CARDIAC PERFORMANCE AFTER DEEP HYPOTHERMIC CIRCULATORY ARREST IN CHRONICALLY CYANOTIC NEONATAL LAMBS

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Objectives: It is controversial whether immature cyanotic hearts are more susceptible to ischemic injury than normoxemic hearts. Acutely induced alveolar hypoxic stress before cardiopulmonary bypass has been used as a model of cyanosis and is reported to worsen recovery of immature hearts after subsequent ischemic insult by means of a free radical injury mechanism. Because of concerns about the relevance of acute alveolar repair to the chronic cyanosis encountered clinically, we assessed the effects of chronic cyanosis without alveolar hypoxia, acute alveolar hypoxia, and normoxemia on recovery of cardiac function after deep hypothermic circulatory arrest.

Methods: A chronic cyanosis model was created in 8 lambs by an anastomosis between the pulmonary artery and the left atrium (cyanosis group). Eight lambs underwent sham operation (control). One week later, the animals underwent cardiopulmonary bypass with 90 minutes of deep hypothermic circulatory arrest at 18°C. Another 8 lambs underwent 45 minutes of hypoxic ventilation before bypass, with arterial oxygen tension being maintained at 30 mm Hg (acute hypoxia group). Cardiac index, preload recruitable stroke work, and tau were measured. Malondialdehyde and nitrate-nitrite, nitric oxide metabolites, were also measured in the coronary sinus. Myocardial antioxidant reserve capacity at 2 hours of reperfusion was assessed by measuring lipid peroxidation in left ventricular tissue samples incubated with t-butylhydroperoxide at 37°C.

Results: Oxygen tension was 35 ± 3 mm Hg in the acute hypoxia group versus 93 ± 7 mm Hg in the control group. In the acute hypoxia group the recovery of cardiac index, preload recruitable stroke work, and tau were significantly worse than that found in both the control and cyanosis groups. Preload recruitable stroke work at 2 hours of reperfusion was slightly but significantly lower in the cyanosis group than in the control group. The postischemic level of nitric oxide metabolites was significantly lower in the acute hypoxia group than in the cyanosis and control groups. However, malondialdehyde levels in the coronary sinus and myocardial antioxidant reserve capacity were not significantly different among the groups.

Conclusion: Recovery of left ventricular function after deep hypothermic circulatory arrest in neonatal lambs with chronic cyanosis was slightly worse than that found in acyanotic animals. Acute hypoxia before bypass was associated with significantly worse recovery of left ventricular function, and the mechanism of injury may be related to an impairment of nitric oxide production. Free radical injury does not appear to explain any differences among cyanotic, acyanotic, and acutely hypoxic animals in recovery of left ventricular function after ischemia. (J Thorac Cardiovasc Surg 2000;120:238-46)

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It remains controversial whether cyanotic hearts are more susceptible to ischemia-reperfusion than normoxic hearts. Comparisons on the basis of clinical results are difficult because many congenital cyanotic lesions also involve pressure overload, volume overload, or both. In addition, postoperative management with inotropic or afterload-reducing agents complicates the assessment of heart function. It has been reported that chronic alveolar hypoxia increases the tolerance of the neonatal heart to ischemia compared with normoxemia.^{1,2} In contrast, Fujiwara and associates³ demonstrated that hearts from neonatal lambs with surgically created cyanosis have slightly worse functional recovery than normoxic hearts after ischemia when evaluated in an isolated heart model. Several authors report that acute alveolar hypoxic stress before cardiopulmonary bypass (CPB) is associated with severe postischemic cardiac dysfunction, and the mechanism of injury has been attributed to oxygen-derived free radicals.⁴⁻⁶ However, it is uncertain that the acute alveolar hypoxia model is relevant to the chronic cyanosis encountered in patients with severe forms of congenital heart disease. The objective of the current study was to assess whether chronic cyanosis without alveolar hypoxia affects the tolerance or susceptibility of neonatal hearts to myocardial ischemia under conditions of deep hypothermic circulatory arrest (DHCA) with CPB. Second, we compared chronic cyanosis with acute hypoxia to evaluate whether acute alveolar hypoxia is an appropriate model as chronic cyanosis.

Methods

Creation of cyanosis. Sixteen neonatal lambs (age 4.6 ± 0.9 days; weight 4.3 ± 0.3 kg [mean $\pm$ SEM]) were anesthetized with intramuscular injection of ketamine hydrochloride (50 mg/kg). After intubation, the anesthesia was maintained by inhalation of isoflurane. A left thoracotomy was performed in the third intercostal space. The ductus arteriosus was ligated with a silk suture after the pericardium was entered. A model of chronic cyanosis was created in 8 of the lambs by an anastomosis between the pulmonary artery (PA) and the left atrium (LA). Two partial side-biting clamps were placed on the left PA and on the LA adjacent to the atrioventricular groove to avoid entering its trabecular portion. The length of the longitudinal incision at the PA was empirically chosen to be the same size as the diameter of the left PA (5-7 mm). The LA was anastomosed to the PA by a continuous 7-0 monofilament suture. A vessel loop was taped around the anastomosis as a marker and to facilitate dissection at the second procedure. The remaining 8 lambs underwent a thoracotomy and a sham operation (control), which was identical except that the PA and the LA were not incised and the anas-

tomosis was not carried out. The sham procedure did include 20 minutes of side clamping of the PA and the LA. The animals were given intramuscular injections of antibiotics during the procedures and 12 hours after the procedures, and buprenorphine hydrochloride (0.01 mg/kg) was given to minimize discomfort and pain every 8 hours for the first 48 hours after the procedures. The animals survived for 7 days.

DHCA study. The lambs were anesthetized with intramuscular injection of ketamine hydrochloride (50 mg/kg). After intubation, the animals' lungs were artificially ventilated with a volume-controlled ventilator (Servo Ventilator 900C; Siemens-Elema, Danvers, Mass) with a tidal volume of 20 mL/kg and 4 cm H₂O of positive end-expiratory pressure at an inspired oxygen fraction (FIO₂) of 100% during the experiment. After a bolus intravenous infusion of 300 μ g/kg fentanyl, anesthesia was maintained by continuous intravenous infusion of ketamine (5 mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹), midazolam (0.2 mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹), pancuronium (mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹), and fentanyl (50 μ g $\cdot$ kg⁻¹ $\cdot$ h⁻¹) throughout the experiment. A 19-gauge catheter was inserted through the left femoral artery and passed into the thoracic aorta for measurements of systemic arterial pressure and for blood gas sampling. Through a median sternotomy, the heart was exposed. A cotton tape was placed around the inferior vena cava (IVC) to allow transient occlusions. A 5F side-holed catheter (Berman angiographic catheter; Arrow International Inc, Reading, Pa) was inserted to measure PA pressure. After systemic heparinization, a 5F polyurethane sheath was inserted into the LA for pressure measurement and blood sampling. A 19-gauge catheter was also inserted into the coronary sinus through the hemiazygos vein. A micromanometer catheter (SPC-350; Millar Instruments Inc, Houston, Tex) was placed into the left ventricular (LV) cavity through the apex to measure LV pressure. Two pairs of ultrasonic transducers (Sonometrics, London, Canada) were implanted in the LV midmyocardium to measure the length of the long and short axes. Another 8 lambs (age 4.4 ± 1.2 days; weight 3.8 ± 0.2 kg [mean $\pm$ SEM]) underwent 45 minutes of hypoxic ventilation before CPB, with arterial oxygen tension (Pao₂) being maintained at 30 mm Hg (acute hypoxia [AH] group) after ligation of the ductus arteriosus and instrumentation (Fig 1). In the cyanosis group the PA-LA shunt was ligated immediately before CPB.

The circuit for CPB consisted of a roller pump and a membrane oxygenator (VPCML; COBE Laboratories, Arvada, Colo). The pump prime consisted of 200 mL of electrolyte solution (Normosol-R; Abbott Laboratories, North Chicago, Ill) and 500 mL of homologous heparinized fresh donor blood to achieve a hematocrit level of 20%. An 8F arterial cannula and a 24F venous cannula were placed into the right femoral artery and right atrium, respectively. The animals were cooled to 18°C over 30 minutes by pH-stat strategy. CPB flow was maintained at 150 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹. During 90 minutes of DHCA, the esophageal temperature was kept at 18°C by means of a temperature-controlled blanket. The patent foramen ovale was closed through a right atriotomy. CPB was reinstituted, and the animals were rewarmed to achieve a rectal temperature of 38°C over 40 minutes. The

Fig 1. Experimental protocol.

heart was defibrillated as necessary at a temperature of 32°C. The animals were then weaned from bypass and observed for 2 hours after reperfusion. The esophageal temperature was kept at 37°C after the termination of CPB. Sodium bicarbonate was added when the base excess exceeded 3.0 mmol/L, and the hematocrit value was kept at 20% during CPB and between 23% and 25% after CPB. Electrolyte solution (Normosol-R) containing glucose (5 g/L) and homologous blood were used for maintenance.

Data analysis. Pulmonary/systemic flow ratio (Qp/Qs) was estimated by the following formula:

$$Qp/Qs = (\text{Sat } O_{2\text{AO}} - \text{Sat } O_{2\text{MV}}) / (\text{Sat } O_{2\text{PV}} - \text{Sat } O_{2\text{PA}})$$

where Sat O_2 is oxygen saturation at various positions (AO = aorta, MV = mixed venous, and PV = pulmonary vein), and it was assumed that oxygen saturation in the pulmonary vein was 100% on 100% oxygen and 98% on 21% oxygen. Cardiac index was calculated by the following equation:

$$(\text{Ved} - \text{Ves}) \cdot \text{Heart rate} / \text{Body surface area}$$

where Ved is end-diastolic LV volume and Ves is end-systolic LV volume. Body surface area was calculated by the following formula⁷:

$$\text{Body surface area (m}^2\text{)} = 0.08 \cdot (\text{Body weight})^{2/3}$$

Digitalized data were obtained at 300 Hz during the transient occlusion of the IVC for 10 seconds and stored in a computer. End-systole was defined as the point of maximal ratio of instantaneous LV pressure to LV volume. End-diastole was defined as the first point at which dV/dt was 0 out of the points of an LV volume more than 80% of the maximum LV volume in each cardiac cycle.

LV volume was calculated by the following ellipsoidal model:

$$\text{LV volume} = (\pi/6)(D_L)(D_S)^2$$

where D_L is long axis length and D_S is short axis length obtained by ultrasonic transducers.

The first derivative of LV pressure (dP/dt) was calculated as a running 7-point method. Stroke work (SW) was calculated as the integral of LV pressure (LVp) and LV volume over each cardiac cycle by the following formula:

$$\text{SW} = \int \text{LVp} \cdot dV$$

Preload recruitable stroke work (PRSW) is defined as the slope of the relation between stroke work (SW) and end-diastolic LV volume (Ved) by the following formula:

$$\text{SW} = \text{PRSW} \cdot (\text{Ved} - \text{Vw})$$

where Vw is the intercept with the volume axis.

Tau is a time constant of isovolumic relaxation from the maximum negative dP/dt to the timing of mitral valve opening and is defined as the inverse slope of linear regression line derived from the relation between natural log of LV pressure (in millimeters of mercury) and time (in milliseconds) by the following equation:

$$P = P_0 \cdot e^{-t/T}$$

where P is LV pressure, t is time after the maximum negative dP/dt, and P_0 is the pressure at the time of the maximum negative dP/dt.

In a preliminary study we found that PRSW and tau were dependent on heart rate (Fig 2). We derived curvilinear regression lines as a normal value from the average PRSW and tau of 10 neonatal lambs at each heart rate from 120 to 260 beats/min. PRSW and tau in this study were expressed by both absolute value and heart rate-normalized data (percentage of normal value).

Biochemistry. Serial plasma samples were taken from the coronary sinus line before CPB; 5 minutes after onset of CPB; and after 5, 20, 60, and 120 minutes of reperfusion. LV tissue was collected from the free wall at the end of the experiment, immediately frozen in liquid nitrogen, and stored at -70°C until analyzed. Total plasma concentrations of NO₂- and NO₃-nitric oxide (NO) products in coronary sinus plasma were measured by the modified Griess reaction (nitrate/nitrite colorimetric assay kit; Cayman Chemical, Ann Arbor, Mich). Malondialdehyde and 4-hydroxyalkenals, the end products of the breakdown of polyunsaturated fatty acids during the process of lipid peroxidation, in the LV tissue and malondialdehyde in coronary sinus plasma were measured by the spectrophotometric method. In brief, LV tissue was homogenized in 20 μmol/L Tris-HCl buffer at pH 7.4 and centrifuged. The supernatant was collected and assayed (LPO-586; Calbiochem, San Diego, Calif). The myocardial antioxidant reserve capacity was assessed by the method of Godin and colleagues.⁸ In brief, homogenated heart tissue was incubated with several different concentrations of t-butylhydroperoxide in saline-azide (0-8 mmol/L) for 30 minutes at 37°C. Reactions were terminated by sodium arsenite (0.1 mol/L) in cold trichloroacetic acid (28% wt/vol). After centrifugation, the supernatant was mixed with thiobarbituric acid solution (0.5% in sodium hydroxide, 0.025 mol/L) and boiled for 15 minutes. The formation of thiobarbituric acid-reactive substances was assessed by the absorbance at 532 nm.

Epinephrine was measured in arterial plasma at baseline and at 1 hour of reperfusion.

Animals in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated

Fig 2. Hemodynamics, including heart rate, mean arterial pressure (AOP), and mean pulmonary pressure (PAP), during 40 minutes of hypoxic ventilation in the AH group. * $P < .05$ compared with value at the beginning of hypoxic ventilation.

by the National Society for Medical Research and “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (National Institutes of Health publication No. 86-23, revised in 1985).

Statistics. All values are expressed as means $\pm$ SEM. Data were compared by use of the 2-tailed unpaired Student t test or repeated-measures analysis of variance.

Results

Validation of cyanosis and baseline measurements. Baseline measurements were performed for validation of cyanosis and acute hypoxia (Table I). The cyanosis model produced a consistent and significantly lower PaO_2 and Qp/Qs at an FiO_2 of 21%. At an FiO_2 of 100%, Qp/Qs and PaO_2 significantly increased compared with values at an FiO_2 of 21% in the cyanotic animals. Acute increase in arterial oxygen resulted in significantly improved LV function (Table I).

Hemodynamics during acute hypoxic stress. Acute hypoxic ventilation resulted in significant hypoxemia (Table I), pulmonary hypertension, tachycardia, and systemic hypotension despite volume adjustment (Fig 3).

Hemodynamics after CPB and cardiac performance. One animal in each group required a very small amount of isoproterenol (INN: isoprenaline; 2-4 $\mu\text{g/kg}$) at weaning from CPB because of bradycardia caused by pulmonary hypertension. Therefore, no inotropic agents were used. Two animals died of severe cardiac dysfunction in the AH group at 90 and 110 minutes after CPB before completion of the experiments.

Postischemic cardiac index was significantly lower in the AH group than in the other 2 groups, and there was

Fig 3. Relationship between PRSW or tau and heart rate in normal neonatal lambs. Baseline heart rates in the neonatal lambs are 160 to 200 beats/min under the same anesthetic methods as this study. Heart rate was changed by right atrial pacing (heart rate > 160 beats/min) or topical cooling of the sinus node with atrial pacing (heart rate < 160 beats/min). PRSW and tau have a peak around heart rates of 180 to 220 beats/min.

a tendency toward a lower heart rate at 2 hours of reperfusion in the AH group ($P = .07$) than in the other groups. The recovery of PRSW (Fig 4) and tau (Fig 5) at 2 hours of reperfusion was significantly worse in the AH group than the others. PRSW at 2 hours of reperfusion was slightly but significantly worse in the cyanosis group than in the control group.

NO metabolites. NO metabolites significantly increased in the early period of reperfusion and reached normal levels by 60 minutes of reperfusion in both the cyanosis and control groups. However, in the AH group NO metabolite levels did not increase in the early period of reperfusion and were significantly decreased at 120 minutes of reperfusion. This level was significantly lower than that found in the cyanosis and control groups (Fig 6).

Coronary sinus malondialdehyde, myocardial lipid peroxidation, and antioxidant reserve capacity. Malondialdehyde levels in the coronary sinus were significantly higher in the early reperfusion period than 5 minutes after the start of CPB in each of the groups (Fig 7). The level of malondialdehyde did not increase with institution of CPB in any groups, although CPB did result in abrupt reoxygenation in the cyanosis group and the AH group. In addition, there were no significant differences in malondialdehyde levels in the coronary sinus among groups at any time. There were no significant differences in 4-hydroxyalkenals and malondialdehyde levels in the myocardial tissue at 2 hours of reperfusion among groups (Fig 8). There were also no significant differences in myocardial antioxi-

Table I. Baseline measurements and validation of cyanosis and acute alveolar hypoxia

Variables F_{IO_2}	Cyanosis		Control		Acute hypoxia	
	21%	100%	21%	100%	10%	100%
PaO_2 (mm Hg)	$35.0 \pm 2.7^{*\ddagger}$	79.6 ± 12.5	92.5 ± 7.4	516.9 ± 29.3	$28.6 \pm 6.4^{*\ddagger}$	489.3 ± 32.3
Saturated O_2 (%)	$70.6 \pm 3.5^{*\ddagger}$	93.6 ± 2.3	97.0 ± 1.0	100.0 ± 0.0	$62.3 \pm 8.9^{*\ddagger}$	100 ± 0.0
Qp/Qs	$0.57 \pm 0.06^{*\ddagger}$	0.83 ± 0.05				
Cardiac function						
PRSW (mm Hg)	$36.9 \pm 3.8^{*\ddagger}$	56.1 ± 3.3	58.6 ± 4.8	62.6 ± 5.0	$33.0 \pm 4.3^{*\ddagger}$	56.1 ± 2.6
Tau (ms)	$30.4 \pm 2.7^{\ddagger}$	25.1 ± 2.4	25.1 ± 2.9	26.3 ± 3.1	$31.6 \pm 3.8^{*\ddagger}$	23.6 ± 0.9

Values are means $\pm$ SEM.

* $P < .005$ compared with value at 100% oxygen within each group.

$\ddagger P < .05$ compared with value at 100% oxygen within each group.

$\ddagger P < .05$ compared with control.

dant reserve capacity at any concentrations of t-butylhydroperoxide (Fig 9).

Catecholamine. Acute hypoxia significantly increased arterial plasma epinephrine, and its level in the AH group was significantly higher than that at 21% oxygen in the cyanosis group (Fig 10).

Discussion

The present study has demonstrated that the recovery of chronically cyanotic neonatal hearts after DHCA was only marginally worse than that of normoxemic hearts. However, acute alveolar hypoxic stress before CPB resulted in significantly worse recovery of both systolic and diastolic cardiac function. The mechanism of injury in the AH group may relate to an impairment of NO production. There was no evidence that oxygen-derived free radicals are related to any of the differences among cyanotic, acyanotic, and acutely hypoxic animals in recovery of LV function after ischemia.

There have been conflicting reports on the effects of chronic cyanosis on the vulnerability to ischemic injury. In several studies chronic alveolar hypoxia increases the tolerance to ischemia in immature hearts.^{1,2} Baker and colleagues¹ demonstrated that 7 to 10 days of alveolar hypoxia beginning at birth was associated with less ventricular dysfunction after ischemia in immature isolated rabbit hearts. In contrast, Nakanishi and colleagues⁹ showed that chronic alveolar hypoxia decreased postischemic cardiac output in isolated rat hearts. However, chronic alveolar hypoxia induces pulmonary vasoconstriction and pulmonary hypertension with right ventricular hypertrophy, and these effects may exert an influence on cardiac function and susceptibility to an ischemic insult. Fujiwara and colleagues³ created a neonatal cyanosis model by creating a similar left PA-LA shunt and reported that chronically cyanotic neonatal hearts had a significantly

worse ventricular functional recovery than normoxemic hearts after 2 hours of hypothermic arrest in the isolated heart model. Silverman and colleagues¹⁰ also created a cyanosis model with a PA-LA shunt and distal PA banding in adult dogs. They demonstrated that after a period of global ischemia, the cyanosis group had a significant depression of ventricular function associated with a reduction of adenosine triphosphate and high phosphate creatine levels in the LV tissue. The results of the current study are consistent with the results reported in Fujiwara and Silverman's studies. However, the recovery of cardiac function after DHCA in the cyanotic animals was marginally (but significantly strategically) worse than that found in the normoxemic animals in this study. In addition, we could not find any differences in biochemical data, lipid peroxidation, and NO metabolites between cyanotic and acyanotic hearts.

Other investigators have used a model with acute (1-2 hours) hypoxic stress before CPB as a cyanosis model.⁴⁻⁶ This acute alveolar model has been reported to be associated with severe myocardial dysfunction after ischemia.⁴ The current study also showed serious ventricular dysfunction after ischemia in the acute alveolar hypoxia group, which underwent a short duration of hypoxia at higher arterial oxygen levels (30 mm Hg of PaO_2) of hypoxia than in the previous reports. We initially attempted to maintain hypoxia for 2 hours and a PaO_2 of 25 mm Hg, but no animals tolerated this level of acute hypoxic stress. Acute hypoxia induced pulmonary hypertension, increased heart rate, and ultimately resulted in severe systemic hypotension despite intravascular volume expansion. For these reasons, we chose a PaO_2 of 30 mm Hg.

Hypoxia is reported to activate glycolysis to shift metabolism from an oxidative pattern to a glycolytic pattern.¹¹ Acute hypoxia has been demonstrated to

Fig 4. Preload recruitable stroke work before and after CPB. *Left panel* shows absolute values, and *right panel* shows value corrected for heart rate and expressed as percentage of normal values. * $P < .05$ compared with control and cyanosis. # $P < .05$ compared with control.

Fig 5. Tau, time constant of isovolumic relaxation, before and after CPB. *Left panel* shows absolute values, and *right panel* shows values corrected by heart rate and expressed as percentage of normal value. * $P < .05$ compared with control and cyanosis.

decrease myocardial glycogen, adenosine triphosphate, and ventricular function in isolated rat hearts.¹² Conversely, myocardial adenosine triphosphate content measured by magnetic resonance spectroscopy is not depleted in cyanotic patients.¹³ In addition, a deterioration of hemodynamics caused by acute hypoxia resulted in an increase in plasma catecholamine levels in the current study. Higher catecholamine levels may increase myocardial oxygen consumption and may accelerate consumption of myocardial energy sources during hypoxia. Subsequent ischemia-reperfusion could then exaggerate the depletion of energy stores. Bolling and colleagues⁶ reported that the adenosine triphosphate/diphosphate ratio was significantly

reduced after ischemia in a model of acute hypoxic stress, and Shimada and colleagues¹⁴ have noted that high concentrations of epinephrine before ischemia caused heart dysfunction after ischemia. Capsi and colleagues¹⁵ observed similar findings that administration of epinephrine before ischemia reduced postischemic recovery of ventricular function in immature piglets. In our study chronic cyanosis did not increase the level of epinephrine before CPB.

Innken and colleagues⁵ showed that acute alveolar hypoxic stress before CPB caused an increase in the generation of oxygen-derived free radicals after the onset of CPB, and myocardial antioxidant reserve capacity was reduced in neonatal animals. In addition,

Fig 6. Nitric oxide metabolites in the coronary sinus plasma before and after CPB. * $P < .05$ compared with control and cyanosis. # $P < .05$ compared with the value at baseline with each group.

Fig 7. Malondialdehyde in the coronary sinus plasma before and after CPB. * $P < .05$ compared with the value at 5 minutes after onset of CPB with each group.

Fig 8. Myocardial lipid peroxidation at 2 hours of perfusion. 4-HN, 4-Hydroxyalkenals; MDA, malondialdehyde.

Fig 9. Myocardial antioxidant reserve capacity at 2 hours of reperfusion. Dotted line shows antioxidant reserve capacity of LV tissue after 5 hours of 100% oxygen ventilation but without ischemia.

Fig 10. Arterial epinephrine level before and after CPB. * $P < .05$ compared with control and cyanosis.

they demonstrated that hypoxemia-reoxygenation was associated with higher levels of lipid peroxidation products and less myocardial antioxidant capacity than normothermic global ischemia-reperfusion. In our studies institution of CPB did not increase the level of lipid peroxidation products in the cyanosis group or the AH group despite abrupt reoxygenation. However, the level of malondialdehyde significantly increased in the early reperfusion period in all 3 groups. In contrast with the results of Ihnken and colleagues, our findings suggested that ischemia-reperfusion caused more production of free radicals than hypoxemia-reoxygenation. We did not find differences in the level of malondialdehyde in the coronary sinus after ischemia or in either lipid peroxidation or antioxidant reserve capaci-

ty in myocardial tissue at 2 hours of reperfusion among the 3 groups. The mechanism by which free radicals decrease antioxidant reserve capacity in tissue is unclear, although free radicals cause protein degradation. In the tissues, the antioxidant system is composed of both enzymatic and nonenzymatic defenses. The antioxidant enzymatic system consists of catalase, superoxide dismutase, and glutathione peroxidase, and the most abundant nonenzymatic scavengers are vitamin E, vitamin C, and glutathione. In the clinical setting, many investigators have reported that antioxidant enzymatic activities are either increased or unchanged after reperfusion.¹⁶⁻¹⁸ Teoh and colleagues¹⁶ reported that these 3 enzymatic activities in cyanotic patients and in those with coronary artery bypass graft procedures were not different before and after ischemia. Kim and colleagues¹⁷ reported that myocardial catalase, superoxide dismutase, and glutathione peroxidase significantly increased after reperfusion in patients with tetralogy of Fallot. Aceto and colleagues¹⁸ also demonstrated an increase in glutathione peroxidase activity from the right atrium of patients undergoing heart operations at reperfusion. Our finding that myocardial antioxidant reserve capacity of the 3 groups after DHCA was not different from that of nonischemic hearts is in agreement with the results of the study by Teoh and colleagues.¹⁶

Another potentially important finding in this study was that postischemic NO production was different in the chronic cyanosis and AH groups. The AH group had lower levels of NO metabolites in the coronary sinus after ischemia. It has been reported that ischemia-reperfusion causes an impairment of the coronary arterial response to endothelium-dependent vasodilation.¹⁹ This endothelial dysfunction may reduce production of NO after ischemia, resulting in a limitation of coronary arterial flow and cardiac dysfunction. We hypothesize that acute hypoxia before ischemia-reperfusion may have exaggerated postischemic endothelial dysfunction, although we did not assess coronary endothelial function in this study.

Several experimental cyanosis models have been reported: a PA-LA shunt,^{3,10,20} an IVC-LA shunt,^{21,22} and PA banding with atrial septal defect creation.²³ We chose the LA-PA shunt for this study because PA banding with atrial septal defect produces a high right ventricular pressure, and the IVC-LA shunt decreases the right ventricular volume, which could affect the geometry of the left ventricle and render the assumption of an ellipsoidal model for the LV volume calculation invalid.

In conclusion, chronic cyanosis without alveolar hypoxia augments the susceptibility to ischemia to

some extent. In contrast, acute hypoxic stress was associated with severe postischemic ventricular dysfunction. Acute hypoxic stress also caused an impairment of postischemic NO production, which was not observed in the animals with chronic cyanosis. Free radical injury does not appear to explain the differences in recovery of cardiac function after ischemia among cyanotic, acyanotic, and acutely hypoxic neonatal animals. Therefore, the acute hypoxic stress model may not be applicable in understanding the effects of ischemia-reperfusion in the clinical setting of cyanotic congenital heart disease.

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Discussion

Dr Verdi J. DiSesa (*Chicago, Ill*). Why did you include a group with acute cyanosis? Were the PO_2 values in the AH group the same as those found in the chronic cyanosis group?

Dr Nagashima. I tried to keep a PO_2 of 30 mm Hg and an absolute PO_2 of 28.6 mm Hg during hypoxic ventilation. Therefore, there was a little bit lower arterial oxygen value in the AH group.

Dr DiSesa. Are you saying that the insult was more severe in the AH group?

Dr Nagashima. That is right.

Dr DiSesa. Did you see compensatory changes in the more chronic cyanotic group? Did they get hyperemic or polycythemic?

Dr Nagashima. The animals were cyanotic for only 7 days. Hematocrit value increased a little bit, but it was not significantly different between the control and cyanotic animals.

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