Reduced endothelial NO-cGMP vascular relaxation pathway during TNF-α-induced hypertension in pregnant rats

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Reduced endothelial NO-cGMP vascular relaxation pathway during TNF-α-induced hypertension in pregnant rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R390–R399, 2002; 10.1152/ajpregu.00270.2001.—Placental ischemia during pregnancy is thought to release cytokines such as tumor necrosis factor-α (TNF-α), which may contribute to the increased vascular resistance associated with pregnancy-induced hypertension. We have reported that a chronic twofold elevation in plasma TNF-α increases blood pressure in pregnant but not in virgin rats; however, the vascular mechanisms are unclear. We tested the hypothesis that increasing plasma TNF-α during pregnancy impairs endothelium-dependent vascular relaxation and enhances vascular reactivity. Active stress was measured in aortic strips of virgin and late-pregnant Sprague-Dawley rats untreated or infused with TNF-α (200 ng·kg⁻¹·day⁻¹ for 5 days) to increase plasma level twofold. Phenylephrine (Phe) increased active stress to a maximum of 4.2 ± 0.4 × 10⁵ and 9.9 ± 0.7 × 10⁵ N/m² in control pregnant and TNF-α-infused pregnant rats, respectively. Removal of the endothelium enhanced Phe-induced stress in control but not in TNF-α-infused pregnant rats. In endothelium-intact strips, ACh caused greater relaxation of Phe contraction in control than in TNF-α-infused pregnant rats. Basal and ACh-induced nitrate/nitrate production was less in TNF-α-infused than in TNF-α-infused pregnant rats. Pretreatment of vascular strips with 100 μM Nω-nitro-L-arginine methyl ester, to inhibit nitric oxide (NO) synthase, or 1 μM 1H-[1,2,4]oxadiazolo[4,3-b]quinoxalin-1-one, to inhibit cGMP production in smooth muscle, inhibited ACh-induced relaxation and enhanced Phe-induced stress in control but not in TNF-α-infused pregnant rats. Phe contraction and ACh relaxation were not significantly different between control and TNF-α-infused virgin rats. Thus an endothelium-dependent NO-cGMP-mediated vascular relaxation pathway is inhibited in late-pregnant rats infused with TNF-α. The results support a role for TNF-α as one possible mediator of the increased vascular resistance associated with pregnancy-induced hypertension.

nitric oxide; cytokines; pregnancy

NORMAL PREGNANCY is often associated with a reduction in systemic vascular resistance and arterial pressure and decreased vascular reactivity to circulating vasoconstrictor substances (15, 28, 32, 36). The hemodynamic and vascular changes observed during normal pregnancy have been explained, in part, by increased nitric oxide (NO) synthesis by various cells, including vascular endothelial cells (1, 14, 38, 41, 46). This is supported by reports that the tissue expression and specific actions of NO synthase are elevated during late gestation (3, 9, 40, 45) and that the metabolic production and plasma level of cGMP, a second messenger of NO and a cellular mediator of vascular smooth muscle relaxation (24, 27), are increased during pregnancy (11).

In 5–7% of pregnancies, women develop a condition called preeclampsia, characterized by increased intravascular coagulation, proteinuria, increased systemic vascular resistance, and pregnancy-induced hypertension (PIH) (19, 35). Although PIH is a major cause of maternal and fetal mortality, the mechanisms of this disorder have not been clearly identified. Because of the difficulty of performing mechanistic studies in pregnant women, several animal models of PIH have been developed (2, 4, 7, 12, 13, 16, 28, 30, 33). Studies in these animal models have proposed that a reduction in the uteroplacental blood flow and the ensuing placental ischemia during late pregnancy initiate a cascade of hemodynamic and vascular changes that lead to increased systemic vascular resistance and PIH (2, 12, 16, 30). In support of this hypothesis, we previously found that reduction in uteroplacental perfusion in pregnant rats results in significant hemodynamic changes and a hypertensive state that closely resembles PIH in women (2). We also found that the reduction in uteroplacental perfusion pressure in pregnant rats is associated with decreased vascular relaxation and enhanced vascular reactivity of the systemic vessels and suggested that these vascular changes could be the cause of the increased vascular resistance and hypertension (12). However, it is not clear how a localized reduction in uteroplacental perfusion pressure could lead to generalized vascular changes in the maternal circulation. For a localized reduction in uterine perfusion pressure to cause generalized vascular...
changes, one would predict possible release of vasoactive factors from the ischemic placenta into the systemic circulation. According to the “cytokine” hypothesis of PIH, the reduction in uteroplacental perfusion pressure and the ensuing placental ischemia are thought to increase the release of cytokines from the placenta into the maternal circulation; the increased plasma cytokines would then lead to the generalized vascular changes and hypertension (8, 10, 29, 43). In support of the cytokine hypothesis, it has been shown that the plasma levels of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, which is activated by TNF-α, are elevated nearly twofold in women with preeclampsia (10, 23, 29, 43). Also, we recently found that a two- to threefold elevation in women with preeclampsia (10, 23, 29, 43). Also, we recently found that a two- to threefold elevation in plasma TNF-α in late-pregnant rats results in significant elevation in vascular resistance and arterial pressure, while elevation of plasma TNF-α to the same level in virgin rats does not cause any significant hemodynamic changes (22). However, the vascular mechanisms underlying the TNF-α-induced increases in vascular resistance and arterial pressure in pregnant rats are still unclear.

The present study was designed to test the hypothesis that a two- to threefold elevation in plasma level of TNF-α, produced by infusing the cytokine into chronically instrumented late-pregnant rats at a rate to mimic the plasma levels observed during PIH in pre eclamptic women (29, 43), is associated with decreased endothelium-dependent vascular relaxation and increased vascular reactivity. We used virgin and late-pregnant rats to investigate 1) whether the vascular reactivity is enhanced in TNF-α-infused pregnant rats compared with control pregnant rats, 2) whether endothelium-dependent vascular relaxation is inhibited in TNF-α-infused pregnant rats compared with control pregnant rats, and 3) whether the reduction in vascular relaxation and enhancement of vascular reactivity in TNF-α-infused pregnant rats involve alterations in the endothelium-dependent NO-cGMP pathway.

METHODS

Animals. Female virgin (nonpregnant; 12 wk, ~200–250 g) and time-pregnant (day 12 of gestation, ~350 g) Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were housed individually in the animal facility and maintained on ad libitum standard rat chow and tap water in a 12:12-h light-dark cycle. The rats were divided into 4 groups of 12 rats each: virgin control, pregnant control, virgin TNF-α-infused, and pregnant TNF-α-infused. On day 14 of gestation or the equivalent in virgin rats, all rats were anesthetized with isoflurane and underwent a surgical procedure for catheter implantation. A section of PE-50 tubing was placed in the carotid artery for measurement of arterial pressure and blood sampling. The catheter was filled with heparin and exteriorized at the back of the neck. The rats were also instrumented with a venous catheter and a miniosmotic pump. TNF-α-treated rats were infused intravenously with TNF-α (Biosource, Camarillo, CA) at a rate of 200 ng·kg⁻¹·day⁻¹ for 5 days to increase plasma levels approximately twofold. Control rats were infused with normal saline. Rats were then housed individually, allowed to recover, and studied 5 days later (day 19–20 of pregnancy or the equivalent in virgin rats). All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center and the American Physiological Society.

Measurement of mean arterial pressure in conscious rats. On the day of the experiment, each rat was placed in a Plexiglas restrainer. The carotid arterial catheter was connected to a Statham pressure transducer, and the mean arterial pressure was continuously recorded on a Grass polygraph (model 7D, Astro-Med, West Warwick, RI).

Measurement of plasma TNF-α. On the day of the experiment, blood samples (0.5 ml) were collected for measurement of plasma TNF-α in control and TNF-α-infused virgin and pregnant rats using a rat TNF-α ELISA system (Cytoscreen, Biosource). This assay is a solid-phase sandwich-type system that utilizes a specific anti-rat TNF-α antibody coated onto the wells of microtiter plates. Serum samples (50 μl) and standards were pipetted in triplicate into appropriate microtiters wells, and the assay was performed according to the manufacturer’s instructions. The sensitivity of this TNF-α ELISA system is 0.7 pg/ml, and the upper limit of detection is 150 pg/ml. The average recovery of TNF-α in serum pools from normal rats is 97%. No significant cross-reactivity was noted with a battery of other human and murine cytokines. With the use of this protocol, the plasma levels of TNF-α were found to be 6.7 ± 2.2 and 13.5 ± 1.8 pg/ml in control and TNF-α-infused rats, respectively.

Tissue preparation. On the day of the experiment (day 19–20 of pregnancy), the rats were anesthetized by inhalation of isoflurane. The thoracic aorta was rapidly excised, placed in oxygenated Krebs solution, and cleaned of connective tissue. The aorta was cut transversely into 3-mm-wide rings. Aortic rings were cut open into strips. For endothelium-intact vascular strips, extreme care was taken through-out the procedure to avoid injury of the endothelium. For endothelium-denuded vascular strips, the endothelium was removed by gentle rubbing of the vessel interior with wet filter paper.

Isometric tension. A thread loop was used to attach one end of the vascular strip to a glass hook, and the other end was connected to a Grass force transducer (model FT03, Astro-Med). Vascular strips were stretched to maximum length (Lmax, i.e., 1.5 times the unloaded initial length). Lmax was measured separately in vascular strips of virgin and pregnant rats. Lmax in virgin rats was not significantly different from that in pregnant rats. Vascular strips were allowed to equilibrate for 1 h in a water-jacketed, temperature-controlled tissue bath filled with 50 ml of Krebs solution continuously bubbled with 95% O₂-5% CO₂ at 37°C. The changes in isometric tension were recorded on a Grass polygraph (model 7D, Astro-Med).

A control contraction was elicited by applying 10⁻⁵ M phenylephrine (Phe) to the tissue bath solution. Once the Phe contraction reached a plateau, the tissue was rinsed three times for 10 min each with Krebs solution. The whole procedure of contraction and washing was repeated twice. Increasing concentrations of Phe were applied, the contractile responses were recorded, and concentration-response curves were constructed.

In other tissues, a contraction to submaximal concentration of Phe was elicited. Increasing concentrations of ACh or sodium nitroprusside were added, and the extent of vascular relaxation was measured. In other experiments, the tissues were pretreated for 30 min with 100 μM N²-nitro-L-arginine methyl ester (L-NAME) to inhibit NO synthase or with 1 μM...
1H-[1,2,4]oxadiazolo[4,3]quinoxalin-1-one (ODQ) to inhibit cGMP production in smooth muscle (25), and the effects on the Phe-induced contraction and on ACh-induced relaxation of the Phe contraction were observed.

Nitrite/nitrate production. Endothelium-intact vascular strips were placed in test tubes containing 1.5 ml of Krebs solution aerated with 95% O2-5% CO2 at 37°C, and the solution was changed every 10 min for 1 h. Samples for basal accumulation of nitrite formed from released NO were first taken. The Krebs solution was replaced, and the strips were stimulated with ACh for 10 min. The vascular strips were rapidly removed, dabbed dry with tissue paper, and weighed. The incubation solutions were assayed for the stable end product of NO, NO3. Briefly, samples of incubation solution (50 μl, in triplicate) were mixed in a 96-well microtiter plate with 100 μl of the Griess reagent (21). The chromophore generated by the reaction with nitrite was detected spectrophotometrically (550 nm) using a microtiter plate reader (BioTek, Winooski, VT). The concentration of nitrite was calculated using a reference calibration curve with known concentrations of NaNO2.

Solutions, drugs, and chemicals. Normal Krebs solution contained (in mM) 120 NaCl, 5.9 KCl, 25 NaHCO3, 1.2 Na2HPO4, 1.15 dextrose, 1.2 MgCl2, and 2.5 CaCl2 at pH 7.4. Stock solutions of L-phenylephrine HCl, ACh, bradykinin, sodium nitroprusside, and L-NAME (Sigma) were prepared in distilled water. ODQ (Calbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide (final concentration <0.1). All other chemicals were of reagent grade or better.

Statistical analysis. The developed force was corrected for the cross-sectional area of each individual strip and expressed as active stress (N/m2) using the following equation: stress = force/cross-sectional area, where cross-sectional area = wet weight/(tissue density × length of the strip) and tissue density = 1.055 g/cm3. Data were analyzed and expressed as means ± SE, with n representing the total number of experiments (12–16) performed on individual vascular strips isolated from six to eight different rats of each group.

Data were compared using ANOVA with multiple classification criteria [rat type (pregnant vs. virgin), condition of endothelium (intact vs. denuded), and treatment (control vs. chronically infused with TNF-α or untreated vs. acutely treated with L-NAME or ODQ)] followed by Bonferroni’s post test to compare selected groups or Dunnett’s post test to compare all groups with the control group. Differences were considered statistically significant if P < 0.05.

RESULTS

On the day of the experiment (day 19–20 of gestation or the equivalent in virgin rats), the mean arterial pressure was 96 ± 3 mmHg in control pregnant rats and was significantly elevated in TNF-α-infused pregnant rats (123 ± 3 mmHg). In contrast, the mean arterial pressure was not significantly different between control virgin rats and TNF-α-infused virgin rats: 107 ± 4 and 109 ± 3 mmHg, respectively.

In endothelium-intact vascular strips of control pregnant rats, Phe caused concentration-dependent increases in contraction (Fig. 1A). The Phe-induced contraction appeared to be greater in TNF-α-infused pregnant rats (Fig. 1B) than in control pregnant rats (Fig. 1A) but did not appear to be different between control virgin rats (Fig. 1C) and TNF-α-infused virgin rats (Fig. 1D). To correct for the difference in the size of the vascular strips, the Phe contraction was normalized for the cross-sectional area of the vascular strip and presented as active stress (see METHODS). The Phe concentration-active stress curve in TNF-α-infused pregnant rats was enhanced compared with that in control pregnant rats (Fig. 2A). The maximal Phe-induced active stress was significantly greater in TNF-α-infused pregnant rats than in control pregnant rats (Table 1). Removal of the endothelium significantly
enhanced the Phe-induced stress in control pregnant rats but caused slight and insignificant increase in Phe-induced stress in TNF-α-infused pregnant rats (Fig. 2A, Table 1). In contrast, the Phe-induced active stress was not significantly different between control virgin rats and TNF-α-infused virgin rats (Fig. 2B). When the Phe response was presented as a percentage of the maximum Phe contraction and the Phe ED₅₀ was calculated, Phe was more potent in causing contraction in endothelium-denuded than in endothelium-intact vascular strips of control pregnant rats (Fig. 2C, Table 1). In contrast, Phe was only slightly more potent in causing contraction in endothelium-denuded than in endothelium-intact strips of TNF-α-infused pregnant rats (Fig. 2C, Table 1). The Phe response as a percentage of maximum and calculation of the Phe ED₅₀ showed that Phe was more potent in causing contraction in l-NAME-pretreated than in untreated vascular strips of control pregnant rats (Fig. 3C, Table 1). In contrast, the maximal Phe-induced stress and the Phe ED₅₀ were not significantly different between l-NAME-pretreated and untreated vascular strips of TNF-α-infused pregnant rats (Fig. 3, B and D, Table 1).

Similarly, in endothelium-intact strips, pretreatment with 1 μM ODQ for 30 min, to inhibit cGMP production in smooth muscle (21, 25), enhanced Phe-induced stress in control pregnant rats (Fig. 3A, Table 1). Also, plotting of the Phe response as a percentage of maximum and calculation of the Phe ED₅₀ showed that Phe was more potent in causing contraction in l-NAME-pretreated than in untreated vascular strips of control pregnant rats (Fig. 3C, Table 1). In contrast, the maximal Phe-induced stress and the Phe ED₅₀ were not significantly different between l-NAME-pretreated and untreated vascular strips of TNF-α-infused pregnant rats (Fig. 3, B and D, Table 1).

In endothelium-intact vascular strips, pretreatment with 100 μM L-NAME for 30 min, to inhibit NO synthase, significantly enhanced the Phe-induced stress in control pregnant rats (Fig. 3A, Table 1). Also, plotting of the Phe response as a percentage of maximum and calculation of the Phe ED₅₀ showed that Phe was more potent in causing contraction in l-NAME-pretreated than in untreated vascular strips of control pregnant rats (Fig. 3C, Table 1). In contrast, the maximal Phe-induced stress and the Phe ED₅₀ were not significantly different between l-NAME-pretreated and untreated vascular strips of TNF-α-infused pregnant rats (Fig. 3, B and D, Table 1).

In endothelium-intact vascular strips of control pregnant rats, ACh caused concentration-dependent relaxation of submaximal Phe (6 × 10⁻⁷ M)-induced contraction (Figs. 4A and 5A). Because the Phe contraction in other groups of rats was greater than that in control

**Table 1. Maximal Phe-induced active stress and ED₅₀ in vascular strips of control pregnant rats and TNF-α-infused pregnant rats**

<table>
<thead>
<tr>
<th></th>
<th>Control Pregnant</th>
<th>TNF-α-Infused Pregnant</th>
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<tr>
<td><strong>Active stress, ×10³ N/m²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Endo</td>
<td>4.2 ± 0.4</td>
<td>9.9 ± 0.7*</td>
</tr>
<tr>
<td>−Endo</td>
<td>6.4 ± 0.6†</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>L-NAME</td>
<td>6.2 ± 0.6†</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>ODQ</td>
<td>6.7 ± 0.6†</td>
<td>10.8 ± 0.8</td>
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<tr>
<td>ED₅₀, ×10⁻⁸ M</td>
<td></td>
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</tr>
<tr>
<td>+Endo</td>
<td>8.5 ± 0.3</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>−Endo</td>
<td>3.5 ± 0.4†</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>L-NAME</td>
<td>2.7 ± 0.2†</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>ODQ</td>
<td>2.2 ± 0.2†</td>
<td>1.0 ± 0.2</td>
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</table>

Values are means ± SE of measurements in 12–16 vascular strips from 6–8 rats of each group that were infused with 10⁻⁵ M phenylephrine (Phe). TNF-α, tumor necrosis factor-α; +Endo, endothelium intact; −Endo, endothelium denuded; L-NAME, N⁶-nitro-L-arginine methyl ester; ODQ, 1H-[1,2,4]oxadiazolo[4,3-b]quinoxalin-1-one. *Significantly different (P < 0.05) from corresponding measurement in control pregnant rats. †Significantly different (P < 0.05) from corresponding measurement in endothelium-intact vascular strips of control pregnant rats.
Fig. 3. Effect of N\(^{\text{\u2013}}\)-nitro-L-arginine methyl ester (L-NAME) and 1H-[1,2,4]-oxadiazolo[4,3-d]quinoxalin-1-one (ODQ) on Phe-induced contraction in endothelium-intact aortic strips of control pregnant rats (A and C) and TNF-\(\alpha\)-infused pregnant rats (B and D). Endothelium-intact aortic strips incubated in normal Krebs solution were untreated or pretreated with 100 \(\mu\)M L-NAME or 1 \(\mu\)M ODQ for 30 min and then stimulated with increasing concentrations of Phe. Phe contraction was measured and presented as active stress (A and B) or as percentage of maximum Phe contraction (C and D). Data points represent means \(\pm\) SE of measurements in 12–16 vascular strips from 6–8 rats of each group.

Fig. 4. Representative traces of ACh-induced relaxation of Phe contraction in aortic strips isolated from control pregnant (A), TNF-\(\alpha\)-infused pregnant (B), control virgin (C), and TNF-\(\alpha\)-infused virgin (D) rats. Endothelium-intact strips were incubated in normal Krebs solution and then stimulated with a submaximal concentration of Phe to produce a contraction roughly equal in magnitude in the different groups of rats. Increasing concentrations of ACh ([ACh]) were added, and relaxation of Phe contraction was measured. At the end of the experiment, 10\(^{-6}\)M sodium nitroprusside (SNP) was added to ensure the ability of the smooth muscle to relax.
pregnant rats, the Phe concentration was adjusted in the TNF-α-infused pregnant rats (3 × 10^{-8} M), control virgin rats (3 × 10^{-7} M), and TNF-α-infused virgin rats (3 × 10^{-8} M) to produce a submaximal contraction that is roughly equal in magnitude to that in control pregnant rats. The ACh-induced relaxation of Phe contraction was reduced in TNF-α-infused pregnant rats compared with control pregnant rats (Figs. 4B and 5A). Also, when the ACh-induced response was presented as a percentage of the maximal ACh-induced relaxation, ACh was less potent in inducing relaxation in TNF-α-infused pregnant rats than in control pregnant rats: ED_{50} = 1.2 ± 0.06 × 10^{-6} and 0.6 ± 0.04 × 10^{-7} M, respectively. ACh-induced relaxation was not significantly different between control virgin rats and TNF-α-infused virgin rats (Figs. 4, C and D, and 5B). Pretreatment of endothelium-intact strips with 100 μM l-NAME or 1 μM ODQ significantly inhibited the ACh-induced relaxation of Phe contraction in control pregnant rats (Fig. 6A) but not TNF-α-infused pregnant rats (Fig. 6B). Removal of the endothelium completely inhibited the ACh-induced relaxation of Phe contraction in all groups of rats.

In endothelium-intact vascular strips of control pregnant rats, the basal nitrite/nitrate production was 42.1 ± 7.5 pmol/mg tissue weight, and ACh caused concentration-dependent increases in nitrite/nitrate production (Fig. 7). The basal and ACh-induced nitrite/nitrate production showed significant reduction in TNF-α-infused pregnant rats compared with control.
significantly greater than that in control pregnant rats, fi
measurements in 12 vascular strips from 6 rats of each group.

DISCUSSION

The main findings of the present study are as follows: 1) the mean arterial pressure in late-pregnant
erats with twofold elevation of plasma level of TNF-α is
significantly greater than that in control pregnant rats,

2) vascular reactivity is greater in TNF-α-infused preg-
nant rats than in control pregnant rats, 3) endo-
thelium-dependent vascular relaxation is less in TNF-α-
influenced pregnant rats than in control pregnant rats,

4) the activity of the endothe

lim-dependent NO- cGMP pathway is reduced in TNF-α-infused pregnant rats compared with control pregnant rats, and 5) the
arterial pressure, vascular reactivity, and vascular relax-
ation are not significantly different between control

virgin rats and TNF-α-infused virgin rats.

Consistent with previous studies from our laboratory
and others, we have found that the mean arterial
pressure and the vascular reactivity to various vaso-
contractors are reduced in pregnant rats compared
with virgin rats (13, 15, 28). The present study has also
shown that the mean arterial pressure and the vas-
ular reactivity to the α-adrenergic agonist Phe are en-
hanced in TNF-α-infused pregnant rats compared with
control pregnant rats. The findings in the TNF-α-in-
fluenced pregnant rats are consistent with previous
studies, which have shown that the arterial pressure and
the vascular reactivity to vasoconstrictors are en-
hanced in other animal models of hypertension during
late pregnancy (7, 12, 13, 28). In search of the possible
mechanisms involved in the observed enhanced vascular
reactivity in TNF-α-infused pregnant rats, we
found that removal of the endothelium significantly
enhanced the Phe contraction in normal pregnant rats
but had minimal effects in TNF-α-infused pregnant
rats. Also, the ACh-induced relaxation was less in

TNF-α-infused pregnant rats than in normal pregnant
rats. These results suggest that an endothelium-de-
pendent relaxation pathway is intact in control preg-
nant rats but is possibly impaired during elevation of
plasma TNF-α in late-pregnant rats.

One important vasodilator released from endothelial
cells is NO (20, 26, 34, 37). The reduced ACh-induced
relaxation in TNF-α-infused pregnant rats could be
due to a decrease in the synthesis and release of NO
from endothelial cells or a change in the sensitivity of
vascular smooth muscle to relaxation by NO. The sen-
tivity of vascular smooth muscle to relaxation by NO
could be evaluated by its sensitivity to relaxation by
exogenous NO donors such as sodium nitroprusside.

The observation that relaxation of endothelium-de-

nuded vascular strips by sodium nitroprusside was not
significantly different between control and TNF-α-in-
fluenced pregnant (Fig. 7).

In endothelium-denuded vascular strips of all groups
of rats, sodium nitroprusside, an exogenous NO donor
and a standard guanylate cyclase activator (24), caused
concentration-dependent relaxation of Phe contraction.
However, no significant differences in the magnitude of
sodium nitroprusside-induced relaxation of Phe con-
traction were observed between control and TNF-α-in-
fluenced pregnant (Fig. 8A) or virgin rats (Fig. 8B).

DISCUSSION

The main findings of the present study are as fol-

lows: 1) the mean arterial pressure in late-pregnant

rats with twofold elevation of plasma level of TNF-α is
significantly greater than that in control pregnant rats,
ation by ACh and enhanced the vascular reactivity to Phe in control pregnant rats but had minimal effects in TNF-α-infused pregnant rats. These results suggest that NO synthesis by endothelial cells is intact in normal pregnant rats but is impaired during elevation of plasma TNF-α in late-pregnant rats. This is further supported by the observation that the basal and the ACh-induced nitrite/nitrate production were significantly reduced in vascular strips of TNF-α-infused pregnant rats compared with control pregnant rats.

The NO produced by endothelial cells is known to promote vascular relaxation by activating guanylate cyclase and increasing cGMP production in vascular smooth muscle (24, 26). We found that ODQ, which is known to inhibit guanylate cyclase and to decrease cGMP production in smooth muscle (21, 25), significantly inhibited the endothelium-dependent vascular relaxation by ACh and enhanced the vascular reactivity to Phe in endothelium-intact strips of control pregnant rats but not TNF-α-infused pregnant rats. These results further support the contention that NO production or release by endothelial cells and, thereby, the activity of the NO-cGMP pathway in vascular smooth muscle are reduced in TNF-α-infused pregnant rats compared with control pregnant rats.

The present results support the hypothesis that placental ischemia contributes to maternal endothelial cell dysfunction by enhancing the synthesis of cytokines such as TNF-α and IL-1 (8, 10, 23). The data are also consistent with the reports that the plasma levels of TNF-α and IL-6, which is activated by TNF-α, are elevated nearly twofold in women with preeclampsia (10, 23, 29, 43). The effects of TNF-α appear to be dependent on the plasma levels of the cytokine. Although high levels of TNF-α, as observed during septic shock or after administration of a high dose of lipopolysaccharide (LPS), activate gene expression of inducible NO synthase, modest levels of TNF-α have been shown to downregulate the mRNA of endothelial NO synthase (47). This is consistent with a recent study by Faas and co-workers (18) that showed that intravenous infusion of a high dose of the endotoxin LPS, which is known to activate TNF-α, decreases blood pressure in conscious pregnant rats, while a very low-dose infusion of LPS results in significant and long-term increase in blood pressure and urinary albumin excretion and significant platelet aggregation (18).

It is important to emphasize the following cautionary remarks regarding the above interpretations. First, although the present results suggest that the decrease in endothelial cell function and the increase in vascular reactivity observed in the TNF-α-infused pregnant rats could contribute to the observed increase in blood pressure, these results should be interpreted with caution, since the changes in endothelial cell function and vascular reactivity may also be secondary to blood pressure elevation. Analysis of the time course of the changes in vascular reactivity and the increase in blood pressure should help determine whether the relationship between these two parameters is causal or associative in nature. Second, the chronic effects of TNF-α in vivo could be due to a direct effect on the vascular endothelium or perhaps indirect effects through the release of other factor(s). Although a recent report suggests that direct exposure to TNF-α and IL-1 causes functional alterations in endothelial cells (39) and reduction in ACh-induced vasodilation and relaxation of vascular strips of normal male rats (44), whether these direct effects of TNF-α on endothelium-dependent vascular relaxation are altered in females, particularly during pregnancy, remains to be investigated. Third, the vascular endothelium has been shown to release other vasodilator substances, in addition to NO, such as endothelium-derived hyperpolarizing factor and prostacyclin (5, 42). This may explain why, in the vascular strips of TNF-α-infused pregnant rats, some relaxation to ACh was still observed and was not completely inhibited by L-NAME or ODQ. On the other hand, the complete absence of ACh-induced relaxation in endothelium-denuded strips of TNF-α-infused pregnant rats still supports the contention that the ACh-induced relaxation is endothelium dependent. Fourth, although the present results provided evidence that the enhanced vascular reactivity in the TNF-α-infused pregnant rats may involve inhibition of an endothelium-dependent NO-cGMP pathway, we cannot rule out the possibility that an increase in the sensitivity of vascular smooth muscle to endothelium-derived contracting factors also occurs. This is supported by reports that long-term inhibition of NO synthesis during mid- to late gestation in rats is associated with elevated plasma levels of endothelin-1 (17) and that TNF-α and IL-1 stimulate the production of endothelium-derived contracting factors including endothelin-1 (31). This is also supported by the present observation that removal of the endothelium or pretreatment of vascular strips of control pregnant rats with L-NAME or ODQ caused an enhancement of Phe-induced vascular reactivity to levels that were still less than that observed in the TNF-α-infused rats. These observations suggest that the elevation of plasma TNF-α during late pregnancy in rats may be associated with additional alterations in the cellular mechanisms of vascular smooth muscle contraction and should represent interesting areas for future experiments. We should note that the causes of the lack of effects of TNF-α in virgin rats and its dramatic vascular effects in pregnant rats are unclear but could be related, in part, to the plasma levels of sex hormones such as estrogen and progesterone and possible synergistic actions of the sex hormones on the vascular effects of TNF-α. This is supported by reports that the plasma estrogen and progesterone levels are elevated during pregnancy and are higher in pregnant than in virgin rats (2). This is also supported by in vitro studies that have shown that estradiol enhances leukocyte binding to TNF-α-stimulated endothelial cells via an increase in TNF-α-induced adhesion molecules (6). Studying the effects of acute and long-term exposure to estrogen and progesterone on the vascular effects of TNF-α should help further identify the mechanisms
underlying the possible synergistic interactions between sex hormones and the cytokine and should represent important areas for future investigations.

In conclusion, the present results suggest that an endothelium-dependent relaxation pathway involving the production and release of NO from endothelial cells and increased cGMP production in smooth muscle is inhibited in systemic vessels of pregnant rats chronically treated with TNF-α. The results suggest a role for TNF-α as one possible mediator of the increased vascular resistance associated with pregnancy-induced hypertension.

Perspectives

The search for the cellular and vascular mechanisms underlying the hypertension induced in pregnant animal models should help us understand better the pathophysiological basis of preeclampsia in pregnant women. Abnormal reduction in uteroplacental blood flow during late pregnancy has been suggested as an initiating event that triggers a cascade of events leading to increased vascular resistance and hypertension. The present results suggest that the release of cytokines such as TNF-α from the ischemic placenta is one factor that may lead to endothelial cell dysfunction, decreased vascular relaxation, and thereby increased vascular resistance and pregnancy-induced hypertension. The present data represent the observed changes in endothelium-dependent vascular relaxation at one specific point in time, namely, day 19 of pregnancy in rats and after 5 days of TNF-α infusion. Future time course studies should be useful to identify the time of onset and the time to peak changes in endothelium-dependent vascular relaxation in TNF-α-infused pregnant rats and to determine whether these vascular changes precede or coincide with the changes in the arterial pressure. We should also note that the observed vascular effects of TNF-α in pregnant rats may not be limited to only this cytokine. Placental ischemia contributes to maternal endothelial cell dysfunction by enhancing the synthesis of not only TNF-α but also IL-1. Also, the plasma levels of the cytokine IL-6 are elevated almost twofold in women with preeclampsia. Whether chronic infusion of other cytokines such as IL-1 or IL-6 during late pregnancy would produce vascular effects similar to those of TNF-α remains to be investigated. In relation to this question, it is not clear whether the observed chronic effects of TNF-α represent direct vascular effects of the cytokine or may be mediated by other factors. Interestingly, TNF-α has been shown to activate IL-6. Therefore, studying the acute vascular effects of not only TNF-α but also other cytokines such as IL-6 should help further delineate the role of cytokines as possible mediators of pregnancy-induced hypertension.

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