Effects of pregnancy and chronic hypoxia on contractile responsiveness to α_1-adrenergic stimulation

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[1]*Women's Health Research Center and* [2]*Division of Cardiology, Department of Medicine, University of Colorado Health Sciences Center, Denver 80262; and* [3]*Department of Anthropology, University of Colorado at Denver, Denver, Colorado 80217-3364*

White, Margueritte Mabry, Robert E. McCullough, Rebecca Dyckes, Alastair D. Robertson, and Lorna G. Moore. Effects of pregnancy and chronic hypoxia on contractile responsiveness to α_1-adrenergic stimulation. *J. Appl. Physiol.* 85(6): 2322–2329, 1998.—Decreased contractile response to vasoconstrictors in uterine and nonuterine vessels contributes to increased blood flow to the uterine circulation during normal pregnancy. Pregnancies complicated by preeclampsia and/or chronic hypoxia show a reversal or diminution of these pregnancy-associated changes. We sought to determine whether chronic hypoxia opposes the reduction in contractile response in uterine and nonuterine vessels during normal pregnancy and, if so, whether decreased basal nitric oxide (NO) activity was involved. We examined the contractile response to phenylephrine (PE) in guinea pig uterine artery (UA), mesenteric artery (MA), and thoracic aorta (TA) rings isolated from nonpregnant or pregnant guinea pigs that had been exposed throughout gestation to either low (1,600 m, $n = 47$) or high (3,962 m, $n = 43$) altitude. In the UA, pregnancy reduced contractile sensitivity to PE and did so similarly at low and high altitude (EC_{50}: 4.0×10^{-8} nonpregnant, 9.3×10^{-8} pregnant at low altitude; 4.8×10^{-8} nonpregnant, 1.0×10^{-8} pregnant at high altitude; both $P < 0.05$). Addition of the NO synthase inhibitor nitro-L-arginine (NLA; 200 mM) to the vessel bath increased contractile sensitivity in the pregnant UA ($P < 0.05$) and eliminated the effect of pregnancy at both altitutes. NLA also raised contractile sensitivity in the nonpregnant high-altitude UA, but contractile response without NLA did not differ in the high- and low-altitude animals. In the MA, pregnancy decreased contractile sensitivity to PE at high altitude only, and this shift was reversed by NO inhibition. In the TA, neither pregnancy nor altitude affected contractile response, but NO inhibition raised contractile response in nonpregnant and pregnant TA at both altitudes. We concluded that pregnancy diminished contractile response to PE in the UA, likely as a result of increased NO activity, and that these changes were similar at low and high altitude. Counter to our hypothesis, chronic hypoxia did not diminish the pregnancy-associated reduction in contractile sensitivity to PE or inhibit basal NO activity in the UA; rather it enhanced, not diminished, basal NO activity in the nonpregnant UA and the pregnant MA.

nitric oxide; vasoreactivity; guinea pig; preeclampsia

NORMAL PREGNANCY is accompanied by decreased vasoconstrictor and increased vasodilator responses that, in turn, contribute to the rise in blood flow in the uterine and other circulations. Preeclampsia is characterized by the absence or reversal of the normal diminution in pressor response (7). Abnormal vascular adjustment to pregnancy is also observed under circumstances of chronic hypoxia in humans and other animals. Women residing at high compared with low altitude have reduced uterine artery blood flow while pregnant and an increased incidence of preeclampsia, and preeclampsic women demonstrate alterations in blood flow redistribution that precede the onset of hypertensive symptoms (31, 32). Hypoxia-related alterations in vascular adjustment to pregnancy are also observed in pregnant guinea pigs housed at high compared with low altitude, in they have a greater systemic vascular resistance at baseline and during angiotensin II infusion and increased contractile response to norepinephrine in isolated thoracic aorta rings (10).

We hypothesized that chronic hypoxia interfered with or opposed the normal decrease in vasoconstrictor response to α_1-adrenergic stimulation during pregnancy. Consistent with this hypothesis are observations of increased α_1-adrenergic activation after weeks of high-altitude residence (17) and augmented blood pressure response to acute hypoxia in preeclamptic compared with normal pregnant women at high altitude (21). Against these observations are those of decreased contractile response in uterine arteries isolated from chronically hypoxic compared with normoxic pregnant sheep (11, 34). However, cerebral vessels from chronically hypoxic sheep had greater contractile responses than did their normoxic counterparts (13), suggesting that the effects of chronic hypoxia on pregnancy-associated alterations in vasoreactivity vary among vascular beds. Studies at low altitude suggest that increased nitric oxide (NO) production and/or activity during pregnancy is an important contributor to the decreased vasoconstrictor response (29) in ways that vary by vascular bed (26). Thus we reasoned that chronic hypoxia might interfere with the pregnancy-associated diminution in vasoconstrictor response and that this variation might be due, at least in part, to differences in basal NO activity.

We studied isolated arterial rings from nonpregnant or late-pregnant guinea pigs exposed to normoxia (the laboratory altitude of 1,600 m) or chronic hypoxia (3,962 m) throughout pregnancy. Contractile response to phenylephrine, an α_1-adrenergic agonist, was determined with and without the addition of nitro-L-arginine (NLA), an inhibitor of NO synthesis, to the vessel bath to assess the contribution of basal NO activity. We used the guinea pig as the experimental animal because we

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(and others) (9, 10, 29) have demonstrated an effect of pregnancy on vascular reactivity in the whole animal and isolated vessel rings and because its small size permits placing it in a hypobaric chamber. Because the decrease in systemic vascular resistance during pregnancy is due to changes in both the uterine and nonuterine circulations (4), we studied both uterine and nonuterine (mesenteric, thoracic) arterial rings from low- and high-altitude, nonpregnant and pregnant guinea pigs. We considered that these studies would be informative about the mechanisms by which vasoconstrictor response is altered by normal pregnancy and/or under circumstances of chronic hypoxia.

METHODS

Animals. Studies were performed in near-term, pregnant (55-to 63-days gestation) and nonpregnant female Hartley guinea pigs (Sasco, Omaha, NE). Pregnancy duration (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 by using a published nomogram (6a). A total of 47 (20 nonpregnant, 27 pregnant) animals were maintained at low altitude (the laboratory altitude of 1,600 m), and 43 (20 nonpregnant, 23 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 in length were cut from the main uterine artery (UA), the approximately equal-sized (second- or third-order branches) superior mesenteric artery (MA), and the thoracic aorta (TA). All vessels were carefully dissected free of connective tissue and fat. Before the vessels were mounted, outer diameter before being mounted was measured with a calibrated ruler through a dissecting microscope (model M7A, Leica, Zurich, Switzerland). A minimum of two UA (513 ± 13 μm) and two MA (460 ± 11 μm) rings per animal were cut and mounted on a linear force-displacement transducer (model FTO-3C, Grass, Quincy, MA) by using a modification designed to minimize trauma. The modification consisted of attaching the vessel to two round, 9-mm Teflon disks, each of which had two screws for anchoring 0.0002-mm-diameter tungsten-iridium wire and a hole for affixing to either the fixed or free end of a force-displacement transducer. One end of each wire was secured with a screw. With use of the dissecting microscope, the other end was threaded through the vessel lumen and anchored to the second screw. The two disks were then secured to a plate for transfer to the fixed and free ends of the force-displacement transducer and were submerged in a vessel bath. A minimum of two segments per animal were cut from the TA (2,108 ± 25 μm) and mounted directly onto 0.43-mm-diameter stainless steel wires attached to a linear force-displacement transducer and submerged in the vessel bath.

Baths were maintained at 37.5°C, pH 7.40, and bubbled with a mixture of 95% O_2-5% CO_2. The chamber was filled and periodically flushed with 10 ml of Earle's balanced salt solution (EBSS) comprising (in mM) 116 NaCl, 5.3 KCl, 0.8 $MgSO_4 \cdot 7\ H_2O$, 21 $NaHCO_3$, 1.01 $NaPO_4$, 5.5 glucose, and 1.8 $CaCl_2$. Vessels were set at resting tensions of 600 mg for the UA and MA and 1,500 mg for the TA and were allowed to equilibrate for 1 h. These resting tensions were previously determined as those that resulted in maximal contractions to 80 mM KCl (Sigma Chemical, St. Louis, MO).

Protocol. Vessels were initially contracted with 80 mM KCl. After 30 min or once a stable contraction was achieved, the chambers were rinsed repeatedly with EBSS and the vessels were allowed to relax to baseline. To assess functional integrity of the endothelium, vessels were preconstricted with phenylephrine (PE) (Elkins-Sinn, Cherry Hill, NJ) (5×10^{-8} M for the UA, 10^{-7} M for the MA and TA) and required to relax at least 50% to 5×10^{-6} M (UA) or 2.5×10^{-8} M (MA) acetylcholine or 10^{-7} M bradykinin for the TA before proceeding. Complete contractile dose responses were obtained to PE (10^{-9} to 10^{-5} M). Vessels with similar PE dose responses were then paired and treated either with NLA (200 mM), an inhibitor of NO synthesis, or vehicle and the dose response to PE was repeated. Vessels were included in the data analysis if successful studies had been completed in both members of a pair (with and without NLA). However, we did not require that usable data be obtained from all three vessel types (UA, TA, and MA) for a given animal.

Contractile response was recorded as absolute and percent maximal contractile response (PE_{max}). To determine whether variation in contractile response was related to vessel dimensions, we analyzed contractile response in relation to vessel size in a total of 96 UA rings from 18 nonpregnant or pregnant animals. Vessel dimensions were determined before the vessels were mounted in rings suspended in EBSS by using an inverted microscope (model CK, Olympus, Tokyo, Japan). Wall thickness was determined as the difference between inner and outer diameter. Cross-sectional area (mm^2) was measured as follows: 2 × wall thickness × length. Tissue volume (mm^3) was calculated as: $\pi \times$ length (outer radius^2 − inner radius^2). Cross-sectional area was larger in the pregnant than in nonpregnant animals (0.74 ± 0.02 vs. 0.49 ± 0.04 mm^2 respectively, $P < 0.05$). However, cross-sectional area was unrelated to variation in PE_{max} ($r = 0.11$, P = not significant). Similar results were obtained if variation in wall thickness or tissue volume was considered in relation to PE_{max}. Thus we did not normalize maximum contractile response by cross-sectional area or tissue volume.

Data reduction and statistics. To be included for analysis, successful studies needed to be completed in at least two vessels of a given type (UA, MA, TA) from a given animal, one of which had received vehicle and the other NLA as described in *Protocol*. An average of four UA, four MA, and two TA were studied per animal. Information from each vessel was recorded for the PE_{max}, the maximum contractile response to KCl (KCl_{max}), the percent maximal contractile response at each dose of PE, and the EC_{50} (the PE dose yielding 50% of the maximum contraction). EC_{50} was determined as the negative ratio of intercept to slope. Vessels from a given animal receiving the same NLA treatment (with, without) were averaged and then grouped by pregnancy (nonpregnant, pregnant) and altitude (low, high) status. Sample size was considered as the number of animals. Contractile sensitivity to PE was analyzed as the entire dose response curve using nonlinear logistic regression analyses (SAS Institute, Cary, NC). EC_{50}, PE_{max}, and KCl_{max} were compared by using paired or Student's *t*-tests as appropriate. Because all comparisons were preplanned, multiple-comparisons procedures were not used (1, 27). Values are expressed as means ± SE or as means and 95% confidence intervals. Comparisons were considered significant when $P < 0.05$ and are reported as trends when $0.05 < P < 0.10$.

RESULTS

UA. At both low and high altitude, pregnancy decreased contractile sensitivity to PE as measured by a

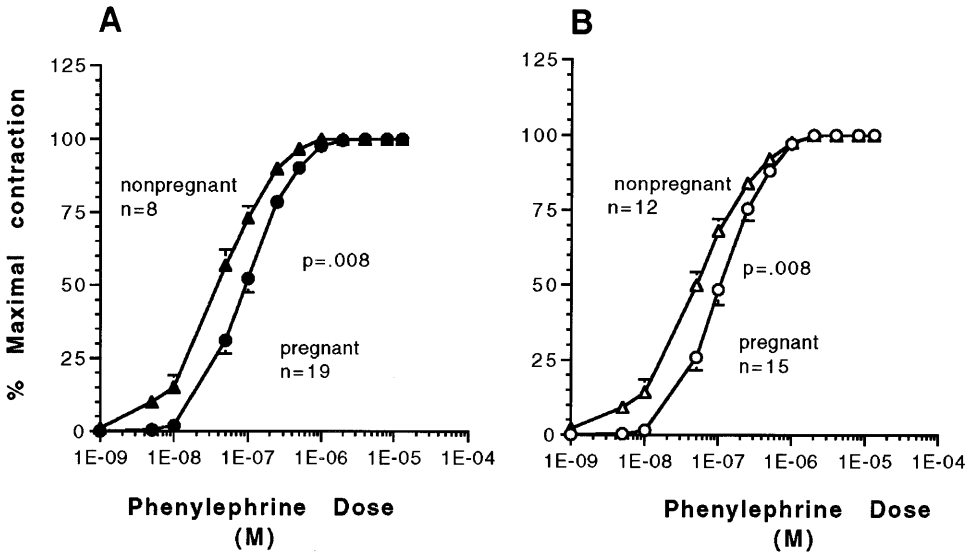

Fig. 1. Pregnancy reduces contractile sensitivity to phenylephrine in uterine artery rings isolated from nonpregnant compared with pregnant guinea pigs that were housed at either low (1,600 m; *A*) or high altitude (3,962 m; *B*) throughout gestation or an equivalent period in the nonpregnant state. Vessels were studied in the absence of nitro-L-arginine. Values are means ± SE; *n*, no. of animals. *P* values indicate comparisons of contractile dose-response curves between nonpregnant and pregnant groups by using nonlinear regression analyses. △, ▲, Vessels from pregnant animals; ○, ●, vessels from pregnant animals; ▲, ●, low-altitude groups; △, ○, high-altitude groups.

rightward shift in the dose-response curve and an increase in the EC_{50} (Fig. 1, Table 1). Pregnancy also increased the PE_{max} and KCl_{max} at both altitudes (Table 1). Contractile response was similar in the vessels from the nonpregnant or pregnant low- vs. high-altitude animals. Addition of the NO inhibitor NLA to the vessel bath left shifted the PE dose-response curves in the nonpregnant high-altitude group and in the pregnant low- and high-altitude animals (Fig. 2). NLA also lowered the EC_{50} in the high-altitude pregnant animals and tended to lower EC_{50} in the low-altitude pregnant group ($P = 0.07$; Table 1). After addition of NLA to the vessel bath, the EC_{50} no longer differed between pregnant and nonpregnant UA at either altitude (Table 1).

MA. Pregnancy did not alter contractile response to PE in MA isolated from low-altitude animals but right shifted the dose-response curve, increased the EC_{50}, and decreased PE_{max} in the high-altitude animals (Fig. 3, Table 2). Contractile sensitivity did not differ in the vessels from nonpregnant low- vs. high-altitude animals, and, although it was somewhat reduced in the high- vs low-altitude pregnant group, this was not statistically significant. KCl_{max} was unaffected by pregnancy at either altitude. NLA treatment left shifted the dose-response curve and reduced the EC_{50} in the pregnant high-altitude animals but did not alter contractile response in the MA of pregnant animals at low altitude or the nonpregnant MA at either altitude (Fig. 4, Table 2). After the addition of NLA to the vessel bath, there was no longer a significant difference between vessels from pregnant and nonpregnant high-altitude animals (Table 2).

TA. Pregnancy did not change contractile response or PE_{max} at either altitude but raised KCl_{max} at low altitude (Table 3). NLA left shifted the dose-response curves to PE and decreased EC_{50} in TA from nonpregnant and pregnant animals at both altitudes (Fig. 5, Table 3). PE_{max} was also increased after NLA addition in all but the low-altitude pregnant vessels (Table 3).

DISCUSSION

We sought to determine whether pregnancy decreased contractile sensitivity to PE, an α_1-adrenergic agonist, in isolated guinea pig UA, MA, and TA rings and whether chronic hypoxia opposed a pregnancy-associated reduction in contractile response. We found that pregnancy decreased contractile sensitivity in the UA and that this reduction appeared to be due to increased basal NO activity at both altitudes. In con-

Table 1. *Uterine artery contractile response characteristics*

	Low Altitude		High Altitude	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
n	8	19	12	15
Phenylephrine EC_{50}, M				
Without NLA	$4.0\ (2.6–6.1)\times10^{-8}$	$9.3\ (7.0–12.3)\times10^{-8}$*	$4.8\ (2.8–8.3)\times10^{-8}$	$1.0\ (0.80–1.4)\times10^{-7}$*
With NLA	$3.5\ (1.7–7.3)\times10^{-8}$	$6.3\ (4.4–9.0)\times10^{-8}$	$3.4\ (2.2–5.2)\times10^{-8}$	$6.9\ (5.0–9.5)\times10^{-8}$†
Phenylephrine maximal contraction, mg				
Without NLA	1,596 ± 150	2,423 ± 152*	1,440 ± 259	2,240 ± 200*
With NLA	1,730 ± 192	2,542 ± 276*	1,381 ± 232	2,295 ± 134*
KCl maximal contraction, mg	638 ± 87	1,154 ± 95*	535 ± 100	997 ± 109*

Values are means ± SE except for phenylephrine EC_{50}, which are means with 95% confidence intervals in parentheses; *n*, no. of animals. NLA, nitro-L-arginine. *Nonpregnant compared with pregnant groups, $P < 0.05$. †Comparison of without vs. with NLA groups in vessels from nonpregnant or pregnant animals, $P < 0.05$.

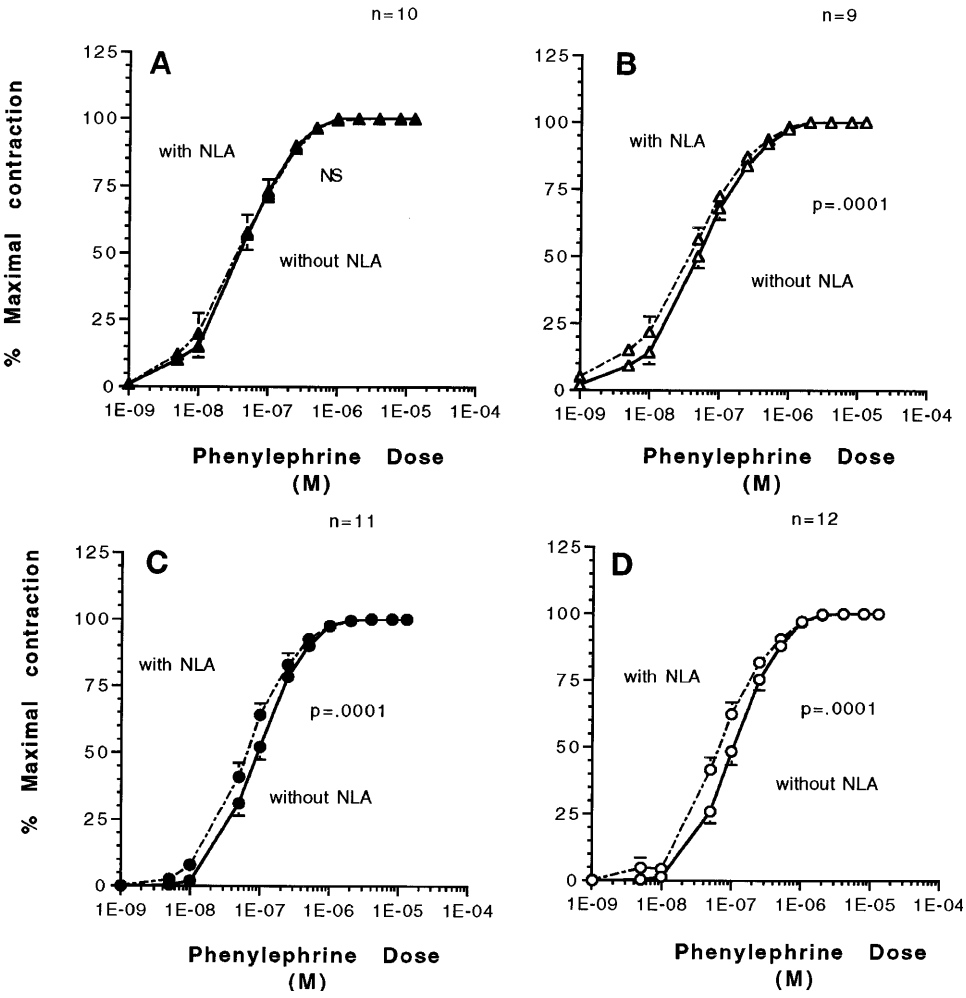

Fig. 2. Addition of nitro-L-arginine (NLA; 200 mM) to vessel bath increased contractile sensitivity to phenylephrine in uterine artery rings from high-altitude nonpregnant (*B*), low-altitude pregnant (*C*), and high-altitude pregnant (*D*) animals. *A*: low-altitude nonpregnant animals. Values are means ± SE; *n*, no. of animals. △, ▲, Vessels from nonpregnant animals; ○, ●, vessels from pregnant animals. Broken lines connect values from vessels treated with NLA; solid lines connect values from vessels without NLA. ▲, ●, Low-altitude groups; △, ○, high-altitude groups. NS, not significant. *P* values indicate comparisons of contractile dose-response curves between vehicle (without NLA) and NLA-treated (with NLA) groups by using nonlinear regression analyses.

trast to our hypothesis, chronic hypoxia did not diminish the pregnancy-associated reduction in contractile sensitivity in the UA. In addition, chronic hypoxia exaggerated the pregnancy-associated reduction in contractile response in the MA, and this also appeared attributable to increased NO activity. Neither pregnancy nor chronic hypoxia effected contractile response or altered basal NO activity in the TA.

Among the uterine and nonuterine vessels examined, we found that pregnancy reduced contractile sensitivity to PE only in the UA at low altitude. These results are consistent with Weiner et al. (29), who demonstrated a pregnancy-associated decrease in contractile sensitivity to adrenergic stimulation in the guinea pig UA but not the carotid artery. Some reports have shown decreased contractile responses in the TA or MA with pregnancy (2, 3, 5, 23, 28), but others have not found reduced contractile sensitivity to PE in mesenteric microvessels (22). Such variation may be due to differences in generation or size of the vessel being studied, agonists employed, use of active vs. passive tension, and conditions of flow vs. no flow. For example, flow-

Table 2. *Mesenteric artery contractile response characteristics*

	Low Altitude		High Altitude	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
n	10	8	9	6
Phenylephrine EC_{50}, M				
Without NLA	4.5 (3.2–6.3)×10^{-7}	5.7 (4.3–7.5)×10^{-7}	4.0 (3.4–4.6)×10^{-7}	8.1 (5.3–12.4)×10^{-7}*
With NLA	5.3 (3.7–7.8)×10^{-7}	5.0 (3.9–6.5)×10^{-7}	4.6 (2.9–5.4)×10^{-7}	5.0 (2.5–10.0)×10^{-7}†
Phenylephrine maximal contraction, mg				
Without NLA	952 ± 83	916 ± 99	1,332 ± 144	706 ± 161*
With NLA	1,092 ± 115	1,195 ± 124	1,565 ± 176	957 ± 122
KCl maximal contraction, mg	539 ± 31	875 ± 88	717 ± 61	975 ± 128

Values are means ± SE except for phenylephrine EC_{50}, which are means with 95% confidence intervals in parentheses. *Nonpregnant compared with pregnant groups, $P < 0.05$. †Without vs. with NLA groups in nonpregnant or pregnant vessels, $P < 0.05$.

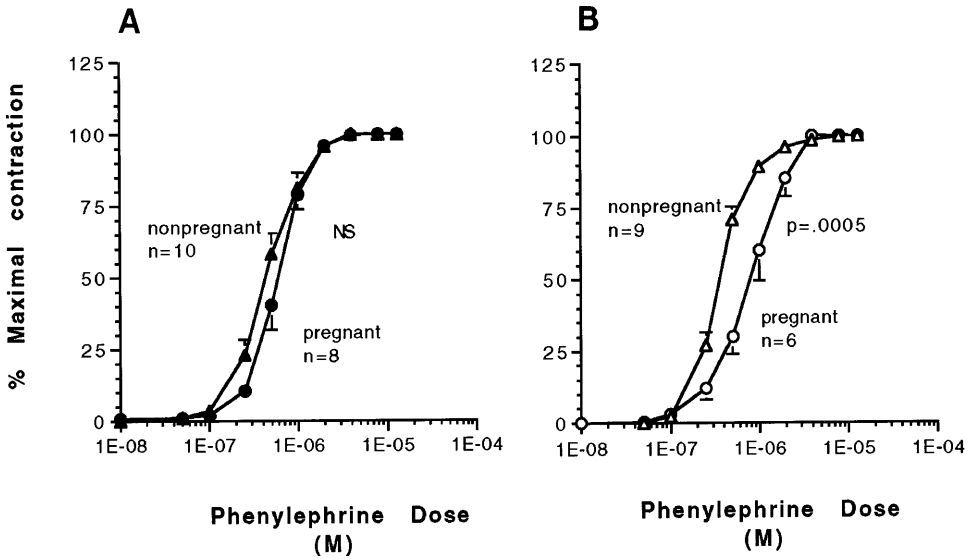

Fig. 3. Pregnancy did not alter contractile sensitivity in mesenteric artery rings isolated from low-altitude animals (*A*) but right-shifted dose-response curve in high-altitude animals (*B*). Values are means ± SE; *n*, no. of animals. *P* values indicate comparisons of contractile dose-response curves between nonpregnant and pregnant groups by using nonlinear regression analyses. △, ▲, Vessels from nonpregnant animals; ○, ●, vessels from pregnant animals; ▲, ●, low-altitude groups; △, ○, high-altitude groups.

stimulated NO release in the perfused mesenteric vascular beds contributes to a pregnancy-associated decrease in contractile response (2). Agonists such as norepinephrine, which bind to both α_1- and α_2-adrenergic receptors, may stimulate NO release through binding of α_2-receptors on endothelial cells (20) and thus decrease contractile response. This may explain why we previously found a pregnancy-associated decrease

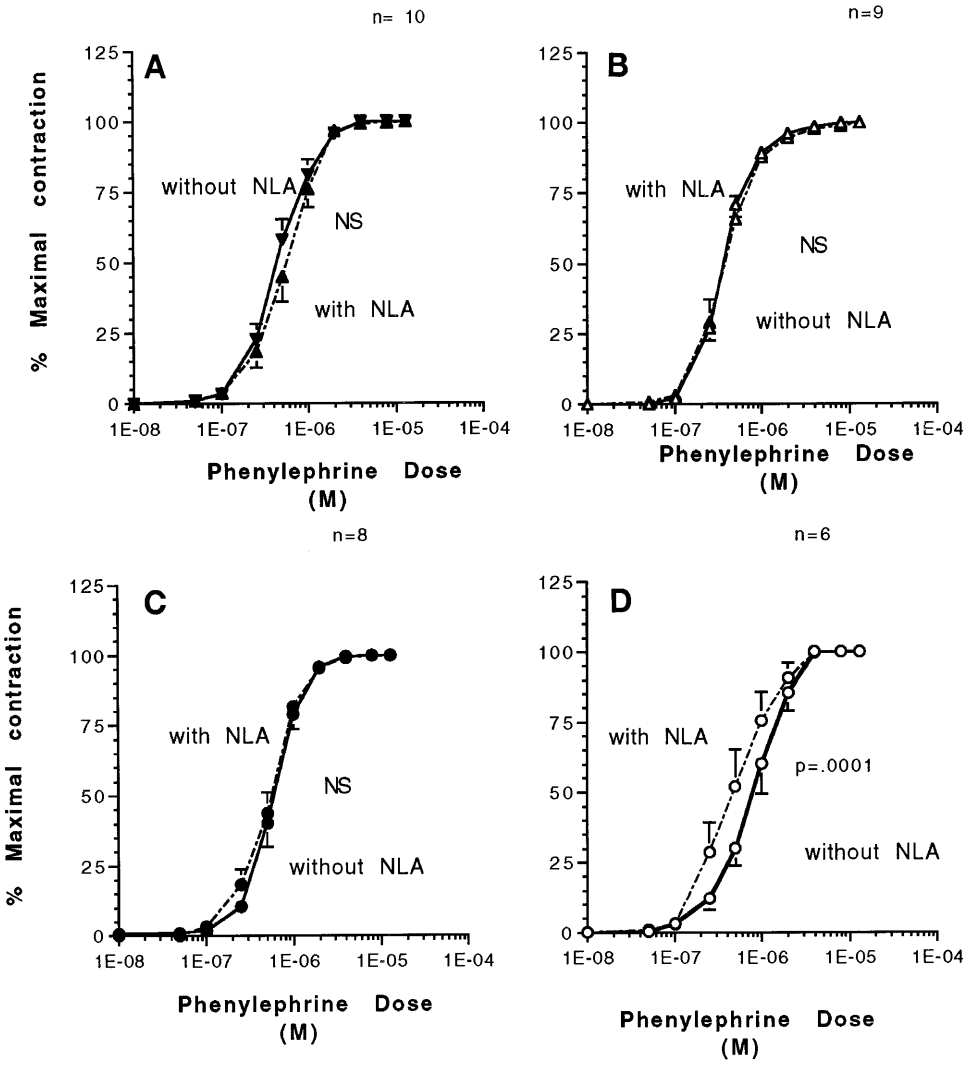

Fig. 4. Addition of NLA (200 mM) to vessel bath did not change contractile sensitivity in mesenteric artery rings isolated from nonpregnant animals or from pregnant animals at low altitude (*A*, *C*). NLA increased contractile sensitivity in vessels from pregnant animals at high altitude (*D*). *A*: low altitude nonpregnant animals. *B*: high-altitude nonpregnant animals. *C*: low-altitude pregnant animals. *D*: high-altitude pregnant animals. Values are means ± SE; *n*, no. of animals. △, ▲, Vessels from nonpregnant animals; ○, ●, vessels from pregnant animals. Broken lines connect values from vessels treated with NLA; solid lines connect values from vessels without NLA. ▲, ●, low-altitude groups; △, ○, high-altitude groups. *P* values indicate comparisons of contractile dose-response curves between vehicle (without NLA) and NLA-treated (with NLA) groups by using nonlinear regression analyses.

Table 3. *Thoracic aorta contractile response characteristics*

	Low Altitude		High Altitude	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
n	10	11	9	12
Phenylephrine EC_{50}, M				
Without NLA	$26\ (1.9–3.4)\times10^{-7}$	$2.6\ (1.8–3.9)\times10^{-7}$	$2.9\ (2.1–4.1)\times10^{-7}$	$2.7\ (1.9–3.8)\times10^{-7}$
With NLA	$1.8\ (1.5–2.2)\times10^{-7}$†	$0.96\ (1.4–2.1)\times10^{-7}$†	$1.9\ (1.3–2.9)\times10^{-7}$†	$1.5\ (1.0–2.2)\times10^{-7}$†
Phenylephrine maximal contraction, mg				
Without NLA	2,491 ± 146	2,704 ± 187	2,678 ± 148	2,461 ± 197
With NLA	3,074 ± 161†	3,097 ± 177	3,207 ± 229†	3,079 ± 217†
KCl maximal contraction, mg	2,315 ± 145	2,769 ± 147*	2,509 ± 147	2,381 ± 214

Values are means ± SE except for phenylephrine EC_{50}, which are means with 95% confidence intervals in parentheses; *n*, no. of animals. *Nonpregnant compared with pregnant groups, $P < 0.05$. †Without vs. with NLA groups in vessels from nonpregnant or pregnant animals, $P < 0.05$.

in contractile sensitivity to norepinephrine in guinea pig TA rings (10), whereas the response to phenylephrine, a pure α_1-adrenergic agonist, was unchanged in the present study. The effects of pregnancy may also vary depending on vessel size. A pregnancy-associated decrease in arterial stiffness, for instance, was present in smaller but not in larger middle cerebral arteries (12).

Both rat and human pregnancy are associated with increased NO biosynthesis (26), but whether increased NO biosynthesis is responsible for the reduction in systemic vascular resistance and vascular reactivity is less clear. In the isolated UA, we found that pregnancy enhanced NO activity as judged by increased contractile response in the presence of a NO inhibitor (NLA). Consistent with a previous report, the greater NO activity appeared to account for the decreased contractile sensitivity to PE (29). Neither this nor previous studies have been able to discern whether increased NO activity in the UA was due to greater expression and/or activity of endothelial nitric oxide synthase (8, 16, 30) or to augmented vascular smooth muscle cell

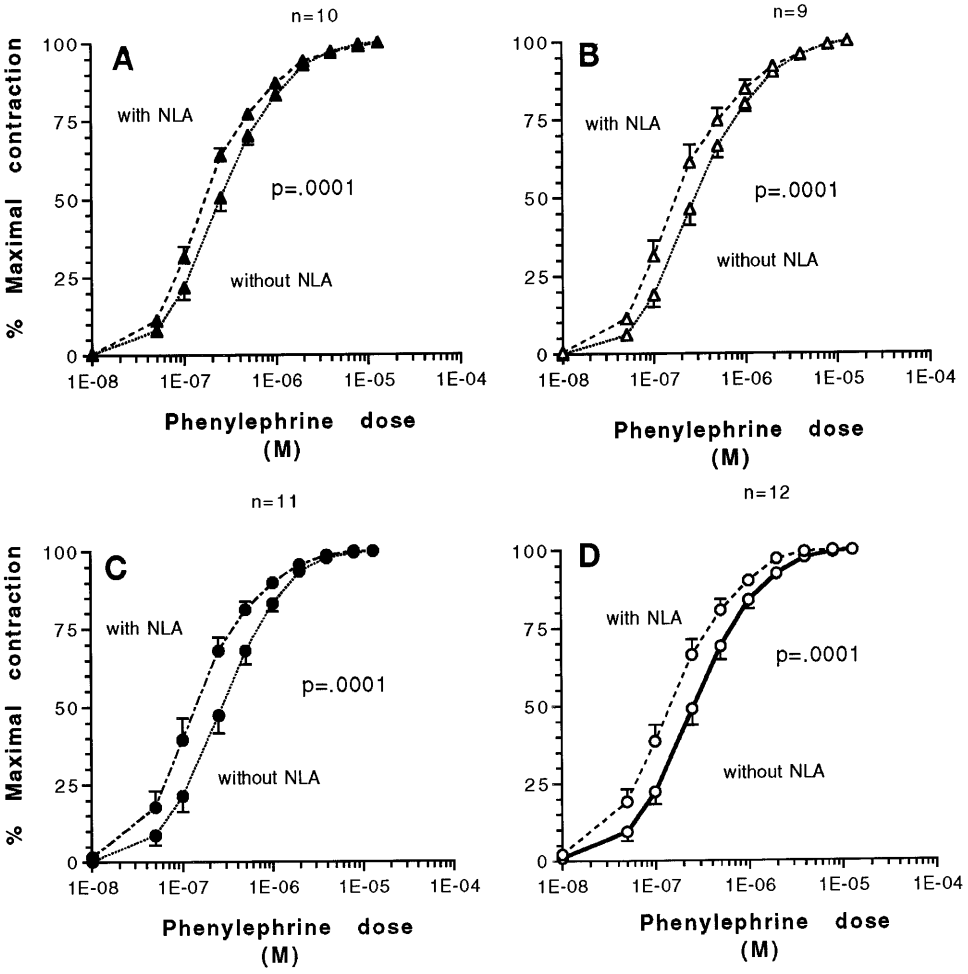

Fig. 5. Addition of NLA (200 mM) to vessel bath increased contractile sensitivity to phenylephrine in thoracic aorta rings isolated from nonpregnant or pregnant, low- or high-altitude animals. *A*: low-altitude nonpregnant animals. *B*: high-altitude nonpregnant animals. *C*: low-altitude pregnant animals. *D*: high-altitude pregnant animals. Values are means ± SE; *n*, no. of animals. △, ▲, Vessles from nonpregnant animals; ○, ●, vessels from pregnant animals. Broken lines connect values from vessels treated with NLA; solid lines connect values from vessels without NLA. ▲, ●, Low-altitude groups; △, ○, high-altitude groups. *P* values indicate comparisons of contractile dose-response curves between vehicle (without NLA) and NLA-treated (with NLA) groups by using nonlinear regression analyses.

responsiveness (19). Increased NO activity was not able to fully account for the decreased contractile responsiveness in nonuterine vessels (2, 5, 27), suggesting that multiple factors are involved in pregnancy-associated changes in vasoreactivity. Previous studies have implicated other endothelial products (e.g., prostacyclin, endothelial-derived hyperpolarizing factor, endothelin-1) as possibly involved.

Previously, we observed that high-altitude pregnant guinea pigs had higher systemic vascular resistance than did pregnant low-altitude animals at baseline as well as during angiotensin II infusion (10). We have also reported that pregnant women residing at high compared with low altitude have lower UA blood flow and less pelvic blood flow redistribution to the uterine circulation (32). These observations suggested to us that chronic hypoxia may oppose the blunting effect of pregnancy on contractile sensitivity and, in turn, serve to increase vascular resistance. However, the present study results indicated that chronic hypoxia did not alter contractile sensitivity to PE or NO activity in the pregnant UA and rather that chronic hypoxia enhanced, not inhibited, the effects of pregnancy in the MA by augmenting NO activity. Effects of hypoxia were also noted in the nonpregnant UA, where isolated rings showed decreased contractile sensitivity and increased NO activity, although to a lesser extent.

The effects of hypoxia observed in the nonpregnant animal are supported by Doyle and Walker (6), who demonstrated that chronic hypoxia decreased contractile sensitivity to PE, angiotensin II, and arginine vasopressin in catheterized rats and decreased maximum tension responses to arginine vasopressin in isolated abdominal aortic rings. Similarly, reduced contractile response has been reported for cerebral vessels isolated from nonpregnant sheep exposed to chronic hypoxia vs. normoxia, accompanied by decreased α_1-adrenergic-receptor density and reduced norepinephrine-induced D-*myo* inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] response (13). These studies suggest that chronic hypoxia downregulates α_1-adrenergic-receptor number and alters excitation-contractile coupling and/or signal transduction in vascular smooth muscle. In addition, our data suggest that another mechanism involves hypoxic stimulation of NO activity. Chronic hypoxia has been shown to stimulate NO activity in the lung and increase endothelial cell NO synthase expression in pulmonary resistance vessels (25), although studies in human umbilical vein endothelial cells exposed to severe hypoxia have shown decreased mRNA and protein expression (18). NO stimulation under conditions of chronic hypoxia in the uterine and mesenteric circulations may reflect, as suggested in the pulmonary circulation, a compensatory mechanism to counteract an underlying increase in baseline tone or resistance. Although our study preparation was not able to evaluate tone, we did not find increased contractile sensitivity in the UA or MA rings isolated from high- compared with low-altitude animals.

Our observation that chronic hypoxia did not oppose the pregnancy-associated reduction in contractile sensitivity or increase in NO activity in the pregnant UA was counter to our hypothesis. We were unaware of any previous studies examining the effect of chronic hypoxia on the pregnancy-associated reduction in UA contractile response. UA isolated from chronically hypoxic compared with normoxic pregnant sheep have less contractile response to norepinephrine (NE) and serotonin (5-HT) (11, 34). In a series of elegant studies, the reduced NE response was attributable to decreased α_1-adrenergic-receptor density and agonist-binding affinity (11) and the diminished 5-HT response to decreased coupling efficiency between activation of 5-HT_2 receptors and Ins(1,4,5)P_3 synthesis, leading to decreased Ins(1,4,5)P_3 levels, decreased Ca^{2+} mobilization, and decreased Ca^{2+} sensitivity of myofilaments (34). It was not possible to evaluate whether the effect of pregnancy was altered by chronic hypoxia in these studies because no vessels from nonpregnant animals were studied. In cerebral vessels, neither chronic hypoxia nor pregnancy altered contractile response to 5-HT or histamine, although chronic hypoxia (but not pregnancy) increased contractile response to KCl and to amines (5-HT and histamine) expressed as a percentage of the KCl response (13). That chronic hypoxia did not inhibit the effects of pregnancy in the mesenteric or uterine circulations suggests that the increased systemic vascular resistance and decreased uterine blood flow reported during pregnancy at high altitude result from factors other than alterations in contractile sensitivity or basal NO activity (10, 33). Such mechanisms might involve effects of hypoxia on endothelial-dependent or -independent vasodilation. Studies in sheep demonstrate that chronic hypoxia decreased relaxation to *S*-nitroso-*N*-acetyl penicillamine, an endothelium-independent vasodilator, in the nonpregnant and pregnant middle cerebral and pregnant basilar arteries (13). Other potential mechanisms may involve hypoxic stimulation of vasoconstrictors such as endothelin-1, as has been demonstrated in cultured human umbilical vein endothelial cells (14a), or alterations in the growth response of the uterine artery and other vessels in the uterine circulation.

In summary, our findings in combination with previous reports indicate that pregnancy enhances NO activity in the UA and that increased NO activity can, in turn, account for the attenuated UA contractile sensitivity of pregnancy. Chronic hypoxia did not inhibit the pregnancy-associated diminution in contractile sensitivity or rise in NO activity in the UA but, rather, appeared to stimulate NO activity in the pregnant MA and nonpregnant UA. Thus, effects of chronic hypoxia on systemic and uterine vascular resistance during pregnancy are likely mediated through mechanisms involving inhibition of endothelium-independent vasodilation, factors affecting vascular smooth muscle cell responsiveness, or the growth response of the uterine vasculature.

Doug Curran-Everett and Don Ellis provided technical assistance in developing the vessel-bath modifications.

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

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Received 9 January 1998; accepted in final form 7 August 1998.

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