Chronic hypoxia increases the NO contribution of acetylcholine vasodilation of the fetal guinea pig heart

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Received 14 October 1999; accepted in final form 19 July 2000

Thompson, Loren P., Kripamoy Aguan, Gerard Pinkas, and Carl P. Weiner. Chronic hypoxia increases the NO contribution of acetylcholine vasodilation of the fetal guinea pig heart. Am J Physiol Regulatory Integrative Comp Physiol 279: R1813–R1820, 2000.—To investigate the effect of chronic hypoxia (HPX) on vasodilation of the fetal heart, we exposed pregnant guinea pigs to room air or 12% O2 for 4, 7, or 10 days. We excised hearts from anesthetized fetuses (60 ± 3 days; 65-day gestation = term) and measured changes in both the coronary artery pressure of the isolated constant-flow preparation and endothelial nitric oxide synthase (eNOS) mRNA of fetal ventricles. Dilator responses to cumulative addition of acetylcholine and sodium nitroprusside in prostaglandin F2α-constricted hearts were similar among normoxia (NMX), 4-, 7-, and 10-day HPX (control). Nitro-L-arginine (L-NA, 10−5M), a NOS inhibitor, inhibited maximal acetylcholine dilation of hearts exposed to 10-day HPX greater than NMX, 4-, 7-, and 7-day HPX. Hypoxia (after 7 and 10 days) increased eNOS mRNA of fetal ventricles compared with NMX and 4-day HPX. 4-Aminopyridine (3 mM), a voltage-dependent K+ channel inhibitor, inhibited acetylcholine- but not sodium nitroprusside-induced dilation of NMX and 10-day HPX hearts to a similar magnitude. Glibenclamide (10−5M), an ATP-sensitive K+ channel inhibitor, had no effect on vasodilation. We conclude that chronic HPX increases the contribution of NO but does not alter K+ channel activation in response to acetylcholine-stimulated coronary dilation. Thus increases in NO production via upregulation of eNOS gene expression may be an adaptive response to chronic HPX in the fetal coronary circulation.

The cardiovascular responses of the fetus to hypoxemia have been well characterized (reviewed in Refs. 9, 15). There is a dramatic redistribution of cardiac output to the heart, brain, and adrenal glands (15, 26). Yet, fetal responses differ depending on the duration and severity of the hypoxemia. During acute hypoxemia, the fetal sheep increases its arterial blood pressure, decreases its heart rate (15, 27), and increases its plasma levels of norepinephrine and epinephrine (26). In response to the prolonged exposure to hypoxia (HPX), arterial blood pressure and heart rate as well as hormone levels return to normoxic values, whereas blood flow to the heart and brain both remain elevated (16). Although reflex mechanisms mediate the acute cardiovascular responses to short-term hypoxemia via the activation of the arterial chemoreceptors (12), it is unclear what mechanisms mediate the sustained cardiovascular responses to prolonged hypoxemia.

Recent studies have shown that remodeling of the coronary microcirculation increases both basal coronary flow and myocardial flow reserve in chronically instrumented fetal sheep during prolonged hypoxemia (5–8 days) (25). Subsequent study from the same laboratory revealed that nitro-L-arginine (L-NA), a nitric oxide (NO) synthase inhibitor, inhibited both the basal flow and the flow increase of chronically instrumented fetal lambs in response to in utero short-term hypoxemia (24). Thus NO release in response to hypoxemia may be an important mechanism for modulating fetal coronary tone. Although NO release has been previously shown to be important in modulating vascular reactivity in a variety of fetal vascular beds (30, 35), its role in the fetal heart has not been fully studied.

NO is a potent endothelium-derived vasodilator and is synthesized by the oxidation of L-arginine to L-citrulline via activation of the calcium-dependent NO synthase type III [endothelial NO synthase (eNOS)]. Endothelium-derived NO relaxes vascular smooth muscle by activating soluble guanylate cyclase and elevating intracellular cyclic GMP (4, 10). It follows that altered NO production secondary to either changes in enzyme activity and/or gene expression could contribute to altered coronary tone in the fetal heart. However, the effect of HPX on NO gene expression in endothelial cells has been largely studied in adult tissues, and the in vitro results are conflicting. HPX (PO2 = 10 mmHg) increases both calcium-dependent NOS activity and protein expression in cultured endothelial cells from porcine coronary resistance arteries (36) and bovine aorta (3). Chronic HPX (3-wk duration, 10% O2) increases eNOS (17, 31) and inducible NOS (iNOS) mRNA (17) and protein expression in the lungs of adult rats. However, HPX decreased NOS gene expression of...
cultured endothelial cells from rat (32) and bovine (18) pulmonary arteries and human umbilical veins (19, 22). Thus, although HPX can alter eNOS transcription in cultured endothelial cells, this effect may be cell type specific and dependent on the conditions of hypoxemia. The mechanisms proposed to mediate these cellular responses in an intact circulation include a direct effect of reduced oxygen levels on gene expression (28) and/or an indirect effect of increased shear stress on the endothelium (29).

We hypothesized that chronic HPX increases NO production by increasing gene expression in the heart and thereby enhances its contribution in mediating flow responses. We tested this hypothesis by measuring dilator responses to both acetylcholine and sodium nitroprusside in the presence and absence of l-NA in the isolated fetal guinea pig heart. Previous studies in the adult guinea pig have shown that acetylcholine stimulates the release of an endothelium-derived hyperpolarizing factor (EDHF) (21) and that NO may activate K+ channels (34). Thus we also considered the effect of chronic HPX on the contribution of K+ channel activation in mediating flow increases to both agonists.

**METHODS**

The methods employed were approved by University of Maryland Animal Care Committee and conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Animal model.** Time-mated pregnant Hartley-Duncan guinea pigs (58- to 62-day gestation; Harlan Sprague Dawley, Indianapolis, IN) were housed in a Plexiglas hypoxic chamber exposed to 12% O2 for either 4-, 7-, or 10-day duration. On the basis of previously published reports (7), maternal arterial P02 under normoxic conditions is estimated at 92 mmHg and fetal umbilical vein P02 is estimated at 27 mmHg. The gas mixture within the chamber passes through two separate canisters containing soda lime and silica gel to remove excess CO2, H2O, and ammonia, respectively. Control animals were housed in room air (estimated 21% O2; normoxia (NMX)) in the same room. At the end of the hypoxic period, pregnant mothers were anesthetized (xyloseine 1 mg/kg ip and ketamine 80 mg/kg ip) and lidocaine was given subcutaneously at the site of an abdominal incision. The anesthetized fetuses were excised through a hysterotomy incision. A thoracotomy was then performed on the first fetus located in the right uterine horn proximal to the cervix, and the fetal heart was removed for study. Blood was collected via cardiac puncture from the remaining fetuses before detaching the placenta for determination of hematocrit. Body weight, placental weight, whole heart weight, and brain weight were measured as indexes of growth.

**Isolated fetal guinea pig heart preparation.** Before fetal heart was removed, ice-cold heparinized saline was injected into the vena cava to prevent clot formation in the coronary circulation. The heart was rapidly excised and immediately weighed in cold oxygenated Krebs-bicarbonate buffer containing (in mM) 118 NaCl; 4.7 KCl; 1.18 MgSO4·7H2O; 1.18 KH2PO4; 5.55 d(+)-glucose; 2 sodium pyruvate; 0.016 EDTA-Na2; 15.8 NaHCO3 (pH = 7.35–7.4)); and 2.2 CaCl2·2H2O. Hearts were then mounted onto a 1.6-mm glass cannula of a perfused heart apparatus (Radnoti Glass Technology, Monrovia, CA) at the base of the aorta and perfused retrograde with oxygenated (95% O2-5% CO2) buffer. Oxygen content of the perfusate was ~1.5 ml O2/ml buffer on the basis of the approximate oxygen tension of the buffer (500–600 mmHg for 95% O2-5% CO2) and 0.003 ml O2/ml buffer dissolved. Given the average perfusion rate is 4.5 ml buffer·min−1·g heart weight−1, we estimated the oxygen delivery rate to be ~6.75 ml O2·min−1·g heart−1. Contractile force was recorded continuously to confirm stability of the preparation with the use of a Grass polygraph by connecting the apex of the heart via suture to a force transducer (Grass FT03 transducer). Hearts were stretched to a previously determined optimal contractile force and allowed to equilibrate for 30 min before initiating the experiment. Optimal force was determined to be ~4.5 g. This was the maximal stretch able to be applied to each heart without tearing the myocardial tissue. Each heart was paced electrically at 235 beats/min with surgical stainless steel electrodes inserted into the ventricles (Grass model SD9 stimulator; stimulus parameters, 1.4 ms, 3 V). Hearts were perfused with temperature-regulated buffer (at 37°C) at a constant-flow rate adequate to produce an arterial pressure of 30–33 mmHg as measured by an in-line Radnoti pressure transducer. Selection of this perfusion pressure was on the basis of an approximate in vivo mean arterial pressure of fetal guinea pigs (20). Selection of such a pressure was also used to ensure that pH and temperature conditions were maintained during perfusion and that drug responses were similar to those observed in vivo.

**Experimental protocol.** After the equilibration period, prostaglandin F2α (PGF2α, 5 × 10−6 M, an EC90 concentration) was infused to increase coronary resistance. After steady state was obtained, the dilator responses to the cumulative addition (10−6–10−5 M) of acetylcholine, an endothelium-dependent dilator, and sodium nitroprusside, an NO donor, were measured. Control concentration-response curves were obtained consecutively in the same heart. After a washout period of ~30 min, l-NA was infused at a rate that achieved 10−6 M in the perfusate. In the presence of l-NA, the PGF2α concentration was reduced to match the control perfusion state was obtained, the dilator responses to the cumulative addition (10−6–10−5 M) of acetylcholine, an endothelium-dependent dilator, and sodium nitroprusside was then repeated in the presence of l-NA. Dilator responses were normalized to the maximal vasodilation produced by papaverine (10−5 M) added at the end of the experiment. Time controls indicate no significant differences in responsiveness to either agonist by replacing l-NA with saline vehicle.

In a separate group of animals, we examined the role of K+ channel activation in mediating dilator responses in normoxic and hypoxic fetal hearts. Hearts of guinea pig fetuses were obtained from normoxic fetuses and fetuses whose mothers were exposed to 10-day HPX. Vasodilator responses to cumulative addition of both acetylcholine and sodium nitroprusside were measured in the presence and absence of the voltage-dependent K+ (K○-ATP)-channel blocker, 4-aminopyridine (3 mM) (23), and the ATP-sensitive K+ (K○-ATP)-channel blocker, glibenclamide (10−5 M) (37). The concentration of each blocker was chosen on the basis of its ability to selectively inhibit its respective K+ channel subtype. Both blockers were administered 30 min before the beginning of the concentration-response curve of each vasodilator and continued throughout the duration of the experiment.

To determine the relative contribution of K+ channel activation in mediating acetylcholine-induced vasodilation, we measured dilator responses of normoxic hearts to cumulative addition of acetylcholine in the presence of l-NA (10−4 M) alone and with l-NA plus 4-aminopyridine (3 mM).

**Ribonuclease protection assay.** eNOS-specific mRNA levels were determined by ribonuclease protection assay as previ-
ously described by Aguan et al. (1). Briefly, both ventricles of hearts from siblings of the same litter were frozen in liquid nitrogen and stored in −80°C until ready for assay. Poly (A+) mRNAs were isolated with the use of a micro-mRNA isolation kit (Pharmacia Biotech, Piscataway, NJ). Partial cDNA clones for eNOS and β-actin were obtained by screening a guinea pig heart cDNA library, and its authenticity was checked by sequencing. Appropriate cDNA fragments (290 bp for eNOS and 157 bp for β-actin) of the clones were subcloned into pCRScript vector (Stratagene, La Jolla, CA) flanking T3 and T7 promoter sites.

32P-labeled antisense RNAs for eNOS and β-actin were generated with the use of MaxiScript T7 kit (Ambion, Austin, TX). Specific activity of β-actin antisense RNA probe was adjusted to a level 10 times less than that of the eNOS probe. About 200 ng of isolated poly (A+) mRNAs were hybridized with 20,000 cpm of eNOS antisense RNA probe along with 12,000 cpm of β-actin probe with the use of the RPA III kit (Ambion). After hybridization over a 16-h period at 45°C and subsequent RNase A/T1 digestion, the protected mRNAs were run on a 6% urea-PAGE gel. The gel was dried down, exposed to film, and the intensity of the different bands of the autoradiograph were recorded with the use of a BioRad GS-525 Phosphoirnager (BioRad, Hercules, CA).

Statistical analysis. Data are expressed as means ± SE. Coronary vascular resistance (mmHg·ml⁻¹·min⁻¹·g heart wet wt⁻¹) was calculated as the perfusion pressure (mmHg) divided by the flow rate (ml·min⁻¹·g heart wet wt⁻¹). Resistance values were normalized to the wet weight of the heart measured before the start of the experiment. Agonist-induced vasodilation was normalized as a percent of the maximal response to papaverine (10⁻⁵ M) infused at the end of the experiment. The log EC₅₀ value was determined as an index of agonist potency and measured as the log concentration producing 50% of maximal vasodilation to each agonist. Responses were compared between normoxic (control) and hypoxic animals with the use of two-way repeated-measures ANOVA with dilator responses as the dependent variable and L-NA, 4-aminopyridine, glibenclamide, or HPX as independent variables. If the mean values for the ANOVA were found to be statistically significant (P < 0.05), a Student-Newman-Keuls or Dunnett’s multiple-comparison test was applied to analyze differences between treatments.

Drugs. L-NA, acetylcholine HCl, sodium nitroprusside dihydrate, 4-aminopyridine, glibenclamide, and PGF₂α Tris salt were purchased from Sigma Chemical (St. Louis, MO). All drugs except for glibenclamide were dissolved in deionized water. Glibenclamide was dissolved in 50:50 NaOH/NaHCO₃ solution. All drugs were made fresh or thawed (PGF₂α) from a frozen stock on the day of the experiment.

RESULTS

Characteristics of the fetal guinea pig HPX model. Exposure to HPX (12% O₂ maternal inhalation) for up to 10 days duration had no significant effect on fetal guinea pig body weight (NMX = 59.5 ± 7.7 g; n = 9). In addition, no significant differences in placenta weight-to-body weight (NMX = 0.0866 ± 0.007), heart weight-to-body weight (NMX = 0.00693 ± 0.0004), and brain weight-to-body weight (NMX = 0.0351 ± 0.002) ratios were measured among the groups. Maternal hematocrit increased significantly (P < 0.05) after 7-day HPX (43.1 ± 0.7, n = 9; 41.5 ± 1.6, n = 9, for 7 and 10 days) compared with normoxic levels (38.1 ± 1.0, n = 9). There were no differences between NMX and 4-day HPX (39.5 ± 0.9, n = 10). Fetal hematocrits of normoxic fetuses (47.1 ± 2.5) were significantly greater than maternal hematocrits of normoxic mothers. There was a small increase in fetal hematocrit after 4-day HPX (53.3 ± 2.3) compared with NMX, but it was not significant and returned to normoxic levels after 7- (49.0 ± 4.7) and 10-day (47.0 ± 1.7) HPX.

Basal coronary responses of the fetal guinea pig heart. This is the first study using the isolated fetal guinea pig heart preparation for examining the effect of chronic HPX on coronary dilator responses. The isolated heart preparation was stable for the entire length of the experiment with contractile force decreasing by ~10% by the end of the experiment. The mean basal flow rate of normoxic hearts perfused at ~32 mmHg was 4.54 ± 0.31 ml·min⁻¹·g wet wt⁻¹ and did not differ significantly from hypoxic hearts perfused at the same pressure regardless of the duration of hypoxic exposure. In normoxic hearts, PGF₂α infusion increased coronary perfusion pressure, and the calculated resistance increased by 80% from basal levels (7.39 ± 0.58 mmHg·ml⁻¹·min⁻¹·g heart⁻¹). There were no statistical differences in PGF₂α-induced increases in coronary artery resistance among groups before and after L-NA administration. L-NA had no significant effect on basal coronary resistance in any of the groups. This is in contrast to an ~25% increase in resistance with an identical protocol using the isolated constant flow-perfused adult guinea pig heart preparation in the same laboratory (unpublished observation).

Effect of chronic HPX on coronary vasodilation. Acetylcholine decreased coronary artery resistance in a concentration-dependent manner in all fetal hearts. There were no differences in EC₅₀ values [7.4 ± 0.1, 7.4 ± 0.1, 7.5 ± 0.2, 7.40 ± 0.1 for NMX (n = 9), 4-day (n = 10), 7-day (n = 9), and 10-day (n = 9) HPX, respectively] or maximal responses to acetylcholine among either normoxic or hypoxic hearts (Fig. 1A). L-NA inhibited the maximal dilator response but not EC₅₀ values to acetylcholine in all hearts. Figure 1B illustrates responses from NMX and 10-day HPX only. L-NA had the same inhibitory effect on the dose-response curve of hearts exposed to 4- and 7-day HPX as normoxic hearts (data not shown). However, L-NA had a greater inhibitory effect in hearts exposed to 10-day HPX compared with those exposed to NMX, 4-, or 7-day HPX.

Sodium nitroprusside produced concentration-dependent vasodilatation in all hearts (Fig. 2A). Similar to acetylcholine, there were no significant differences in EC₅₀ values or maximal responses among the groups regardless of hypoxic exposure. There was no significant effect of L-NA on sodium nitroprusside-induced dilation in any of the hearts measured. Figure 2B illustrates the response of L-NA to NMX and HPX for 10 days only.

The effect of 4-aminopyridine was measured on a separate series of fetal hearts (Figs. 3 and 4). Both EC₅₀ values and maximal dilator responses to acetylcholine were similar between NMX (n = 7) and 10-day (n = 8) HPX. 4-Aminopyridine completely inhibited the
Acetylcholine-induced dilation at concentrations as low as $10^{-8}$ and $10^{-7}$ M (Fig. 3). In addition, 4-aminopyridine inhibited $10^{-5}$ M acetylcholine response approximately twofold greater than l-NA at the same concentration. However, the inhibitory effect of 4-aminopyridine alone at $10^{-5}$ M acetylcholine was similar to that with l-NA alone at the same concentration. 4-Aminopyridine inhibited the sodium nitroprusside dilation only at the highest ($10^{-4}$ M) concentration tested (Fig. 4). In the presence of l-NA plus 4-aminopyridine (Fig. 5), acetylcholine-induced dilation was completely inhibited at concentrations of $10^{-9}$ to $10^{-6}$ M. At $10^{-5}$ M, the maximal response was inhibited to a level significantly greater than measured with each inhibitor alone.

In a separate series of experiments, glibenclamide had no effect on either EC$_{50}$ values (6.76 ± 0.1 vs. 6.73 ± 0.2, n = 3 for both groups, NMX vs. 10-day HPX, respectively) or maximal dilation to acetylcholine (Fig. 6). In addition, there were no differences in acetylcholine-induced responses between normoxic and hypoxic hearts under control conditions.

**Effect of chronic HPX on fetal guinea pig heart NOS mRNA.** Figure 7 illustrates an autoradiograph of eNOS and β-actin mRNA samples of fetal guinea pig hearts from normoxic and hypoxic (4, 7, and 10 days)
animals. Each lane represents a single fetal heart. mRNA levels were determined by measuring the density of the bands corresponding to each sample by densitometry. eNOS-to-β-actin mRNA ratios were calculated, and the mean values are plotted in the graph. eNOS mRNA levels were significantly increased in fetal guinea pig ventricles by 7 days of HPX and remained elevated in hearts from animals exposed for at least 10 days.

DISCUSSION

Chronic HPX increases the contribution of NO in acetylcholine-induced vasodilation in the fetal guinea pig heart. K^+^-channel activation also contributes to acetylcholine-induced dilation yet is unaffected by chronic HPX. The increase in eNOS mRNA expression of the fetal heart by chronic HPX suggests that NO production by the heart may be upregulated under these conditions as an adaptive response to the hypoxic stress.

Fig. 4. Effect of chronic HPX on sodium nitroprusside-induced dilation of the fetal guinea pig heart in the presence and absence of 4-AP (3 mM). Dilator responses were measured in hearts of animals exposed to NMX and 10-day HPX as a percent maximal dilation to papaverine (10^{-5} M). *P < 0.05 compared with normoxic controls.

Fig. 5. Effect of L-NA plus 4-AP on acetylcholine-induced dilation of fetal guinea pig hearts. Responses to cumulative addition of acetylcholine were measured in normoxic fetal hearts in the presence and absence of L-NA (10^{-4} M) alone and L-NA plus 4-AP (3 mM). *P < 0.05 compared with the untreated control and ‘+’ to L-NA alone.

Fig. 6. Effect of glibenclamide (10^{-5} M) on acetylcholine-induced dilation of the fetal guinea pig heart. Responses were measured as percent vasodilation of fetal hearts exposed to NMX and 10-day HPX. There were no significant differences among the groups.

Effects of HPX on fetal growth and hematocrit.

Chronic HPX does not significantly alter fetal growth under the conditions of our study. Heart weight, brain weight, and placenta weight-to-body weight ratios were not significantly affected by HPX in the present study. Previous studies have shown, however, that fetal guinea pig growth restriction can occur with longer duration of HPX and at earlier gestational ages (5). The lack of growth effect in our study is likely due to a later gestational age and shorter duration of HPX. Fetal hematocrit, although greater than maternal hematocrit under normoxic conditions, did not increase significantly with continued hypoxic exposure. Although this demonstrated that the mother responded to HPX with increased hematocrit, the fetus did not. This may reflect a relatively mild hypoxic stress to the fetus despite significant changes in coronary artery responsiveness.

Effects of HPX on dilator responses.

In the fetal heart, we do not see differences in basal tone among the hypoxic and normoxic groups. Additionally, L-NA does not alter basal tone. This differs from the adult guinea pig heart, which demonstrates a significant increase in coronary resistance (~25%) for the same L-NA concentration as used in the present study. This suggests that in the fetal coronary microcirculation, there may be a maturational difference in basal NO release between the fetal and the adult heart.

Chronic HPX had no significant effect on coronary artery responsiveness to either agonist under control conditions. However, L-NA had a greater inhibitory effect on acetylcholine-induced dilation in hearts exposed to HPX for 10 days compared with NMX and shorter durations (4 and 7 days) of HPX. Because L-NA inhibits NOS, this suggests that chronic HPX after 10 days increases the contribution of NO in acetylcholine-induced dilation. The increase in eNOS mRNA supports the hypothesis that enhanced NO production is mediated by increased eNOS gene expression. Although the magnitude of change in eNOS mRNA seems relatively small, this is similar to that measured in
other studies. For example, eNOS mRNA increased 2.5-fold (31) and 1.6-fold (17) in the adult rat lung in response to HPX, and eNOS mRNA increased 1.6-fold in the fetal sheep brain in response to chronic HPX (1). Thus, although the hypoxic challenge may seem relatively mild, the changes in eNOS mRNA levels in the fetal guinea pig heart are similar to those previously reported.

Previous study has shown that although eNOS mRNA of intact adult rat hearts localizes to both the coronary vascular endothelium and the cardiac endocardium (2), vascular eNOS distribution is more extensive in the fetal heart (33). The presence of eNOS in the coronary artery endothelium is necessary for acetylcholine-induced NO release to modulate coronary tone. Because the acetylcholine-induced dilation is inhibited by L-NA, our results suggest that the effect of L-NA is on the coronary microcirculation rather than the myocardial cells of the fetal guinea pig heart where eNOS could also be expressed (11). If the HPX-induced increase in eNOS mRNA occurs in the coronary endothelium, this would indicate a local vascular response to reduced oxygen levels. Further work is needed to identify the cell type expressing the increased eNOS mRNA of the intact fetal guinea pig heart. Thus enhanced agonist-stimulated NO production by the coronary endothelium via upregulation of eNOS may be an important adaptive response to increase coronary blood flow during an oxygen deficit. Yet, it should also be considered that the increased dependence of the fetal heart on NO under conditions of HPX or acute ischemia could prove maladaptive with reperfusion/reoxygenation at the time of birth due to the enhanced oxidative stress.

Effects of HPX on K\(^{+}\) channel-related dilation. Acetylcholine-induced vasodilation is mediated by multiple substances such as NO as well as EDHF in the adult guinea pig heart (13). However, the mechanism of acetylcholine-induced vasodilation has not been previously studied in the fetal guinea pig heart. Our results demonstrate that acetylcholine-induced vasodilation is mediated by both an NO-dependent mechanism and by K\(^{+}\)-channel activation because the combination of L-NA plus 4-aminopyridine was greater than each treatment alone. Thus acetylcholine may additionally stimulate the release of an EDHF from the fetal guinea pig heart and dilate the microcirculation via activation of K\(^{+}\)-ATP channels. Yet, chronic HPX had no effect on the ability of 4-aminopyridine to inhibit acetylcholine-induced dilation. This is in contrast to L-NA, where chronic HPX after 10 days increased its inhibitory effect. Furthermore, dilation to sodium nitroprusside, an NO donor, is unaffected by 4-aminopyridine (except at the highest concentration of 10\(^{-4}\) M), suggesting that NO does not directly activate K\(^{+}\)-channels in the fetal guinea pig heart. Thus chronic HPX may have a specific effect on altering the contribution of NO independent of K\(^{+}\)-channel activation of vascular smooth muscle. Furthermore, NO production by the fetal coronary endothelium in vivo may be more sensitive than NO sensitivity of vascular smooth muscle to chronic HPX. This suggests a site-specific effect of prolonged hypoxemia in modulating perfusion of the coronary microcirculation.

It should be noted that all coronary responses were measured under conditions of constant flow. Although this preparation has the advantage of testing vascular responsiveness of the coronary microcirculation under well-controlled conditions of drug administration, it does not allow the heart to increase its flow in a physiological manner. As with all in vitro preparations, the data provide information regarding the changes in sensitivity to most treatment effects, but they do not provide a quantitative measure of the absolute change that could occur in vivo. Thus the ability to test the influence of NO in regulating coronary flow in this preparation is limited because flow does not change. Yet, we are still capable of quantifying the reactivity of the microvasculature in the fetal heart and find it to be altered after 10-day hypoxic exposure. We do not know if the relatively small change in coronary responsiveness is due to a limitation of the experimental preparation. Furthermore, we do not know whether the measured hypoxic effect in vitro is reduced by the relatively high in vitro PO\(_2\) levels. An additional consideration, however, is that multiple complex compensatory mechanisms may be occurring simultaneously (both adaptive and maladaptive), resulting in a relatively small change in coronary responsiveness.

Previous studies have reported that chronic exposure to HPX increases maximal flow responses due to
increased vessel growth in the newborn rabbit heart (14) and the fetal sheep heart (24, 25). In the fetal guinea pig heart, HPX increased the NO contribution of acetylcholine-induced vasodilation but not the magnitude of vasodilation. Furthermore, the overall response of hematocrit and organ weight changes of the fetal guinea pig to HPX was modest relative to responses of other species. Whether this is related to the ability of the guinea pig to better tolerate HPX than other species or due to other differences related to gestational age, hypoxic levels, duration of exposure, or even a more efficient placental gas exchange (7) is unknown. Although the wild guinea pig (Cavia lutetiae) is considered well acclimated to high altitude, its domesticated relative, the adult Hartley-Duncan guinea pig, exhibits morphological and hematologic changes to hypoxic stress (6, 8). Thus comparisons of fetal guinea pig responses to those of other species or newborn animals must be made with caution because it is not clear what constitutes the differences among different species. Thus the fetal guinea pig, despite being a relative of a high-altitude species, exhibits an adaptive coronary response to HPX by enhancing the NO contribution to vasodilatation of the microcirculation.

In summary, chronic HPX alters fetal coronary responsiveness by increasing its NO contribution. This may be mediated by an increase in NOS gene transcription because eNOS mRNA was increased. Chronic HPX does not alter the contribution of K⁺-channel activation in mediating coronary dilator responses to acetylcholine. Thus the fetal heart responds to hypoxic stress by increasing the availability of NO for enhancing coronary perfusion to agonist stimulation as an important coronary adaptation to chronic HPX.

Perspectives

Chronic HPX alters the NO contribution of acetylcholine-induced vasodilation but not NO sensitivity of the coronary microcirculation in the fetal guinea pig heart. Our data suggest that this may be mediated by an upregulation of eNOS gene expression by HPX. This altered response is independent of the level of oxygen tension the coronary circulation is exposed to, suggesting a permanent change in the vasculature to HPX. The effect of chronic HPX on coronary responses of the fetal guinea pig heart suggests an adaptive response to reduced oxygen levels in utero. Although this is important for understanding how the fetus responds physiologically to hypoxic stress in utero, it is more critical to understand the long-lasting effects on the coronary circulation postnatally. Recent evidence suggests that fetuses exposed to stress at a critical gestational period may exhibit increased arterial blood pressure in adult life. We propose that chronic HPX may be sufficient to alter gene expression of eNOS and contribute to permanent changes in vascular reactivity. Whether this is manifested postnatally is a critical area that needs to be investigated to understand the impact of fetal programming in the juvenile and the mature adult. This study provides the basis for investigating the effects of chronic HPX in utero as a potential stress for inducing fetal programming in the coronary microcirculation. Furthermore, this animal model provides advantages over other species for studying, longitudinally, the impact of fetal programming on the cardiovascular mechanisms underlying increased arterial blood pressure in the affected adult.

These studies were supported by the National Institutes of Health Grants HL-49999 to L. P. Thompson and HL-49041 and HD-22294 to C. P. Weiner.

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