Coronary vasoconstriction mediated by endothelin-1 in neonates

Reversal by nitroglycerin

To determine the role of the vasoconstrictor peptide endothelin-1 in cardiopulmonary bypass in neonates, we measured plasma endothelin-1 concentrations in infants before and after cardiopulmonary bypass for arterial switch procedures and studied the effects of endothelin-1 on coronary tone and contractility in normal and reperfused neonatal pig hearts. Endothelin-1 blood concentrations (picograms per milliliter, mean $\pm$ standard error) were significantly higher in neonates with arterial transposition and in umbilical venous blood (22.9 ± 2.3 and 19.2 ± 2.9 , respectively) than in older children with atrial septal defects (13.2 ± 1.6) or in healthy adults (10.7 ± 2.5). After cardiopulmonary bypass, endothelin-1 concentrations increased 29% in neonates undergoing arterial switch procedure and 28% in children undergoing atrial septal defect repair ($p < 0.05$ versus before bypass). In isolated, blood-perfused neonatal pig hearts, endothelin-1 had dose-related coronary constrictor and inotropic effects between 25 and 100 pmol. Endothelin-1 concentrations that did not increase coronary perfusion pressure (5 to 10 pmol) caused significant coronary constriction in the presence of norepinephrine (10 nmol/L). During reperfusion after 30 minutes of global normothermic ischemia, the coronary vasoconstrictor effects of both endothelin-1 alone and endothelin-1 plus norepinephrine were significantly enhanced. Nitroglycerin reversed vasoconstriction produced by endothelin-1 and endothelin-1 plus norepinephrine both before and after ischemia-reperfusion. We conclude that endothelin-1 concentrations are significantly elevated in neonates and are further increased after cardiopulmonary bypass. Coronary vasoconstriction caused by endothelin-1 is enhanced by ischemia-reperfusion and by norepinephrine present in concentrations typically observed after neonatal cardiopulmonary bypass. Nitroglycerin reverses coronary vasoconstriction induced by endothelin-1 and may therefore be beneficial in the postoperative management of neonates after cardiac operations. (J THORAC CARDIOVASC SURG 1995;109:88-98)

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The role of vascular endothelium as a source of substances that contribute to control of vascular tone and to its interaction with blood elements has gained increasing recognition.¹⁻³ Endothelial cells (as well as airway epithelium and kidney) release endothelins, which are a family of peptides composed of 21 amino acids. Endothelins are believed to act primarily in either a paracrine or autocrine fashion in target tissues, where local concentrations may be high. The presence of endothelin in plasma suggests the possibility of endocrine actions, as well. Despite rapid removal of circulating endothelin (half-life 30 to 60 seconds), the biologic actions of endothelin can be prolonged. Prominent among

these effects is vasoconstriction: endothelin is perhaps the most potent endogenous vasoconstrictor identified thus far.⁴⁻⁸ Other actions include positive inotropic and chronotropic effects, increased systemic vascular resistance, bronchoconstriction, renal vasoconstriction, decreased glomerular filtration rate, and increased plasma levels of atrial natriuretic peptide, renin, aldosterone, and catecholamines.^{3,4,7} Endothelin is also a potent mitogen, stimulates protooncogene expression, and may be involved in cellular hypertrophy, vascular remodeling, and the inflammatory response to injury.^{4,7}

Endothelin expression and release can be stimulated by epinephrine, thrombin, cytokines such as interleukin-1, and hypoxia.³ Increased circulating concentrations of endothelin-1 (ET-1) have been found in neonates and patients and in experimental animals with congestive heart failure, angina, myocardial infarction, septic and hemorrhagic shock, as well as after cardiopulmonary bypass (CPB) and major surgery.⁹⁻¹⁸ Endothelin has also been implicated in the pathogenesis of cerebral vasospasm, atherosclerosis, myocardial hypertrophy, and pulmonary hypertension resulting from hypoxia, respiratory distress syndrome, and large left-to-right intracardiac shunts.^{4,7,18,19} Whether increased endothelin concentrations contribute directly to the pathophysiology of any of these conditions or are simply a reflection of tissue damage and disease severity remains controversial.

The purposes of the present study were (1) to measure circulating endothelin concentrations in neonates with transposition of the great arteries and (2) to investigate the effects of ET-1 on the neonatal heart. Because the effects of cytokines such as endothelin are "contextual,"¹⁹ that is, dependent on their physiologic milieu and the presence of other regulatory factors, we also sought to examine the effects of ET-1 in a defined model of neonatal myocardial ischemia-reperfusion and in combination with norepinephrine concentrations representative of those measured after CPB in neonates.

Methods

ET-1 measurements in human plasma. These experiments were approved by the Human Subjects Committee of the Children's Hospital of Pittsburgh. Plasma endothelin concentrations were measured in four groups of subjects (Table I): (1) infants with transposition of the great arteries (TGA) undergoing arterial switch repair; (2) children undergoing repair of secundum atrial septal defect (ASD); (3) umbilical vein blood (obtained from the placenta within 5 minutes of delivery) from neonates born

Table I. Plasma endothelin concentrations (pg/ml)

	Age	Control	Post-CPB
TGA (<i>n</i> = 14)	14 ± 2 days	22.9 ± 2.3* (14.8 – 28.2)	29.6 ± 3.1† (21.4 – 39.4)
ASD (<i>n</i> = 6)	3.4 ± 0.8 years	13.2 ± 1.6 (8.8 – 17.3)	16.9 ± 2.2† (10.7 – 21.1)
Cord blood (<i>n</i> = 8)	—	19.2 ± 2.9 (12.7 – 24.8)	
Adult (<i>n</i> = 14)	26 ± 3 years	10.7 ± 2.5 (5.2 – 15.9)	

Data are mean ± standard error of the mean (range).

**p* < 0.05 versus ASD and adult.

†*p* < 0.05 versus control (pre-CPB).

after term, uncomplicated pregnancy, labor, and vaginal delivery; and (4) healthy, nonsmoking adults of both sexes (eight male, six female). In surgical patients, samples were drawn (1) after anesthetic induction, sternotomy, and pericardiotomy (pre-CPB); (2) 10 minutes after institution of CPB; (3) immediately before CPB termination; and (4) 1 hour after termination of CPB (post-CPB). Simultaneous blood samples were obtained from the superior vena cava and a pulmonary vein before and after CPB and from the arterial and venous limbs of the perfusion circuit during CPB. In healthy volunteers, venous blood was obtained from an antecubital vein (without use of a tourniquet).

Blood samples (4 ml) were aspirated into iced syringes, transferred into pre-chilled tubes containing ethylenediaminetetraacetic acid, and centrifuged at 3000 *g* at 4° C for 10 minutes. Plasma was aspirated, frozen at –70° C, and analyzed in batches within 4 weeks of collection. Plasma endothelin was measured with a commercially available radioimmunoassay kit (Peninsula Laboratories, Belmont, Calif.). The assay is based on the competition of endothelin in the sample with exogenous ¹²⁵I-labeled endothelin for a finite amount of specific anti-endothelin antibody. Plasma extraction was performed on C₁₈ cartridges (Bond-Elut, Varian, Harbor City, Calif.) that were activated by multiple washes with 60% acetonitrile/0.1% trifluoroacetic acid followed by 0.1% trifluoroacetic acid. Samples of plasma were acidified with an equal volume of 0.1% trifluoroacetic acid, centrifuged (5000 *g* at 4° C for 10 minutes), and the supernatant slowly loaded onto preactivated C₁₈ columns. Columns were washed (5 ml twice each) with 0.1% trifluoroacetic acid, deionized water, and hexane; endothelin was eluted by washing (1 ml three times) with 60% acetonitrile/0.1% trifluoroacetic acid. Elutriates were concentrated to dryness by evaporation (SpeedVac, Savant Instruments, Farmingdale, N.Y.; medium heat setting), reconstituted in assay buffer, and incubated with rabbit anti-endothelin antiserum for 24 hours at 4° C. Subsequently, ¹²⁵I-labeled endothelin was added and incubated for a second 24-hour period. On the third day, bound and free ligands were separated by centrifugation after addition of second antibody (goat antirabbit immunoglobulin G). Radioactivity was measured by gamma counting. Assay sensitivity was 1 pg per tube. All samples were analyzed in duplicate. Recovery of

added ET-1 was $76\% \pm 3\%$; values presented were corrected for this recovery rate. Intraassay variability and interassay variability were $5\% \pm 1\%$ and $9\% \pm 2\%$, respectively. No attempt was made to correct for hemodilution accompanying CPB. The anti-endothelin antibody used in this study cross-reacted with big endothelin (35%) and endothelin-3 (7%). The physiologic effects of big endothelin are entirely dependent on its conversion to ET-1.²⁰

Isolated, perfused hearts. Animal experiments were performed with the approval of the institutional Animal Care and Use Committee and conformed to the Helsinki Declaration. Piglets aged 4 to 8 days were obtained from a commercial breeder. General anesthesia was induced with sodium pentobarbital (25 to 30 mg/kg intraperitoneally), a tracheotomy was performed, and mechanical ventilation was begun. After anticoagulation with heparin (1000 U intravenously), hearts were rapidly excised by transection of the pulmonary hilae, venae cavae, and great vessels; the aorta was cannulated rapidly (<1 minute) and antegrade perfusion was begun with a Langendorff column. Details of the preparation have been described elsewhere.^{21, 22}

Arterial gas exchange was carefully controlled with a mixture of 95% oxygen and 5% carbon dioxide passed through the perfusate in the Langendorff column. Aortic blood gas tensions were determined frequently; oxygen and carbon dioxide tensions were maintained at 300 to 350 torr and 30 to 40 torr, respectively, and pH at 7.38 to 7.45. Perfusate temperature was measured continuously and maintained at $36.5^\circ \pm 0.5^\circ \text{C}$ with a heat exchanger.

After initiation of retrograde perfusion, the pulmonary artery was cannulated and a fluid-filled latex balloon passed into the left ventricle via a small incision in the left atrium. A ligature was placed around the left atrium so as to close the atrial incision, maintain balloon position within the left ventricle, and prevent shunting across a patent foramen ovale. With this arrangement, pulmonary arterial effluent is composed solely of coronary venous blood. Balloons of different sizes were prepared, and for each experiment balloon volume was chosen to be greater than estimated left ventricular volume (the estimate was based on pilot experiments and measured external left ventricular dimensions). The balloon was connected to a micromanometry catheter (Millar Instruments, Inc., Houston, Tex.) via a short length of polyethylene tubing. A calibrated syringe was used to vary balloon volume and thus left ventricular end-diastolic pressure. The isolated heart was enclosed in a close-fitting water jacket that maintained right ventricular temperature at $36.5^\circ \pm 1.0^\circ \text{C}$.

A red cell-enhanced Krebs-Henseleit buffer solution was used as the perfusion medium. Human erythrocytes were filtered through a $40 \mu\text{m}$ blood filter (Pall Corporation, East Hills, N.Y.), washed thrice with 0.9% sodium chloride, and then twice with a solution consisting of 2% bovine serum albumin (Cohn fraction V; endotoxin-free) in Krebs buffer. The washed erythrocytes were added to filtered, modified Krebs solution containing 2% bovine albumin, glucose 5 mmol/L, lactate 1.5 mmol/L, palmitate about 0.5 mmol/L, L-arginine, 50 $\mu\text{mol/L}$, NaCl 118 mmol/L, KCl 4.7 mmol/L, MgSO_4 2.4 mmol/L, KH_2PO_4 1.2 mmol/L, NaHCO_3 25 mmol/L, and CaCl_2 2.4 mmol/L.

A final hematocrit value of 22% to 25%, comparable with that of newborn piglets, was used. Insulin (porcine) was added to produce a final concentration of 25 $\mu\text{U/ml}$, equivalent to that found in fasted piglets in vivo (unpublished observation). A nonrecirculating mode of perfusion was used to avoid contamination from metabolites released by the heart. A $20 \mu\text{m}$ filter (Statlabs, Nashville, Tenn.) was placed in-line in the perfusion apparatus to remove microaggregates.

Oxygen content of coronary arterial and venous samples drawn anaerobically from the aortic root and pulmonary artery was measured with a Co-Oximeter device (Lexington Instrument Co., Waltham, Mass.). Myocardial oxygen consumption was determined by the coronary arteriovenous oxygen difference divided by coronary flow rate and expressed as millimoles of oxygen per minute per gram wet heart weight.

All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise noted and were of cell-culture grade (where obtainable). ET-1 (Peninsula Laboratories, Belmont, Calif.) was dissolved in filtered Krebs buffer containing 1% bovine serum albumin, frozen at -70°C , and used within 2 weeks of preparation. Dilutions of ET-1 were freshly prepared from thawed stock solution with perfusion buffer.

On completion of the preparation, a 30-minute period was allowed for stabilization. Heart rate was kept constant at 150 beats/min (approximately 10% to 20% above intrinsic rate) by atrioventricular pacing (atrioventricular delay 80 to 90 msec) with needle electrodes secured to the right atrium and right ventricle. Coronary flow rate was then set at 2.0 ml/min per gram heart weight, and thus changes in coronary perfusion pressure directly reflected changes in coronary vascular resistance. This coronary flow rate (1) exceeds that required for oxygen and substrate delivery, (2) produces coronary perfusion pressure of 50 to 60 mm Hg, and (3) maintains stable mechanical function, myocardial metabolism, and high-energy phosphates.^{21, 22} Of particular relevance to the present experiments, coronary perfusion pressure and coronary dilator responses to adenosine and bradykinin are stable for a minimum of 3 hours in this preparation.²²

After stabilization and measurement of control function, the dose-responses of coronary perfusion pressure to norepinephrine, 5 to 30 nmol/L, and ET-1, 1 to 100 pmol, were determined. The effects of endothelin treatment (1 to 100 pmol) in the presence of norepinephrine 10 nmol/L were also determined. Separate hearts ($n = 6$) were used for each of these sets of experiments. Norepinephrine was added directly to the perfusate. Because endothelin may adhere to glassware and plastic, it was administered by infusion into the aortic root.

In six additional hearts, the stabilization and control period was completed, aortic perfusion terminated, and pacing discontinued. A 30-minute period of zero-flow global ischemia was instituted; myocardial temperature (measured by a thermistor in the right ventricle) was maintained at 35° to 36°C by a water jacket. At the end of ischemia, coronary flow was begun at 0.5 ml/min per gram (25% of control coronary flow) and systematically increased to 2 ml/min per gram over 6 minutes. Ventricular fibrillation was terminated with a direct current of 5

Table II. Effects of endothelin and norepinephrine on myocardial function

	Peak developed LV pressure (mm Hg)		Myocardial oxygen consumption ($\mu\text{mol}/\text{min}$ per gram)	
	Nonischemic	Reperfused	Nonischemic	Reperfused
Control	87 $\pm$ 3	78 $\pm$ 4	2.1 $\pm$ 0.2	1.8 $\pm$ 0.2
ET-1, 10 pmol	93 $\pm$ 3	76 $\pm$ 4	2.5 $\pm$ 0.2	1.8 $\pm$ 0.2
ET-1, 100 pmol	112 $\pm$ 4*	68 $\pm$ 3*	2.9 $\pm$ 0.3*	1.9 $\pm$ 0.3
Norepinephrine, 10 nmol/L	91 $\pm$ 4	78 $\pm$ 4	2.2 $\pm$ 0.3	1.8 $\pm$ 0.3
ET-1 + norepinephrine†	94 $\pm$ 3	75 $\pm$ 3	2.3 $\pm$ 0.3	1.8 $\pm$ 0.3
ET-1 + norepinephrine‡ + nitroglycerin	94 $\pm$ 2	76 $\pm$ 3	2.3 $\pm$ 0.2	1.8 $\pm$ 0.3

* $p < 0.05$ versus control.

†ET-1, 10 pmol, plus norepinephrine, 10 nmol/L.

‡ET-1, 10 pmol, norepinephrine, 10 nmol/L, and nitroglycerin, 200 nmol/L.

watt-seconds, and pacing was resumed at 150 beats/min. Left ventricular end-diastolic pressure was maintained at about 5 cm by adjusting balloon volume. As shown in Table II, this sequence of ischemia-reperfusion resulted in an approximately 10% reduction in contractile function that is paired with a similar reduction in myocardial oxygen consumption. Coronary perfusion pressure was significantly reduced during early reperfusion (to 34 to 42 mm Hg) but had recovered to control, nonischemic values by 30 to 45 minutes of reperfusion. Mechanical function and coronary perfusion pressure were subsequently stable at this level for a minimum of 2 hours during reperfusion. In prior studies, we²² have shown that even longer periods of global normothermic ischemia in this model (1 to 2 hours) result in essentially complete recovery of adenosine triphosphate, phosphocreatine, and oxidative metabolism.

On the basis of this information, coronary perfusion pressure responses to norepinephrine, ET-1, and norepinephrine and ET-1 in combination were investigated at 45 to 60 minutes of reperfusion. Separate hearts were used for these experiments to avoid tachyphylaxis or injury caused by the agents studied.

Statistical analysis. Values were expressed as mean plus or minus standard error. Multiple group comparisons were made with analysis of variance for repeated measures followed by Scheffe's procedure. Differences between pre-CPB and post-CPB endothelin concentrations were analyzed by paired t tests (two-tailed). Differences between preischemic and postischemic responses in isolated hearts were analyzed by unpaired t tests (two-tailed). A p value less than 0.05 was considered significant. All data analysis was performed with Statview/SuperANOVA computer software (Abacus Concepts, Berkeley, Calif.) in consultation with a statistician (Wayne DellaMaestra, BS).

Results

Human plasma endothelin measurements. Preoperative arterial oxygen saturation (measured by pulse oximetry) was 77% $\pm$ 4% in infants with TGA; six of 14 had ventricular septal defects. The durations of CPB and deep hypothermic circulatory

arrest in patients with TGA were 102 $\pm$ 14 minutes and 22 $\pm$ 6 minutes, respectively. The duration of CPB was 42 $\pm$ 6 minutes in children undergoing ASD repair. Nitroglycerin was used to facilitate cooling and rewarming in all patients with TGA and was not used in patients with ASD. No patients required inotropic agents after CPB.

High plasma concentrations of ET-1 were found in neonatal cord blood and preoperatively in patients with TGA (see Table I). No correlation existed between preoperative ET-1 concentration in infants with TGA and the presence of a ventricular septal defect, arterial oxygen saturation, or the use of prostaglandin E₁. Measured ET-1 concentrations did not change significantly during CPB in either group of patients (data not shown). Endothelin concentrations were increased significantly and to a similar degree 1 hour after CPB both in patients with ASD and in those with TGA, although post-CPB ET-1 concentrations were significantly higher in infants with TGA.

Preoperatively, net extraction of ET-1 across the pulmonary vascular bed (defined as superior vena cava minus pulmonary vein endothelin concentration) was 0.6 $\pm$ 0.3 pg/ml in children with ASD, whereas infants with TGA had net ET-1 release preoperatively (1.4 $\pm$ 0.5 pg/ml, $p < 0.05$ versus patients with ASD). The post-CPB period was characterized by net pulmonary release of ET-1 both in patients with ASD (0.4 $\pm$ 0.3 pg/ml, $p < 0.05$ versus pre-CPB) and in those with TGA (2.5 $\pm$ 0.5 pg/ml, $p = 0.14$ versus pre-CPB, $p < 0.01$ versus post-CPB values in patients with ASD).

Effects of endothelin in isolated, perfused hearts. A 100 pmol dose of ET-1 caused significant coronary constriction in the nonischemic neonatal heart, increasing coronary perfusion pressure by approxi-

Fig. 1. Effects of ET-1 on coronary perfusion pressure (CPP) in control (circles) and ischemia-reperfused (squares) neonatal pig heart. Data are mean $\pm$ standard error; $n = 6$ each. * $p < 0.05$ versus no endothelin-1. # $p < 0.05$ versus ischemia-reperfused preparation.

mately 49% (Fig. 1). The constrictor effects of 1 and 10 pmol were minimal, whereas 25 pmol increased coronary perfusion pressure approximately 18%. Increases were sustained for at least 15 minutes after discontinuation of ET-1. ET-1 doses between 1 and 25 pmol also caused an early, transient decrease in coronary perfusion pressure of 8% to 17% that began within 6 seconds of administration and resolved within 15 to 20 seconds.

After ischemia-reperfusion, coronary constriction induced by ET-1 was markedly enhanced (see Fig. 1). A 10 pmol dose of ET-1 increased coronary perfusion pressure by approximately 40% during reperfusion, and the constrictor effects of 100 pmol were increased nearly twofold. ET-1 stimulated coronary dilation was absent during reperfusion.

The effects of a 5 to 30 nmol/L concentration of norepinephrine (which are similar concentrations to those observed after CPB and hypothermic circulatory arrest in neonates²³) are shown in Fig. 2. Coronary perfusion pressure in the nonischemic piglet heart was decreased somewhat by a 5 to 10 nmol/L dose of norepinephrine and increased modestly by higher concentrations. Dose-dependent vasoconstriction was observed in reperfused hearts,

although the magnitude of this effect was not different from that observed before ischemia.

On the basis of these results, the effects of threshold amounts of ET-1 (1 to 10 pmol) were investigated in the presence of a 10 nmol/L concentration of norepinephrine. Before ischemia, the combination of 1 pmol ET-1 and 10 nmol/L norepinephrine increased coronary perfusion pressure from 51 ± 3 mm Hg (10 nmol/L norepinephrine alone) to 64 ± 3 mm Hg ($p < 0.05$); during reperfusion, this combination increased coronary perfusion pressure from 58 ± 3 mm Hg to 78 ± 4 mm Hg ($p < 0.05$). The effects of norepinephrine on 10 pmol ET-1 are shown in Fig. 3. Coronary perfusion pressure was significantly increased by 10 pmol ET-1 in conjunction with norepinephrine, and this effect was enhanced by ischemia-reperfusion. Nitroglycerin, 200 nmol/L (which would be roughly equivalent to an infusion of 3 μ g/kg per minute in a patient with a cardiac index of 2.5 L/min per square meter), effectively reversed coronary constriction as a result of either ET-1 alone or ET-1 plus norepinephrine.

ET-1 also caused dose-dependent increases in peak developed left ventricular pressure and oxygen consumption in the nonischemic heart (Table II). In

Fig. 2. Effects of norepinephrine on coronary perfusion pressure (CPP) in control (circles) and ischemia-reperfused (squares) neonatal pig heart. Data are mean $\pm$ standard error; $n = 6$ each.

contrast, 100 pmol ET-1 decreased left ventricular peak developed pressure during reperfusion; myocardial oxygen consumption was maintained despite the decrease in peak developed pressure caused by ET-1. Neither norepinephrine nor nitroglycerin altered these effects.

Discussion

The present study demonstrates that circulating ET-1 concentrations are significantly higher in neonates with arterial transposition than in older children with ASDs or in healthy adults. CPB, with or without deep hypothermic circulatory arrest, increased plasma endothelin concentrations in conjunction with a tendency toward decreased net pulmonary endothelin extraction. ET-1 caused minimal cardiac effects when infused into the neonatal heart in low concentrations but was a significant coronary vasoconstrictor and inotropic agent when present in higher concentrations. The vasoconstrictive effects of ET-1 were significantly enhanced by ischemia-reperfusion and norepinephrine and were effectively reversed by nitroglycerin.

Plasma ET-1 concentrations similar to those observed in infants with TGA and in cord blood in the present study have been previously reported in healthy neonates during the first few days after

birth; values decline to approximately 12 to 20 pg/ml within 5 to 10 days of birth and approach adult values by 3 to 6 months of age.^{12, 24-26} The mechanism for the sustained increase in plasma ET-1 observed preoperatively in infants with TGA is uncertain but may be related to hypoxemia, which is a known stimulus for endothelin synthesis and release.³ Increased plasma endothelin has been measured in children during acute hypoxia,⁹ as well as in neonates with bronchopulmonary dysplasia and persistent pulmonary hypertension.^{25, 27} A correlation between plasma endothelin and pulmonary hypertension and/or pulmonary blood flow in children with congenital heart disease has been reported in some^{15, 18, 28} but not all studies.^{29, 30}

Prior studies in adult patients have also documented increased ET-1 both during and after CPB.^{11, 31} The mechanisms of CPB-induced increase in circulating endothelin are uncertain. Hypoxia, ischemia, abnormal flow patterns, hypotension, cytokines, and catecholamines have been shown to stimulate endothelin synthesis and release under other circumstances.^{3, 7, 16, 17, 32-34} It is likely that our measurements made at 1 hour after CPB underestimate the maximal increase in ET-1 levels. Endothelin is stored in small amounts in endothelial cells, and additional peptide synthesis (which re-

Fig. 3. Comparison of the effects of ET-1 and norepinephrine, alone and in combination, on coronary perfusion pressure (CPP) in control and ischemia-reperfused neonatal pig hearts. Data are mean $\pm$ standard error; $n = 6$ each. * $p < 0.05$ versus control. # $p < 0.05$ versus corresponding response in nonischemic hearts.

quires 2 to 6 hours) would probably be necessary to permit large and sustained increases in plasma concentrations.³⁵ Komai and associates³⁰ recently studied increased plasma ET-1 concentrations in older children (aged 1.6 to 6.2 years) after CPB. They demonstrated ET-1 concentrations that peaked 3 to 6 hours after CPB and were similar to those found in the present study. The magnitude of CPB-induced increase in ET-1 was greater in children with increased as compared with normal pulmonary blood flow. Also noted was a significant positive correlation between the post-CPB increase in ET-1 and the maximal pulmonary artery/systemic pressure ratio after the operation.

After CPB, the present study found a modest but significant conversion from pulmonary ET-1 extraction to release in patients with ASD and a trend toward increased pulmonary release of ET-1 in patients with TGA. The pulmonary endothelium is a major organ of endothelin clearance, as well as synthesis.³⁶ Reduced pulmonary endothelin removal may be a factor after CPB, which is believed to cause pulmonary endothelial injury through many mecha-

nisms including neutrophil and complement activation and free radical generation. Endothelin may itself stimulate free radical production by neutrophils.³⁷ Pulmonary uptake of other substances such as catecholamines and serotonin are decreased after lung injury and CPB.^{38,39} Significant net pulmonary endothelin release and decreased pulmonary removal of exogenously infused endothelin has been demonstrated in patients with adult respiratory distress syndrome.^{10,40} Impaired pulmonary uptake of endothelin after CPB, although modest in the present study, may indicate a generalized derangement of the metabolic functions of the lung. Pulmonary endothelium also modulates the effects of constricting substances such as ET-1 and catecholamines via the release of vasodilators such as prostacyclin and nitric oxide. Endothelial injury, especially in conjunction with increased catecholamine and ET-1 concentrations, may increase susceptibility to pulmonary vasoconstriction and postoperative pulmonary hypertensive crises.⁴¹

The vascular effects of ET-1 were examined in an isolated, blood-perfused neonatal heart preparation.

The heart was chosen for study because little is known about neonatal cardiac effects of ET-1 and also because myocardial dysfunction can be an important problem after arterial switch repair of TGA. Endothelin was a potent vasoconstrictor of the normal neonatal coronary circulation and also caused significant inotropic effects. These results are consistent with observations in a wide variety of adult preparations.^{3, 17, 19, 42} The mechanisms responsible for the effects of endothelin on vascular smooth muscle and ventricular muscle are not entirely clear. In general, it appears that ET-1 binding to specific receptors results in opening of Ca^{++} channels and activation of phospholipase C, with subsequent formation of inositol triphosphate (which increases intracellular Ca^{++}) and diacylglycerol (which activates protein kinase C). Activation of protein kinase C stimulates the membrane Na/H exchanger to promote intracellular alkalinization, which increases myofilament sensitivity to Ca^{++} .^{3, 19} The end result of these mechanisms is increased contractile amplitude.

An important finding of the present study was that ischemia-reperfusion increased endothelin-induced coronary constriction. There are several possible explanations for this result. Ischemia-reperfusion more than doubles the number of cardiac endothelin binding sites,⁴³ and thus endothelin effects may be increased in reperfused hearts. Distinct endothelial endothelin receptors are linked to prostacyclin and nitric oxide production,³ and nitric oxide and prostacyclin release are known to counterbalance ET-1 in a variety of vascular beds, including the coronary circulation.^{5, 44, 45} This is the likely explanation for transient coronary dilation in response to ET-1 observed before ischemia. Endothelial dysfunction and reduced endothelial release of vasodilating substances such as nitric oxide and prostacyclin during reperfusion may lead to unopposed vasoconstricting actions of ET-1 on vascular smooth muscle. Of the two compounds, it is likely that nitric oxide release is more important in the neonatal heart.²² Decreased basal and receptor-stimulated nitric oxide synthesis in the coronary circulation during reperfusion has been reported.^{22, 46} The function of smooth muscle guanylate cyclase, which is the target of nitric oxide and exogenous nitrovasodilators that produce vasodilation, appears to be intact after a reversible ischemia-reperfusion injury.²² Coronary vasodilation in response to nitroglycerin is therefore preserved.

Norepinephrine is an endothelium-dependent va-

sodilator that acts by similar mechanisms. It can also cause contraction of vascular smooth muscle by increasing intracellular calcium and activating protein kinase C (which is coupled to α -adrenergic receptors).⁴⁷ Once again, the balance of these actions will determine the final effect on coronary vascular resistance. The concentrations of norepinephrine used in this study have been observed in neonates during the course of CPB/deep hypothermic circulatory arrest.²³ These concentrations had modest effects on coronary perfusion pressure in the normal and reperfused neonatal heart. However, norepinephrine enhanced endothelin-induced coronary constriction and this effect was increased by ischemia-reperfusion. It is likely that norepinephrine potentiated the intracellular actions of endothelin in vascular smooth muscle. These effects were also reversed by nitroglycerin.

The negative inotropic effects of ET-1 in reperfused myocardium were unexpected. As noted previously, endothelin produces positive inotropic effects in a variety of isolated muscle and intact animal preparations. Decreased myocardial function as a result of ET-1 has been reported^{16, 41}; this has usually been attributed to increased systemic vascular resistance and myocardial ischemia resulting from reduced coronary blood flow. Neither effect is likely in the present study because isolated hearts and controlled coronary flow were used. It is possible that increased calcium cycling, intracellular alkalinization, or some other consequence of endothelin signaling was poorly tolerated by reperfused myocardium. Endothelin has also been noted to stimulate degradation of membrane phospholipids,⁴⁸ and perhaps this effect was particularly deleterious when superimposed on ischemia-reperfusion injury.

Myocardial dysfunction is among the most frequent and difficult complications after cardiac surgery in neonates; impaired coronary blood flow can be a particular issue after arterial switch repair of TGA. Although these problems are most often related to technical difficulties or inadequate myocardial preservation, this study demonstrates that circulating compounds such as endothelin may have an important role and that nitroglycerin effectively inhibits the arterial constriction that may result. Because of the diffuse nature of the injury that accompanies CPB and deep hypothermic circulatory arrest, these results have important implications not only for the coronary circulation, but for the pulmonary, cerebral, and renal vascular beds as well.

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Discussion

Dr. Antonio F. Corno (Milan, Italy). You are working with Pedro del Nido and must be well aware of the damage induced by reoxygenation at the beginning of CPB on both endothelium and myocardium, because Dr. del Nido published this clinical data years ago. The damage is directly correlated with the difference between the oxygen tension before bypass and the oxygen tension at the beginning of bypass. What level of oxygen tensions did you use in these patients at the beginning of bypass? What was the difference between the oxygen tension at the beginning of bypass between the cyanotic neonates with TGA and the acyanotic patients with ASD? Did you see a difference because of these discrepancies in reoxygenation at the beginning of bypass?

Dr. McGowan. Thank you very much. Certainly the arterial oxygen saturation was lower in the infants with TGA (their mean saturation was approximately 75% to 80%). Children with ASDs were, of course, normally saturated. Hypoxia is one of the stimuli known to induce endothelin synthesis and release. It is therefore likely that relative hypoxemia was one cause of the abnormal persistence of high endothelin levels in the neonates with transposition. Whether restoration of normal oxygen saturation by CPB caused a reoxygenation injury leading to increased ET-1 levels after bypass is unknown. There are reports in adult patients (who were not cyanotic before CPB) that demonstrate increased ET-1 concentrations after CPB; this would suggest that reoxygenation is unlikely to be the only factor involved. Reoxygenation/free radical injury to the coronary endothelium is a likely component of the enhanced endothelin-mediated constriction observed in the reperfused piglet heart preparation.

Dr. Corno. What was the oxygen tension at the beginning of bypass? We generally start bypass at an oxygen tension of more than 200 mm Hg. I know your method is different.

Dr. McGowan. I cannot tell you exactly what the early bypass oxygen tension was. It is usually in the range of 200 to 400 mm Hg. The arterial oxygen saturation before CPB in the patients with TGA was maintained within their preoperative range. As you know, we try to avoid hyperventilation and hyperoxia in these patients to balance their systemic and pulmonary blood flows.

Mr. Magdi Yacoub (London, England). What do you think the mechanism of the negative inotropic effect is

during the reperfusion? Do you think that is ischemic? Can you repeat that, for example, on isolated myocytes?

Dr. McGowan. In the isolated, perfused heart preparation, coronary blood flow was controlled and constant. Therefore, despite the marked increase in coronary vascular resistance caused by endothelin after ischemia-reperfusion, endothelin-induced ischemia would seem to be an unlikely cause of the negative inotropic effect observed during reperfusion.

The positive inotropic effects of endothelin have been attributed to increased cytosolic calcium and/or intracellular alkalinization, which can increase the sensitivity of the contractile proteins to calcium. We can only speculate that perhaps calcium overload is occurring at a time the

myocyte is ill-equipped to deal with excess calcium, and/or that endothelin effects on the contractile proteins are different after ischemia. Another important mechanism may be endothelin-induced acceleration of cell membrane phospholipid breakdown resulting from activation of membrane phospholipases.

Mr. Yacoub. Have you tried using endothelin antagonists to block some of these effects and dissect out whether it is a cellular or a vascular effect?

Dr. McGowan. No. That is certainly an excellent point. Relatively specific endothelin receptor antagonists have become available and would be a logical choice for the next phase of these studies.

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