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Effects of Chronic Hypoxia on Maternal Vasodilation and Vascular Reactivity in Guinea Pig and Ovine Pregnancy

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ABSTRACT

White, Margueritte M., and Lubo Zhang. Effects of chronic hypoxia on maternal vasodilation and vascular reactivity in guinea pig and ovine pregnancy. *High Alt Med Biol* 4:157–169, 2003.—During pregnancy, exposure to chronic hypoxia is thought to be associated with an increased risk of preeclampsia and fetal intrauterine growth restriction (IUGR). While some studies suggest that this process may be mediated through effects of chronic hypoxia on uterine artery vasodilation and growth, these observations are likely to be species specific and may represent genetic variability in maternal adaptation to hypoxia. This review is a comparative analysis of the effects of chronic hypoxia on vascular reactivity in pregnant and nonpregnant guinea pig and sheep. Data suggest that exposure to chronic hypoxia is associated with enhanced uterine artery blood flow in the sheep, whereas, in the guinea pig, blood flow is decreased.

Key Words: high altitude; vascular function; animal models; comparative analysis

INTRODUCTION

HYPOXIA IS A COMMON stress that affects an organism's homeostasis. Although much is known of the mechanisms of cellular and biochemical responses to acute hypoxia, relatively little is known of the mechanisms underlying responses to prolonged or chronic hypoxia. During pregnancy, exposure to chronic hypoxia is thought to be associated with an increased risk of preeclampsia and fetal intrauterine growth restriction (IUGR) (Moore et al., 1982; Zamudio et al., 1995a). Studies in humans have suggested that exposure to chronic hypoxia during pregnancy may inhibit the normal pregnancy-mediated vasodilation and remodeling that characterize the uteroplacental circulation. For instance, in pregnant women residing at high altitude (3100 m) throughout pregnancy, uterine blood flow at 36 weeks is decreased in comparison with those at low altitude (the Denver altitude of 1600 m), primarily due to a decrease in vessel diameter (Zamudio et al., 1995b). This reduction in vessel diameter may result in part from hypoxic inhibition of the normal remodeling that occurs in the uterine artery during pregnancy, as suggested by studies in pregnant guinea pigs. Uterine arteries from animals exposed to chronic hypoxia (3962 m) for their entire gestation (63 days) had a diminished growth response as measured by DNA synthesis com-

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pared to their low altitude counterparts (Keyes et al., 1997).

The regulation of uterine blood flow is crucial for growth and survival of the fetus and for maternal cardiovascular well-being. However, the adaptive mechanisms of the uterine vasculature to chronic hypoxia during pregnancy are likely to be species dependent and may represent interspecies variability in genetic adaptation to high altitude. For example, while the cardiovascular adaptation to normal pregnancy is similar for the guinea pig and sheep, in the former, maternal exposure to chronic hypoxia is associated with growth retardation (Rockwell et al., 2000), which is not evident in the sheep after similar exposure (Kamitomo, 1992). The purpose of this review is to further explore interspecies differences in maternal adaptation to chronic hypoxia by presenting a comparative analysis of the effects of chronic hypoxia on vascular function in both the uterine and nonuterine circulations in the guinea pig and sheep. Data are presented from a variety of study preparations, including whole animal, isolated vessels, and endothelial cell cultures.

EFFECTS OF PREGNANCY AND CHRONIC HYPOXIA ON CARDIOVASCULAR PHYSIOLOGY IN THE GUINEA PIG AND SHEEP

Whole-animal studies

Both ovine and guinea pig pregnancies are characterized by changes in cardiovascular physiology that mirror certain alterations in human pregnancy. In the guinea pig, pregnancy increased cardiac output and uteroplacental blood flow in conjunction with a marked decrease in systemic vascular resistance (SVR) (Curran-Everett et al., 1991). The increase in uteroplacental blood flow was accounted for by similar corresponding increases in cardiac output of 43% and 35% in the guinea pig and sheep, respectively (Rosenfeld, 1977; Curran-Everett et al., 1991). In both guinea pig and sheep, the cardiac output increases progressively and reaches the maximum in late gestation (Rosenfeld, 1977; Myers and Hsui-Yu, 1985). In evaluating regional contributions to the pregnancy-associated decrease in SVR, Curran-Everett concluded that most (70%) of the decrease in SVR in the pregnant guinea pig was attributed to decreased SVR of the nonuteroplacental circulation (Curran-Everett et al., 1991).

A hallmark of human pregnancy is the well-characterized decrease in vasoconstrictor response to vasopressors such as angiotensin II (AII) (Benjamin et al., 1991; Ramsay et al., 1992). Early studies in catheterized guinea pigs and sheep demonstrated that the nonuteroplacental conductance response to AII (measured as blood flow) is increased in pregnant compared to nonpregnant animals, supporting a refractoriness to the constrictor effects of AII (Annibale et al., 1989; Curran-Everett et al., 1991; Hariharan et al., 1987) . In the guinea pig, pregnancy did not affect conductance response to AII in the uteroplacental circulation, whereas in sheep the uteroplacental conductance was slightly decreased in response to AII, albeit less than the rest of the maternal circulation (Magness and Rosenfeld, 1989). However, despite the decrease noted in sheep, overall uteroplacental blood flow was preserved in both sheep and guinea pig during AII infusion.

Studies in guinea pigs have evaluated the effect of chronic hypoxia on several aspects of these normal systemic vascular changes during pregnancy. In these studies, low altitude is defined as the Denver altitude of 1600 m. Pregnant and nonpregnant guinea pigs maintained at low (1600 m) (Pa_{O_2} 70 mmHg) and high (3962 m) (Pa_{O_2} 35 mmHg) altitude for 6 weeks or the entire length of gestation were catheterized and, after full recovery, studied in a quiet awake state. Cardiac output, blood pressure, and heart rate were monitored at baseline and in response to a slow infusion of vasoactive substances. In the nonpregnant animals, there was no difference in SVR or change in SVR response to PE or AII between the normoxic and hypoxic group (Harrison et al., 1986). Addition of the prostaglandin inhibitor meclofenamate increased the SVR response to PE, but had no effect on the response to AII, suggesting that in the nonpregnant guinea pig chronic hypoxia enhanced the production of dilator prostaglandins during α-adrenergic stimulation.

In pregnant guinea pigs exposed to chronic hypoxia, baseline SVR was increased compared to normoxic pregnant animals (Harrison and Moore, 1990). However, chronic hypoxia did not alter the SVR response to AII. Addition of meclofenamate did not equalize the difference in SVR between low and high altitude pregnant animals, suggesting that during pregnancy chronic hypoxia does not inhibit dilating prostaglandin production. Results from these study preparations suggest that in the guinea pig, while chronic hypoxia increases SVR during pregnancy, this effect is mediated through mechanisms other than increased sensitivity to PE or AII or through an inhibition of vasodilating prostaglandins.

Maternal responses to long-term hypoxemia were studied in chronically catheterized sheep at 110 to 115 days gestation (term 140 days) and normoxic controls (Kitanaka et al., 1989). Arterial P_{O_2} was maintained at about 60 mmHg for up to 28 days. In previous studies in pregnant sheep, exposure to high altitude (3820 m) from 30 to 120 days gestation was shown to decrease maternal P_{O_2} from 102 to 64 mmHg (Kamitomo, 1992). In hypoxic pregnant ewes, there was a significant decrease in cardiac output by 14% throughout the experimental period. Within the first 24 h of the onset of hypoxemia, uterine blood flow decreased about 15% from 1180 to 990 mL/min. Thereafter it increased significantly to 1360 mL/min by day 21 of hypoxemia. Thus, during pregnancy, chronic hypoxemia is associated with increased SVR in the guinea pig. In the sheep, hypoxia was associated with a gradual increase in uterine artery blood flow.

Isolated vessel rings studies

To further characterize the effects of pregnancy and hypoxic exposure on vascular function and to determine regional variability, numerous studies have been carried out in isolated rings. Through measurement of isometric tension, it has been consistently demonstrated in the guinea pig uterine artery that pregnancy decreases the contractile response to a number of agonists, including PE, norepinephrine (NE), epinephrine (EPI), serotonin and the thromboxane analog U46619 (Weiner et al., 1989; Weiner et al., 1992a; Weiner et al., 1992b; Kim et al., 1994; White et al., 1998). Effects of pregnancy have also been noted in nonuterine vessels, though results vary depending on the pharmacologic agent employed, as well as vessel type and size. While the carotid and mesenteric artery segments from pregnant animals showed a decrease in the contractile response to serotonin and the thromboxane mimetic U46619, respectively, (Weiner et al., 1992b), no effect of pregnancy was noted in thoracic and mesenteric artery response to PE (White et al., 1998). Pregnancy has also been shown to enhance relaxation to acetylcholine (ACH), A23187, and sodium nitroprusside (SNP) in the guinea pig uterine artery, suggesting an effect on receptor- and nonreceptor-mediated, endothelium-dependent relaxation, as well as downstream signals in the smooth muscle (Weiner et al., 1991; White et al., 2000). In an attempt to create a more physiologic preparation, a dual pipette system was used in which both ends of a vessel are cannulated and flow through the vessel can be carefully controlled (Sillau et al., 2002). Under these conditions, uterine artery segments from pregnant guinea pigs showed diminished contractile sensitivity to PE and enhanced vasodilator response to ACH and to flow when preconstricted with PE (Mateev et al., 2002).

In sheep, uterine artery relaxation to A23187 was enhanced during pregnancy (Xiao et al., 2001b). In these studies, however, unlike in the guinea pig, uterine artery relaxation to SNP was unaltered by pregnancy. Further evaluation of pregnancy-related alterations in smooth muscle cell function in sheep have revealed that, although pregnancy increases endothelial-mediated relaxation and decreases protein kinase C-mediated contraction in the uterine artery, it paradoxically increases acute contractile response of the uterine artery to adrenergic stimulation. During ovine pregnancy, the uterine artery when denuded of endothelium was less responsive to the circulating vasoconstrictor angiotensin II, but increased its contractile sensitivity by threefold to α-agonists (Annibale et al., 1989; Xiao and Zhang, 2002). Similarly, uterine arteries from late pregnant rats increased the contractile response to α-adrenergic stimulation (D'Angelo and Osol, 1993, 1994). These

studies suggest that in sheep and other species decreased vascular tone in the uterine artery in pregnancy is accompanied by an increase in vasoconstriction reserve and contractile capability to α-agonists in the smooth muscle cell.

Chronic hypoxia has been shown to alter responses to agonist stimulation in uterine and nonuterine isolated vessel preparations, but the effects are quite different in the guinea pig and sheep and vary among vascular beds even within the same species. In evaluating changes in isometric tension, chronic hypoxic exposure did not alter the pregnancy-associated decrease in contractile response to PE in term guinea pig uterine artery segments (White et al., 1998). Furthermore, though the pregnancy-associated enhanced relaxation to ACH was less in hypoxic compared to normoxic pregnant animals, this difference did not reach statistical significance (White et al., 2000) (Fig. 1). In more recent studies using the

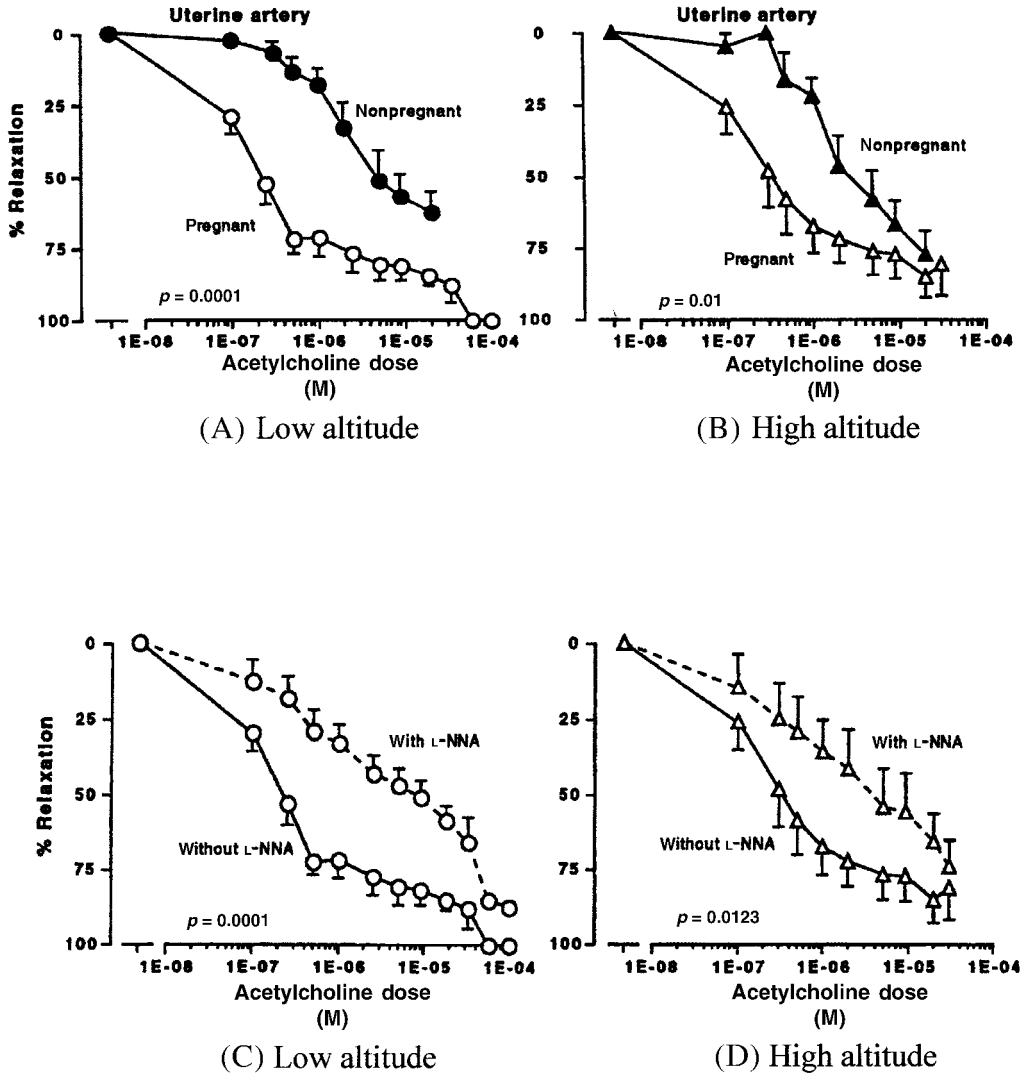

FIG. 1. Pregnancy-enhanced relaxation to Ach in the guinea pig uterine artery at both altitudes: (A) low altitude; (B) high altitude. *p*-Values indicate comparisons of relaxation dose–response curves between nonpregnant and pregnant groups by nonlinear regression analyses. Solid symbols, nonpregnant vessels; open symbols, pregnant vessels. Circles, low altitude vessels; triangles, high altitude vessels. Addition of 200-μM L-NNA to vessel bath diminished relaxation response to Ach in the uterine artery from (C) low altitude and (D) high altitude pregnant animals, but the 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; Δ, high altitude vessels (n = 10 low altitude nonpregnant; n = 16 low altitude pregnant; n = 13 high altitude nonpregnant; n = 9 high altitude pregnant). (Reprinted with permission from White et al., 2000.)

cannulated vessel preparation described above, chronic hypoxia similarly did not alter contractile sensitivity to PE or inhibit relaxation to ACH (Mateev et al., 2002) in uterine artery segments from mid-gestation animals (day 30). However, uterine artery segments from hypoxic pregnant animals exhibited a decreased vasodilator response (Fig. 2) and increased wall stress to flow compared to their normoxic counterparts. Unknown is whether, in the latter preparation, the effects of hypoxia on responses to PE and ACH would have been similar in uterine arteries from term as opposed to mid-gestation animals; however, these studies suggest that local mechanisms controlling blood flow are potentially important targets through which chronic hypoxia inhibits uterine artery blood flow in pregnant women at high altitude (Zamudio et al., 1995b). Responses in the nonuterine circulation are differentially altered by chronic hypoxia such that the middle cerebral artery exhibits increased contractile response to the thromboxane mimetic U-46619 (Sillau et al., 2002), while the mesenteric artery demonstrates decreased contractile response to PE (White et al., 1998).

In pregnant ewes exposed to chronic hypoxia (3820 m) from day 30 to approximately day 140 of gestation (maternal Pa_{O_2} values decreased from 102 ± 2 to 64 ± 2), uterine artery segments exhibited decreased contractile response to a number of agonists, such as 5-HT and NE (Fig. 3), and enhanced relaxation to A23187 in precontracted vessels (Hu et al., 1996b; Hu and Zhang, 1997; Xiao et al., 2001c). The decreased contractile response was attributed to decreased α1-adrenergic and 5-HT receptor density and agonist binding affinity in uterine arteries from hypoxic versus normoxic animals (Hu et al., 1996a). In pregnant sheep, in nonuterine circulations such as cerebral vessels, chronic hypoxia enhanced endothelium-dependent relaxation to A23187 similar to effects in the uterine artery (Longo et al., 1993). Unlike the uterine artery, however, in these studies cerebral arteries from hypoxic sheep demonstrated decreased relaxation to SNAP, whereas hypoxia had no effect on SNAP or 8-Br-cGMP-mediated relaxation in the uterine artery (Xiao et al., 2001a). Because SNAP directly releases NO, which in turn stimulates vascular guanylate cyclase and cGMP synthesis, these results suggest that chronic hypoxia may affect some component of the cGMP relaxation pathway in cerebral but not uterine arteries (Xiao et al., 2001a).

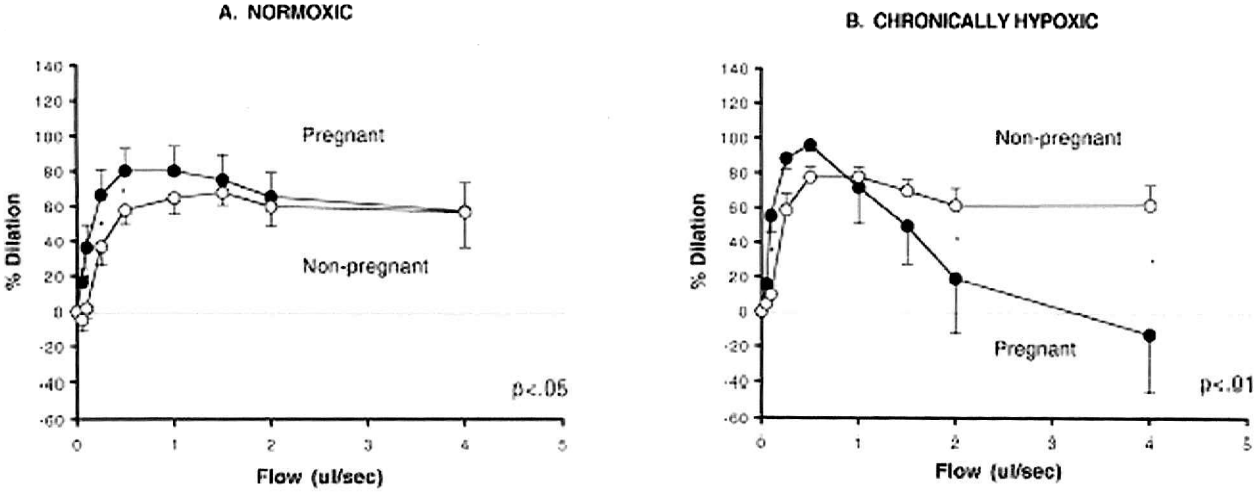

FIG. 2. The percent maximal vasodilation at each level of flow was measured in uterine arteries (UtA) 50% maximally preconstricted with phenylephrine from pregnant (closed circles) and nonpregnant (open circles) guinea pigs that had been housed under (A) normoxic and (B) chronically hypoxic conditions. Pregnancy increased UtA flow vasodilation in the normoxic group animals, but the vasodilator response to flow was not sustained in the vessels from chronically hypoxic animals, resulting in net vasoconstriction at high flow. *p*-values refer to comparisons between groups using a linear mixed effects modeling approach, which accounts for variability between animals as well as multiple measurements on the same animal. * = $p < 0.05$ for specified comparisons. $n = 7$ low altitude nonpregnant, 5 low altitude pregnant; $n = 6$ high altitude nonpregnant, 6 high altitude pregnant). (Reprinted with permission from Mateev et al., 2002.)

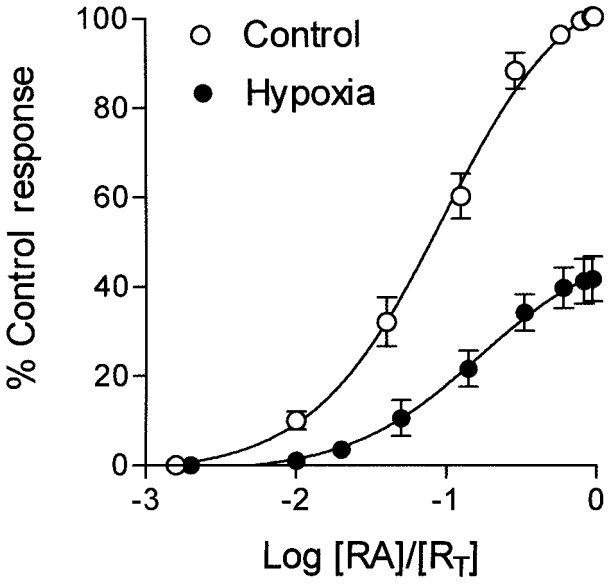

FIG. 3. Pregnant (day 30) sheep were divided between normoxic control and chronic hypoxic (maintained at high altitude, 3820 m, Pa_{O_2}: ~60 mmHg for 110 days) groups ($n = 7$ per group). The fourth branch of main uterine arteries was obtained from near-term (140 day) pregnant sheep, and isometric contractions by norepinephrine were measured. The fraction of α_1-adrenoceptors occupied at each NE concentration ([RA]/[R_T]) was calculated by Furchgott's method. The relative intrinsic efficacy of NE was obtained from the antilog of the distance between the two curves along the abscissa at 30% of the maximal control response. The relative intrinsic efficacy of NE in chronic hypoxic, as compared with control arteries, was 14%.

In summary, data from guinea pig isolated vessels suggest that the effects of chronic hypoxia are primarily aimed at inhibiting flow-mediated relaxation, with no significant effects on agonist-induced contractile or relaxation responses. In sheep, however, the net effect of chronic hypoxia appears to be enhanced uterine artery relaxation as evidenced by a decrease in contractile and an increase in relaxation responses to a number of agonists.

MECHANISM UNDERLYING EFFECTS OF PREGNANCY AND CHRONIC HYPOXIA ON MATERNAL VASCULAR ADAPTATION

Role of the endothelium

Nitric oxide (NO) is a potent endothelium-derived vasodilator whose role in the control of vascular tone and reactivity has been extensively studied in a number of vascular beds. The contributions of NO to the enhanced relaxation and depressed contractile responses during pregnancy vary among vascular beds and with specific agonists employed. In the guinea pig uterine artery, addition of the NOS inhibitor NLA abolished the difference in contractile response to PE between term pregnant and nonpregnant vessels, suggesting that basal NO accounted for the pregnancy-associated decrease in contractile response to PE (White et al., 1998). However, NLA did not completely reverse the relaxation response to ACH in the pregnant vessels, implicating a role for additional vasodilators to the enhanced endothelium-dependent relaxation during pregnancy (White et al., 2000). In recent flow studies, while NLA decreased the dilator response to flow, considerable residual vasodilation remained after both NO and cyclooxygenase inhibition, implicating another dilator, such as EDHF, as contributing to flow-mediated vasodilation (Mateev et al., 2002). Even within the same vessel, results vary depending on the agonist employed. While NO partially contributes to the decreased contractile response of guinea pig uterine arteries to thromboxane (Weiner et al., 1992a), it is not responsible for the decreased sensitivity to serotonin observed in uterine and carotid arteries (Weiner et al., 1992b).

In sheep, several additional lines of evidence support the importance of NO in pregnancy-mediated vascular alterations. Plasma nitrate concentrations as determined by a chemiluminescence assay in pregnant ewes were significantly elevated compared with those of nonpregnant ewes (Zhang et al., 1998) (Fig. 4A). In perfused ovine uterine arteries (Xiao et al., 1999), basal NO release was significantly higher in pregnant than in nonpregnant uterine arteries. The agonist (A23187 and ATP)-induced NO release was also significantly enhanced in the pregnant uterine artery. Subsequent studies point to a mechanism through which pregnancy increases NO that may involve alterations in $[Ca^{2+}]$ homeostasis (Xiao et al., 2001c). Simultaneous measurement of tension and $[Ca^{2+}]_i$ in smooth muscle demonstrated a linear correlation with the slope of unity between A23187-induced relaxation and the reduction of $[Ca^{2+}]_i$ in both nonpregnant and pregnant uterine arteries.

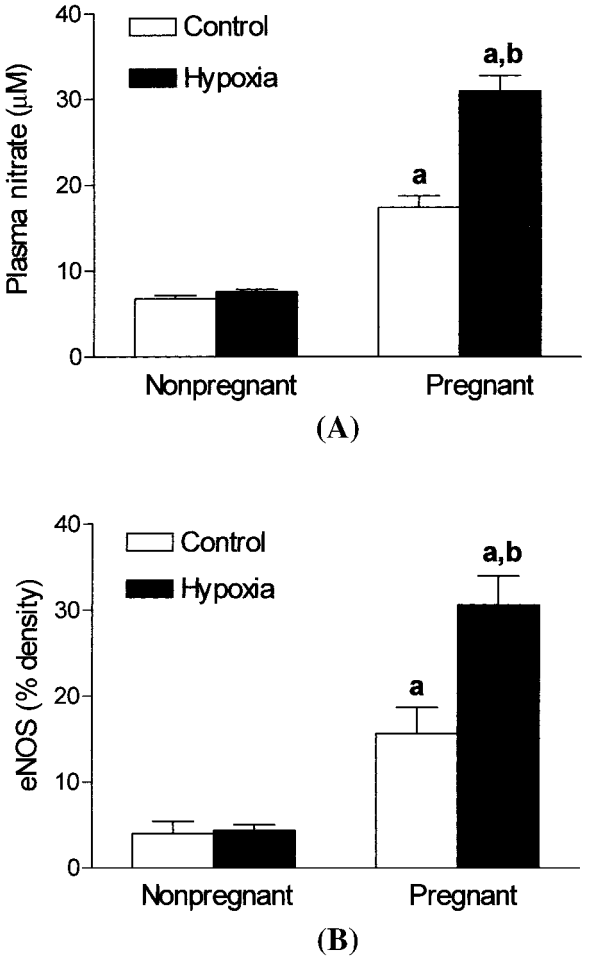

FIG. 4. (A) Nonpregnant ($n = 7$) and pregnant (day 30) ($n = 17$) sheep were divided between normoxic control and chronic hypoxic (maintained at high altitude, 3820 m, Pa_{O_2}: ~60 mmHg for 110 days) groups. Blood samples were taken from the jugular veins in nonpregnant and near-term (140 day) pregnant sheep, and plasma nitrate concentration was measured by the chemiluminescence method. (B) Western analysis of eNOS was performed in freshly isolated endothelial cells of uterine arteries obtained from the four groups of animals: control nonpregnant ($n = 6$), control pregnant ($n = 12$), hypoxic nonpregnant ($n = 6$), and hypoxic pregnant ($n = 12$) sheep. **a,** $p < 0.05$, pregnant versus nonpregnant; **b,** $p < 0.05$, hypoxia versus control.

The A23187-induced reduction of $[Ca^{2+}]_i$ was significantly enhanced in pregnant uterine arteries. The results indicated that pregnancy increased NO release, which, through decreasing $[Ca^{2+}]_i$ in smooth muscle, accounted for the increased endothelium-dependent relaxation of the uterine artery.

In both guinea pig and sheep, the effect of pregnancy on NO is associated with increased eNOS protein expression and activity (Weiner et al., 1994; Magness, 1997; Xiao et al., 2001a). eNOS protein expression in pregnant sheep uterine artery was 197% of that in nonpregnant artery (Xiao et al., 1999), while pregnancy increased eNOS protein expression fivefold in guinea pig uterine but not thoracic arteries (White et al., 2001).

Mechanisms underlying increases in NO during pregnancy may also involve modifications of downstream signaling pathways such as in the ERK-1/2. While ERK-2 is increased in pregnant ovine uterine artery (Xiao and Zhang, 2002), studies in cultured ovine uterine artery endothelial cells confirmed that ERK-1/2 and Ca^{2+} were both required for NO and PGI_2 production in pregnant uterine artery endothelial cells, as well as for PGI_2 production in nonpregnant cells (Bird et al., 2000; Di et al., 2001). They also revealed that $cPLA_2$ was much more Ca^{2+} sensitive than eNOS in nonpregnant uterine artery endothelial cells and that both eNOS and $cPLA_2$ activation showed further increases in Ca^{2+} sensitivity in association with ERK-phosphorylation in pregnant uterine artery endothelial cells. These studies suggest that Ca^{2+} sensitivity of eNOS may be regulated in an ERK-1/2 sensitive manner and that changes in Ca^{2+} and ERK-1/2 signaling are potentially a major underlying factor in the observed pregnancy-specific changes in NO and PGI_2 production in uterine artery endothelial cells.

In addition to the selective increase in plasma nitrate levels in pregnant sheep, chronic hypoxia has been associated with increased (1) eNOS protein and mRNA in uterine artery endothelium (Fig. 4B), (2) basal and the calcium ionophore A23187-induced NO release from the uterine artery, and (3) endothelium-dependent relaxation of phenylephrine-precontracted uterine artery rings (Xiao et al., 2001c). Furthermore, the effects of chronic hypoxia appeared to be tissue specific, since eNOS was unaffected in femoral and renal arteries from either pregnant or nonpregnant sheep. In pregnant uterine artery endothelium, chronic hypoxia was associated with a ninefold increase in steady-state eNOS mRNA levels, with a onefold increase in eNOS protein. The finding that chronic hypoxia did not change the apparent translational efficiency of eNOS mRNA, but increased eNOS mRNA levels, suggests that increased

eNOS protein expression in the pregnant uterine artery endothelium may not be regulated at the translational level. In addition, it is unlikely that increased steady-state eNOS protein levels found in hypoxic, compared with control, pregnant ovine uterine artery endothelium are due to increased protein stability, because that eNOS protein versus message is constant among different groups. It is not clear, however, from these studies to what extent increased steady-state mRNA levels resulted from increased transcription or enhanced message stability. Furthermore, whether the disproportional reduction in eNOS protein relative to mRNA may be due to decreased eNOS protein stability in hypoxic tissues remains unclear.

In the sheep uterine artery, chronic hypoxia produced a close correlation of onefold increases in both basal NO release and eNOS protein levels, suggesting that the increased basal NO release is predominantly due to the increased eNOS protein in the hypoxic arteries. On the other hand, the fold-increase of NO release induced by the calcium ionophore A23187 was higher than that of eNOS protein expression in hypoxic uterine arteries, suggesting a hypoxic-mediated increase in the sensitivity of the calcium signaling pathway of eNOS in addition to the enhanced protein expression. Although chronic hypoxia increased eNOS mRNA in the endothelium of nonpregnant ovine uterine arteries, it had no effect on their NOS protein levels, NO production, or endothelium-dependent relaxation, suggesting that the effect of moderate chronic hypoxia is selective to pregnant uterine arteries in sheep. The reason that chronic hypoxia increased eNOS mRNA by threefold, but did not significantly change eNOS protein levels in nonpregnant uterine artery endothelium, may have resulted at least in part from the low apparent translational efficiency (0.12) of eNOS mRNA determined in ovine uterine artery. In fact, it has been shown that many mammalian genes translate relatively poorly (Kozak, 1991).

In the guinea pig uterine artery, the overall effect of chronic hypoxia during pregnancy appears to be consistent with a decrease in the contribution of NO, depending on the study preparation and vessel bed examined. While there was no significant difference in ACH-mediated relaxation, the effect of NO inhibition was significantly less in chronically hypoxic versus normoxic uterine artery segments, suggesting an inhibitory effect of hypoxia on NO (White et al., 2000). This is consistent with a finding of decreased eNOS protein expression in uterine arteries from hypoxic versus normoxic pregnant animals (White et al., 2001). In addition, the increase in eNOS protein expression associated with pregnancy in normoxic animals was not evident in uterine arteries from hypoxia animals. In the guinea pig middle cerebral artery, increased contractile response to U-46619 in vessels from hypoxic animals was completely eliminated by the addition of NLA, suggesting that in this vessel decrease in basal NO may be responsible for the increase in contractile sensitivity (Sillau et al., 2002). In the evaluation of flow-mediated vasodilation in the uterine artery, the effect of chronic hypoxia does not appear to be mediated through NO. Addition of the NO inhibitor NLA increased rather than decreased the vasodilator response to flow in uterine arteries of the chronically hypoxic pregnant guinea pigs (Mateev et al., 2002). The addition of meclofenamate further raised, not lowered, flow vasodilation. Both of these observations indicate that decreased NO and prostaglandin vasodilator production were not responsible for the reduced vasodilator response to flow in chronic hypoxia and suggest that a possible contribution of another vasodilator such as EDHF to flow-mediated vasodilation. The rise in uterine artery flow vasodilation following NOS inhibition may have been due to restoration of EDHF production, given the ability of NOS inhibition to stimulate a compensatory increase in EDHF.

Mechanism of endothelium-dependent effect

It is not clear why chronic hypoxia has variable effects on eNOS protein expression and activity and NO production among different species and vascular beds. In whole-lung homogenates from chronically hypoxic piglets, eNOS protein levels were decreased in conjunction with decreased plasma and lung perfusate NO_x measurements (Fike et al., 1998). In rat pulmonary vasculature, chronic hypoxia increased endothelial NO release and upregu-

lated endothelial (eNOS) and inducible (iNOS) gene and protein expression (Isaacson et al., 1994; Le Cras et al., 1996; Resta and Walker, 1996). In contrast, in rat aorta it has been shown that chronic hypoxia results in a decrease in eNOS protein and mRNA and impaired endothelium-dependent relaxation (Toporsian et al., 2000), while in the rat pulmonary circulation chronic hypoxia is associated with upregulation of eNOS protein (Resta et al., 1999). Furthermore, numerous studies in cultured endothelial cells have yielded conflicting results on the effects of hypoxia (24 h) on eNOS protein and mRNA (McQuillan et al., 1994; Liao, 1995; Arnet, 1996; Le Cras et al., 1996, Phelan and Faller, 1996; Toporsian et al., 2000). Whether chronic hypoxia has been associated with up- or downregulation of eNOS activity, similar underlying mechanisms have been proposed. In a recent study, chronic hypoxia increased eNOS production of NO by increasing hsp90 association and eNOS serine phosphorylation (Shi et al., 2002), while in pulmonary artery endothelial cells hypoxia decreased NOS activity by reducing hsp90 levels (Su and Block, 2000). In addition to hypoxic modulation of regulators of eNOS activity such as hsp90, substrate availability represents another level of control. Studies in lung suggest that chronic hypoxia impairs L-arginine uptake (Fike et al., 2000). In the guinea pig MCA, addition of L-arginine to the drinking water had no effect on contractile sensitivity of MCA to U-46619 in vessel segments from chronically hypoxic animals, suggesting that this was not a factor to the decreased production of NO.

Previous studies in the sheep have determined the importance of Ca^{2+} and ERK-1/2 signaling in the pregnancy-specific changes in NO and PGI2. Recent findings that chronic hypoxia upregulated ERK-2 levels and increased Ca^{2+} sensitivity of endothelium-dependent relaxation of pregnant ovine uterine artery indicate a potential role for the ERK-1/2 signaling pathway in hypoxic-mediated regulation of sheep uterine artery endothelial function.

While hypoxia per se may have different regulatory effects on eNOS protein and mRNA expression, other factors that result from systemic in vivo hypoxia are also likely to be important contributors to the selective responses of vascular beds to hypoxia. Human studies have clearly demonstrated that uterine artery blood flow velocity (hence the shear stress) is significantly increased by chronic moderate high altitude hypoxia in pregnant women (Zamudio et al., 1995b). This increase in blood flow is thought to be due to decreased vessel diameter due to hypoxic inhibition of vessel growth (Rockwell et al., 2000). Consistent with this observation is the finding in recent studies that uterine artery vasodilation response to flow is reduced in vessels from pregnant animals exposed to chronic hypoxia. The reduction in dilator response to flow in the hypoxic guinea pig uterine artery is due to factors other than hypoxic inhibition of NO and may be associated with structural changes in vessel growth, inhibition of EDHF, or possible hypoxic stimulation of vasoconstrictors (Mateev et al., 2002). In sheep, uterine blood flow is increased in response to chronic hypoxia in pregnant sheep (Kitanaka et al., 1989). Shear stress has been shown to increase eNOS mRNA and protein levels in cultured endothelial cells (Ranjan et al., 1995), and vessels exposed to chronic elevations in shear stress exhibit augmented endothelium-dependent relaxation (Miller and Vanhoutte, 1988). Thus, in the sheep uterine artery, this may represent one mechanism contributing to hypoxia-mediated enhancement of vasodilation.

Effects of pregnancy on vascular smooth muscle function in sheep

The vascular smooth muscle cell plays an important role in mediating response to chronic hypoxia. A number of elegant studies in sheep have suggested that chronic hypoxia alters receptor-mediated excitation–contraction coupling and/or signal transduction in the vascular smooth muscle. A decrease in agonist binding affinity to the receptor may be a generalized effect of chronic hypoxia on vascular smooth muscle. Decreased binding affinities of noradrenaline and serotonin to α_1-adrenoceptors and serotonin$_2$ receptors, respectively, have been observed in uterine and umbilical arteries in response to chronic hypoxia (Hu et al., 1996a; Hu et al., 1996b; Hu and Zhang, 1997). In addition to the effects of chronic hypoxia on

agonist–receptor interaction, for a given number of α_1-adrenoceptors occupied, less IP3 was produced in the hypoxic tissues, suggesting that chronic hypoxia attenuated coupling efficiency of α_1-adrenoceptors to IP_3 synthesis in the uterine artery. The mechanisms underlying the decreased coupling efficiency of α_1-adrenoceptors to IP_3 synthesis are not clear, but may involve decreases in signal amplification for G protein coupled receptors at various levels. G proteins coupling α_1-adrenoceptors to phospholipase C may be decreased in response to hypoxia. This is supported by the finding that chronic exposure to catecholamines causes downregulation of α_1-adrenoceptor coupled G proteins (Hadcock and Malbon, 1993; Zhou et al., 1995).

Release of intracellular Ca^{2+} from the sarcoplasmic reticulum by IP3 is a major mechanism of pharmacomechanical coupling in smooth muscle (Somlyo and Somlyo, 1994; Zhang et al., 1995). Through the development of a method to estimate coupling efficiency of the receptor to its second messenger, it was demonstrated that hypoxia decreased the intrinsic ability of α_1-adrenoceptors to couple to IP_3 synthesis. Chronic hypoxia reduced both the potency and the maximal response to norepinephrine-induced IP_3 synthesis. Furthermore, a reduction in tension development for a given amount of IP_3 formation in response to chronic hypoxia was noted. In pregnant sheep uterine artery, while chronic hypoxia did not change the density of IP_3 receptors, it significantly decreased IP_3 binding affinity to its receptors (Hu et al., 1999).

Chronic hypoxia also affects Ca^{2+} mobilization and Ca^{2+} sensitivity of myofilaments in pregnant ovine uterine arteries (Zhang and Xiao, 1998). Chronic hypoxia suppressed both Ca^{2+} mobilization and agonist-mediated Ca^{2+} sensitization induced by 5-HT in pregnant uterine arteries. In contrast, chronic hypoxia differentially regulated NE-induced Ca^{2+} mobilization and Ca^{2+} sensitization by increasing NE-mediated Ca^{2+} release, but decreasing NE-induced Ca^{2+} sensitization of myofilaments in pregnant ovine uterine arteries.

SUMMARY

In sheep, moderate high altitude exposure during pregnancy alters endothelial and smooth muscle cell function in such a way as to enhance vasodilation and inhibit contractile responses to

Table 1. Comparison of the Effects of Chronic Hypoxia and Pregnancy on the Constrictor and Relaxation Responses in the Guinea Pig and Sheep Uterine and Middle Cerebral Arteries

	Guinea pig		*Sheep*		
	Uterine artery	*Middle cerebral artery*	*Uterine artery*	*Middle cerebral artery*	*Author*
Vasoconstrictor response					
PE or NE	No change		Decrease		Hu et al., 1996; Hu and Zhang, 1997 White et al., 1998
5HT			Decrease		Hu et al., 1996; Hu and Zhang, 1997
U46619		Increase			Sillau et al., 2002
Vasodilator response					
Ach	No change		Increase	Increase	Longo, 1993 White et al., 2000; Xiao et al., 2001
A23187			Increase	Increase	Long et al., 1993 Xiao, 2001
SNP			No change	Decrease	Longo et al., 1993; Xiao et al., 2001
Flow-mediated dilation					
U46619	Decrease				Mateev et al., 2002

a number of pharmacologic agents (Table 1). These effects are in part mediated through factors that increase (1) eNOS message, protein levels, and enzyme activity, (2) alter receptor number and affinity, and (3) modulate downstream signaling pathways. In sheep, these factors may serve to maintain uterine artery blood flow, thereby sparing the development of intrauterine growth retardation. Guinea pig adaptation to high altitude exposure suggests that chronic hypoxia opposes uterine artery flow-mediated vasodilation seen with normal pregnancy and inhibits the contribution of NO to agonist-mediated dilation (Table 1). While hypoxic inhibition of additional vasodilators such as EDHF are also implicated, inhibitory effects occur in conjunction with decreased vessel growth. These alterations are likely contributors to the development of intrauterine growth retardation in the guinea pig fetus. We speculate that these differing adaptive responses may represent interspecies variability in the ability of the maternal vascular system to adjust to chronic hypoxia. This adaptability may be genetically determined, as demonstrated by studies in humans in which long-term inhabitants of high altitude regions do not demonstrate the reduction in uterine artery blood flow or growth retardation that is evident in more recent inhabitants (Zamudio et al., 1993; Moore et al., 2001).

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