Cardioprotection in chronically hypoxic rabbits persists on exposure to normoxia: role of NOS and K$_{\text{ATP}}$ channels

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FORTY THOUSAND INFANTS are born in the United States annually with a congenital heart defect, many of whom will require surgery (1). However, heart failure due to poor pump function remains the leading problem after pediatric cardiac surgery. Surgical ischemia and reperfusion may complicate postoperative recovery of these infants’ hearts despite successful repair of the heart defect. Pediatric cardiac repair is different from in the adult with acquired disease because the majority of infant hearts with congenital defects are chronically perfused with hypoxic blood. Understanding the impact that chronic hypoxia exerts on resistance to myocardial ischemia may help devise better strategies to protect the infant heart against surgical ischemia.

A reproducible, nonsurgical model of cyanosis from birth has been developed by our laboratory in which the myocardium is chronically perfused with hypoxic blood. Our model has proved to simulate the essential characteristics of cyanotic congenital heart disease. The model is characterized by decreased arterial oxygen levels, polycythemia, right ventricular hypertrophy, decreased weight gain, and overall failure to thrive (3), characteristics similar to those seen in children with cyanotic congenital heart disease (14). The resulting changes in the physiology and biochemistry of the heart are achieved without surgical manipulation to create an anatomic abnormality.

Nitric oxide (NO) is an important mediator of adaptation to chronic hypoxia. At 10 days of age, nitric oxide synthase (NOS) 3 protein levels are increased in hearts chronically hypoxic from birth. Chronic hypoxia also increases the amount of heat shock protein-90 (Hsp90) in the heart that associates with NOS3 to increase NO generation (18). These events occur at the same time that chronic hypoxia increases NOS3 catalytic activity, cGMP accumulation, and nitrite plus nitrate release (19). ATP-dependent potassium (K$_{\text{ATP}}$) channels also serve as important mediators of adaptation to chronic hypoxia (5). NO activates the K$_{\text{ATP}}$ channel in normoxic and chronically hypoxic hearts by a cGMP-dependent mechanism (4). However, the impact of exposure of chronically hypoxic infant rabbits to subsequent normoxia during postnatal development on resistance to ischemia and the underlying mechanisms that mediate resistance to ischemia are unknown. Exposure to chronic hypoxia in adult rats conferred resistance against acute hypoxic injury 4 mo after removal from the hypoxic environment (16); however, the underlying mechanism was not defined. We hypothesized that the memory of increased protection against ischemia programmed into infant hearts chroni-
cally hypoxic from birth persists following subsequent development in a normoxic environment and involves increased activity of NOS and activation of K\textsubscript{ATP} channels.

The objectives of our study were to determine whether the increased cardioprotection programmed into hearts chronically hypoxic from birth persists following subsequent exposure to a normoxic environment and involves the activation of NOS and K\textsubscript{ATP} channels.

**METHODS**

Animals used in the study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals formulated by National Institutes of Health in 1996.

*Creation of hypoxia from birth.* Pregnant New Zealand White rabbits were obtained from a commercial breeder. The mother was maintained in a normoxic environment (Fi\textsubscript{O\textsubscript{2}} = 0.21). The hypoxic kits were born in a normoxic environment. After the first feeding, they were transferred to a hypoxic chamber (Fi\textsubscript{O\textsubscript{2}} = 0.12). The kits were returned to the mother for a 30-min feeding period daily. This period of time was sufficient for the mother to nurse and care for her kits and did not result in dehydration and undernutrition compared with kits kept continuously with their mother. After 10 days of hypoxic exposure, the kits were removed from the hypoxic chamber and returned to the mother (Fi\textsubscript{O\textsubscript{2}} = 0.21). They remained with the mother until 30 days of age at which point they were weaned and allowed to develop independently up to 60 days of age. Kits were allowed free access to the mother for nursing as they grew from days 10 to 30 (Fig. 1).

*Isolated heart perfusion.* Modified Krebs-Henseleit bicarbonate buffer was prepared daily and contained the following components (in mM): 118.5 NaCl, 25.0 NaHCO\textsubscript{3}, 4.8 KCl, 1.2 MgSO\textsubscript{4}·7H\textsubscript{2}O, and 1.2 KH\textsubscript{2}PO\textsubscript{4} (pH 7.4 when gassed with 95% O\textsubscript{2}-5% CO\textsubscript{2}) in which the calcium content was 1.8. Glucose (11.1 mM) was added to the perfusate. Before use, all perfusion fluids were filtered through cellulose acetate membranes with a pore size of 5 \(\mu\)m. To this perfusate, drugs were added as needed. Animals were weighed, and heparin was administered intraperitoneally based on weight (150 IU/kg). Anesthesia was induced with pentobarbital sodium (30 mg/kg ip). Once adequate anesthesia was obtained, the abdomen was opened, and the ribs were cut bilaterally along the anterior axillary lines. The diaphragm was separated from its chest wall attachments and the pericardium opened. The aorta was clamped and the heart excised. The heart then underwent a 30-min global ischemic period. After ischemia, heartbeats were averaged into 30-s intervals. Recovery was expressed as a ratio of the postischemic value over the preischemic value for developed pressure, end-diastolic pressure, and heart rate. Thus each heart served as its own control. End-diastolic pressure and peak systolic pressures were expressed as millimeters of mercury. Data points collected on each heart for both the right and left ventricle included preischemic developed pressure, percent recovery of postischemic right and left ventricular developed pressure, positive and negative derivative of pressure (dP/dt), and end-diastolic pressure. Heart rates (in beats/min) were determined by beat-to-beat averages at 30-s intervals. Coronary flow rate (in mL/min \(\times 10^{-3}\) g wet heart wt \(^{-1}\)) was collected as the overflow from the bath used to immerse the heart. Coronary perfusate was collected during preischemia and during reperfusion at 5, 10, 15, 25, and 35 min.

*Resistance to myocardial ischemia.* Resistance to myocardial ischemia was determined in rabbits raised from birth to 10 days of age in a normoxic (Fi\textsubscript{O\textsubscript{2}} = 0.21) or hypoxic (Fi\textsubscript{O\textsubscript{2}} = 0.12) environment and subsequently exposed to normoxia (Fi\textsubscript{O\textsubscript{2}} = 0.21) from 10 to 60 days of age (Fig. 1). Resistance to myocardial ischemia was determined using an isolated perfused heart model. Isolated hearts (\(n = 8\) group) were perfused with bicarbonate buffer and subjected to 30 min of global ischemia followed by 35 min of reperfusion. Recovery of LVDP and right ventricular developed pressure monitored continuously during 35 min of reperfusion was used to assess resistance to ischemia (6).

*Drug perfusion.* After steady-state levels of function were reached, hearts were perfused with drugs for 20 min before a 30-min global ischemic period. Glibenclamide (3 \(\mu\)M) (Calbiochem no. 356310), a general K\textsubscript{ATP} channel blocker, was dissolved in two different vehicles and tested in separate experiments. The vehicles used were DMSO or a combination of 1 part NaOH, 1 part polyethylene glycol 400, 2 parts 0.9% normal saline, and 1 part 95% EtOH. The final concentration of DMSO in the perfusate was <0.1% (vol/vol). These vehicles did not exert any effect on resistance to myocardial ischemia. A general NOS inhibitor L-NAME, Sigma N-5751, was dissolved in distilled water and added to the coronary perfusate to achieve a final concentration of 200 \(\mu\)M.

*Nitrite and nitrate measurements.* Nitrate plus nitrite content in hearts (\(n = 7\) group) was measured using a Sievers model-280 NO analyzer. The detection limit for nitrate plus nitrite was 25 \(n\)M, representing an increase in sensitivity over the Greiss reaction. A

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**Fig. 1.** Experimental protocol used to study resistance to myocardial ischemia in rabbits subjected to chronic hypoxia followed by a return to a normoxic environment.
solution of 0.08 g VC11, 0.8 ml HCl, and 9.2 ml distilled water was prepared and filtered. Three milliliters of solution were added to the reaction chamber, and the temperature was raised to 92°C. To this system, 50 µl of effluent were added and the NO levels measured. The detection system is based on the reaction -NO + O2 → NO3 and O2. The extra energy of the electronically excited nitrogen dioxide is released as a photon and emitted as a detectable wavelength. The emitted light is proportional to and sensitive for -NO content in a specimen. Data (means ± SD) are shown as moles of nitrate plus nitrite per gram wet weight.

**Immunoprecipitation and Western blot analysis of NOS isoforms.** Hearts were analyzed for NOS1, NOS2, and NOS3 protein content and Hsp90 association with NOS3 by immunoprecipitation and Western blot analysis using isoyme-specific monoclonal antibodies as described previously (18, 19).

**Data collection and statistical analysis.** One hundred seconds of continuous recording during steady-state function were averaged to calculate preischemic function. Recovery of developed pressure was expressed as a percentage of its preischemic predrying value. Thirty-second intervals were averaged from the start of reperfusion (time = 0) to the end (time = 35 min). Heart rates were calculated using a beat-to-beat interval. The peak and trough derivatives were calculated using the DataQ acquisition system. The end-diastolic pressure was calculated from this baseline. Eight hearts were used for each of the conditions studied, and results were expressed as means ± SD. Excel was used for statistical analysis using paired t-tests and ANOVA, where appropriate. Significance was accepted at P < 0.05. Excel and Sigma plot were used to graph multiple comparisons.

**RESULTS**

**Gross changes.** Table 1 shows the effect that hypoxia from birth to 10 days of age followed by normoxic development to 30 and 60 days of age exerted on body weight, heart weight, and hemodynamics. At 10 days of age there were no differences in body or heart weight between normoxic and hypoxic rabbits. At 30 days of age, normoxic rabbits weighed more but had a smaller heart weight compared with rabbits hypoxic for the first 10 days of life. At 60 days of age, rabbits previously hypoxic weighed more and also had a larger heart weight than normoxic rabbits.

**Preischemic function.** Heart rate was unaffected by adaptation to chronic hypoxia in 10-day-old rabbits. Heart rate declined in both normoxic and previously hypoxic rabbits at 30 days of age and continued to decline at 60 days of age (Table 1). Coronary flow rate was higher in chronically hypoxic hearts at 10 days of age but was no different from normoxic controls at 30 and 60 days of age (Table 1). Preischemic LVDP was comparable at 10 days of age in normoxic and hypoxic hearts and was unchanged at 30 and 60 days of age. A measure of contractility was the rate of rise in pressure during each heartbeat and labeled as dP/dt + ve. A measure of relaxation was the rate of fall in pressure and labeled dP/dt – ve. Left ventricular dP/dt + ve and dP/dt – ve were greater in chronically hypoxic hearts at 10 and 30 days of age compared with normoxic hearts. However, at 60 days of age, left ventricular dP/dt + ve and dP/dt – ve were no different between chronically hypoxic and normoxic hearts. Right ventricular developed pressure in hypoxic hearts compared with normoxic hearts was higher in rabbits at 10 days of age. Increased pressures observed in the right ventricle in chronically hypoxic hearts at 10 days of age persisted at 30 and 60 days of age. Right ventricular dP/dt + ve and dP/dt – ve were greater in chronically hypoxic hearts compared with normoxic hearts at 10 and 30 days of age. By 60 days of age values for right ventricular dP/dt + ve and dP/dt – ve were no different between normoxic and previously hypoxic hearts (Table 1).

The hemodynamic stability of the model was assessed by perfusing six nontreated normoxic and hypoxic hearts at 10, 30, and 60 days of age in the Langendorff mode for 120 min with nonrecirculating perfusate. No statistically significant changes in developed pressure or heart rate from the values reported in Table 1 occurred until after 85 min of perfusion. In the protocol that tested our hypothesis, hearts were perfused in the Langendorff mode for a maximum of 80 min, well within the stability limits of the preparation.

**Resistance to myocardial ischemia.** Rabbit hearts exposed to 10 days of chronic hypoxia from birth were more resistant to ischemia compared with age-matched normoxic controls as manifest by increased recovery of LVDP (68 ± 4% vs. 43 ± 4%). At 30 days of age, resistance to ischemia in normoxic hearts declined (36 ± 5%). However, in hearts subjected to chronic hypoxia from birth to 10 days of age then exposed to normoxia until 30 days of age, increased resistance to ischemia persisted (63 ± 4%) (Figs. 2 and 3). Recovery of postischemic right ventricular function regardless of exposure to hypoxia was higher than in the left ventricle (Fig. 3). At 60 days of age, recovery of LVDP and right ventricular developed pressure persisted (63 ± 4%) (Figs. 2 and 3). Recovery of postischemic right ventricular function regardless of exposure to hypoxia was higher than in the left ventricle (Fig. 3). At 60 days of age, recovery of LVDP and right ventricular developed pressure.....
was greater in normoxic hearts compared with those hypoxic from birth to 10 days of age and then allowed to develop in a normoxic environment (Fig. 3). Thus by 60 days of age, increased cardioprotection conferred by chronic hypoxia in the perinatal period was lost.

The change in pressure over time for each beat of the ventricles was compared as an additional measure of function. Because there was no change in preload, these values can be used to support the findings with the recovery of function. At 10 and 30 days of age, left ventricular $\frac{dP}{dt}$ was greater in animals exposed to 10 days of hypoxia than those in the normoxic controls. In addition, 10 days of chronic hypoxia resulted in a significantly greater $\frac{dP}{dt}$ between hypoxic hearts and hypoxic hearts exposed to L-NAME. There was no change in end-diastolic pressure. At 10 days of age, L-NAME abolished the cardioprotective effects of chronic hypoxia (40 ± 4%) but had no effect on normoxic hearts (39 ± 5%) (Fig. 4). These results confirmed our previous observations in hearts at 10 days of age (7). At 30 days of age, L-NAME also abolished cardioprotection in hearts previously hypoxic but then exposed to normoxia (39% ± 4%) but had no effect on protection in normoxic hearts (36 ± 6%) (Fig. 4). Nitrite and nitrate content, an index of the catalytic activity of NOS3, increased threefold in hypoxic hearts at 10 and 30 days of age versus normoxic controls (Fig. 5). In the present study, we found by Western blot analysis that chronic hypoxia from birth to 10 days of age increased NOS3 levels in heart homogenates by 2.2 ± 0.7-fold (Fig. 6). However, in 30-day-old hearts either normoxic or hypoxic from birth for the first 10 days and then allowed to develop in a normoxic environment until 30 days of age, there was no difference in NOS3 protein content (Fig. 6). We then probed for NOS1 and NOS2. With the use of Western blot analysis, NOS1 and NOS2 proteins were undetectable in hearts at 10 and 30 days of age. Because Hsp90 increases NO generation from NOS3 (18), we next determined the extent to which Hsp90 was associated with NOS3 in 10-day-old and in 30-day-old hearts. At 10 days of age chronic hypoxia increased the association of Hsp90 with NOS3 compared with normoxic hearts more than threefold (Fig. 6). These data demonstrate how important Hsp90 is to coupling NOS3 activity to L-arginine metabolism.

**Role of NOS.** To determine whether increased resistance to myocardial ischemia in hearts subjected to chronic hypoxia was mediated by NOS, L-NAME (200 μM) was added to the perfusate for 20 min before ischemia. Perfusion of hearts from both normoxic and chronically hypoxic rabbits at 10 days of age with L-NAME (200 μM) before ischemia decreased coronary flow rate. There was no effect on $\frac{dP}{dt}$ but a significant difference with $\frac{dP}{dt}$ between hypoxic hearts and hypoxic hearts exposed to L-NAME. There was no change in end-diastolic pressure. At 10 days of age, L-NAME abolished the cardioprotective effects of chronic hypoxia (40 ± 4%) but had no effect on normoxic hearts (39 ± 5%) (Fig. 4). These results confirmed our previous observations in hearts at 10 days of age (7). At 30 days of age, L-NAME also abolished cardioprotection in hearts previously hypoxic but then exposed to normoxia (39% ± 4%) but had no effect on protection in normoxic hearts (36 ± 6%) (Fig. 4). Nitrite and nitrate content, an index of the catalytic activity of NOS3, increased threefold in hypoxic hearts at 10 and 30 days of age versus normoxic controls (Fig. 5). In the present study, we found by Western blot analysis that chronic hypoxia from birth to 10 days of age increased NOS3 levels in heart homogenates by 2.2 ± 0.7-fold (Fig. 6). However, in 30-day-old hearts either normoxic or hypoxic from birth for the first 10 days and then allowed to develop in a normoxic environment until 30 days of age, there was no difference in NOS3 protein content (Fig. 6). We then probed for NOS1 and NOS2. With the use of Western blot analysis, NOS1 and NOS2 proteins were undetectable in hearts at 10 and 30 days of age. Because Hsp90 increases NO generation from NOS3 (18), we next determined the extent to which Hsp90 was associated with NOS3 in 10-day-old and in 30-day-old hearts. At 10 days of age chronic hypoxia increased the association of Hsp90 with NOS3 compared with normoxic hearts more than threefold (Fig. 6). These data demonstrate how important Hsp90 is to coupling NOS3 activity to L-arginine metabolism.

**Fig. 2.** Time course of recovery of postischemic developed pressure in left ventricle (LV) following 30 min ischemia in hearts hypoxic from birth to 10 days of age and then exposed to normoxia until 30 and 60 days of age. Data are means from 8 hearts in each experimental group.

**Fig. 3.** Recovery of postischemic developed pressure in LV and right ventricle (RV) after 30 min ischemia and 35 min reperfusion in hearts hypoxic from birth to 10 days of age and then exposed to normoxia until 30 and 60 days of age. Data are means ± SD; n = 8 rabbits per group. +P < 0.05 hypoxic vs. normoxic; *P < 0.05 vs. RV.

**Fig. 4.** Effect of perfusion of hearts with Nω-nitro-L-arginine methyl ester (L-NAME, 200 μM) for 15 min before 30 min ischemia and 35 min reperfusion on recovery of postischemic LV developed pressure. Data are means ± SD; n = 8 rabbits per group. +P < 0.05 L-NAME vs. control.
for the efficient generation of \( \cdot \text{NO} \) (9, 10) and confirms our previous findings (18). However, at 30 days of age, despite elevated nitrite and nitrate content in these hearts, there was no increased association of Hsp90 with NOS3. To determine whether the increase in association of Hsp90 with NOS3 is due to a change in Hsp90 content in 10-day-old hypoxic rabbits, Western blot analysis of Hsp90 in total heart homogenates was performed. Figure 6 shows that at 10 and 30 days of age chronic hypoxia does not appreciably change the total content of Hsp90 in the heart. Taken together, these data support the notion that the association of Hsp90 plays an important role in helping NOS3 generate \( \cdot \text{NO} \), which protects against ischemic injury at 10 days of age. In contrast, there is no increased expression of NOS3 protein or association of Hsp90 with NOS3 at 30 days of age. Thus the mechanisms responsible for increased \( \cdot \text{NO} \) generation at 10 and 30 days of age appear different.

**Role of \( K_{\text{ATP}} \) channels.** To determine whether increased resistance to myocardial ischemia in hearts subjected to chronic hypoxia was mediated by \( K_{\text{ATP}} \) channels, glibenclamide (3 \( \mu \text{M} \)) was added to the perfusate for 20 min before ischemia. Perfusion of hearts with glibenclamide before ischemia decreased coronary flow rate in both normoxic and chronically hypoxic hearts at 10 and 30 days of age. At 10 days of age, glibenclamide abolished the cardioprotective effects of chronic hypoxia (40 \( \pm \) 4\%) but had no effect on normoxic hearts (39 \( \pm \) 5\%) (Fig. 7). These results confirmed our previous observations in hearts at 10 days of age (6). At 30 days of age, glibenclamide also abolished cardioprotection in hearts previously hypoxic but then exposed to normoxia (39\% \( \pm \) 4\%) but had no effect on protection in normoxic hearts (36 \( \pm \) 6\%) (Fig. 7). Two vehicles (DMSO and ethanol) were tested with and without the presence of glibenclamide, and there were no differences in the responses between vehicles in the normoxic or hypoxic groups; therefore, these data were pooled for analysis. There was no effect on the recovery of function with vehicle alone.

**DISCUSSION**

Our study demonstrates that the memory of increased resistance against ischemia programmed into infant hearts chronically hypoxic from birth to 10 days of age persists following subsequent development in a normoxic environment to 30 days of age. This memory of increased cardioprotection persists at least 20 days following removal from the stimulus of chronic hypoxia. This contrasts with the memory of increased protection against ischemia conferred by the stimulus of ischemic preconditioning, which persists for only 3–4 days. The memory of increased resistance to myocardial ischemia was lost in this species by 60 days of age. The mechanism underlying the memory of increased resistance to myocardial ischemia conferred by adaptation to previous hypoxia appears to involve activation of both NOS and enhanced current through \( K_{\text{ATP}} \) channels. This is the first report to our knowledge where improved recovery of postischemic left and right ventricular function has been documented weeks after the termination of exposure to chronic hypoxia.

At 10 days of age, chronic hypoxia from birth conferred increased resistance to myocardial ischemia compared with
age-matched normoxic controls. L-NAME and glibenclamide abolished increased cardioprotection in hearts hypoxic from birth. In contrast, L-NAME and glibenclamide had no effect on resistance to ischemia in normoxic hearts. Previous studies showed that chronic hypoxia from birth increased NOS3 activity but not message levels (2). We found that nitrite plus nitrate content (an index of NOS activity) was elevated in chronically hypoxic hearts and correlated with increased NOS3 protein expression as well as increased association of Hsp90 with NOS3. We (4) previously demonstrated that L-NAME abolishes cardioprotection in hearts previously hypoxic. The current study extends these findings and suggests NOS and KATP channels may act in concert to mediate increased cardioprotection in rabbit hearts hypoxic from birth to 10 days of age.

At 30 days of age resistance to ischemia in hearts normoxic from birth declined compared with 10-day-old normoxic hearts. However, resistance to ischemia in hearts previously hypoxic from birth and then exposed to normoxia until 30 days of age persisted. L-NAME abolished increased cardioprotection in hearts that were previously hypoxic. L-NAME had no effect on normoxic hearts at 30 days of age. Elevated nitrite plus nitrate content persisted in hearts previously hypoxic, indicating increased NOS activity. Thus increased NO production was associated with increased resistance to ischemia. To identify which NOS isoform is responsible for increased NO production, we probed for all three NOS isoforms using Western blot analysis. NOS1 and NOS2 protein expression was not detected in hearts at 10 and 30 days of age. NOS3 was the only isoform detected. At 30 days of age, although hearts previously hypoxic produce more NO than normoxic hearts (Fig. 5), the mechanism by which this occurs did not involve increased expression of NOS3 protein or Hsp90 association with NOS3. Thus the mechanisms by which increased NO production protect the heart against ischemia appear to be different at 10 and 30 days of age. NO production by NOS3 can be regulated in other ways. For example, association of caveolin-3 with NOS3 (19) and tetrahydrobiopterin levels (21) in the heart both control NO production from NOS3. These other mechanisms may be playing a role in controlling increased NO production in 30-day-old hearts previously exposed to hypoxia. However, KATP channels still appear to function as mediators of increased cardioprotection at 30 days of age as glibenclamide abolished cardioprotection in hearts previously hypoxic. Glibenclamide had no effect on normoxic hearts at 30 days of age. At 60 days of age the memory of increased cardioprotection was lost.

The first 10 days of life and especially the first 24–72 h are marked by significant changes in the cardiopulmonary dynamics. Previous studies have documented a failure of the ductus arteriosus to close in rabbits exposed to hypoxia for 10 days (3). There is also an increased hematocrit in these chronically hypoxic hearts as an adaptive response to increase oxygen delivery. We suggest that this adaptation to increase oxygen delivery may prepare the heart for the low oxygen conditions encountered during subsequent surgical ischemia. Thus chronically hypoxic hearts preserve their ability to function under conditions of low oxygen thereby recovering more function following global ischemia compared with normoxic hearts. In support of this notion, we (20) recently showed that erythropoietin, an essential component required for increasing hematocrit, exerts a protective effect against ischemia in hearts from rabbits normoxic from birth to 10 days of age.

To date, the timing for repair of congenital hearts defects is based on the size and medical condition of the infant rather than on optimal protection of the heart (13). Although not specifically investigated, we found that the impact of chronic hypoxia from birth to 10 days of age exerted a profound effect on body weight and heart weight in rabbits subsequently exposed to normoxia to 60 days of age. At 10 days of age body weight and heart weight were unaffected by chronic hypoxia. At 30 days of age body weight was decreased and heart weight increased in rabbits previously exposed to hypoxia compared with normoxic hearts. At 60 days of age heart weight and body weight increased in rabbits previously hypoxic from birth to 10 days of age compared with normoxic age-matched rabbits. Our results indicate hypoxia from birth to 10 days of age appears to act as a stimulus for growth during subsequent development over the period from 30 to 60 days of age. The underlying mechanisms are not addressed in the current study but appear directly related to the stress of chronic hypoxia during the first 10 days of age. Animals were matched for age without exclusion by weight in an attempt to capture a representative sample of the population. Thus it is possible that NO availability may be related to heart size as well as age in our study. Chronic hypoxia from birth results in the persistence of right ventricular hypertrophy that regresses with time, and increased heart weight may reflect increased mass of the right ventricle. In adults the reversibility of pulmonary hypertension and right ventricular hypertrophy following removal from a chronically hypoxic environment has been described (12, 17). Further studies are required to uncover the underlying mechanism and to determine whether this is related to the ability of the heart to resist ischemia.

Increased resistance of the right ventricle to ischemia. Decompensating children with congenital cyanotic heart disease generally display right-sided heart failure. Most studies to
date have investigated failure of the left side of the heart. Our model is unique in its ability to simultaneously measure the recovery of both the right and left ventricles in infant rabbit hearts and make comparisons between them. Our study indicates that the rate and extent of posts ischemic recovery in the right ventricle from normoxic and chronically hypoxic hearts is greater than for the corresponding left ventricle. Recovery of function in the right ventricle for hearts treated with a $K_{\text{ATP}}$ channel blocker and a NOS inhibitor paralleled the response of the left ventricle.

Clinically, cyanotic children undergoing repairs of heart defects manifest symptoms of right heart failure (8, 15). There are little animal data analyzing the response of the right ventricle in response to ischemia and reperfusion. The right ventricle has both favorable supply and demand characteristics. The right ventricle is thinner and under less pressure than the left ventricle. There is coronary blood flow present during systole as well as diastole. The right ventricle is more compliant than the left ventricle and dilates following ischemia. There is also a smaller right ventricle mass (11). In our study, right ventricular recovery remains high in normoxic and hypoxic rabbits following ischemia at 10 and 30 days of age. Glibenclamide decreased recovery of the right ventricle in 30-day-old rabbits that were hypoxic from birth to 10 days of age. This would suggest that the $K_{\text{ATP}}$ channel had been activated in the right ventricle of these 30-day-old rabbits and contributes to the improved recovery. Our results also imply that the $K_{\text{ATP}}$ channel is not normally active in the unstrassed state in the right ventricle. 1-NAME decreased recovery of posts ischemic right ventricular function in previously hypoxic 30-day-old rabbits, suggesting that -NO is constitutively active and contributes to baseline function in both hypoxic and normoxic rabbits at this age. Because this effect was not seen in the 10-day-old normoxic rabbits, it is possible that there is a fundamental change in the right ventricle related to age and -NO production that has not developed in the first 10 days of life.

In summary, the work presented here provides a foundation for investigating the endogenous mechanisms mediating resistance to myocardial ischemia in hearts adapted to chronic hypoxia and then switched to normoxia that have the potential for clinical application. By understanding how hearts adapt to chronic hypoxia and readapt to subsequent normoxia, we can better understand how to protect patients with cyanotic heart defects that present for cardiac repair, thereby providing more success in the outcome of these patients. Many improvements in clinical technology have allowed the opportunity for young patients with complicated congenital heart diseases to have repairs. The end point of a successful repair need not be ventricular failure due to ischemic cellular damage. This system and our understanding of it are incomplete and require further study.

In conclusion, the memory of increased cardioprotection conferred by adaptation to hypoxia from birth persists on subsequent normoxic development and is associated with enhanced NOS activity and activation of $K_{\text{ATP}}$ channels.

REFERENCES