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Chronic Hypoxia Increases Inducible NOS-Derived Nitric Oxide in Fetal Guinea Pig Hearts

LOREN THOMPSON, YAFENG DONG, AND LASHAUNA EVANS

Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, Maryland 21201

ABSTRACT: Intrauterine hypoxia impacts fetal growth and organ function. Inducible nitric oxide synthase (iNOS) and neuronal NOS (nNOS) expression was measured to assess the response of fetal hearts to hypoxic (HPX) stress. Pregnant guinea pigs were housed in a hypoxic chamber (10.5% O_2 for 14 d, $n = 17$) or room air [normoxic (NMX), $n = 17$]. Hearts of anesthetized near-term fetuses were removed. mRNA [hypoxia-inducible factor, (HIF)-1α, 1β, 2α, 3α, iNOS, and nNOS] and protein levels (HIF-1α, iNOS, and nNOS) of fetal cardiac left ventricles were quantified by real time polymerase chain reaction (PCR) and Western analysis, respectively. Cardiac nitrite/nitrate levels were measured in the presence/absence of L-N^6-(1-iminoethyl)-lysine (L-NIL), an iNOS inhibitor, administered to pregnant sows. Hypoxia significantly increased fetal cardiac HIF-1α and -2α mRNA, HIF-1α protein but not HIF-3α or -1β mRNA levels. Hypoxia increased both iNOS mRNA (by 5×) and protein (by 23%) levels but had no effect on nNOS levels. Nitrite/nitrate levels were increased in HPX hearts by 2.5× and decreased with L-NIL by 67 ± 14%. Thus, up-regulation of iNOS-derived nitric oxide (NO) generation is an important mechanism by which fetal hearts respond to chronic hypoxic stress. **(*Pediatr Res* 65: 188–192, 2009)**

The adaptive response of the fetal heart to intrauterine stress is critical for its survival. Several studies using high altitude (1,2) exposure to acute and chronic hypoxia (2,3), and anemia (4) have demonstrated how the fetal cardiovascular system responds to hypoxic (HPX) stress. Depending on the severity and duration of the HPX conditions, as well as the gestational age of the fetus, cardiac adaptations have been associated with altered coronary blood flow (2,5,6), increased heart size (1), and decreased contractile performance (7). The underlying mechanisms mediating these changes in fetal heart morphology and function are not fully understood.

Hypoxia is a potent stimulus for gene activation of several genes (8), including nitric oxide synthase (NOS), the synthetic enzyme that generates nitric oxide (NO) from L-arginine oxidation (9). NO is derived from three isoforms of NOS [endothelial NOS (eNOS); neuronal NOS (nNOS); and inducible NOS (iNOS)], all of which are expressed in the heart (10,11). Specifically, eNOS is expressed constitutively in both endothelial cells and cardiomyocytes (10), nNOS in both cardiomyocytes and conducting tissue of cardiac ventricles (12), and iNOS in cardiomyocytes (12) and in mature hearts under conditions of hypoxia (12–14), heart failure (15), left ventricular hypertrophy (16) and cardiac cyanosis in children (17). NOS gene expression has been reported to be oxygen-sensitive, with levels varying among cardiac cells (18,19) and endothelial cells of differing vascular origin (20–22). For example, hypoxia increases eNOS expression in porcine coronary artery endothelial cells (20), newborn (23–25) and adult rabbit heart ventricles (26), and adult guinea pig ventricles (27), and decreases expression in pulmonary artery endothelial cells (28) and fetal guinea pig hearts (27,29). Thus, the severity and duration of the HPX stimuli, as well as, the tissue type, likely dictate the effect of hypoxia on NOS gene expression.

The ability of the cell to sense changes in oxygen concentration is mediated by the transcriptional regulator, hypoxia-inducible factor (HIF) (30,31). Three isoforms of HIF (1α, 2α, and 3α) are characterized as basic helix-loop-helix (bHLH) PAS (PER-ARNT-SIM) heterodimeric proteins made up of α and constitutive β (aryl hydrocarbon nuclear factor – ARNT) subunits and expressed in most cell types, including cardiomyocytes (31). Hypoxia has been shown to induce iNOS gene expression through the HIF-1α pathway (8,32). HIF-2α (EPAS) and HIF-3α have also been ascribed important transcriptional roles under HPX conditions (33) associated with the vascular endothelium (34) and heart (35), respectively.

The present study investigates the effect of chronic hypoxia on both HIF and NOS isoform expression in fetal guinea pig heart ventricles. We hypothesize that chronic hypoxia induces iNOS expression in the fetal heart, identifying iNOS-derived NO synthesis as an important pathway under conditions of HPX stress. This is supported by a hypoxia-induced increase in HIF-1α mRNA and protein, iNOS mRNA and protein, and LNIL-sensitive nitrite/nitrate levels in near-term fetal guinea pig hearts.

METHODS

Animal model. Time-mated pregnant guinea pigs (Dunkin-Hartley, term = 65 d) were placed in a HPX chamber [10.5%O_2 for 14 d; HPX; $n = 17$] as previously described (27) or in room air [normoxic; (NMX); $n = 17$]. To test the role of iNOS in NO generation, L-N6-(1-Iminoethyl)-lysine (L-NIL) was

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Correspondence: Loren Thompson, Ph.D., Department of Obstetrics, Gynecology, and Reproductive Sciences, Bressler Research Building, Rm 11-029, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore, MD 21201; e-mail: lthompson1@umm.edu

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Yafeng Dong, Ph.D. is currently at Department of Obstetrics, Gynecology, University of Kansas School of Medicine, Kansas City, KS 66160.

Abbreviations: NMX, normoxic; **HPX,** hypoxic; **HIF,** hypoxia-inducible factor; **L-NIL,** L-N^6-(1-iminoethyl)-lysine

Table 1. *Nucleotide sequences of PCR primers for amplification of HIF1α, HIF1β, HIF2α, HIF3α, iNOS, and nNOS*

Primers	Forward	Reverse	Accession No.	Reference
HIF1α	5′-AAGAAACCGCCTATGACGTG-3′	5′-CCACCTCTTTTTGCAAGCAT-3′	AF057308	35
HIF1β	5′-GCAGGATCAGAACACAGCAA-3′	5′-CCTGGGTAAGGTTGGAGTGA-3′	U61184	35
HIF2α	5′-CCCCAGGGGATGCTATTATT-3′	5′-GGCGAAGAGCTTCTCGATTA-3′	AJ277828	35
HIF3α	5′-AGAGAACGGAGTGGTGCTGT-3′	5′-ATCAGCCGGAAGAGGACTTT-3′	NM_022528	35
iNOS	5′-TGGATGCAACCCCATTGTC-3′	5′-CCCGCTGCCCCAGTTT-3′	XM_034166	50
nNOS	5′-GGATCACATGTTCGGTGTTCAG-3′	5′-CCCAACTTTGCGCTTGAAGA-3′	NM_000620	50

PCR, polymerase chain reaction; HIF, hypoxia inducible factor; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase.

administered to HPX animals ($n = 4$) in their drinking water at a dose of 1–2 mg/kg/d for 10 d. L-NIL selectively inhibits iNOS without affecting eNOS and nNOS activity at doses used in the present study (36). At 60 d gestation, pregnant mothers were anesthetized (ketamine, 1 mg/kg; xylazine, 80 mg/kg) and fetuses were removed *via* hysterotomy. Fetal body and organ weights of heart, brain, and placenta were measured. The methods were approved by University of Maryland Animal Care Committee.

Quantification of HIF isoforms (1α, 1β, 2α, and 3α), iNOS, and nNOS mRNA levels. Animals were randomized into two groups (NMX; $n = 6$ and HPX; $n = 6$). Gene expression of HIF1α, 1β, 2α, 3α, iNOS, and nNOS of NMX and HPX fetal hearts were quantified by real time polymerase chain reaction (PCR), similar to that previously described (27). In brief, total RNA was isolated from NMX and HPX fetal left cardiac ventricles by RNeasy Mini Kit and RNase-free DNase Set (Qiagen, Valencia, CA) and reverse-transcribed using Omniscript RT Kit (Qiagen). mRNA was quantified by real-time PCR (Bio-Rad, iCycler iQ Real-Time PCR Detection System) and the SYBR Green I labeling method. 18S primers were acquired from Applied Biosystems (Forster City, CA), and primer sequences of HIF-1α, 1β, 2α, 3α, iNOS, and nNOS obtained from GenBank (Table 1). The amplification protocol of the resulting cDNA consisted of 95°C for 2.5 min, followed by 45 cycles of amplification, each cycle consisting of 95°C for 30 s (denaturation), 60°C for 25 s (annealing), and 72°C for 7 min (extension). mRNA levels of target genes were measured as $2^{-\Delta\Delta CT}$ values and normalized to 18S rRNA generated from the same sample. Data were obtained as C_T values (cycle number at which PCR product crosses threshold) and normalized to 18S rRNA. To compare differences between groups, mRNA levels were expressed as relative expression using $2^{-\Delta\Delta CT}$ values derived from delta C_T NOS HPX group – delta C_T NMX 18S (37).

Western blot analysis. iNOS, nNOS ($n = 5$ from each group), and HIF-1α ($n = 3$ from each group) protein levels were quantified from NMX and HPX animals using standard methods, as previously reported (27). Briefly, the apical sections of fetal hearts were frozen in liquid N_2, homogenized in ice-cold lysis buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Igepal Ca 630, 0.1% SDS, 0.5% SDC, Protease inhibitor, Phosphatase inhibitor cocktail I, Phosphatase inhibitor cocktail II), placed on ice for 1 h, and spun at 1000 ×*g* at 4°C for 10 min. Protein concentration of the supernatant was analyzed by the RC-DC Protein Assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein of NMX and HPX fetal ventricles (70 μg for iNOS and nNOS; 60 μg for HIF1α) were loaded onto 7.5% Tris/glycene polyacrylamide gels and separated by gel electrophoresis. Proteins were transferred to Immun-Blot poly(vinylidene fluoride) (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA), blocked for 2 h, and probed overnight at 4°C. Membranes were incubated with a polyclonal antibody specific for either iNOS, nNOS, and HIF-1α (rabbit antibody for iNOS and nNOS, 1:200, Transduction Laboratories, San Diego, CA; HIF-1α, 1:1000, Novus, Littleton, CO) and then with the second antibody (1:5000, horseradish peroxidase-conjugated goat antirabbit and rabbit antigoat IgG for iNOS/nNOS and HIF-1α, respectively) after extensive washing. Protein bands were detected by enhanced chemiluminescence (ECL) Western Blotting Analysis System (Amersham, Piscataway, NJ). The relative quantity of each band was determined by densitometry (GS-700 Imaging system, Bio-Rad Laboratories, Hercules, CA) and compared with NMX controls loaded onto the same gel. HIF-1α protein levels were normalized to β-actin as a loading control. Because NO has previously been shown to stabilize HIF-1α (38), protein levels were also measured in fetal hearts of animals treated with LNIL.

Total NO product measurement. Nitrite/nitrate levels ($NO2^-$ and $NO3^-$) of fetal hearts of NMX ($n = 4$), HPX ($n = 5$), and HPX+L-NIL ($n = 4$) treated animals were measured using a Fluorometric NO Assay kit (EMD Biosciences, San Diego, CA). Left cardiac ventricles were excised, frozen in liquid nitrogen, and stored at −80°C. Frozen tissue was homogenized, centrifuged at 16,000 ×*g* for 20 min, and the supernatant filtered through a 10 kD cutoff filter at 14,000 ×*g* for 1 h. Enzyme cofactors and nitrate reductase were added to each sample according to the manufacturer's protocol. The fluorescing reagent, DAN (2,3-diaminonaphthotriazole) was added to tissue samples loaded onto a plate and read (excitation wavelength, 360–365 nm; emission 430–450 nm) using a Biotek Synergy HT Multi-Detection microtiter plate reader.

Statistics. Responses are expressed as mean ± SEM Student's *t* test was used to compare differences between NMX and HPX groups with a significant difference of $p < 0.05$.

RESULTS

Maternal and fetal guinea pig characteristics. Chronic hypoxia induces fetal adaptations consistent with HPX stress. For age-matched NMX (62.5 ± 0.4 d) and HPX (61.5 ± 0.7 d) fetuses, hypoxia significantly ($p < 0.05$) reduced body weight (85.6 ± 4.9 g *versus* 66.8 ± 4.8 g,) and brain weight (2.55 ± 0.07 g *versus* 2.32 ± 0.09 g, NMX *versus* HPX, respectively). The placental (0.059 ± 0.0024 *versus* 0.076 ± 0.005) and brain weight to body weight (0.031 ± 0.002 *versus* 0.037 ± 0.002) ratios were increased in HPX compared with NMX fetuses, consistent with that previously measured in a separate group of animals (27). The heart weight to body weight ratio was slightly increased with hypoxia although not significantly different from NMX controls.

HIF mRNA expression induced by chronic hypoxia in fetal guinea pig hearts. Chronic hypoxia alters the mRNA levels of HIF subunits (1α, 1β, 2α, and 3α) in fetal guinea pig hearts exposed to NMX and chronically HPX conditions. To determine the expression of RNA encoding HIF subunits, real time PCR was performed using total RNA of fetal ventricles of NMX and HPX animals. Figure 1 illustrates that hypoxia significantly increased HIF-1α by 4.8 fold (Fig. 1*A*), but had no significant effect on HIF-1β (Fig. 1*B*) in fetal hearts. In the same heart samples, hypoxia significantly increased HIF-2α (Fig. 1*C*) by 6.1 fold, but had no effect on HIF-3α (Fig. 1*D*) levels.

Chronic hypoxia increases HIF-1α protein expression in fetal guinea pig hearts. HIF-1α protein levels of NMX and HPX fetal cardiac ventricles were quantified by Western immunoblot analysis (Fig. 2). HPX significantly increased ($p < 0.05$) HIF-1α protein compared with NMX controls. LNIL had no significant effect ($p = 0.39$) on HIF-1α protein levels between groups (1.13 ± 0.02 *versus* 1.10 ± 0.02, HIF-1α/β-actin ratios of optical density (OD) values, HPX ($n = 3$) *versus* HPX+LNIL ($n = 3$), respectively).

Chronic hypoxia increases iNOS mRNA and protein expression in fetal guinea pig hearts. To quantify the effect of chronic hypoxia on iNOS expression, mRNA and protein levels were quantified using real time PCR and Western immunoblotting, respectively, (Fig. 3). Chronic hypoxia significantly increased both iNOS mRNA by 5.0-fold (A) and protein levels by 23% (B) compared with NMX controls. The immunoblot shows single bands of iNOS from NMX and HPX fetal hearts, each represented by a single band.

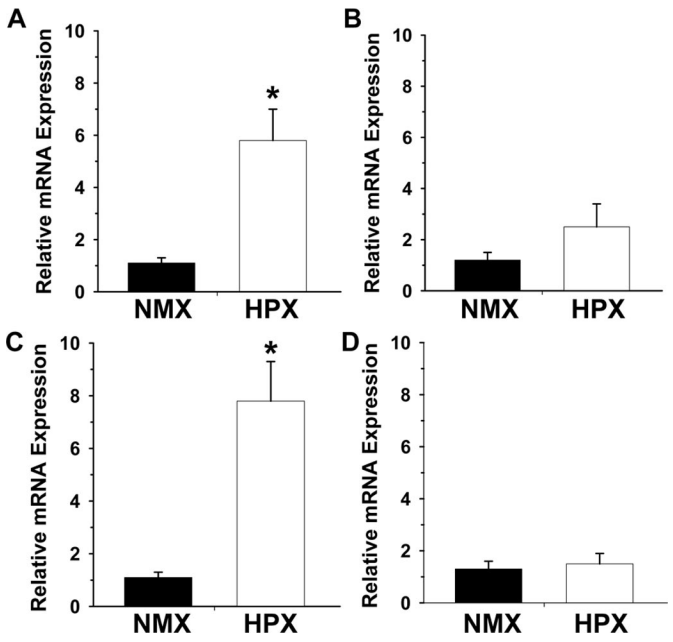

Figure 1. Effect of chronic hypoxia on hypoxia inducible factor (HIF) mRNA levels of fetal cardiac ventricles. HIF-1α (*A*), -2α (*C*), -3α (*D*) and HIF-1β (*B*) mRNA levels of normoxic (NMX, $n = 6$, ■) and hypoxic [(HPX), $n = 6$, □] fetal hearts were measured by real time PCR and quantified as $2^{-\Delta\Delta CT}$ values. HIF, hypoxia inducible factor. Values are mean ± SE. Asterisk indicates $p < 0.05$.

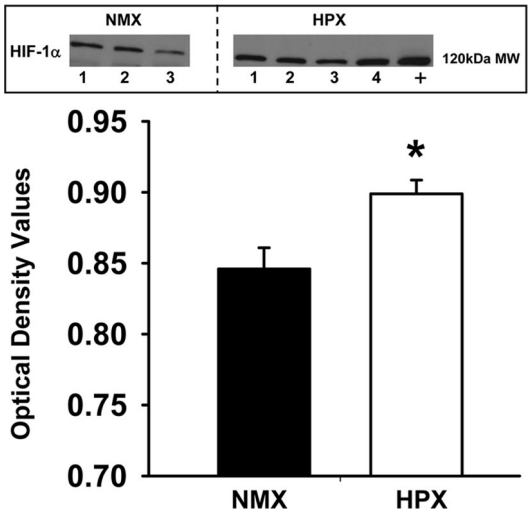

Figure 2. Effect of chronic hypoxia on HIF-1α protein levels of normoxic [(NMX), $n = 3$, ■] and hypoxic (HPX, $n = 4$, □) fetal hearts. Protein levels of HPX hearts were significantly ($p < 0.05$) increased compared with NMX controls. Each lane of the immunoblot represents a single fetal heart and the "+" indicates HIF-1α positive control. Values are mean ± SE. Asterisk indicates $p < 0.05$.

Effect of hypoxia on nNOS expression in fetal guinea pig hearts. To quantify the effect of hypoxia on nNOS expression, mRNA and protein levels were measured by real time PCR and Western analysis, respectively (Fig. 4). Figure 4 illustrates no significant effect of hypoxia on either mRNA or protein levels of fetal cardiac ventricles. The immunoblot illustrates single bands representing individual NMX and HPX heart samples.

Effect of hypoxia on nitrite/nitrate levels. Nitrite/nitrate levels (pmoles/mg tissue, total NO product) of NMX and HPX fetal cardiac ventricles were measured in animals with and without L-NIL treatment. HPX significantly ($p < 0.05$) increased NO product in cardiac ventricles (78.7 ± 13.3 pmoles/mg tissue) by 2.5× compared with the NMX controls (31.8 ± 9.8 pmoles/mg tissue) (Fig. 5). L-NIL significantly ($p < 0.05$) decreased total NO levels (78.65 ± 13.3 *versus* 25.6 ± 11.6 pmoles/mg tissue) in HPX animals by 67 ± 14% (% inhibition from HPX controls).

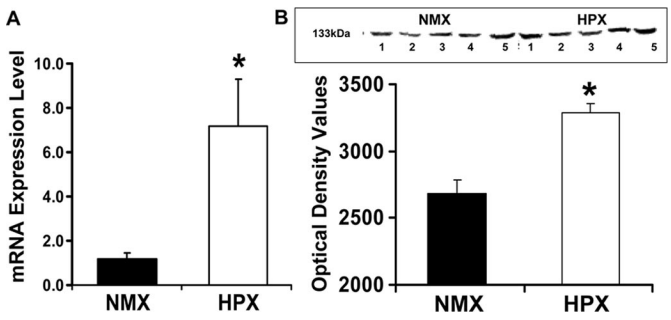

Figure 3. Effect of chronic hypoxia on mRNA and protein expression of inducible NOS (iNOS) in fetal guinea pig hearts. Both mRNA (*A*) and protein (*B*) levels were significantly ($p < 0.05$) increased in hypoxic (HPX, $n = 6$, □) compared with normoxic (NMX, $n = 6$, ■) hearts. In the immunoblot of iNOS protein, each lane represents an individual fetal heart. Values are mean ± SE. "*" indicates $p < 0.05$.

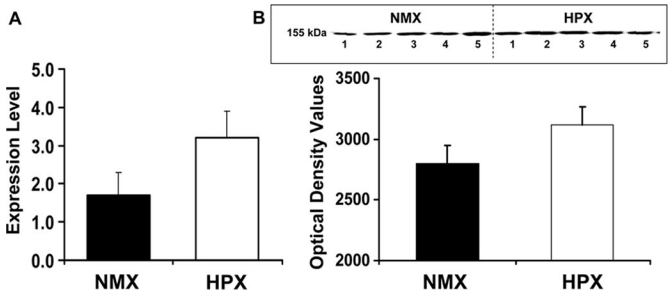

Figure 4. Effect of chronic hypoxia on mRNA and protein expression of neuronal NOS (nNOS) in fetal guinea pig hearts. mRNA (*A*) and protein (*B*) levels were similar between normoxic (NMX, $n = 6$, □) and hypoxic (HPX, $n = 6$, ■) hearts. In the immunoblot of nNOS protein, each lane represents an individual fetal heart. Values are mean ± SE. "*" indicates $p < 0.05$.

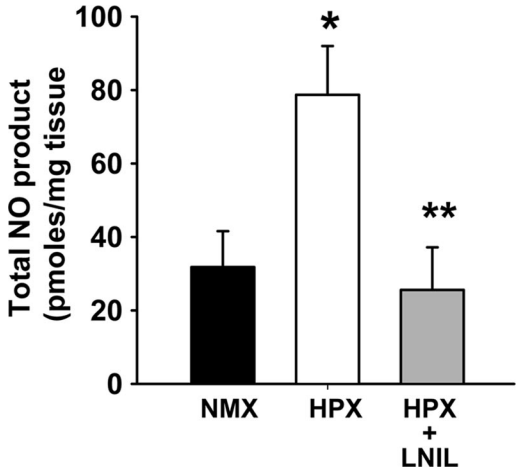

Figure 5. Effect of chronic hypoxia on total NO product (NO_2^- and NO_3^-) of fetal guinea pig hearts. Total nitrite/nitrate levels (pmol/mg tissue) were measured in fetal cardiac ventricles of NMX ($n = 4$, ■), HPX ($n = 5$, □) and HPX+L-NIL ($n = 4$, ▨) guinea pigs. Values are mean ± SE. "*" indicates $p < 0.05$ *versus* NMX control, "**" indicates $p < 0.05$ *versus* HPX.

DISCUSSION

In the present study, we have shown that chronic intrauterine hypoxia up-regulates the iNOS pathway in fetal guinea pig cardiac ventricles. Chronic hypoxia increased HIF-1α mRNA and protein, iNOS mRNA and protein, and an LNIL-sensitive increase in NO product (nitrite/nitrate) levels in fetal guinea pig hearts. No significant changes were measured in nNOS expression (*i.e.* mRNA and protein) between groups. Previously, we reported that hypoxia decreased both eNOS mRNA and protein in fetal guinea pig hearts under identical conditions of study (29). Together, these data suggest that maternal hypoxia alters fetal cardiac NOS expression in an isoform specific manner and that up-regulation of iNOS is an important pathway in HPX fetal hearts.

HPX regulation of NOS expression. The hypoxia-induced increase in iNOS expression may be mediated by HIF-1α. Gene expression in response to changes in oxygen is regulated by the hypoxia transcriptional complex, HIF, made up of α- (*i.e.*, 1α, 2α, and 3α) and β-subunits (8,32). Although the functional roles of each of the three α-subunits in heart tissue are unknown, transcriptional regulation of HIF isoforms is associated with tissue hypoxia (34). In the present study, all three of the HIF α-subunits are expressed in fetal guinea pig heart ventricles under baseline (*i.e.*, NMX) conditions, similar to that previously measured in fetal ovine (39) and adult rat hearts (35). In addition, our study shows a significant increase in both HIF-1α and 2α mRNA levels in HPX fetal ventricles compared with NMX controls, whereas HIF-3α and the constitutively expressed β-subunit was unchanged, as expected. HIF-1α protein levels were also increased in HPX fetal hearts. Although we expected a greater increase in HIF-1α protein levels given a 4.8 fold increase in mRNA levels in response to hypoxia, this could be attributed to either rapid degradation of HIF proteins during tissue dissection under NMX conditions, or a relatively mild HPX stimulus in the fetal cardiac ventricle. Interestingly, HIF-2α mRNA was also increased with hypoxia. HIF-2α is an HIF-1α analogue, up-regulated by low levels of oxygen, and previously reported to be expressed in the ovine fetal heart (39). Increased expression of both HIF-1α and -2α levels supports the hypothesis that global hypoxia of the pregnant mother induces local tissue hypoxia in fetal cardiac ventricles. The present study is the first to measure HIF-1α, -2α, and -3α mRNA levels in fetal guinea pig hearts demonstrating an association of HIF specific isoforms and fetal hypoxia.

HIF-1α is a transcriptional factor for iNOS and others such as VEGF and erythropoietin (31). Evidence supports a hypoxia-induced up-regulation of iNOS expression in several cell types, including macrophage (40), pulmonary endothelial cells (41), and cardiomyocytes (8,14,40). In cardiac myocytes of rat hearts, both *in vivo* and *in vitro* hypoxia increases iNOS and HIF-1α mRNA levels (14). After transient transfection of an iNOS promoter, hypoxia alone increased iNOS promoter activity of isolated cardiomyocytes and demonstrated that the HIF-1 binding site is required for transcriptional regulation of iNOS gene expression (14). In addition, chronic hypoxia (10% O_2 for up to 21 d) increases iNOS expression and NOS activity in enzymatically isolated cardiomyocytes of adult rat hearts (42). Thus, the increase in HIF-1α may contribute to the hypoxia-induced increase in iNOS expression in the HPX fetal heart.

NO itself has been shown to have opposing effects on HIF-1α protein levels, depending on the cell type and NO concentration. For example, NO has been reported to either inhibit HIF-1α activation in cancer cells (43) or increase HIF-1α protein levels *via* protein stabilization in vascular endothelial cells (38). It could be suggested that the HPX-induced increase in HIF1α protein is mediated by NO-mediated protein stabilization rather than by local tissue hypoxia. Our results show that LNIL inhibits NO levels, but has no effect on HIF-1α protein in HPX fetal hearts. Although further study is needed to identify the HIF-1α regulation in HPX hearts, these data support an increase in HIF-1α gene expression by hypoxia rather than because of protein stability by increased NO.

Functional role of increased iNOS expression. The role of NO in cardiac function is complex, dependent on the level of NO produced, the cell type in which it is synthesized and the NOS isoform from which it is derived. In the present study, chronic hypoxia alters NOS expression in an isoform specific manner and the up-regulation of iNOS expression is accompanied by increased NO production that was inhibited by L-NIL, a selective iNOS inhibitor. This is supportive evidence that iNOS-derived NO is up-regulated by chronic hypoxia in the fetal heart. Although further study is needed to confirm the selectivity of L-NIL in the guinea pig, previous study has identified the selected dose range to be effective in inhibiting iNOS over eNOS and nNOS (36). Further, we propose that neither eNOS nor nNOS likely contributes to the HPX-induced NO generation in cardiac ventricles, because hypoxia did not increase the expression of either isoform. In addition, iNOS has a catalytic activity 10 fold higher than either eNOS or nNOS isoforms (44), implicating its potential role in generating a high NO output under conditions of hypoxia.

NO is an important modulator of both fetal (45,46) and adult coronary artery vasodilation (5,23–25) and cardiac contraction (12,18,19,47). Yet, few studies have focused on the effects of intrauterine hypoxia on NOS gene expression in HPX fetal hearts. Increased NO has been associated with relaxation of cardiac cells by an increase in cyclic GMP (cGMP) levels and likely plays an important role in modulating myocardial contractile function (47). Fetal hypoxia reduces myocardial force generation by mechanisms poorly understood. Given the multiple actions of NO on ion channel activation, regulation of intracellular calcium, phosphorylation of contractile proteins, and induction of apoptotic mechanisms (10,18,19,47), iNOS-derived NO would be expected to have an important physiologic role in HPX fetal hearts.

In adult rabbits, exposure to hypoxia from birth to 10 d of age increased NOS activity in heart ventricles (23). At 30 d of age, the hypoxia-exposed hearts exhibited improved recovery of left ventricular pressure in response to subsequent myocardial ischemia, which was reversed by nitro-L-arginine, a nonselective NOS inhibitor (23). Although Fitzpatrick *et al.* (23) did not identify an iNOS-specific effect, this study suggests a cardioprotective response that may be NO-dependent. In addition, increases in iNOS-derived NO of adult hearts also contribute a cardioprotective role in late phase of ischemic preconditioning

(48,49). On the other hand, extensive study has demonstrated increased iNOS expression in response to infiltrating inflammatory mediators, ischemia/reperfusion injury, and infection of adult hearts (9), identifying iNOS as a major pathophysiologic mediator of cardiac injury. Although these studies ascribe NO as having both a cardioprotective and damaging role in the adult, it is difficult to simply extrapolate these findings to the fetal heart, whose response to hypoxia is dependent on the gestational age, severity/duration of the HPX stimulus, and the unique environmental milieu, in which the fetal heart exists. Thus, the functional role of iNOS-derived NO in fetal hearts remains unclear, although identifying it as an important pathway during chronic hypoxia.

In summary, chronic hypoxia up-regulates the iNOS pathway in fetal guinea pig hearts, which likely contributes to the increase in NO production. Given that eNOS expression is decreased with hypoxia (29) and that nNOS expression is unchanged, this study demonstrates that iNOS induction, in association with HIF-1α, may be an important cardiac response in the fetal guinea pig heart to HPX stress. Further study is needed to confirm the functional consequences of the enhanced iNOS-derived NO levels in the HPX fetal heart.

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