Chronic hypoxia, pregnancy, and endothelium-mediated relaxation in guinea pig uterine and thoracic arteries

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1Women’s Health Research Center and 3Division of Cardiology and Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Sciences Center, Denver 80262; and 2Department of Obstetrics and Gynecology, Denver Health Medical Center, Denver, Colorado 80204

White, Margueritte Mabry, Robert E. McCullough, Rebecca Dyckes, Alastair D. Robertson, and Lorna Grindlay Moore. Chronic hypoxia, pregnancy, and endothelium-mediated relaxation in guinea pig uterine and thoracic arteries. Am J Physiol Heart Circ Physiol 278: H2069–H2075, 2000.—Vasodilation that occurs during normal pregnancy is associated with enhanced relaxation and decreased contractile response to agonists, which are in part due to increased stimulated and basal nitric oxide (NO). In preeclampsia and/or pregnancies carried at high altitude (HA), this normal vascular adjustment is reversed or diminished. We previously reported that HA exposure did not inhibit the pregnancy-associated decrease in contractile response to agonist or basal NO in guinea pig uterine arteries (UA). We therefore sought to determine whether altitude interfered with effects of pregnancy on endothelium-dependent relaxation through a reduction in stimulated NO. We examined the relaxation response to ACh in UA and bradykinin in thoracic arteries (TA) and effects of NO inhibition with 200 µM N ω-nitro-L-arginine (L-NNA) in arterial rings isolated from nonpregnant and pregnant guinea pigs exposed throughout gestation to low altitude (LA, 1,600 m, n = 26) or HA (3,962 m, n = 22). In pregnant UA, relaxation to ACh was enhanced (P < 0.05) at both altitudes and NO inhibition diminished, but did not reverse, ACh relaxation. The effect of L-NNA on the relaxation response to ACh was less in HA than in LA animals (P = 0.0021). In nonpregnant UA, relaxation to ACh was similar in LA and HA animals. L-NNA reversed the relaxation response to ACh at HA but not at LA. In TA, relaxation to bradykinin was unaltered by pregnancy or altitude and was completely reversed by NO inhibition. These data suggest that effects of NO inhibition are diminished in UA during pregnancy at HA. Additional studies are needed to confirm whether these effects are mediated through inhibition of stimulated NO. HA exposure did not inhibit relaxation to ACh, perhaps because of stimulation of other vasodilators.

nitric oxide; vasoreactivity; preeclampsia

During normal pregnancy, a marked rise in blood flow to the uterine and other circulations is associated with decreased vasoconstrictor and increased vasodilator responses. Studies in whole animals and isolated vessel rings have demonstrated that pregnancy decreases contractile sensitivity to α-adrenergic stimulation and enhances endothelium-dependent vasodilation in certain vascular beds (21, 23, 26). These effects are due in part to increased basal and stimulated endothelium-derived nitric oxide (NO) (3, 6, 22, 26).

Preeclamptic women demonstrate an absence or reversal of the normal diminution in blood pressure and greater vasoconstrictor response to ANG II, suggesting that preeclampsia interferes with the normal vascular adjustment to pregnancy (28). Several lines of evidence suggest that residence at high altitude also interferes with the normal vascular adjustment to pregnancy. Pregnancies of women residing at high altitude are complicated by intrauterine growth retardation, reduced uterine artery blood flow, and a fourfold increase in the incidence of preeclampsia (27).

Mechanisms by which high-altitude exposure opposes the effects of pregnancy on vascular reactivity are unknown. We previously reported that pregnancy reduced the contractile response to phenylephrine (PE) similarly in uterine artery rings isolated from guinea pigs subjected to gestation at low and high altitude (3,962 m) (26). Furthermore, the pregnancy-associated increase in uterine artery basal NO was equivalent at both altitudes. We therefore hypothesized that the effects of high altitude on vascular reactivity during pregnancy are mediated through inhibition of endothelial-dependent relaxation due, in part, to decreased stimulated NO. Effects of high altitude on NO production or activity are unclear. Studies in cultured endothelial cells suggest that the effects of chronic hypoxia are mediated at the level of transcription and/or translation of endothelial NO synthase (NOS 3), but the results are conflicting, demonstrating an inhibitory and a stimulatory effect of hypoxia on gene and protein expression (1, 5, 15, 19, 20). How hypoxia may alter effects of pregnancy on NO production or activity is unknown.

We studied uterine and thoracic arterial rings from nonpregnant or near-term pregnant guinea pigs housed at low (1,600 m) and simulated high (3,962 m) altitude. We chose the guinea pig as a model with which to study effects of high-altitude exposure on the basis of our previous studies demonstrating increased systemic vascular resistance response to ANG II in high- compared with low-altitude animals (9). Relaxation to ACh was measured in vessels preconstricted with PE in the presence and absence of N ω-nitro-L-arginine (L-NNA),
an inhibitor of NO synthesis. We reasoned that these studies would be informative as to whether high altitude interfered with the normal vascular adjustment to pregnancy through alterations in stimulated NO in uterine and nonuterine vessels.

METHODS

Animals. Studies were performed in near-term, pregnant (55–63 days gestation) and nonpregnant female Hartley guinea pigs (Sasco, Omaha, NE). Pregnancy duration (full term = 63 days) was calculated as the number of days after conception as judged by the appearance of a vaginal plug and confirmed by fetal assessment with a published nomogram. A total of 26 (10 nonpregnant and 16 pregnant) animals were maintained at low altitude (the laboratory altitude of 1,600 m), and 22 (13 nonpregnant and 9 pregnant) animals were kept in a hypobaric chamber at a simulated high altitude (3,962 m). Animals were placed in the altitude chamber within 3–5 days of conception and remained at altitude throughout gestation except for brief (<30 min), triweekly descents as required for cage cleaning.

Vessel preparation. Vessel segments 2 mm long were cut from the main uterine artery and the thoracic aorta. All vessels were carefully dissected free of connective tissue and fat. Outer diameter before mounting was measured with a calibrated ruler through a dissecting microscope (model M7A, Leica, Zurich, Switzerland). A minimum of two uterine artery vessels were carefully dissected free of connective tissue and from the main uterine artery and the thoracic aorta. A minimum of two segments per animal were cut from each vessel, and all descents as required for cage cleaning.

RESULTS

Effects of high-altitude exposure in the nonpregnant vessels. In the nonpregnant uterine artery and thoracic aorta, the dose-dependent relaxation to ACh or bradykinin was similar in vessels from high- and low-altitude nonpregnant animals. Addition of the NO inhibitor L-NNA diminished the relaxation response to ACh in the nonpregnant uterine artery at high (Fig. 1B; P = 0.0001) but not at low altitude (Fig. 1A). There was a significant interaction between the effects of altitude and L-NNA on the relaxation response in the nonpregnant uterine artery, consistent with a greater effect of L-NNA at high than at low altitude (P = 0.001).

In the nonpregnant thoracic aorta, L-NNA eliminated the bradykinin relaxation response at both altitudes (Fig. 1, C and D; P = 0.0001).

Effects of high-altitude exposure in the pregnant vessels. Dose-dependent relaxation to ACh was similar in uterine arteries from pregnant high- and low-altitude animals. Pregnancy enhanced the ACh relaxation response in the uterine artery at high and low altitude (P < 0.0001 and P < 0.01, respectively; Fig. 2, A and B). Pregnancy did not alter bradykinin-induced relaxation in the thoracic aorta at either altitude (Fig. 2, C and D).

L-NNA diminished, but did not eliminate, ACh relaxation in the pregnant uterine artery at both altitudes (Fig. 3, A and B; P < 0.0001 for high altitude and P < 0.01 for low altitude). There was significant interaction between altitude and L-NNA in pregnant uterine artery from high-altitude animals, supporting a decreased effect of L-NNA in the pregnant high- vs. low-altitude uterine artery (P < 0.0021; Fig. 3, A and B). Also consistent with this finding, L-NNA abolished the difference in ACh relaxation between pregnant and nonpregnant uterine arteries at low (Fig. 4A) but not at high altitude (Fig. 4B).

In the pregnant thoracic aorta, NO inhibition completely reversed bradykinin-induced relaxation at high and low altitude (Fig. 5, A and B).
DISCUSSION

We sought to determine whether the stimulatory effect of pregnancy on endothelium-dependent relaxation in the isolated guinea pig uterine artery was decreased at high altitude. Our results suggest that the stimulatory effect of pregnancy on NO was diminished at high compared with low altitude, as judged by the effect of NO inhibition on the relaxation response to ACh. However, altitude did not impair the effect of pregnancy on the relaxation response or the maximum relaxation to ACh in isolated uterine arteries, suggesting that some other vasodilator may have compensated for the diminution in NO, with the result that ACh relaxation was unchanged. Altitude exposure was associated with an increased effect of NO inhibition in the uterine artery from nonpregnant animals, whereas in the thoracic aorta the effect of NO inhibition was unaltered by pregnancy or altitude.

Effects of chronic hypoxia on NO-mediated relaxation in the uterine artery. We based our hypothesis that chronic hypoxia opposes the normal vascular adjustment to pregnancy on our previous studies demonstrating increased systemic vascular resistance and response to ANG II in pregnant guinea pigs subjected to gestation at high altitude (9) and decreased uterine artery blood flow in pregnant women residing at high altitude (29). These factors may contribute to the increased frequency of intrauterine growth retardation and preeclampsia previously reported at high altitude (28). A possible explanation for such a decrease in uterine artery blood flow could be diminished vasodilation in the uterine artery and/or other arteries during pregnancy at altitude. Because NO is implicated in the increased vasodilatation in the uterus and other circulations during normal pregnancy in this and other studies (3, 14, 16, 26), we hypothesized that high-altitude exposure led to decreased NO stimulation and reduced ACh relaxation in the uterine artery during pregnancy. In support of this hypothesis, we found that L-NNA treatment had less inhibitory effect on the relaxation response to ACh in the uterine artery from pregnant high- than low-altitude animals. One limitation of our study design is our use of a pharmacological method of assessing NO by competitive inhibition of NOS 3 with
Fig. 2. Pregnancy enhanced relaxation to ACh in uterine artery at both altitudes, but relaxation to bradykinin was not altered in thoracic aorta at either altitude. A and C: low altitude; B and D: high altitude. P values indicate comparisons of relaxation dose-response curves between nonpregnant and pregnant groups by nonlinear regression analyses. Solid symbols, nonpregnant vessels; open symbols, pregnant vessels; circles, low-altitude vessels; triangles, high-altitude vessels.

Fig. 3. Addition of 200 µM L-NNA to vessel bath diminished relaxation response to ACh in uterine artery from low (A) and high-altitude (B) pregnant animals, but effect of L-NNA was diminished at high altitude. P values indicate comparisons of relaxation dose-response curves between vehicle (without L-NNA) and L-NNA-treated (with L-NNA) groups by nonlinear regression analyses. Dashed lines connect values from vessels treated with L-NNA; solid lines connect values from vessels without L-NNA. ○, Low-altitude vessels; ▲, high-altitude vessels.
L-NNA. We did not directly measure NO production or NOS 3 enzyme activity, and we did not control for indirect effects of L-NNA on cellular functions that may decrease enzyme activity (4). However, L-NNA is among several L-arginine analogs shown to inhibit NOS 3 enzyme activity and, therefore, NO production (7). That the effect of L-NNA was due to indirect effects on cellular functions is less likely because of the vessel specificity of our findings; i.e., a decreased effect of NO inhibition was seen only in uterine arteries from high-altitude animals and not in vessels from other treatment groups. To our knowledge, there are no previous studies examining the effects of high altitude on NO-mediated relaxation in guinea pig uterine arteries during pregnancy, but hypoxic inhibition of NOS 3 transcript and protein has been reported in cultured bovine aortic and human umbilical vein endothelial cells (15, 19). Our previous report of decreased NOS 3 protein expression by Western blot analysis in uterine arteries from high- vs. low-altitude animals supports an inhibitory effect of altitude on NOS 3 (25). Taken together, although these findings suggest that the stimulatory effect of pregnancy on NO is inhibited in uterine arteries from pregnant animals exposed to high altitude, additional studies measuring effects of high-altitude exposure on NO production and/or enzyme activity are needed.

Contrary to our hypothesis, decreased stimulatory effect of pregnancy on NO was not associated with impaired relaxation to ACh in uterine arteries from high- compared with low-altitude pregnant animals. One potential explanation for failure to detect a significant difference in relaxation response at altitude may involve the smaller number of animals studied in the high (n = 9)- than in the low (n = 16)-altitude pregnant group. This was primarily due to the exclusion of a greater number of studies from the high-altitude group because of failure to satisfy one or more of the inclusion criteria.

**Fig. 4.** Addition of 200 µM L-NNA abolished difference in relaxation response to ACh between pregnant and nonpregnant uterine arteries at low (A) but not at high altitude (B). P values indicate comparisons of relaxation dose-response curves between vehicle (without L-NNA) and L-NNA-treated (with L-NNA) groups by nonlinear regression analyses. Solid symbols, nonpregnant vessels; open symbols, pregnant vessels. Dashed lines connect values from vessels treated with L-NNA. Circles, low-altitude vessels; triangles, high-altitude vessels.

**Fig. 5.** Addition of 200 µM L-NNA reversed relaxation to bradykinin in pregnant thoracic artery at high (B) and low (A) altitude. Dashed lines connect values from vessels treated with L-NNA; solid lines connect values from vessels without L-NNA. O, Low-altitude vessels; Δ, high-altitude vessels.
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criteria (see METHODS). Alternatively, in the presence of reduced NO, high-altitude exposure may stimulate the release of other endothelial dilators during pregnancy that compensated for decreased NO and resulted in a similar augmentation of ACh relaxation in the pregnant high- and low-altitude animals. In the guinea pig basilar artery, for instance, the contribution of endothelium-derived hyperpolarizing factor (EDHF) and NO to ACh-mediated vasodilation is altered by acute hypoxia, such that EDHF alone mediates ACh relaxation (18). However, although some observations suggest that EDHF may play a role in vasodilation during pregnancy (13, 24), this remains to be determined. Our finding that NO inhibition diminished but did not completely reverse relaxation in uterine arteries from pregnant animals at both altitudes supports a role for other ACh-mediated dilators. However, additional studies need to be performed to determine which endothelial factors may be contributing to uterine artery relaxation during pregnancy and whether their effects are enhanced by high-altitude exposure. Finally, we did not assess effects of flow or other physiological agonists on NO-mediated relaxation. Studies in subcutaneous arteries from pregnant women show enhanced flow-mediated relaxation due to NO that is diminished in vessels from preeclamptic women (2). Thus flow-induced dilation may be a more sensitive measure of reduced NO effect than agonist-induced dilation.

A number of studies have suggested an inhibitory effect of low Po2 on NO production. Extreme chronic hypoxia and moderate levels of acute hypoxia have been shown to inhibit endothelium-dependent relaxation in rat and rabbit pulmonary arterial rings (4, 12). Subsequent studies suggest that hypoxia alters NOS 3 transcription and translation, but the results are conflicting. In cultured endothelial cells, severe hypoxia for up to 24 h has been associated with increased (1, 5, 20) and decreased (15, 19) NOS transcript, protein levels, and activity. These discrepant findings may be explained by variability in the types of endothelial cells studied as well as the duration and degree of hypoxia. In support of our observation of decreased NO effect at altitude, it was demonstrated that exposure of human umbilical vein and bovine endothelial cells to physiologically low O2 levels, similar to those obtained in our guinea pigs housed at altitude, decreased NOS mRNA (9), protein levels, and bradykinin-induced NO release (19).

Prolonged exposure to high altitude may also modulate receptor function and/or postreceptor intracellular pathways during pregnancy that lead to NO release. ACh and bradykinin induce vascular endothelial cells to make and release NO, which in turn leads to vascular smooth muscle cell relaxation. Binding of these agonists to their G protein-linked receptor activates an intracellular pathway that leads to intracellular Ca2+ release, which in turn catalyzes the formation of NO. Studies in isolated uterine arteries from pregnant sheep suggest that chronic hypoxia decreases α1-receptor density and ligand-binding affinity (10), resulting in decreased contractile response to α1-receptor stimulation, but whether ACh receptors are similarly affected is unknown. Our findings that uterine arteries from pregnant high- and low-altitude animals relaxed similarly to ACh, however, do not support a reduction in ACh receptor number or affinity. Other studies have suggested an effect of chronic hypoxia on intracellular Ca2+ homeostasis. Zhang and Xiao (30) reported decreased inositol trisphosphate synthesis and a reduced surge in intracellular Ca2+ in uterine artery vascular smooth muscle cells from pregnant sheep exposed to high vs. low altitude. Although these results were reported for smooth muscle cells, hypoxic inhibition of endothelial intracellular Ca2+ may also decrease activity of the Ca2+-calmodulin-dependent enzyme NOS. In the nonpregnant uterine artery, NO was enhanced in vessels from high- vs. low-altitude animals, a finding consistent with effects of hypoxia in other circulations independent of pregnancy (11, 20). Furthermore, ACh-induced relaxation was similar in vessels at both altitudes and appeared wholly dependent on increased NO. We previously reported enhancement of uterine artery basal NO in nonpregnant animals exposed to high altitude that did not alter contractile response to agonist stimulation (26). Taken together, these observations suggest that, in the nonpregnant state, similar uterine artery responsiveness to agonist stimulation in vessels from low- and high-altitude animals is due to hypoxic stimulation of NO at altitude. It is tempting to speculate that this stimulation of NO in the nonpregnant uterine artery at high altitude may limit the effect of pregnancy on further NO stimulation.

Effects of chronic hypoxia on NO-mediated relaxation in the thoracic aorta. In the thoracic aorta, our data suggest that the relaxation response to bradykinin is not influenced by pregnancy or high-altitude exposure, although pregnancy has been shown to enhance relaxation to ACh in the thoracic aorta in another report (8). This may reflect differential effects of bradykinin vs. ACh on receptor-mediated vasodilation, as has been suggested by observations in omental arteries from normotensive and preeclamptic women (17). Alternatively, given that, within the thoracic aorta, sensitivity to agonist-induced vasodilation increases in the more distal segment (8), the lack of a pregnancy effect in the thoracic aorta in our studies may be a result of sampling the more proximal segment of the vessel. Contrary to our findings in the uterine artery, bradykinin-induced relaxation appeared to be entirely due to NO. Consistent with other reports (8), our data support a varying effect of pregnancy and high-altitude exposure on NO-mediated relaxation among vascular beds, although these differences in uterine and thoracic responses may be due to differences in ACh and bradykinin receptors.

In summary, the pregnancy-associated increase in stimulated NO is attenuated in uterine arteries from high-altitude animals. However, this decrease in NO during pregnancy does not inhibit ACh-induced relaxation in high-altitude vessels, suggesting a potential role for other dilators that may function in a compensatory fashion. High-altitude exposure appears to stimu-
late NO in the uterine artery from nonpregnant animals, which may limit the effects of pregnancy on NO-induced vasodilation.

Doug Curran-Everett and Don Ellis (Spiderwort Design, Colorado Springs, CO) provided technical assistance in developing the vessel bath modifications.

This work was supported by National Heart, Lung, and Blood Institute Research Grants HL-14985 and HL-07171.

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Received 20 April 1999; accepted in final form 7 December 1999.

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