Effect of prenatal hypoxia on heat stress-mediated cardioprotection in adult rat heart

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Li, Guohu, Soochan Bae, and Lubo Zhang. Effect of prenatal hypoxia on heat stress-mediated cardioprotection in adult rat heart. Am J Physiol Heart Circ Physiol 286: H1712–H1719, 2004. First published January 8, 2004; 10.1152/ajpheart.00898.2003.—Fetal programming has profound effects on cardiovascular function in later adult life. We tested the hypothesis that chronic hypoxic exposure during fetal development downregulates endogenous cardioprotective mechanisms in adult rats. Time-dated pregnant rats were divided between normoxic and hypoxic (10.5% O2 from days 15 to 21 of gestation) groups. The male progeny were studied at 2 mo of age. Rats were subjected to heat stress (42°C for 15 min). After 24 h, hearts were excised and subjected to 30 min of global ischemia and 1 h of reperfusion. Prenatal hypoxia did not change adult rat body weight and heart weight, but significantly increased the cross-sectional area of a left ventricular (LV) myocyte. Heat stress significantly improved postsischemic recovery of LV function in normoxic control rats, but not in prenatally hypoxic rats. The infant size in the LV resulting from ischemia-reperfusion was reduced by the heat stress pretreatment in control rats, but not in prenatally hypoxic rats. In accordance, heat stress significantly increased LV myocardial content of heat shock protein 70 only in normoxic control rats. In addition, there was a significant decrease in the LV myocardial content of the PKC-ε isoform in prenatally hypoxic rats compared with control rats. We conclude that prenatal hypoxia causes in utero programming of hsp70 gene in the LV, leading to an inhibition of its response to heat stress and a loss of cardioprotection in later adult life.

fetal programming; heat shock protein 70; protein kinase C-ε; ischemia-reperfusion injury

RECENT EPIDEMIOLOGICAL STUDIES have suggested that prenatal factors may be linked with the development of adult diseases, such as hypertension and ischemic heart disease in later life (3, 10). Chronic hypoxia during the course of pregnancy is one of the most common insults to the fetal development and is thought to be associated with fetal intrauterine growth retardation (12, 26). Previous studies (24, 27, 41) showed that chronic hypoxia induced an asymmetric cardiac growth in fetal lambs and rats. Recently, we have demonstrated that maternal chronic hypoxic exposure stimulates hypoxia-inducible factor-1α expression and increases apoptosis in the fetal rat heart (2). Moreover, this prenatal hypoxic exposure leads to an increase in cardiac vulnerability in later adult life, and hearts from adult rats that were exposed to hypoxia before birth show greater myocardial damage after ischemia and reperfusion than do control hearts (21). The mechanisms underlying the increased cardiac vulnerability in adult rats are not clear, although it was associated with changes in basal levels of several endogenous proteins in the heart, including decreases in endothelial nitric oxide synthase and heat shock protein 70 (HSP70) levels and an increase in β2-adrenergic receptor/stimulatory G protein levels (21). Because HSP70 is one of the most important endogenous protective mechanisms in the heart in response to ischemia-reperfusion injury (35), the present study was designed to determine the effect of prenatal hypoxia on HSP70 synthesis in adult hearts and its cardiac protective effect.

The synthesis of HSP70 in the heart can be regulated by heat stress, acute hypoxia, and short transient ischemia. Although ischemic preconditioning may increase HSP synthesis in the heart, it mediates a completely different route for activating endogenous cardiac protective mechanisms not related to HSPs (35). In general, HSP-mediated cardioprotection does not occur before 24 h after pretreatment, the ischemic preconditioning-associated protection occurs within the first hours after the ischemic trigger. The molecular changes after ischemic preconditioning are complex and involve changes in protein activity and transcriptional activity, and involvement of HSPs in early protection by ischemic preconditioning is not clear. It has been well documented that whole body hyperthermia increases myocardial HSP70 content, which confers cardioprotection (9, 15, 23, 40, 44). Not only is HSP70 necessary, but also the amount of HSP70 produced by heat stress is directly related to the level of protection that is afforded against ischemia-reperfusion injury in the heart (15). Therefore, the present study tested the hypothesis that prenatal chronic hypoxia inhibits myocardial HSP70 synthesis in response to whole body heat stress, leading to a loss of cardioprotection against ischemia-reperfusion injury in the adult rat heart.

MATERIALS AND METHODS

Experimental animals. Time-dated pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Portage, MI) and were randomly divided into two groups: normoxic control and continuous hypoxic exposure (10.5% oxygen) from day 15 to day 21 of gestation. Hypoxia was induced by a mixture of nitrogen gas and air as previously described (21). Experiments from our laboratory have shown that an ambient oxygen level of 10.5% lowers maternal arterial PO2 to approximately 50 mmHg (34). The hypoxic group was housed identically with only room air flowing through the chambers. Food and water were provided as desired. Two-month-old male progeny rats that were raised in room air were used in the study. The normoxic control and prenatally hypoxic rats were randomly divided into four groups: 1) normoxic without heat stress, 2) normoxic with heat stress, 3) hypoxic without heat stress, and 4) hypoxic with heat stress. All procedures and protocols used in the present study were approved by the Institu-
tutional Animal Care and Use Committee of Loma Linda University and followed the guidelines in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of myocyte size. Hearts were isolated from normoxic control and prenatally hypoxic rats and weighed. To measure myocyte size, tissue slices (4 μm thick) obtained from the middle position of the left ventricle (LV) were stained with hematoxylin and eosin, viewed, and photographed by the microscope with the SPOT digital camera (Diagnostic Instruments; Sterling Heights, MI). The cross-sectional area of myocytes was quantified by computerized planimetry (Image-Pro Plus) in a double-blind manner.

Heat stress treatment. Whole body hyperthermia was achieved as previously described (19). Rats were anesthetized with 40 mg/kg pentobarbital sodium intraperitoneally. Anesthetized rats were placed in a heat incubator. The temperature of the incubator can be adjusted according to rat rectal temperature. Rectal temperature was measured with the use of a rectal probe with a digital thermometer. Rectal temperature was maintained at 42°C for 15 min. After treatment, the rats were returned to their cages and monitored until they woke from anesthesia. Sham control rats were given anesthesia only. After 24 h, the hearts were excised for perfusion, cardiac histology, and protein measurement.

Ischemia-reperfusion protocol. Twenty-four hours after heat stress or sham treatment, rats were anesthetized by intramuscular injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). Hearts were excised rapidly and were retrogradely perfused via the aorta in a modified Langendorff apparatus under constant pressure (70 mmHg) with gassed (95% O₂, 5% CO₂) Krebs-Henseleit buffer at 37°C, as previously described (21). A pressure transducer connected to a saline-filled balloon inserted into the LV was used to assess ventricular function by measuring ventricular pressure (mmHg) and its first derivative (dP/dt). LV end-diastolic pressure was set to 5 mmHg. After the baseline recording, hearts were subjected to 30 min of global ischemia and 1 h of reperfusion. Pulmonary artery effluent was collected as an index of coronary flow.

Measurement of LV infarct size. At the end of reperfusion, LV was collected, cut into four slices, incubated with 1% 2,3,5-triphenyltetrazolium chloride solution for 15 min at 37°C, and immersed in formalin for 30 min. Each slice was then photographed (Kodak digital camera) separately, and the areas of myocardial infarction in each slice were analyzed by computerized planimetry (Image-Pro Plus), corrected for the tissue weight, summed for each heart, and expressed as a percentage of the total LV weight.

Table 1. Body weight and heart weight

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>HW, g</th>
<th>LVW, g</th>
<th>HW/BW, mg/g</th>
<th>LVMCSA, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic control</td>
<td>395.3±16.9</td>
<td>1.61±0.05</td>
<td>0.77±0.04</td>
<td>4.2±0.2</td>
<td>295.2±17.3</td>
</tr>
<tr>
<td>NHS</td>
<td>377.8±12.2</td>
<td>1.63±0.06</td>
<td>0.73±0.04</td>
<td>4.3±0.1</td>
<td>374.0±12.8</td>
</tr>
<tr>
<td>Prenatal hypoxic</td>
<td>412.2±17.8</td>
<td>1.68±0.10</td>
<td>0.84±0.05</td>
<td>4.1±0.1</td>
<td>370.6±21.1*</td>
</tr>
<tr>
<td>HHS</td>
<td>391.7±16.5</td>
<td>1.60±0.08</td>
<td>0.78±0.03</td>
<td>4.1±0.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats/group. NHS, normoxic with heat stress; HHS, prenatal hypoxic with heat stress; BW, body weight; HW, heart weight; LVW, left ventricle (LV) weight; LVMCSA, LV myocyte cross-sectional area; *P < 0.05, hypoxic vs. normoxic control.

Caspase activity assay. Caspase-3 activity was determined using the corresponding caspase activity detection kit (R&D Systems), as described previously (2). Briefly, 100 μg proteins isolated from the LVs were added to 50 μl of reaction buffer and 5 μl of substrates of Asp-Glu-Val-Asp-fluoromethylketone-p-nitroanilide. Samples were incubated at 37°C for 8 h, and the enzyme-catalyzed release of p-nitroanilide was measured at 405 nm with the use of a microtiter plate reader. The values of ischemic samples were normalized to the controls, allowing determination of the fold increase in the caspase activity.

DNA fragmentation determination by ELISA. DNA fragmentation was measured by specific determination of cytosolic mononucleosomes and oligonucleosomes using an ELISA kit (Boehringer Mannheim) as described previously (21). Briefly, LV samples (40 mg) were put into 500 μl of lysis buffer supplied in the kit, homogenized in a tissue grinder, and incubated for 30 min at room temperature. After centrifugation at 200 g for 10 min, the supernatant (cytosolic fraction) was used as the antigen source in the sandwich ELISA. The absorbance was measured at 405/490 nm, and the background value of the immunoassay was subtracted.

Quantitative analysis of apoptotic cells. Fluorescent DNA-binding dyes were used to define nuclear chromatin morphology as a quantitative index of apoptosis as described previously (21). Briefly, LVs collected at the end of reperfusion were sectioned (4 μm thick) horizontally at two positions: apex and middle. The slices were stained with Hoechst 33258 (5 μg/ml) for 10 min. To confirm myocyte apoptosis, a combination of nuclear Hoechst 33258 staining and cardiac sarcomeric actin staining with monoclonal antibody was used on the same slice. The nuclei with DNA fragmentation stained blue amid the surrounding green color of actin staining developed by FITC-conjugated second antibody. Nuclei without DNA fragmentation had clear nuclear regions. The nuclear morphology was examined by fluorescence microscopy with the SPOT digital camera imaging system, and myocytes were scored as apoptotic if they showed unequivocal nuclear chromatin condensation or fragmentation. To quantify apoptosis, 2,000 nuclei from each heart were analyzed, and apoptotic cell counts were expressed as a percentage of the total number of counted nuclei.

Western blot analysis. Protein levels of HSP70, HSP27, and PKC isoforms in LVs from normoxic control and prenatally hypoxic rats were determined by Western blot analysis. Briefly, proteins (20–70 μg) isolated from the LV were loaded on 7.5% (HSP70 and PKC isoforms) and 12% (HSP27) SDS-PAGE, respectively, transferred to nitrocellulose membranes, and incubated with antibodies specific for each protein. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies, and the bands were visualized using an enhanced chemiluminescence detection system.

Table 2. Preischemic LV functional parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDP, mmHg</th>
<th>HR, beats/min</th>
<th>PRP, beats/min</th>
<th>dP/dtmax, mmHg/s</th>
<th>dP/dtmin, mmHg/s</th>
<th>CF, ml/min</th>
<th>LVEDP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic control</td>
<td>111.3±4.4</td>
<td>257.0±5.5</td>
<td>28,555.4±1076.7</td>
<td>3,355.3±209.2</td>
<td>2,232.5±122.4</td>
<td>13.4±1.0</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>NHS</td>
<td>106.8±5.6</td>
<td>254.7±5.5</td>
<td>27,119.8±1209.8</td>
<td>2,988.3±170.5</td>
<td>1,979.8±158.8</td>
<td>11.2±1.1</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>Prenatal hypoxic</td>
<td>122.8±1.6</td>
<td>250.3±5.9</td>
<td>30,748.1±938.0</td>
<td>3,439.7±234.3</td>
<td>2,196.0±106.0</td>
<td>11.9±0.9</td>
<td>5.2±0.7</td>
</tr>
<tr>
<td>HHS</td>
<td>113.3±7.9</td>
<td>248.7±4.8</td>
<td>28,172.9±2076.2</td>
<td>3,247.5±307.7</td>
<td>2,090.7±182.3</td>
<td>11.6±1.3</td>
<td>5.3±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats/group. LVEDP, LV developed pressure; HR, heart rate; PRP, pressure-rate product; CF, coronary flow; LVEDP, LV end-diastolic pressure; dP/dtmax and dP/dtmin, maximum and minimum first derivatives of LV pressure.

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Fig. 1. Postischemic recovery of left ventricular (LV) function. Data were obtained from normoxic control (normoxia; A) and prenatally hypoxic (hypoxia; B) animals 24 h after sham treatment or heat stress treatment. LVDP, LV developed pressure; dP/dt, the first derivative of LVDP; PRP, pressure-rate product. Data were analyzed by two-way ANOVA with ischemia-reperfusion as one factor and heat stress as the other. *P < 0.05, heat stress vs. sham control, n = 6.
nitrocellulose membranes, and incubated with the primary antibodies against inducible HSP70, HSP27 (Amersham; Arlington Heights, IL), and PKC isoforms (α, β1, β2, δ, ζ, ε) (Santa Cruz Biotechnology; Santa Cruz, CA), respectively. The secondary antibody was horseradish peroxidase-conjugated goat anti-mouse (Amersham). Proteins were visualized with an enhanced chemiluminescence detection system. Results were quantified with Kodak Electrophoresis Documentation and Analysis System and Kodak 1D Image Analysis Software.

**Statistical analysis.** Data are expressed as means ± SE and were analyzed by two-way ANOVA or by Student’s t-test. *P < 0.05 was considered significant.

**RESULTS**

**Body, heart weight, and myocyte size.** As we reported previously, the birth weights of animals from hypoxic mothers (4.87 ± 0.06 g) were significantly reduced compared with those from normoxic mothers (6.28 ± 0.07 g). There was no significant difference in body weight, heart weight, and heart weight-to-body weight ratio of the male progeny at 2 mo of age among the four experimental groups (Table 1). However, there was a significant increase in the cross-sectional area of LV myocytes from 295.2 ± 17.3 μm² in normoxic control rats to 370.6 ± 21.1 μm² in prenatally hypoxic animals (*P < 0.05, n = 5) (Table 1).

**Postischemic recovery of LV function.** LV developed pressure, heart rate, pressure-rate product (PRP), maximum dP/dt, minimum dP/dt, and coronary flow were not significantly different among the four groups at baseline (Table 2). As shown in Fig. 1, whole body heat stress 24 h before ischemia-reperfusion significantly improved postischemic recovery of LV developed pressure, PRP, maximum dP/dt, and minimum dP/dt in normoxic control rats. Postischemic recovery of heart rate and coronary flow was not affected (data not shown). In contrast to normoxic control rats, heat stress failed to improve postischemic recovery of LV function in prenatally hypoxic rats (Fig. 1). Ischemia-reperfusion-induced increase in LV end-diastolic pressure was significantly reduced by the heat stress treatment in normoxic control rats, but was not affected by heat stress in prenatally hypoxic rats (Fig. 2).

**Myocardial infarction and apoptosis.** As shown in Fig. 3, ischemia-reperfusion-induced LV myocardial infarct size was significantly decreased by the heat stress pretreatment in normoxic control rats (40.7 ± 4.7.9% vs. 23.3 ± 2.1%, *P < 0.05). In contrast, the heat stress treatment had no effect on LV myocardial infarct size after ischemia and reperfusion in prenatally hypoxic rats (Fig. 3). Figure 4 shows that ischemia-reperfusion significantly increased caspase-3 activity and DNA fragmentation in the LV, demonstrating an increase in apoptotic cell death. Assessment of nuclear chromatin morphology by the Hoechst 33258 staining using fluorescence microscopy indicated that ischemia-reperfusion increased condensed and segmented apoptotic nuclei in the LV. The nuclear staining performed in the combination with staining for α-cardiac sarcomeric actin using the monoclonal antibody confirmed that apoptosis was confined to the myocytes (data not shown). As shown in Fig. 5, quantification of ischemia-reperfusion induced apoptotic nuclei defined by the fluorescent DNA-binding dye Hoechst 33258 demonstrated a significant decrease in LV myocyte apoptosis in heat stress-treated rats (29.0 ± 2.2% vs. the sham control (47.8 ± 2.1%) in normoxic control rats (*P < 0.05). However, there was no difference in LV myocyte apoptosis between heat stress-treated rats and the sham control in prenatally hypoxic rats (Fig. 5).

**Western blot.** There were no significant difference in HSP70 and HSP27 protein levels in the LV between normoxic control rats and prenatally hypoxic rats. After 24 h of whole body heat stress treatment, myocardial HSP70 content in LV was significantly increased in normoxic control rats (*P < 0.05), but not in prenatally hypoxic animals (Fig. 6). Heat stress did not significantly increase HSP27 content in LV in either normoxic control or hypoxic rats (Fig. 6). Among six PKC isoforms examined, only PKC-ε isoform protein levels in the LV were significantly decreased in prenatally hypoxic rats compared with those in normoxic control rats (Fig. 7).

**DISCUSSION**

The novel finding of the present study is that prenatal hypoxia inhibits the heat stress-induced increase in myocardial HSP70 content and abolishes heat stress-mediated cardioprotection in adult rats. This provides clear and conclusive evidence that suppression of myocardial HSP70 expression plays a key role in the prenatal hypoxic-induced increase in myocardial vulnerability to ischemia and reperfusion injury in the
It has been clearly demonstrated that whole body hyperthermia increases myocardial HSP70 content, which confers cardioprotection (9, 15, 23, 40, 44). Not only is HSP70 necessary, but also the amount of HSP70 produced by heat stress is directly related to the level of protection that is afforded against ischemia-reperfusion injury in the heart (15). The direct cause-and-effect relationship of HSP70 expression and myocardial protection during ischemia-reperfusion injury in vivo has been demonstrated (16, 31). We have demonstrated recently that chronic hypoxia during fetal development decreases HSP70 expression in the near-term fetal rat heart (2).

In the present study, hearts from prenatally hypoxic rats showed similar basal LV function compared with those from control animals, suggesting that prenatal hypoxia does not influence contractility in the resting adult heart. The same finding was obtained in our previous studies in 6-mo-old rats (21). Because the hearts were perfused at a constant pressure, and the end-diastolic pressure was set at ~5 mmHg, the present study was not subject to differences in afterload, preload, or endogenous sympathetic tone. Previous studies (21) demonstrated that hearts from 6-mo-old rats that were exposed to hypoxia before birth showed greater myocardial damage after ischemia and reperfusion than did control hearts. In the present study, ischemia-reperfusion caused similar damage to the hearts from both prenatally hypoxic and control rats. This may be due to two factors. One factor is that 2-mo-old rats were used in the present study compared with 6-mo-old rats in the previous study (21). The other is that 30-min ischemia was used in the present study compared with 25-min ischemia in the previous study (21). These findings would suggest that either the increased susceptibility of adult heart to ischemia-reperfusion injury observed in 6-mo-old rats has not been developed yet in the younger hearts of 2-mo old rats or the longer ischemic treatment in the present study masks the potential differences in myocardial injury and postischemic recovery of LV function between the control and prenatally hypoxic rats. Compared with 25-min ischemic treatment in the previous study (21), 30-min ischemia in the present study caused a larger myocardial infarct size and a lower postischemic recovery of LV function in rats.

Acute ischemic injury resulting from coronary artery disease leads to myocardial cell death, much of which is accomplished...
through apoptosis (11, 13). Unlike necrosis, apoptosis is a highly selective process controlled and regulated by intracellular signal transduction that involves the sequential activation of cysteine proteases known as caspases, resulting in protein cleavage and breakdown of DNA molecules. In the present study, ischemia-reperfusion-induced myocardial infarction was associated with an increase in myocardial apoptosis. Similar findings were obtained in 6-mo-old rats (21). Other studies (22, 45) in rabbit and rat hearts demonstrated that apoptosis played a key role in ischemia-reperfusion-induced cardiac functional injury. Administration of a p38 mitogen-activated protein kinase inhibitor, SB-203580, markedly reduced postischemic myocardial apoptosis and significantly improved cardiac function recovery after reperfusion in Langendorff-perfused rabbit hearts (22). On the other hand, PD-98059, an extracellular signal-regulated kinase inhibitor, significantly increased postischemic myocardial apoptosis and markedly diminished functional recovery after reperfusion in rat hearts (45). The present finding of ∼45% apoptosis rate after 30-min ischemia is consistent with previous findings showing that 30-min ischemia caused 20–46.7% myocyte apoptosis in the rat heart (28, 30, 36, 37). Whole body hyperthermia significantly decreased ischemia-reperfusion-mediated myocardial apoptosis and reduced infarct size in the control animals, suggesting that suppressing apoptotic cell death may contribute significantly to heat stress-mediated cardioprotection in rats. It has been shown that heat-targeted overexpression of caspase-3 in mice increases ischemia-reperfusion-induced myocyte infarct size and depresses cardiac function (7). On the other hand, myocardial delivery of peptide inhibitors of caspase-3 decreased the infarct size in rats (43). The present finding that heat stress had no effect on ischemia-reperfusion-induced myocardial apoptosis and infarct size in animals that were exposed to hypoxia before birth suggests a loss of cardioprotection in these hearts. One of the potential mechanisms may be due to a dysregulation of HSP70 synthesis in the hearts of prenatally hypoxic animals. It has been well demonstrated in both cultured cardiomyocytes and the intact heart of experimental animal models that the HSP70 plays an important role in protection against ischemia, and that the degree of the early postischemic functional recovery correlates with the cardiac HSP70 tissue content (35). The use of HSP70 antisense molecules in cultured cardiomyocytes showed a specific inhibition of HSP70 synthesis and decreased tolerance to stress compared with control cells, even when the stress was mild (29). HSP70 has been demonstrated to suppress apoptosis (5), and heart-targeted overexpression of HSP70 decreased myocardial cell death (14, 17).

In the present study, we found that cardiomyocyte size in the LV was significantly increased in the hearts of 2-mo-old rats that were exposed to hypoxia before birth. However, both body weight and heart weight were not changed; hence, the ratio of heart weight to body weight remained the same between the two groups. Similar findings were obtained in 6-mo-old rats (21). We have recently demonstrated that chronic hypoxia during fetal development causes an increase in myocardial apoptosis in the term fetal rat heart, leading to a premature exit

![Fig. 6. LV myocardial heat shock protein (HSP)70 (A) and HSP27 (B) content after heat stress in normoxic control and prenatally hypoxic animals. Data were obtained from normoxic control and prenatally hypoxic animals 24 h after sham treatment or heat stress treatment. Data were analyzed by two-way ANOVA. *P < 0.05, heat stress vs. sham control, n = 3–4 animals.](http://ajpheart.physiology.org/)

![Fig. 7. LV myocardial PKC-ε content in normoxic control and prenatally hypoxic animals. Data were obtained from normoxic control and prenatally hypoxic animals. Data were analyzed by Student’s t-test. *P < 0.05, normoxia vs. hypoxia, n = 4.](http://ajpheart.physiology.org/)
of the cell cycle of cardiomyocytes and myocyte hypertrophy (2). We speculate that myocyte hypertrophy observed is a compensatory response to the loss of myocyte. The finding in the present study that hearts of prenatally hypoxic animals showed myocyte hypertrophy but had the same heart weight-to-body weight ratio compared with that of control animals is probably due to fewer myocyte numbers in the hypoxic heart. Although the myocyte hypertrophy observed may compensate for the loss of myocyte and maintain LV function at the resting level, it may cause increased ischemic vulnerability at the same time. It has been demonstrated that hypertrophied heart decreases the tolerance of global ischemia and the recovering of posts ischemic cardiac function (1, 25, 39). In animal models of long-standing cardiac hypertrophy due to aortic banding, heat shock-induced cardiac HSP70 protein levels were depressed (8). Similarly, hypertrophied rat hearts attenuated the HSP70 expression after coronary occlusion (38). Given that the characteristic of hypertrophied heart is myocyte hypertrophy, it is possible to speculate that myocyte hypertrophy in the animals that were exposed to hypoxia before birth may lead to suppression of HSP70 gene expression and attenuation in self-protective responses in the heart.

Although the mechanisms involved in the suppressed HSP70 gene expression in prenatally hypoxic hearts are not clear at present, previous studies (6, 20, 40, 42) showed that inhibition of PKC blocked heat stress-mediated protection and expression of HSP70 in the heart. This suggests that PKC may play a vital role in the regulation of HSP70 gene expression in the heart. In the present study, we have found that, among the PKC isoforms examined, only PKC-δ isoform protein levels in the LV were significantly decreased in prenatally hypoxic rats compared with those in normoxic control animals. Because the basal levels of HSP70 in the heart are the same between normoxic control and prenatally hypoxic animals in the present study, it does not appear that PKC-δ plays a role in the regulation of HSP70 in the resting heart. However, given the findings that heat stress-induced HSP70 expression is regulated by activation of PKC in the heart (6, 20, 40, 42), we speculate that the decreased PKC-δ isoform may play a role in the suppressed HSP70 expression in response to heat stress in prenatally hypoxic hearts. It has been suggested that PKC-δ plays an important role in the late phase of cardioprotection (4, 18, 32, 33, 42).

In summary, we have demonstrated for the first time in a rat model that prenatal hypoxia results in an inhibition of heat stress-induced increase in myocardial HSP70 synthesis and abolishes heat stress-mediated cardioprotection in later adult life. The results support the idea that prenatal hypoxia is a primary programming stimulus in the heart, leading to cardiac vulnerability in later adult life, and provide clear and conclusive evidence that suppression of myocardial HSP70 expression plays a key role in the increased myocardial vulnerability. Although the mechanisms underlying this suppression of HSP70 expression and cardioprotection in the heart from adult rats that were exposed to hypoxia before birth are not known, and are likely to be multiplex, it is proposed that a possible mechanism of hypoxic-induced DNA methylation of the promoter region of the hsp70 gene in the fetal heart presents an intriguing avenue for future investigation.

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