVR fell slightly (5.8±0.9 versus 5.1±0.8 U·m², P<.05) in response to the single dose of 10⁻⁴ M ACH. There was a fall in systemic blood pressure but no statistically significant change in cardiac index or systemic vascular resistance (Table 4). ACH had no effect on other hemodynamic or arterial blood gas variables.

Comparative Effects of Acetylcholine
The marked fall in pulmonary artery pressure and PVR with the 10⁻⁶ M infusion of ACH in preoperative patients was significantly attenuated in postoperative patients. Fig 1 shows the percentage change in pulmonary artery pressure compared with baseline following ACH infusion in preoperative patients (−27±4%) and postoperative patients (+3±4%). Similarly, preoperative patients had a 46±5% reduction in PVR, but postoperative PVR fell by only 5±8% (Fig 2). Even if baseline pulmonary artery pressure were high postoperatively, the ACH-induced fall in PVR after CPB was less than half that observed before CPB (P<.02).

The fall in PVR in preoperative patients could not be ascribed to a rise in pulmonary blood flow since flow increased by less than 10% in 9 of 12 patients and pulmonary artery pressure fell in all. There were no
significant differences in baseline pulmonary blood flow in preoperative versus postoperative patients that could account for the differential sensitivity of pulmonary artery pressure and PVR to ACH.

Since atrial pressures rose slightly during sequential infusions of ACH in postoperative patients (Table 2), the reduction in calculated PVR might be attributed in part to the rise in left atrial pressure associated with multiple infusions. Therefore, postoperative patients (group 2) received a single dose of $10^{-6}$ M ACH, analogous to the preoperative patient protocol. This produced a mild fall in pulmonary artery pressure without change in cardiac index or atrial pressures; similarly, PVR fell slightly (Table 4). However, ACH-induced pulmonary vasodilation was substantially less than the dilution seen in preoperative patients at the same dose of ACH. Fig 3 compares the percentage fall in pulmonary artery pressure in preoperative patients with postoperative patients (group 2) (27±4% versus 9±2%, $P<.003$). Fig 4 compares the percentage fall in PVR in preoperative patients with postoperative patients (group 2) (46±5% versus 11±4%, $P<.002$). Attenuated response to ACH among postoperative patients could not be attributed to statistically significant differences in baseline PVR.

**Response to Nitric Oxide**

In contrast to ACH, breathing 80 ppm nitric oxide for 15 minutes reduced mean pulmonary artery pressure (34.4±2.6 versus 25.8±2.4 mm Hg, $P<.0001$) and PVR (6.8±1.2 versus 4.5±0.8 U · m$^{-2}$, $P<.005$) in all nine postoperative patients (group 2). This was associated with a statistically significant although clinically small decrease in right atrial pressure (9.8±0.9 versus 8.9±0.9 mm Hg, $P<.05$) and systemic blood pressure (65.8±2.4 versus 60.9±2.6 mm Hg, $P<.02$) but no changes in other hemodynamic variables (Table 5). Arterial blood gases were unaffected by nitric oxide. The decrease in pulmo-

### Table 2. Postoperative (Post-CPB) Patients—Group 1 (ACH Only) Hemodynamic Dose-Response to ACH

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10$^{-9}$</th>
<th>10$^{-8}$</th>
<th>10$^{-7}$</th>
<th>10$^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>9.1±0.6</td>
<td>10.2±0.6*</td>
<td>10.7±0.7*</td>
<td>11.5±0.6*</td>
<td>11.3±0.6*</td>
</tr>
<tr>
<td>Mean systemic blood pressure, mm Hg</td>
<td>67.7±2.1</td>
<td>67.0±2.5</td>
<td>69.0±3.0</td>
<td>70.0±2.6</td>
<td>69.0±2.8</td>
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<tr>
<td>Left atrial pressure, mm Hg</td>
<td>9.2±0.6</td>
<td>9.8±0.7</td>
<td>10.2±0.7*</td>
<td>10.7±0.8*</td>
<td>10.9±0.7*</td>
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<td>Mean pulmonary artery pressure, mm Hg</td>
<td>23.9±1.5</td>
<td>24.5±1.6</td>
<td>25.3±2.0</td>
<td>24.5±1.6</td>
<td>24.1±1.4</td>
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<td>Cardiac index, L · min$^{-1} · m^{-2}$</td>
<td>4.05±0.20</td>
<td>3.90±0.19</td>
<td>3.85±0.20</td>
<td>3.91±0.20</td>
<td>4.01±0.21</td>
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<td>Heart rate, bpm</td>
<td>130±4</td>
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<td>128±4</td>
<td>127±4</td>
<td>128±5</td>
</tr>
<tr>
<td>Systemic vascular resistance, U · m$^{-2}$</td>
<td>15.3±0.9</td>
<td>15.2±0.8</td>
<td>15.8±0.9</td>
<td>15.9±1.0</td>
<td>14.7±0.8</td>
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<td>Pulmonary vascular resistance, U · m$^{-2}$</td>
<td>3.8±0.4</td>
<td>4.0±0.5</td>
<td>4.2±0.6</td>
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<td>pH</td>
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<td>PCO$_2$, mm Hg</td>
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<td>38±1</td>
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<td>PO$_2$, mm Hg</td>
<td>144±8</td>
<td>133±7</td>
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</table>

CPB indicates cardiopulmonary bypass; and ACH, acetylcholine. n=22.
*P<.05 compared with initial baseline using Dunnett's test.

### Table 3. Postoperative (Post-CPB) Patients—Group 1, Subgroup With High Pulmonary Artery Pressure Hemodynamic Dose-Response to ACH

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10$^{-9}$</th>
<th>10$^{-8}$</th>
<th>10$^{-7}$</th>
<th>10$^{-6}$</th>
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<tbody>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>8.8±0.9</td>
<td>9.7±1.0</td>
<td>10.7±1.1*</td>
<td>10.6±0.9*</td>
<td>11.2±1.1*</td>
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<tr>
<td>Mean systemic blood pressure, mm Hg</td>
<td>66.8±3.3</td>
<td>67.8±4.6</td>
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<td>70.1±5.1</td>
<td>70.4±5.4</td>
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<tr>
<td>Left atrial pressure, mm Hg</td>
<td>10.3±1.1</td>
<td>11.6±1.3</td>
<td>11.7±1.5</td>
<td>12.4±1.7*</td>
<td>12.4±1.4*</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>30.8±1.4</td>
<td>31.2±2.0</td>
<td>32.9±3.0</td>
<td>30.3±2.3</td>
<td>28.9±2.1</td>
</tr>
<tr>
<td>Cardiac index, L · min$^{-1} · m^{-2}$</td>
<td>3.94±0.32</td>
<td>3.65±0.28</td>
<td>3.68±0.34</td>
<td>3.68±0.32</td>
<td>3.86±0.38</td>
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<tr>
<td>Heart rate, bpm</td>
<td>127±5</td>
<td>125±5</td>
<td>127±4</td>
<td>124±5</td>
<td>125±5</td>
</tr>
<tr>
<td>Systemic vascular resistance, U · m$^{-2}$</td>
<td>15.3±0.9</td>
<td>16.3±0.7</td>
<td>16.5±1.3</td>
<td>16.8±1.3</td>
<td>15.0±0.8</td>
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<tr>
<td>Pulmonary vascular resistance, U · m$^{-2}$</td>
<td>5.4±0.6</td>
<td>5.8±0.8</td>
<td>6.1±1.06</td>
<td>4.8±0.6</td>
<td>4.2±0.5*</td>
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<td>pH</td>
<td>7.45±0.01</td>
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<tr>
<td>PCO$_2$, mm Hg</td>
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<tr>
<td>PO$_2$, mm Hg</td>
<td>137±11</td>
<td>128±10</td>
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</table>

CPB indicates cardiopulmonary bypass; and ACH, acetylcholine. n=9.
*P<.05 compared with baseline using Dunnett's test.
nary artery pressure and PVR with nitric oxide was greater than that with ACH (P<.0003 and P<.0002, respectively). However, the fall in PVR and pulmonary artery pressure with nitric oxide in postoperative patients was comparable to the response to ACH in preoperative patients (Figs 3 and 4).

**cGMP and Methemoglobin**

Blood levels of cGMP in postoperative patients (group 2) showed no change in response to ACH (10.9±3.7 versus 11.2±3.9 pmol/mL, P=NS) but rose more than threefold after 15 minutes of inhaled nitric oxide (12.4±3.2 versus 52.2±11.9 pmol/mL, P<.0001). After 15 minutes of nitric oxide inhalation, methemoglobin levels were all within normal range (0.8±0.3%).

**Discussion**

Children with many forms of congenital heart disease are prone to develop perioperative elevations in PVR.\(^1,^4\) This may complicate the postoperative course, when transient myocardial dysfunction requires optimal control of right ventricular afterload.\(^5,^6,^8,^11\) Several factors peculiar to CPB may raise PVR: microemboli, pulmonary leukosequestration, excess thromboxane production, atelectasis, hypoxic pulmonary vasoconstriction, and adrenergic events have all been suggested to play a role in producing postoperative pulmonary hypertension.\(^7,^8\) Postoperative pulmonary vascular reactivity has been related not only to the presence of preoperative pulmonary hypertension and left-to-right shunts\(^1,^6,^43\) but also to the duration of total CPB.\(^31,^32\) Treatment of postoperative pulmonary hypertensive crises has been only partly addressed by surgery at earlier ages, pharmacologic intervention, and other postoperative management strategies. It is likely that improved understanding of post-CPB pulmonary hypertension would improve survival after open-heart surgery in children.

**Endothelial Damage and Pulmonary Hypertension**

Loss of endothelium-dependent relaxation has been demonstrated in several models of pulmonary hypertension, including rat lungs exposed to chronic hypoxia,\(^44\) and suggested in various forms of pulmonary hypertension in humans.\(^26,^28,^45,^46\) Human pulmonary vascular ring preparations from lung transplant recipients with cystic fibrosis, chronic obstructive pulmonary disease, and Eisenmenger syndrome have a diminished in vitro response to ACH.\(^26\) This loss of physiologic function has

### Table 4. Postoperative (Post-CPB) Patients—Group 2 (ACH and Nitric Oxide) Hemodynamic Response to ACH

<table>
<thead>
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<th>Baseline</th>
<th>10⁻⁶ M ACH</th>
<th>P</th>
</tr>
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<tr>
<td>Right atrial pressure, mm Hg</td>
<td>10.0±0.8</td>
<td>10.4±1.0</td>
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<tr>
<td>Mean systemic blood pressure, mm Hg</td>
<td>65.9±3.5</td>
<td>61.4±3.7</td>
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<tr>
<td>Left atrial pressure, mm Hg</td>
<td>11.6±1.7</td>
<td>11.8±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>32.9±3.0</td>
<td>30.0±2.7</td>
<td>.005</td>
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<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>3.94±0.46</td>
<td>3.86±0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>124±5</td>
<td>123±4</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic vascular resistance, U·m²</td>
<td>15.3±2.2</td>
<td>14.5±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, U·m²</td>
<td>5.8±0.9</td>
<td>5.1±0.8</td>
<td>.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.01</td>
<td>7.43±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Paco₂, mm Hg</td>
<td>38±1</td>
<td>38±1</td>
<td>NS</td>
</tr>
<tr>
<td>Po₂, mm Hg</td>
<td>150±9</td>
<td>163±11</td>
<td>NS</td>
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</table>

CPB indicates cardiopulmonary bypass; and ACH, acetylcholine. NS, P>.05. n=9.

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![Fig 1. Percentage change in pulmonary artery (PA) pressure with 10⁻⁶ M dose of acetylcholine (ACH) in preoperative (Pre-CPB) patients and postoperative (Post-CPB) patients—group 1 (Postop). The vasodilating response to ACH is attenuated in Post-CPB patients. *P<.001 compared with preoperative response.](image1)

![Fig 2. Percentage change in pulmonary vascular resistance (PVR) with 10⁻⁶ M dose of acetylcholine (ACH) in preoperative (Pre-CPB) patients and postoperative (Post-CPB) patients—group 1. The vasodilating response to ACH is attenuated in Post-CPB patients. *P<.001 compared with preoperative response.](image2)
Fig 3. Percentage change in pulmonary artery (PA) pressure with $10^{-6}$ M dose of acetylcholine (ACH) in preoperative (Pre-CPB) patients and postoperative (Post-CPB) patients—group 2. The vasodilatory response to acetylcholine is attenuated in Post-CPB patients, but the capacity for vasodilation, as indicated by the response to inhaled nitric oxide (NO), is retained. *$P<.003$ compared with preoperative ACH response. **$P<.0003$ compared with postoperative ACH response.

an anatomic correlate, with demonstrable changes in endothelial structure in both the laboratory-induced condition and human disease. Abnormalities in pulmonary vascular endothelial structure have been described in children with congenital heart disease.23,31,37,39,47 Alterations in preoperative endothelial structure and function may predispose this population to postoperative abnormalities of the regulatory mechanisms mediated by the endothelium.27,46,49 Evidence is also accumulating that the abnormal pulmonary endothelium is further damaged when its normal blood supply via the pulmonary artery is removed by instituting total CPB.31,32,46,50 Pulmonary endothelial blood supply from the vasovasorum via the bronchial circulation may not be adequate on CPB; ischemic damage to the endothelium has been described after CPB.27,48 Postoperative pulmonary hypertensive crises promoted by an injured endothelium may be analogous to vasospasm in the coronary circulation resulting from supersensitivity to circulating catecholamines occurring in the context of endothelium damaged by atherosclerotic disease.22,51 Elevation in endogenous catecholamines and sensitivity of the pulmonary circulation to stimuli are common postoperative profiles in children with congenital heart disease.52,53 Endothelial dysfunction after a transient ischemic event has been demonstrated in other organ systems and may be further affected by ischemic reperfusion injury.54 Thus, prior data would suggest that postoperative conditioning of the pulmonary bed, perioperative vasospastic stimuli, and increased postoperative adrenergic tone may conspire with the pulmonary endothelium, damaged by intraoperative ischemia, to increase PVR after CPB.

Site of Impaired Vasodilation After CPB: Current Studies

Impairment of pulmonary vasodilation, as assessed by an attenuated response to ACH after CPB, may occur at the level of the endothelial cell or the vascular smooth muscle or may represent a diffusion barrier between the two cells imposed by CPB. This study supports the hypothesis that CPB damages pulmonary endothelium in children, causing a failure of endothelium-dependent pulmonary vasodilation. This conclusion is based on the findings that (1) despite abnormal pulmonary resistance, young children with congenital heart disease respond to ACH prior to surgery; (2) similar patients respond poorly to the same doses of ACH following CPB; and (3) they retain the ability to dilate in response to nitric oxide. However, the conclusion rests on certain assumptions: (1) that ACH causes pulmonary vasodilation by stimulating endothelial cell production of nitric oxide with its attendant effect on intracellular smooth muscle cGMP; (2) that inhalation of nitric oxide provides a vasodilatory stimulus similar to nitric oxide released in vivo; and (3) that the differences in the preoperative and post-CPB patient responses to ACH vasodilation cannot be ascribed to other parallel events.

ACH infusion. Acetylcholine is thought to combine with a muscarinic receptor on the endothelial surface, stimulating formation of nitric oxide from l-arginine. Nitric oxide is then released from the cell by diffusion.55 In this study, we cannot differentiate receptor failure on the endothelial surface from intracellular impairment of nitric oxide production. Inhalational anesthetics may impair nonreceptor-mediated endothelium-dependent relaxation of rat thoracic aortic rings in a reversible fashion.56 Since our postoperative patients had intraoperative anesthesia maintained without inhalational agents and were sedated postoperatively with a narcotic-benzodiazepine regimen similar to that used for preoperative patients studied in the cardiac catheterization laboratory, the confounding effect of such agents seems minimal. A similar proportion of preoperative and postoperative patients had their narcotic/relaxant anesthetic continued throughout the study period, making differences in anesthetic agents or sedation unlikely to account for postoperative attenuation in endothelium-dependent vasodilation. Similarly, we cannot exclude the possibility that other long-acting drugs, intravenous solutions, or blood products administered as part of CPB were bound selectively to the muscarinic receptor or otherwise inhibited postoperative actions of ACH. However, the presence of normal heart rate variability in our patients and the absence of tachycar-
dia would further argue against unrecognized muscarinic blockade. A study design using multiple endothelium-dependent vasodilators in addition to ACH may provide additional information in this regard.

**Inhaled nitric oxide.** Although the effects of inhaled nitric oxide gas in man were studied nearly 20 years ago, the use of nitric oxide to treat pulmonary hypertension awaited the demonstration in 1987 that nitric oxide was an important endothelium-derived relaxing factor. Because of the extremely rapid reaction of nitric oxide with oxyhemoglobin to form small amounts of methemoglobin, low concentrations of nitric oxide gas have been inhaled by humans to measure lung diffusion capacity. The feasibility of using inhaled nitric oxide in animal models of pulmonary hypertension was demonstrated recently by Zapol and colleagues, using awake animals with hypoxia, acidosis, and thromboxane- or protamine-induced pulmonary hypertension. The first published experience with nitric oxide treatment of pulmonary hypertension in humans was provided by Pepke-Zaba et al in adults with primary pulmonary hypertension, where pulmonary vascular resistance fell by 5% to 68%, but pulmonary artery pressure was not reported. Recent preliminary reports suggest a possible therapeutic role for inhaled nitric oxide in the treatment of persistent pulmonary hypertension of the neonate.

**Comparability of groups.** The importance of baseline vascular tone in assessing response of the pulmonary circulation to vasodilators has been known for many years. Vasodilation with ACH may be absent when pulmonary artery pressure is normal, even when the endothelium is intact. The hemodynamic response of an intact pulmonary circulation with an injured endothelium and low pulmonary artery pressure is unknown. Therefore, in our initial 22 postoperative patients, we included those with mean pulmonary artery pressures less than 25 mm Hg who were at risk of having postoperative pulmonary hypertension by virtue of their diagnosis. Paradoxical pulmonary hypertension with ACH was not reliably produced in either high or low pulmonary artery pressure patients postoperatively, although there was a tendency for pulmonary artery pressure to rise slightly in some patients at lower doses of ACH.

To compare groups with similar baseline vascular tone, we analyzed the subgroup of postoperative patients with mean pulmonary artery pressure of at least 25 mm Hg and compared their capacity to vasodilate at 10⁻⁶ M ACH to preoperative patients with elevated pulmonary artery pressure. Vasodilation was present at the highest dose of ACH but was significantly reduced compared with preoperative patients. However, there are limitations in human studies, including constraints on the duration of hemodynamic protocols in patients undergoing elective cardiac catheterization, that prevented investigation of the dose-response relationship for ACH in preoperative patients. In this regard, the response to the 10⁻⁶ M ACH dose in postoperative patients was not exactly comparable to preoperative studies since it was preceded by three lower doses of ACH, each having a small but finite volume infusion associated with it. This may have produced the small rise in left atrial pressure seen in postoperative patients and passively distended the pulmonary vasculature, thus perhaps overestimating the vasodilating influence of ACH attributable to changes in active tone.

Post-hoc selection of postoperative patients with high pulmonary artery pressure and increased PVR for comparison to preoperative patients introduces potential bias from arbitrary inclusion criteria. Although the absence of any features other than pulmonary artery pressure and PVR that differentiated postoperative high- and low-pressure groups was reassuring, we proceeded to prospectively enroll another set of patients, postoperative (post-CPB) patients (group 2) with elevated pulmonary artery pressure, who received only a single dose of 10⁻⁶ M ACH, analogous to the protocol in the catheterization laboratory. We also used this group to measure the capacity to vasodilate through endothelial independent mechanisms by giving them nitric oxide gas to breathe. The results from this group confirmed the attenuated postoperative response to ACH but verified the integrity and responsiveness of the vascular smooth muscle.

**TABLE 5. Postoperative (Post-CPB) Patients—Group 2 (ACH and Nitric Oxide) Hemodynamic Response to Nitric Oxide**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Nitric Oxide, 80 ppm</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>9.8±0.9</td>
<td>8.9±0.9</td>
<td>.04</td>
</tr>
<tr>
<td>Mean systemic blood pressure, mm Hg</td>
<td>65.8±2.4</td>
<td>60.9±2.6</td>
<td>.02</td>
</tr>
<tr>
<td>Left atrial pressure, mm Hg</td>
<td>11.9±1.9</td>
<td>10.4±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>34.4±2.6</td>
<td>25.8±2.4</td>
<td>.0001</td>
</tr>
<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>4.02±0.49</td>
<td>3.97±0.46</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>123±6</td>
<td>122±6</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic vascular resistance, U·m²</td>
<td>16.4±2.5</td>
<td>15.3±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, U·m²</td>
<td>6.8±1.2</td>
<td>4.5±0.8</td>
<td>.005</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.02</td>
<td>7.43±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Pco₂, mm Hg</td>
<td>38±1</td>
<td>36±1</td>
<td>NS</td>
</tr>
<tr>
<td>Po₂, mm Hg</td>
<td>163±0.7</td>
<td>168±8.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

CPB indicates cardiopulmonary bypass; and ACH, acetylcholine.

NS, P>.05. n=9.
cGMP. In animals, endothelium-derived nitric oxide, induced by exposure to ACH, diffuses to intrapulmonary vascular smooth muscle and raises the intracellular level of cGMP.17 Smooth muscle relaxation temporally coincides with a threefold to fourfold rise in measured cGMP levels within the first 1 to 2 minutes after exposure to 10-8 M ACH.17 Similarly, we observed more than a threefold rise in cGMP levels measured in the pulmonary venous blood of our patients after exposure to inhaled nitric oxide, coinciding with vasodilation, consistent with the purported role of cGMP as the second messenger in this process. The failure of cGMP levels to rise with ACH infusion in post-CPB patients may reflect endothelial dysfunction and inadequate formation of nitric oxide as the initial messenger. Alternatively, the rise in intracellular smooth muscle cGMP in response to physiologic doses of ACH acting on an intact endothelium may be too small to be reflected in pulmonary venous blood, whereas 80 ppm inhaled nitric oxide may represent a comparatively massive activation of guanylate cyclase. It cannot be stated with certainty what the source of cGMP is, although it is presumably from the lung or pulmonary circulation. Many potential sources of cGMP exist within the lung, including tracheal and bronchial smooth muscle, epithelial cells, macrophages, and so on. Nitric oxide from alveolar gas, which diffuses in excess beyond smooth muscle and endothelium into the lumen of pulmonary arterioles and capillaries, may activate platelets or leukocytes and release cGMP before being inactivated by hemoglobin.

**Therapeutic and Toxic Implications**

We have shown that there are potent pulmonary vasodilating effects of inhaled nitric oxide with minimal systemic vasodilation in patients with elevated PVR after CPB. The potential therapeutic importance of these data is evident but must be considered in conjunction with possible toxic effects.

The dose of inhaled nitric oxide of 80 ppm (0.008%) was chosen based on the animal work of Frostell et al.4 and on our own preliminary experience suggesting no significant advantage of higher dosages. Little evidence of pulmonary toxicity exists when nitric oxide is delivered to nonprimate animals in concentrations of less than 100 ppm.69 Others have reported no alterations in lung water or histopathology when rats were exposed to 1500 ppm nitric oxide for 15 minutes.70 The dose of nitric oxide effective in achieving pulmonary vasodilation was not addressed in this study and may be considerably less than 80 ppm, perhaps approaching the 25 ppm standard allowable in the workplace by the US Occupational Safety and Health Administration.71 Nitrogen dioxide is formed from nitric oxide and oxygen and has considerably greater potential pulmonary toxicity than nitric oxide, but its formation can be limited by restricting the concentration of nitric oxide in the source, eliminating exposure of nitric oxide to oxygen prior to delivery, and minimizing gas residency time in the delivery circuit. If necessary, nitrogen dioxide can be chemically absorbed from the inspired gas.

Formation of methemoglobin has been described in animals and humans exposed to nitric oxide gas57 and accounts for its rapid inactivation beyond the pulmonary circuit. We did not see abnormal levels during this short trial in our patients. Our protocol used ACH and inhaled nitric oxide as probes to clarify endothelial and smooth muscle function after CPB. We limited the nitric oxide trial to 15 minutes and therefore minimized toxicity that may be associated with long-term exposure.

**Summary**

This study suggests that CPB is responsible for pulmonary endothelial dysfunction. The results focus attention on the endothelium as an important organ to address in the management of pulmonary hypertension. These findings also suggest a potentially important diagnostic and therapeutic role for inhaled nitric oxide as a novel selective pulmonary vasodilator with minimal systemic hemodynamic effects in children with congenital heart disease. Before long-term clinical use of nitric oxide can be recommended, further studies should be performed to ensure efficacy without toxicity.

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**References**


71. MMWR August 26, 1988;37:S-7(21).
Use of inhaled nitric oxide and acetylcholine in the evaluation of pulmonary hypertension and endothelial function after cardiopulmonary bypass.
D L Wessel, I Adatia, T M Giglia, J E Thompson and T J Kulik

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