L-Arginine and Substance P Reverse the Pulmonary Endothelial Dysfunction Caused by Congenital Heart Surgery

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Background**—**The increase in pulmonary vascular resistance (PVR) seen in children after cardiopulmonary bypass has been attributed to transient pulmonary endothelial dysfunction (PED). We therefore examined PED in children with congenital heart disease by assessing the L-arginine–nitric oxide (NO) pathway in terms of substrate supplementation (L-arginine [L-Arg]), stimulation of endogenous NO release (substance P [Sub-P]), and end-product provision (inhaled NO) before and after open heart surgery.

Methods and Results**—**Ten patients (aged 0.62±0.27 years) with pulmonary hypertension undergoing cardiac catheterization who had not had surgery and 10 patients (aged 0.65±0.73 years) who had recently undergone cardiopulmonary bypass were examined. All were sedated and paralyzed and received positive-pressure ventilation. Blood samples and pressure measurements were taken from catheters in the pulmonary artery and the pulmonary vein or left atrium. Respiratory mass spectrometry was used to measure oxygen uptake, and cardiac output was determined by the direct Fick method. PVR was calculated during steady state at ventilation with room air, during F_{IO_2} of 0.65, then during additional intravenous infusion of L-Arg ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and Sub-P ($1 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and finally during inhalation of NO (20 ppm). In preoperative patients, the lack of an additional significant change of PVR with L-Arg, Sub-P, and inhaled NO suggests little preexisting PED. Postoperative PVR was higher, with an additional pulmonary endothelial contribution that was restorable with L-Arg and Sub-P.

Conclusions**—**Postoperatively, the rise in PVR suggested PED, which was restorable by L-Arg and Sub-P, with no additional effect of inhaled NO. These results may indicate important new treatment strategies for these patients. **(*Circulation*. 1999;100:749-755.)**

Key Words: endothelium ■ hypertension, pulmonary ■ amino acids ■ heart disease, congenital ■ nitric oxide

Pulmonary vascular resistance (PVR) is often increased in children with congenital heart disease, and this may be exacerbated after cardiac surgery.[1] This increase in PVR is explained in part by a loss of the ability of the pulmonary endothelium to maintain the production of vasodilating substances,[2] ie, pulmonary endothelial dysfunction (PED). Endothelial function is examined conventionally by comparing the vasodilating effects of infused endothelium-dependent (eg, acetylcholine) versus endothelium-independent (eg, nitroglycerin or nitroprusside) agents[3–5] or, in the pulmonary vasculature, by using inhaled nitric oxide (NO) as the endothelium-independent vasodilator.[3,6–8] Such studies in children have shown a blunted pulmonary vasodilator response to infused acetylcholine in preoperative patients[9] and similar but more severe PED after surgery.[6]

The raised PVR seen in these patients can be attenuated by inhaled NO, and its use has become widespread as therapy for postoperative PED and other pulmonary disorders.[6,10–14] Nonetheless, although the impressive short-term effects of inhaled NO are accepted, its use is not without problems. Some patients fail to respond,[15] and its efficacy with prolonged use in some conditions has been questioned.[16] Furthermore, life-threatening rebound pulmonary hypertension may occur with abrupt withdrawal.[17,18]

Although the use of inhaled NO may effectively circumvent the problem of PED, a more systematic assessment of the underlying components of PED is necessary if we are to fully understand its pathogenesis. We used a protocol that isolated these components by optimization of ventilation and then by sequential administration of the substrate for NO

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production (L-arginine), a stimulator of an endothelial receptor (substance P), and direct smooth vascular muscle relaxation (inhaled NO) to assess the reversibility of the PED described in these patients.

Methods

Patients

The study was approved by our Hospital Research Ethics Committee, and written informed consent was obtained from the parents of each child. Preoperative patients with isolated intracardiac left-to-right shunting defects and clinical and echocardiographic signs of pulmonary hypertension were studied in the catheterization laboratory under general anesthesia. Postoperative patients at risk for postoperative pulmonary hypertension were studied immediately after heart surgery in the intensive care unit.

All patients were sedated, mechanically ventilated, and paralyzed (by use of vecuronium, propofol, ketamine, and midazolam in the catheterization laboratory and vecuronium, midazolam, and morphine in the postoperative patients) throughout the study. They were intubated with a cuffed endotracheal tube (Mallinckrodt) to exclude any respiratory gas leaks. Volume controlled ventilation was delivered by a Siemens 900 C ventilator.

The preoperative patient group studied in the cardiac catheterization laboratory first underwent a diagnostic study, and a catheter was then passed into the pulmonary artery and the left atrium for subsequent pressure monitoring and blood sampling. The study protocol (see below) in the postoperative group was instituted 2 hours after cardiopulmonary bypass. This delay allowed for sufficient time for central rewarming, adjustment of sedation and inotropic agents, and tracheal suctioning after transfer to the pediatric intensive care unit. Thereafter, additional handling and therapeutic intervention during the study protocol were minimized. Intracardiac shunts were excluded by echocardiography. In both patient groups, the cuff of the endotracheal tube was then inflated with a pressure below the systemic diastolic blood pressure for the duration of the study protocol, during which continuous monitoring of surface ECG, pulse oximetry, end-tidal carbon dioxide concentration, and hemodynamic pressures (see below) was performed.

Special Metabolic and Hemodynamic Measurements

Oxygen consumption ($\dot{V}O_2$ [mL · kg^{-1} · min^{-1}]) was continuously determined with respiratory mass spectrometry by the mixed-expiration inert-gas dilution method[19] with our previously described modification[20] for use in ventilated patients. Special care was taken to detect and exclude any air leaks or carbon dioxide contamination of the monitoring and ventilatory circuits. The mass spectrometer was calibrated directly before the study and then every 30 minutes to exclude any measurement drift.

Systemic arterial and pulmonary arterial pressures, as well as right and left atrial (or pulmonary wedge) pressures, were measured, and blood samples were taken from the pulmonary artery and pulmonary vein or systemic artery. Partial pressures for oxygen and carbon dioxide and hemoglobin saturation were measured by the spectral absorption method (Chiron 270 CO oximeter), and the arteriovenous oxygen content difference (avDO$_2$ [mL/L]) and cardiac output by the Fick principle ($\dot{V}O_2$/avDO$_2$) were calculated. PVR (PVR; mm Hg · L^{-1} · min^{-1}) and PVR index (PVRI; PVR×m^2) were derived from the transpulmonary pressure gradient by standard formula[20] and reported in Wood Units indexed to body surface area (WU · m^2). Alveolar dead-space ventilation and intrapulmonary shunt flow were assessed for each stage of the protocol.[21]

Study Protocol

The study protocol was instituted after a cardiorespiratory steady state was confirmed during 5 to 10 minutes of monitoring. Measurements of oxygen consumption, cardiac output, and hemodynamic pressures were made at steady state during the last 2 minutes of each new condition (Figure 1): (1) baseline measurements during ventilation with air (F_{IO_2} at 0.21); (2) ventilation in increased oxygen (F_{IO_2} of 0.65), which was continued to the end of the protocol, to obviate the possible confounding effects of alveolar hypoxia; (3) intravenous infusion of L-arginine (Fresenius) 15 mg · kg^{-1} · min^{-1}, which was continued to the end of the protocol, as the substrate for endogenous NO production; (4) intravenous infusion of substance P (Clinalfa AG) 1 pmol · kg^{-1} · min^{-1}, which was continued to the end of the protocol, to stimulate endothelial NO production; and (5) inhalation of NO (BOC) 20 ppm, which was continued to the end of the protocol, to provide direct pulmonary vascular smooth muscle relaxation.

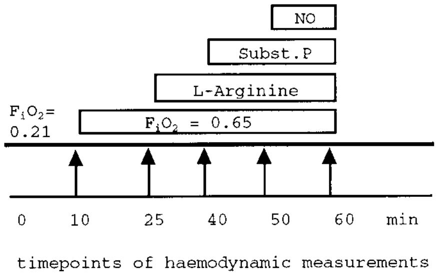

Figure 1. Study protocol. Each measurement was performed after a new steady state was achieved (arrow), and each substance was added to the preexisting ones and continued to the end of the protocol.

Statistical Analysis

All data are expressed as mean±SD. A repeated-measures ANOVA was used to analyze serial hemodynamic and metabolic data over time, and a post hoc paired *t* test with Bonferroni correction was applied when appropriate to evaluate for significant differences between conditions and individual time points. A P value of <0.05 was considered statistically significant.

Results

Ten patients were examined in the catheterization laboratory, and 10 were examined after surgery in the pediatric intensive care unit (Table 1), with 3 patients having both a preoperative and postoperative study. Cardiorespiratory stability was confirmed in all. P_{aCO_2} as well as base excess showed appropriate normal ventilation and metabolic state (average values for all study conditions before versus after surgery: P_{aCO_2} 4.72±0.54 versus 4.82±0.82 kPa, and base excess 3.5±2.5 versus 0.5±1.3 mmol/L). Variability was <0.6% for ventilation (L/min) and <5% for oxygen consumption (mL · kg^{-1} · min^{-1}) throughout the study (Figure 2). The measured PVR returned to its baseline level after completion of the protocol.

Baseline Measurements With Ventilation in F_{IO_2} of 0.21

Oxygen consumption was similar in both patient groups (6.8±1.1 mL · kg^{-1} · min^{-1} before surgery versus 6.6±1.2 mL · kg^{-1} · min^{-1} after surgery). Transpulmonary blood flow index and transpulmonary pressure gradient were higher in the preoperative group (8.57±3.85 versus 2.26±0.89 L · min^{-1} · m^{-2} and 27.1±6.5 versus 13.5±4.7 mm Hg, $P<0.001$; Figure 3). Thus, PVRI was moderately elevated before and further increased after cardiopulmonary bypass (4.16±1.87 WU · m^2 before versus 6.66±2.29 WU · m^2 after surgery; $P<0.001$).

TABLE 1. Patient Data

PreOP	Diagnosis	Age, y	Weight, kg	PostOP	Diagnosis	Age, y	Weight, kg
1	T21, VSD	0.90	7.3	1	T21, VSD	0.66	5.9
2	T21, AVSD	0.42	4.7	2	VSD	0.64	7.5
3	T21, AVSD	1.02	7.5	3	VSD	0.42	5.3
4	VSD	0.41	5.2	4	VSD	0.23	4.4
5	T21, VSD, PDA	0.41	4.9	5	T21, AVSD	0.64	4.6
6	T21, VSD	0.59	5.3	6	VSD	0.26	5.9
7	T21, AVSD	0.30	4.1	7	VSD	2.64	9.8
8	T21, AVSD	0.41	5.1	8	AVSD	0.23	4.3
9	T21, VSD	0.92	6.3	9	VSD	0.26	5.1
10	ASD	0.82	7.3	10	VSD	0.56	4.8
Mean		0.62	5.83			0.65	5.79
Median		0.51	5.25			0.49	5.21

PreOP indicates patients examined in catheterization laboratory; PostOP, patients examined on pediatric intensive care unit; T21, trisomy 21; ASD, VSD, and AVSD, atrial, ventricular, and atrioventricular septal defects; and PDA, persistent arterial duct.

Ventilation With F_{IO_2} of 0.65

Pa_{O_2} increased and remained stable throughout the remainder of the protocol (12.4±3.1 to 37.1±7.0 kPa before surgery; 10.1±1.4 to 29.9±5.9 kPa after surgery; Table 2). $\dot{V}_{O_2}$ increased in postoperative patients (to 7.2±1.3 mL · kg^{-1} · min^{-1}; $P<0.05$) but not in the preoperative group. PVRI fell significantly in both groups, to 2.8±1.6 WU · m^2 (37% change, $P<0.001$) in preoperative patients and 4.5±1.9 WU · m^2 (33% change, $P<0.001$) in postoperative patients, with a concomitant increase in cardiac output (Table 2, Figure 3).

Substrate Provision With Infusion of L-Arginine

There was an additional significant fall (Figure 3) in PVRI in the postoperative group (to 3.84+1.48 WU · m^2; $P<0.01$) but not in the preoperative group. However, although mean arterial pressures remained unchanged before and after surgery (Table 2), there was a rise in cardiac output after surgery, leading to a trend ($P=0.12$) of a fall in systemic vascular resistance (Table 2).

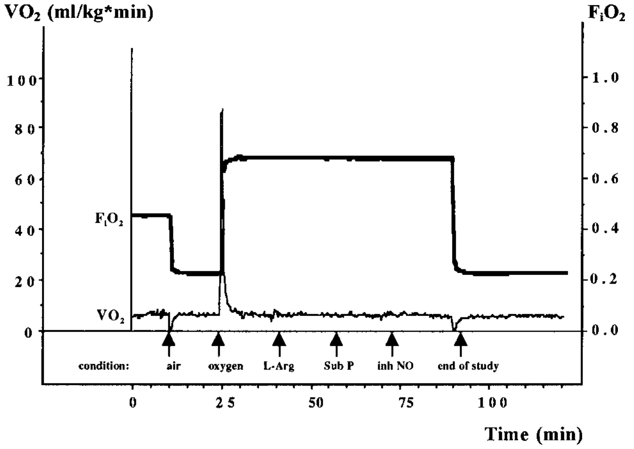

Figure 2. Oxygen consumption and F_{IO_2} during study protocol. The technique of oxygen consumption measurement with respiratory mass spectrometry is illustrated in a typical postoperative patient. F_{IO_2} shows tracing of inspired oxygen concentration, and $\dot{V}_{O_2}$ shows tracing of oxygen uptake (baseline corresponds to 6.6 mL · kg^{-1} · min^{-1}). Air indicates F_{IO_2} 0.21; oxygen, F_{IO_2} 0.65; L-Arg, L-arginine infusion; Sub P, substance P infusion; and inh NO, inhaled NO. Each arrow indicates time point of new condition. The apparent peaklike changes in $\dot{V}_{O_2}$ on changes in F_{IO_2} are an artefact caused by the delay in the gas equilibration in the expired-gas–mixing chamber.

Pulmonary Endothelial Stimulation With Intravenous Substance P

The biological effect of additional substance P was evident in all patients by an immediate and transient reduction in systemic blood pressure, which reached a steady state after 2 to 3 minutes. The heart rate remained unchanged, and no patient required specific support. PVRI fell again significantly in postoperative patients (to 3.23±1.47 WU · m^2; $P<0.05$) but not in preoperative patients.

Endothelium-Independent Pulmonary Vasodilatation With Exogenous NO

The application of additional inhaled NO had no significant effect on PVRI in either patient group (Figure 3). Interestingly, there was a fall in Pa_{CO_2} that failed to reach significance in the preoperative patients (5.1±0.5 to 4.8±0.5 kPa; $P=0.14$) but was significant in postoperative patients (5.2±0.7 to 4.7±0.7 kPa; $P<0.001$). Despite this, there was no significant change in intrapulmonary shunting or alveolar dead-space ventilation (maximum change from baseline 4.5%) during any of the conditions of the protocol.

Discussion

This study is the first to systematically examine the effects of L-arginine, substance P, and inhaled NO on the pulmonary vasculature in both preoperative and postoperative patients with congenital heart disease. It demonstrates that in a relatively young cohort of preoperative patients, there appears to be little preexisting pulmonary endothelial damage resulting from intracardiac left-to-right shunting. Postoperatively, there is an increase in PVR, which is reversed when endogenous NO release is maximally stimulated with intravenous

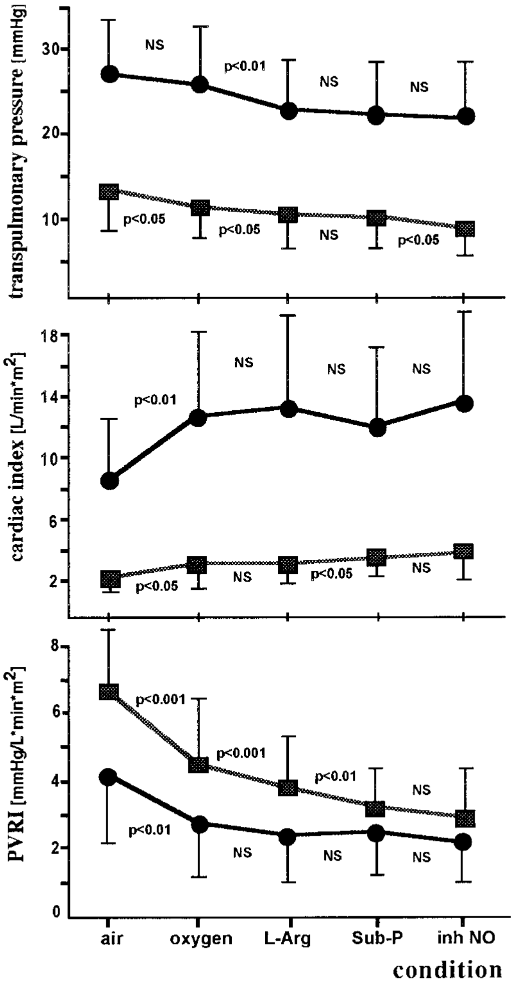

Figure 3. Pulmonary hemodynamics during study protocol. Response of transpulmonary pressure gradient, cardiac index, and PVRI in both preoperative (●) and postoperative (■) patient groups during each of the conditions of the study protocol. All values are given as mean±SEM, and NS (not significant) and probability values refer to differences between different conditions within each of the patient groups. Air indicates Fio_2 0.21; oxygen, Fio_2 0.65; L-Arg, L-arginine infusion; Sub-P, substance P infusion; and inh NO, inhaled NO.

L-arginine and substance P. There is little additional effect of inhaled NO.

PED in Congenital Heart Disease

PED in congenital heart disease is a new and important pathophysiological concept. It has been used to explain the rise in PVR seen in children with long-standing left-to-right shunts[9] and also the postoperative increase and crisis-like rise in PVR that occurs in children after open heart surgery.[6] Although many other components of pulmonary endothelial function have been studied in the past,[22–26] contemporary studies have focused on the L-arginine–NO pathway, which arguably is the most clinically important system for vasodilation. Furthermore, abnormalities of this pathway probably reflect early endothelial dysfunction, preceding irreversible, histological changes.[27] Defining the function of the pulmonary endothelium should pave the way to a better understanding and ultimately a more focused treatment of this important phenomenon.

Clinical Assessment of PED

The PVR reflects a multitude of structural and functional conditions of the pulmonary vascular bed. Nonetheless, in the absence of other pathophysiological changes, it has been shown to be exquisitely sensitive to changes in pulmonary endothelial function.[22–26] Its accurate assessment is fraught with difficulties, however. When PVR is measured under clinical conditions, it must be borne in mind that both PVR and cardiac output are influenced by sedation and type of ventilation.[28–30] In the present study, by using mechanical ventilation, we controlled the nonspecific, non–endothelium-related effects of alveolar hypoventilation and hypercarbia on PVR occurring in patients breathing spontaneously.[31–33] We measured pulmonary blood flow by the direct Fick method. This method, unlike thermodilution, is not confounded by intracardiac shunting[34] but requires the continuous determination of oxygen uptake as indicator. Because the error of assuming oxygen uptake can be large,[35] the measurement of oxygen uptake in the present study was performed by high-precision respiratory mass spectrometry.[19] However, because extremely high levels of Fio_2 can make mass spectrometric measurements imprecise, we used a maximal Fio_2 of 0.65. Taking all these factors into account, we believe that ours are the most sensitive measurements of PVR available in clinical practice.

Early Increase in PVR in Congenital Heart Disease and Relation to PED

An increase in PVR is a not uncommon finding in unoperated congenital heart disease associated with left-to-right shunting. However, it is not known how much of the initially reversible increase in PVR is related to the development of PED. Contrary to our results, Celermajer et al[9] suggested that in older patients, a large component of the rise in PVR in preoperative patients was due to PED that was reversed with infused sodium nitroprusside. However, in their study, PVR and vasodilator response in segmental pulmonary arteries were measured by a technique that examines the local vasomotor responses in the lung, which may not accurately reflect the vasodilator response of the whole lung.[36] Furthermore, there was no information regarding Fio_2 in their patients. Our preoperative patients did not show such an overt endothelial contribution to increased PVR: nearly all of the pulmonary vasodilation occurred with supplemental oxygen alone, and there was little additional benefit from L-arginine, substance P, or inhaled NO. It should be stressed that our patients were much younger; nonetheless, they are more typical of the type of patients being assessed before surgery in the contemporary era.

PED After Cardiopulmonary Bypass

Our postoperative findings confirm previous data suggesting an adverse effect of cardiopulmonary bypass on the pulmonary vascular bed.[37,38] Cardiopulmonary bypass causes structural and functional impairment to the pulmonary endothelium in many ways, including complement activation,[39] neutrophil adhesion facilitated by a lack of NO production, and oxygen free-radical injury.[40] However, the precise nature of the ensuing increase in PVR remains unclear. Wessel et al[6]

TABLE 2. Oxygen Consumption and Hemodynamic Data

	F_iO_2=0.21	F_iO_2=0.65	L-Arginine	Substance P	Inhaled NO
$\dot{V}O_2$, mL · kg^{-1} · min^{-1}					
PreOP	6.8±1.1	6.8±1.3	6.9±1.1	6.8±1.1	6.9±1.1
PostOP	6.6±1.2	7.2±1.3	7.2±1.2	7.3±1.2	7.4±1.1
PaO_2, kPa					
PreOP	12.4±3.1	37.1±7.0	33.9±7.2	34.9±7.2	35.3±5.7
PostOP	10.1±1.4	29.9±5.9	27.2±6.4	27.7±6.3	28.5±5.4
CI_{pulm}, L · min^{-1} · m^{-2}					
PreOP	8.6±3.9	12.7±5.5	13.3±6.1	11.9±5.2	13.6±5.9
PostOP	2.3±0.9	3.2±1.5	3.2±1.2	3.5±1.1	3.9±1.7
PAP, mm Hg					
PreOP	38.7±7.2	36.5±8.3	33.7±6.7	32.6±7.1	32.3±7.8
PostOP	20.8±4.6	19.1±3.6	18.5±3.8	17.9±3.6	16.6±3.2
MAP, mm Hg					
PreOP	52.3±19.1	55.1±24.3	53.7±7.8	48.5±20.9	50.1±21.2
PostOP	61.5±6.8	59.8±7.1	58.9±5.9	50.6±9.4	53.2±10.5
LAP, mm Hg					
PreOP	10.9±3.9	10.6±4.0	10.9±3.9	10.4±3.6	10.3±3.3
PostOP	7.3±1.9	7.6±1.7	7.8±1.6	7.7±1.7	7.7±1.7
PVRI, WU · m^2					
PreOP	4.2±1.9	2.8±1.6	2.4±1.4	2.5±1.3	2.2±1.2
PostOP	6.7±2.3	4.5±1.9	3.8±1.5	3.2±1.5	2.9±1.5
SVRI, WU · m^2					
PostOP	28.3±11.5	21.5±8.9	18.7±6.2	13.5±4.5	14.3±5.0

PreOP indicates patients examined in catheterization laboratory; PostOP, patients examined on pediatric intensive care unit. All data are mean±SD. CI pulm, pulmonary blood flow (in PreOP) and cardiac index (in PostOP), respectively; PAP, MAP, and LAP, pressures in pulmonary artery, systemic arteries, and left atrium; and PVRI and SVRI, pulmonary and systemic vascular resistance indexes.

attributed this to PED by showing failure of acetylcholine-induced pulmonary vasodilation, which was reversed by inhaled NO. The mechanism of this dysfunction was not explored in that study, and it was not known whether substrate deficiency or failure of NO production or its release was the cause of the endothelial dysfunction. In both the preoperative and postoperative patients, oxygen led to a fall in PVR associated with an increase in cardiac output and only a modest fall in pulmonary arterial pressure, but in the latter group, PVR after oxygen administration remained significantly elevated. Our subsequent data confirm Wessel's suggestion of PED as the major cause for this residual elevation in PVR, but the response seen to L-arginine and substance P suggests that this is a reversible defect.

A reduction in plasma levels of L-arginine has been shown in children after cardiopulmonary bypass.[41] It is unlikely that such a reduction is enough to fully explain our findings. Although supplemental L-arginine has been shown to be of benefit in neonates with persistent pulmonary hypertension[42] in whom L-arginine deficiency is quite common,[43] its response in older patients is less predictable: L-arginine administration in normal subjects[44] and those with pulmonary hypertension due to systemic sclerosis[45] had no effect on hemodynamic variables or cGMP production, whereas it lowered PVR in congestive heart failure and improved vasodilatory function in the older age group[45–47]; all of these groups had normal plasma levels of L-arginine. Although speculative, these data suggest that in some way, an increase in NO synthase activity is responsible for the marked recovery in PED with supplemental L-arginine. An endothelium-independent vasodilator effect of L-arginine, however, cannot be completely excluded but seems improbable in view of the lack of any vasodilator response shown previously in normal subjects.[44] L-Arginine supplementation did not completely reverse the PED in our patients, however.

Our data showing an additional effect of substance P underscore the potential viability of the endothelium after cardiopulmonary bypass and suggest that cellular mechanisms for NO production are intact but inadequate without stimulation. The previous data[6,9] showing a lack of response to acetylcholine thus need to be interpreted with caution in this regard. Acetylcholine, in the presence of endothelial dysfunction, is able to cause vasoconstriction via vasoconstrictive prostanoids, which is not the case for substance P.[48,49] Both acetylcholine and substance P eventually activate NO synthase, leading to the production of NO and cGMP, but substance P reacts with a neurokinin-1h receptor[50] in the pulmonary vascular bed causing vasodilation[51,52] via a mechanism involving the inositol phosphate pathway, not, as acetylcholine, by binding to G proteins.[53] A loss of G proteins has been described not only after cardiopulmonary bypass[54] but also in pulmonary hypertension[55,56] and may indicate reduced signal transfer from endothelial receptor to NO synthase.[57,58]

Study Limitations

Because of the blood-sampling requirements already imposed by the physiological measurements, we were unable to make measurements of L-arginine or cGMP levels, which would have been desirable.[6,41] Only 3 patients were studied both before and after surgery. Nonetheless, the difference noted in preoperative and postoperative groups was reflected in the individual data from this small subset. We believe, therefore, that these limitations do not undermine the general conclusions that we have drawn from our study; rather, they highlight areas for future investigations.

Summary

We have shown that pulmonary endothelial function after cardiopulmonary bypass can be restored to preoperative levels with L-arginine and substance P. Although not a therapeutic trial, this study may stimulate further studies aimed at replacing or supplementing the current treatment strategies, which merely replace endothelial function.

Acknowledgments

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