

Modulation of Ceramide Content and Lack of Apoptosis in the Chronically Hypoxic Neonatal Rat Heart

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ABSTRACT

To assess the effect of chronic hypoxia on cardiomyocyte apoptosis, we used an animal model that mimics cyanotic heart disease. Rats were placed in a hypoxic environment at birth, and oxygen levels were maintained at 10% in an air-tight Plexiglas chamber. Controls remained in room air. Animals were killed, and the hearts were harvested at 1 and 4 wk. Significant polycythemia developed in the hypoxic rats at 1 and 4 wk. Right ventricular mass in the hypoxic rats was 192% and 278% that of controls, and hypoxic left ventricular mass was 140% and 178% that of the controls at 1 and 4 wk, respectively. The increase in cardiac mass was paralleled by only mild hypertrophy (10 to 20%). Contrary to previous reports showing increased apoptosis in response to hypoxia in cultured cardiomyocytes, there was no difference in the number of apoptotic cardiomyocytes between the chronically hypoxic rats and controls, as assayed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling and Hoechst staining. We then examined the role of the sphin-

golipid ceramide because of its reported role in the stress response, growth suppression, and apoptosis. We found that the right ventricular ceramide content was significantly decreased in the hypoxic rats to 73% of control levels at the age of 4 wk. We suggest that the decrease in the ceramide content in the hypoxic right ventricular rat heart may be an adaptive response to chronic hypoxia and pulmonary hypertension. Lower ceramide levels may help suppress apoptosis and allow compensatory right ventricular cardiomyocyte proliferation. (*Pediatr Res* 51: 144–149, 2002)

Abbreviations

LV, left ventricle
RV, right ventricle
TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

Apoptosis, or programmed cell death, is a mechanism of regulated cell death that plays a major role during development and homeostasis, and in many disease states. Apoptosis in cardiomyocytes has been demonstrated after injury caused by ischemia and reperfusion (1, 2), myocardial infarction (3, 4), cardiac aging (5), ventricular pacing (6), heart failure, and coronary embolization (7, 8).

Conflicting data regarding apoptotic changes, in response to hypoxia, have been described (9–12). The failing myocardium is subject to regional ischemia and hypoxia, an abnormality that can trigger cardiomyocyte apoptosis (13). Long *et al.* (14) reported that cultured neonatal rat cardiomyocytes exposed to

in vitro hypoxia for 48 h exhibited apoptotic changes. However, Bishopric *et al.* (15) found that induction of apoptosis of cardiac myocytes by hypoxia required acidosis, and that chronic hypoxia alone did not cause apoptosis of cardiomyocytes in culture. Hypoxia can induce cellular adaptive responses, such as c-Jun expression, that overcome apoptosis signals to minimize injury or damage (16). The *in vivo* effect of chronic hypoxia on the neonatal heart has not been investigated, and differentiating the effect of hypoxia and ischemia from that of hypoxia alone on apoptosis in the neonatal heart is important.

Although no universal definition of apoptosis currently exists, morphologic and biochemical characteristics of apoptosis have been described that distinguish it from necrosis. The molecular events that drive the apoptotic response are rapidly being discovered. One of the central regulators of the apoptotic response appears to be ceramide, a sphingolipid breakdown product (17, 18). Ceramide has been shown to regulate the apoptotic response in several cell systems, including myocar-

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dial cells (19–22). Treatment of cardiomyocytes in culture with synthetic ceramides induces apoptosis and inhibition of proliferation (20, 23). Also, cardiomyocyte apoptosis induced by ischemia-reperfusion or tumor necrosis factor- α treatment is preceded by significant elevation in ceramide levels (19, 20, 22). In addition to apoptosis, ceramide induces terminal differentiation and a specific arrest of cells in the G₀/G₁ phases of the cell cycle as a result of activation of the retinoblastoma protein through its dephosphorylation (24). Inducers of ceramide accumulation include a number of stressors such as various cytokines, chemotherapeutic agents, irradiation, or serum deprivation (18). Moreover, up-regulation of p53 in response to chemotherapeutic agents or γ -irradiation has been found to induce the accumulation of cellular ceramide (25). These observations led to the suggestion that ceramide may play a role as a coordinator of the cellular responses to stress and act as a biostat of cell stress (26).

The neonatal myocardium is adapted to hypoxemia *in utero*. Normally, adaptation to normoxemia occurs within 24 h postnatally. The decrease in pulmonary vascular resistance and pressure and increase in systemic blood pressure that occur after birth are later followed by a shift from RV to LV predominance. This is marked by gradual loss of RV muscle mass and increase in the LV muscle mass. The exact molecular events that help the heart adapt to the postnatal environment are unknown (27). For example, it is not clear whether apoptosis in RV cardiomyocytes occurs during this transition period and whether this contributes to the decrease in RV mass. In neonates with cyanotic congenital heart disease, this transition to normoxemia does not occur and *in utero* adaptive mechanisms to hypoxia continue to operate. This may explain the remarkable absence of severe symptoms in children whose blood oxygen level would be incompatible with life in an adult. Therefore, we hypothesized that the apoptotic response of the neonatal myocardium to hypoxia may differ from the response of the adult myocardium, and we decided to explore the role of ceramide in modulating this response. Identifying the molecular regulators of this response may help us understand the age-dependent differences in adaptive mechanisms in response to stress.

In this study, we used an animal model of chronic neonatal hypoxia that mimics some forms of cyanotic heart disease and examined the response of the neonatal heart to chronic hypoxia with respect to apoptosis and ceramide content.

METHODS

Experimental design. We used an animal model to mimic conditions of cyanotic heart disease. Sprague-Dawley rats raised in our established breeding colony were studied. They were placed in a hypoxic environment within the first few minutes of life. Control animals remained in room air. Nitrogen and oxygen tanks with gauged flow were attached into a specially built air-tight Plexiglas chamber at standard cage dimensions. Oxygen levels were monitored continuously by an oximeter and maintained at 10% in the Plexiglas chamber. Air within the chamber was recycled after being passed through an anhydrous calcium sulfate and charcoal mixture to remove

ammonia and moisture. Soda lime was placed inside the chamber to absorb CO₂ (28). All animals received care in accordance with approved institutional animal care guidelines and according to the Guide for the Care and Use of Laboratory Animals of the National Academy of Science and the Principles of Laboratory Animal Care of the National Society of Medical Research.

Rats were randomly divided into four groups. Group A included seven control rats raised in room air and studied at 1 wk; group B included eight rats raised in hypoxic environment since birth and studied at 1 wk of age. Group C included eight control rats raised in room air and studied at 4 wk, and group D included nine hypoxic rats raised in hypoxic environment since birth and studied at 4 wk of age. The dam stayed with its rat pups in the Plexiglas chamber and tolerated the hypoxic environment.

The rat pups were killed under deep pentobarbital anesthesia (50 mg/kg, i.p.). The hearts were exposed through a midline sternotomy. The heart was excised, and the RV and LV were carefully identified and weighed. Hematocrit levels were assessed in all studied groups.

Tissue fixation. The heart was excised and cross-sectioned from the LV and RV in similar areas in both control and hypoxic animals. Samples were collected and fixed in 4% buffered formalin or kept frozen (–80°C). Formaldehyde-fixed tissues were processed for hematoxylin and eosin staining or for apoptosis studies. Routine light microscopy slides from paraffin-embedded sections of both hypoxic and control hearts were stained by Masson's trichrome stain (29). Using this stain, edema or fibrosis can be identified and nonmyocytes can be differentiated from myocytes histologically. The pathologist (A.T.) analyzed the slides from control and hypoxic animals while blinded to the origin of the slide (*i.e.* not knowing whether a slide was from a control or a hypoxic animal) with emphasis on changes related to fibrosis, edema, signs of inflammation, or significant hypertrophy.

To determine whether cardiomyocyte hypertrophy was present, the number of cells in heart sections was estimated in representative samples from control and hypoxic rats. Sections (4 μ m) were prepared and stained with the nuclear dye Hoechst 33258 (Molecular Probes, Eugene, OR, U.S.A.). The number of nuclei per unit area ($\times 20$ power field photographed by a Nikon camera) of myocardial tissue was determined by averaging counts of at least three to four fields from corresponding areas from hypoxic and control hearts. The decrease in the number of nuclei per unit area and in the absence of edema, fibrosis, or inflammatory cells indicated the presence of hypertrophic changes.

Lipid extraction. Lipids were extracted by the method of Bligh and Dyer (30). Briefly, cardiac tissue was homogenized after washing with PBS, then extracted with 3 mL of chloroform-methanol (1:2, vol/vol) in 13-mm \times 100-mm screw-top glass tubes. The monophasic mixture was mixed, then 0.7 mL of water was added and the samples were rested for 10 min. The organic and aqueous phases were subsequently separated by addition of 1 mL of chloroform and 1 mL of water followed by vigorous shaking and centrifugation at 1000 rpm. The organic phase was carefully removed and transferred to a new tube, and the

samples were dried under nitrogen. Lipids were then resuspended in 1 mL of chloroform.

Ceramide measurement. Ceramide levels were measured using a modified diacylglycerol kinase assay using external standards (31). Briefly, 80% of the lipid sample was dried under nitrogen. The dried lipid was solubilized in 20 μL of an octyl- β -D-glucoside–dioleoyl phosphatidylglycerol micellar solution (7.5% octyl- β -D-glucoside, 25 mM dioleoyl phosphatidylglycerol) by two cycles of sonication in a bath sonicator for 60 s followed by resting at room temperature for 15–20 min. The reaction buffer contained 100 mM imidazole HCl, pH 6.6, 100 mM LiCl, 25 mM MgCl_2 , and 2 mM EGTA. To the lipid micelles, 50 μL of 2 \times reaction buffer was added, 0.2 μL of 1 M DTT, 5 μg of diglycerol kinase membranes, and dilution buffer (10 mM imidazole, pH 6.6, 1 mM diethylenetriaminepentaacetic acid, pH 7) to a final volume of 90 μL . The reaction was started by adding 10 μL of 2.5 mM [γ - ^{32}P]ATP solution (specific activity of 75,000–200,000 cpm/nmol). The reaction was allowed to proceed at 25°C for 30 min. Lipids were extracted as described above, and a 1.5-mL aliquot of the organic phase was dried under nitrogen. Lipids were then resuspended in a volume of 100 μL of methanol-chloroform (1:20, vol/vol), and 20 μL was spotted on a 20-cm silica gel thin-layer chromatography plate. Plates were developed with chloroform-acetone-methanol-acetic acid-water (50:20:15:10:5), air-dried, and subjected to autoradiography. Radioactive spots corresponding to ceramide-phosphate, the phosphorylated product of ceramide, were scraped into a scintillation vial containing 4 mL of scintillation fluid and counted on a scintillation counter. Linear curves of phosphorylation were produced for a concentration range of 0–960 pM of external standards (dioleoyl glycerol and C-III ceramide; Sigma Chemical Co., St. Louis, MO, U.S.A.). Ceramide levels were always normalized to lipid phosphate.

Lipid phosphates. Lipid phosphates were measured according to the method of Rouser *et al.* (32). Briefly, 20% of the lipid sample was dried under nitrogen and oxidized with 70% perchloric acid on a heating block at 160°C for 45 min. The tubes were allowed to cool, then water was added, followed by 2.5% ammonium molybdate and 10% ascorbic acid with vortexing after each addition. The tubes were then incubated at 50°C for 15 min and allowed to cool, and the absorbency was read at 820 nm and compared with standards.

Apoptosis index determination. Serial cardiac tissue from hypoxic and control hearts were cut and collected on slides. The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was used to monitor the extent of DNA fragmentation as a measure of apoptosis in paraffin-embedded sections. The assay was performed according to the recommendations of the manufacturer (Boehringer Mannheim, Germany). Using paraffin sections that were 4 μm thick, the TUNEL assay was performed as described previously with minor modification (33). Fluorescein-conjugated dUTP incorporated in nucleotide polymers was detected and quantified using fluorescence microscopy (Zeiss LSM 410, Germany). Positive and negative controls were used to verify the specificity of the TUNEL assay. Positive controls were

treated with DNase I (Sigma Chemical Co.) to enzymatically induce DNA fragmentation. TUNEL-positive nuclei were distinguished from the TUNEL-negative nuclei by counter staining with Hoechst 33258 and were counted after being photographed. The count was done blindly. Briefly, the slides were prepared and TUNEL-labeled by one author and given random numbers, and then another author, who had no indication as to whether the slide represented a control or hypoxic tissue sample, performed the scoring of apoptotic cells based on TUNEL-positivity. After the blind count, the result was allocated to each group. The percent of nuclei labeled by TUNEL per unit of cells stained with Hoechst nuclear dye reflected the apoptotic index.

Statistical assessments. Values were expressed as arithmetic mean \pm SEM, and differences were considered significant when $p < 0.05$ in comparing two measurements. Experimental groups were compared by the two-way ANOVA with the Bonferroni *post hoc* test.

RESULTS

To examine the effects of chronic hypoxia on apoptosis and ceramide content in the neonatal rat heart, we used a model for chronic neonatal hypoxia mimicking some forms of cyanotic congenital heart disease. To verify the occurrence of chronic hypoxia in our experimental groups, we examined several variables. First, we determined the hematocrit of hypoxic rats and compared it with that of control animals. We found that significant polycythemia developed in the hypoxic rats (Table 1). This polycythemia is expected as an adaptive response to chronic hypoxia and validates our hypoxic model. Hypoxic rats did not thrive well as evidenced by their poor weight gain. The body weight of the hypoxic rats was significantly less than control rats at 1 and 4 wk (Table 1).

When the hearts of hypoxic and control rats were compared, we found that there was a significant increase in the RV and LV masses corrected for body weight in the hypoxic rats at 1 and 4 wk of age. This increase was more significant in the RV, in which its mass reached almost 2-fold that of control group after 4 wk of hypoxia (Table 2). This indicated that in response to chronic hypoxia, the cardiac mass might increase as a compensatory mechanism to improve cardiac output. The more pronounced increase in the RV mass compared with LV mass is most probably secondary to persistent pulmonary hypertension seen in our model owing to the failure to decrease pulmonary vascular resistance after birth because of continued

Table 1. Hematocrit levels and body weight in the control and hypoxic groups at the age of 1 and 4 wk

Variable	1 wk	4 wk
Hematocrit (%)		
Control group	35.2 \pm 0.3	41.3 \pm 0.3
Hypoxic group	51.2 \pm 0.7*	55.0 \pm 1.2*
Body weight (g)		
Control group	14.4 \pm 0.5	78.4 \pm 2.1
Hypoxic group	9.5 \pm 0.2*	58.5 \pm 3.1*

Significant polycythemia developed in the hypoxic rats and body weight of the hypoxic rats was significantly less than controls.

* Significantly different from controls, $p \leq 0.05$.

Table 2. Comparison of cardiac mass and estimated cardiac hypertrophy between hypoxic and control rats

Variable	RV		LV	
	1 wk	4 wk	1 wk	4 wk
Estimated hypertrophy in hypoxic rats (% over control)	15	19.4	10	12.3
Cardiac mass in hypoxic rats (% over control)	192*	278*	140**	178***

Hypertrophy was estimated by counting the number of cells/unit area as described in "Methods." Significantly different from controls, * $p < 0.00001$, ** $p = 0.001$, *** $p = 0.002$.

hypoxia. Moreover, these findings lend additional support to the validity of the chronic hypoxia model used in our experiments.

Next, we decided to determine the cause of the increase in cardiac mass observed in the hypoxic rats. The possibilities included 1) hypertrophy of existing myocytes, 2) decreased apoptosis of myocytes that may normally occur in the normal heart in this transition period but is prevented in response to hypoxia, 3) interstitial edema, 4) fibrosis, 5) inflammatory cell infiltration, and 6) increased proliferation of myocytes in response to hypoxia. When evaluated with Masson's trichrome stain, there was no difference between the hypoxic animals and their respective controls at 1 and 4 wk in either the RV or the LV regarding edema, inflammatory cell infiltration, or fibrosis. However, there was mild hypertrophy noted in hypoxic animals. To quantify the degree of hypertrophy, we counted the number of nuclei per unit area, including myocyte as well as nonmyocyte nuclei, in the hypoxic and control RV and LV at 1 and 4 wk of age. In the absence of edema, fibrosis, and inflammatory cell infiltration, a smaller number of nuclei per unit area would reflect the presence of larger cells (*i.e.* hypertrophy) compared with the control group. We found that the average cell count per unit area in the hypoxic myocardium was less than in control, such that in the RV there were 2388 ± 108 cells/unit area in hypoxic rats *versus* 2960 ± 186 cells/unit area in control rats at 1 wk and 1375 ± 60 cells/unit area in hypoxic rats *versus* 1569 ± 72 cells/unit area in controls at 4 wk. In the LV, the average cell count per unit area at 1 wk was 2245 ± 168 in the hypoxic rats *versus* 2635 ± 133 in controls, and at 4 wk it was 1563 ± 91 in hypoxic rats *versus* 1730 ± 98 in controls. When compared with controls (Table 2), these findings suggest that there is only mild hypertrophy (approximately 10 to 20%) in hypoxic cardiomyocytes.

The contribution of apoptosis to the remodeling of the heart in the transition from *in utero* hypoxemia to normoxemia has not been studied (27). In our model, two scenarios for the involvement of apoptosis were possible. First, it was possible that in the nonhypoxic heart, apoptosis would contribute to the loss of cell mass in the RV that occurs during this transition period. In that case, cell loss would be attenuated in response to chronic hypoxia, thus resulting in a higher number of surviving cells that leads to the larger RV mass in hypoxic rats. The second possibility was that in response to hypoxia, more apoptosis would occur in the hypoxic rat heart irrespective of the baseline apoptosis that occurs in the control rat heart but that the resulting cell loss would be compensated for by other pathways that lead to cardiomyocyte proliferation and hypertrophy. To determine which of these two scenarios was operating in our model, we decided to examine the degree of

apoptosis in cardiomyocytes in both control and hypoxic rats. We used two techniques to determine the occurrence of apoptosis. First, we used the TUNEL assay, which relies on the fragmentation of DNA that is pathognomonic of apoptosis. We found that the hypoxic and control RV and LV did not display any significant TUNEL reactivity, with apoptotic cells being $<1\%$ in both. There was no difference in the percentage of apoptotic cells in the chronically hypoxic rats compared with controls using the TUNEL assay. The average number of apoptotic cells per $\times 20$ power field in the RV was 0.8 ± 0.3 (control, $n = 6$) *versus* 0.7 ± 0.2 (hypoxic, $n = 6$) at 1 wk and 0.3 ± 0.2 (control, $n = 6$) *versus* 0.2 ± 0.2 (hypoxic, $n = 6$) at 4 wk. In the LV, the average number of TUNEL-positive cells per unit area was not significantly different between both groups. The average number of apoptotic cells per $\times 20$ power field in the LV was 0.2 ± 0.3 (control, $n = 6$) *versus* 0.2 ± 0.2 (hypoxic, $n = 6$) at 1 wk and 0.1 ± 0.1 (control, $n = 6$) *versus* 0.1 ± 0.2 (hypoxic, $n = 6$) at 4 wk. We confirmed our results by using another apoptosis detection technique, namely Hoechst 33258 chromatin staining, which reveals the chromatin condensation and fragmentation that occur in apoptosis. No significant differences were found in the number of apoptotic cells counted in hypoxic *versus* control RV and LV. In both hypoxic and control animals at 1 and 4 wk, the number of apoptotic cells was rare.

The previously described roles for the sphingolipid ceramide in the stress response and growth control led us to investigate its role in the response to chronic hypoxia. Therefore, we compared the ceramide content of both ventricles in hypoxic and control rats. We found that ceramide content in the RV was the same in both hypoxic and control rats at 1 wk of age (Fig. 1). However, by 4 wk of age, ceramide content in the RV was significantly lower in the hypoxic compared with control rats [ceramide/phosphate ratio (pmole/nmole) was 1.39 ± 0.14 (control) *versus* 1.19 ± 0.21 (hypoxic) at 1 wk, and 2.35 ± 0.16 (control) *versus* 1.72 ± 0.05 (hypoxic) at 4 wk]. In the LV, ceramide content was not significantly different in the hypoxic and control groups at the ages of 1 and 4 wk (ceramide/phosphate ratio was 0.48 ± 0.17 in control rats *versus* 0.41 ± 0.23 in hypoxic rats at 1 wk and 0.88 ± 0.08 in control *versus* 0.88 ± 0.15 in hypoxic rats at 4 wk). The ceramide content of the RV was higher than that of the LV in both hypoxic and control animals at 1 and 4 wk (Fig. 1).

DISCUSSION

The response of myocardial cells to chronic hypoxia may differ from that to acute hypoxia and the *in vivo* response may be different from the *in vitro* response. The fetal heart is well

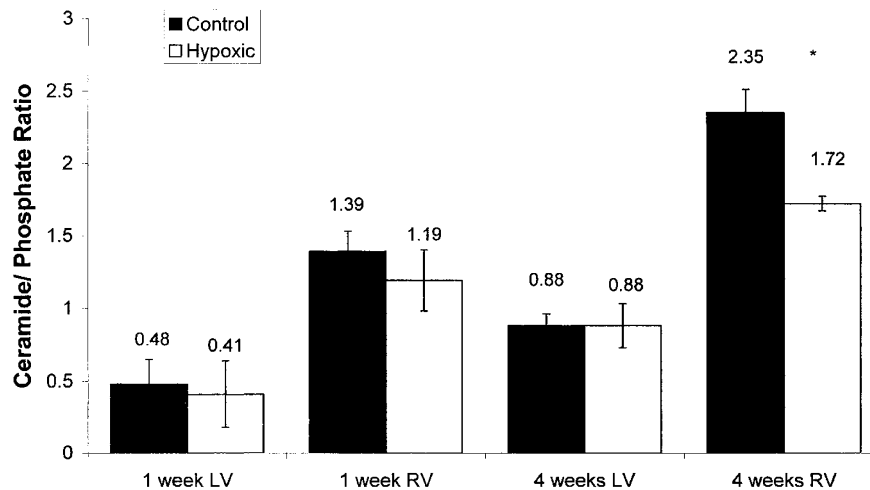


Figure 1. Ceramide/phosphate ratio in the RV and LV of the hypoxia and control groups at the age of 1 and 4 wk. Data shown are mean \pm SEM. * $p = 0.004$ vs control.

adapted to hypoxia, and these yet to be molecularly defined adaptive mechanisms may persist in the event of neonatal hypoxia as in our model. On the other hand, additional adaptive mechanisms may become activated in response to hypoxia in the neonatal period. These may include responses, such as the activation of mitogen-activated protein kinases or the transcription factor nuclear factor- κ B, that overcome apoptosis signals to minimize hypoxic injury or loss of cells that would otherwise be committed to die (34–36). Although the hypoxia achieved in our model was severe, as evidenced by the polycythemia observed in hypoxic rats, there was no evidence of increased apoptosis, as assayed by morphologic or biochemical criteria, in the LV and RV in either hypoxic rats or controls. Our findings suggest that, within the detection limits of our assays, the baseline levels of apoptosis in the neonatal rat heart are very low and that chronic hypoxia does not alter these levels. This is in contrast to findings in a model of ischemia-reperfusion injury in which, after ligation of the adult rat heart left coronary artery and subsequent reperfusion, significant apoptosis occurred (20).

Our findings support earlier studies (15) that factors in addition to hypoxia, *e.g.* acidosis, must be present for the apoptotic process to occur. The previously reported apoptotic changes in response to hypoxia in neonatal cultured cardiomyocytes (10–14) may not adequately represent the *in vivo* situation as well as our model does. This could possibly be because of the loss of survival signals emanating from the extracellular matrix in the intact heart when cardiomyocytes are grown in culture (37, 38). Moreover, many of the previous reports that demonstrated cardiac apoptosis in cell culture experiments used shorter duration, but more severe, hypoxia compared with our model. Thus, cardiomyocyte apoptosis may be related to the severity of hypoxia as well as the duration of exposure to the hypoxic insult.

In our rat model of chronic neonatal hypoxia, there was a significant increase in the cardiac mass. Although both ventricles were involved, the RV was more affected than the LV. In attempting to explain this finding, we found no evidence for edema, fibrosis, or inflammatory cell infiltration and only mild

hypertrophy. We speculate that cardiomyocyte proliferation occurs in the neonatal rat heart in response to chronic hypoxia and accounts for the significant increase in cardiac mass. More specific experiments are required to evaluate the role of cardiomyocyte proliferation in this response.

Recently, multiple lines of evidence have shown a role for ceramide in inducing apoptosis and cell cycle arrest (26, 39). The intracellular levels of ceramide increase shortly before biochemical events typical of apoptosis appear (40). For example, in the ischemia-reperfusion rat model, ischemic cardiac tissue ceramide levels increased by 1.5- to 4.5-fold after ligation of the left coronary artery for 30–210 min, followed by massive apoptosis (20). In our model, there was neither apoptosis nor ceramide elevation in response to chronic hypoxia. Instead, we found a significantly lower level of ceramide in the hypoxic RV at 4 wk compared with the control RV. This was accompanied by a significant increase in RV mass in the hypoxic *versus* control rats. No such differences were found in the LV. These findings are compatible with the proposed role of ceramide as a negative regulator of cell growth. We speculate that the lower ceramide content in the RV may serve to suppress any ceramide-mediated apoptosis and cell cycle arrest that could be triggered in the RV during the perinatal transitional period. This would allow the survival, and possibly enhanced proliferation, of cardiomyocytes that would have otherwise undergone apoptosis. Thus, the lower ceramide content may be an adaptive, biochemically regulated process that allows myocyte growth and proliferation in the dominant ventricle in response to chronic hypoxia and pulmonary hypertension. Curiously, we also found that ceramide levels were significantly increased in control ventricles at 4 wk compared with 1 wk of life. This raises the possibility that ceramide may be playing a role in the tissue remodeling that normally occurs in this transition period. More specific experiments will be needed for defining this role.

The cyanotic congenital heart diseases are associated with significant morbidity and mortality. Better understanding of the changes in the myocyte secondary to chronic hypoxemia, in particular cell death and its timing and severity in relationship

to the duration and level of cyanosis, will allow us to better understand the approach and timing of interventional surgery. Also, it may help in improved understanding of the adaptive changes in the myocyte associated with hypoxia and thus the development of novel cardioprotective strategies for the child with cyanotic congenital heart disease, or an adult with chronic hypoxia secondary to lung disease.

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