Role of peroxynitrite in altered fetal-placental vascular reactivity in diabetes or preeclampsia

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Division of Maternal-Fetal Medicine, 1Department of Obstetrics and Gynecology, 2Department of Pediatrics, and 3Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0526

Kossenjans, Wilhelm, Annie Eis, Rashmi Sahay, Diane Brockman, and Leslie Myatt. Role of peroxynitrite in altered fetal-placental vascular reactivity in diabetes or preeclampsia. Am J Physiol Heart Circ Physiol 278: H1311–H1319, 2000.—Oxidative stress may increase production of superoxide and nitric oxide, leading to formation of prooxidant peroxynitrite to cause vascular dysfunction. Having found nitrotyrosine residues, a marker of peroxynitrite action, in placental vessels of preeclamptic and diabetic pregnancies, we determined whether vasoactivity is altered in these placentas and treatment with peroxynitrite produces vascular dysfunction. The responses of diabetic, preeclamptic, and normal placentas to increasing concentrations of the vasoconstrictors U-46619 (10-9–10-7 M) and ANG II (10-8–10-6 M) and the vasodilators glyceryl trinitrate (10-8–10-7 M) and prostacyclin (PGI2; 10-8–10-6 M) were compared as were responses to these agents in normal placentas before and after treatment with 3.16 × 10-4 M peroxynitrite for 30 min. Responses to both vasoconstrictors and vasodilators were significantly attenuated in diabetic and preeclamptic placentas compared with controls. Similarly, responses to U-46619, nitroglycerin, and PGI2, but not ANG II, were significantly attenuated following peroxynitrite treatment. The presence of nitrotyrosine residues confirmed peroxynitrite interaction with placental vessels. Overall, our data suggest that peroxynitrite formation is capable of attenuating vascular responses in the human placenta.

reactive oxygen species; nitric oxide; superoxide; oxidative injury

PREECLAMPSIA IS CONSIDERED one of the most significant health problems in human pregnancy (12), complicating ~5–7% of pregnancies, and is a leading cause of fetal growth restriction, indicated premature delivery, and maternal death. The disorder is characterized by maternal hypertension, proteinuria, and edema with accompanying platelet aggregation, vasoconstriction of the maternal vascular bed, and increased resistance of the fetal-placental circulation, with characteristic reduced uteroplacental and fetal-placental blood flows. Increasingly, preeclampsia is recognized to be a syndrome characterized by profound dysfunction of the vascular endothelium (31), perhaps secondary to oxidative stress (32).

In the United States, 16 million people are affected by diabetes mellitus, and pregestational diabetes mellitus complicates 2–5% of pregnancies. Despite improved perinatal morbidity over the last several decades, considerable morbidity related to growth aberrations, including a wide range of structural and biological abnormalities, still exists in infants of insulin-dependent diabetic mothers. A number of placental lesions have been described, particularly in cases of poorly controlled glucose homeostasis, including edema, chorangiosis, edema, hypo- and hyperamification of the terminal villi, infarcts, fetal-placental sclerosis, fibrotic villi, and villous basement membrane changes (6). Such changes are likely to affect placental vascular resistance and vascular volume and therefore may lead to chronic disturbances in fetal-placental blood flow. In addition, diabetes mellitus in pregnancy is accompanied by an increased incidence of preeclampsia and pregnancy-induced hypertension (9), which like diabetes are characterized as being states of endothelial dysfunction (33).

Recently, evidence has accumulated which suggests that reactive oxygen species play an important role in both diabetes- and preeclampsia-related complications. In diabetes, oxygen free radicals are thought to be produced as a result of prolonged periods of exposure to hyperglycemia, which is known to cause nonenzymatic glycation of plasma proteins (39). The glycated products undergo further spontaneous reactions leading to the production and release of free radicals, including superoxide (O2-·) (35). Gillery et al. (14) have demonstrated that glycated protein prepared from diabetic serum is able to generate O2-· at physiological pH, which in the absence of appropriate levels of scavengers may lead to an imbalance between prooxidants and antioxidants and produce a state of oxidative stress. Pregnancies complicated by preeclampsia are associated with elevated blood and tissue levels of lipid peroxidation products (21, 25), thus implicating increased oxidative stress in the etiology of preeclampsia. Increased lipid peroxide levels in preeclampsia may also be the result of decreased antioxidant activities, such as superoxide dismutase, glutathione peroxidase, and vitamin E, which have been shown to be decreased...
in placental tissues from preeclamptic pregnancies (41).

As the human fetal-placental vasculature lacks autonomic innervation, autocrine and/or paracrine agents such as nitric oxide radical (NO•) play an important role in the regulation of fetal-placental blood flows, being shown to maintain low-basal tone and attenuate the vasoconstrictive effects of thromboxane and endothelin (27). However, NO• is inactivated by superoxide anion (O2•−), therefore limiting its activity, but this interaction yields peroxynitrite anion (ONOO•−), a powerful oxidant of a variety of biomolecules (2). Peroxynitrite is known to cause lipid peroxidation, inhibit the expression of nitrotyrosine residues, formed from the interaction of ONOO•− with tyrosine moieties, in the fetal vasculature and villous stroma of preeclamptic and diabetic placentas (22, 28). These findings suggest involvement of ONOO•− in the pathological processes of diabetic and preeclamptic placental injury.

However, the functional significance associated with the presence of altered tyrosine residues on proteins in the fetal-placental vasculature remains unknown. The present study was therefore undertaken to determine whether the responsiveness to vasoactive agents of the fetal-placental vasculature of pregnancies affected by diabetes or preeclampsia is compromised. In addition, we sought to directly determine whether ONOO•− causes functional deficit in this vascular bed by perfusing the fetal-placental vasculature with authentic ONOO•− and determining its effects on vascular reactivity.

MATERIALS AND METHODS

List of chemicals. Polyvinylpyrrolidone K30 was purchased from Acros (Geel, Belgium). Peroxynitrite was obtained from Alexis (San Diego, CA) and stored at −80°C until used for experiments. The exact concentration of the peroxynitrite was determined spectrophotometrically before use at 302 nm using a molar absorptivity of E302 = 1.67 × 104 M−1·cm−1 and was infused directly into the inflow line of the perfused placenta to give a final concentration of 3.16 × 10−4 M. All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Tissue procurement. Placentas from uncomplicated pregnancies and those affected by either pregestational insulin-dependent diabetes mellitus or by preeclampsia were obtained after vaginal delivery or cesarean section at term in the labor and delivery service at University Hospital under a protocol approved by the Institutional Review Board. For the purpose of our study, preeclampsia was defined as occurrence of hypertension (sustained blood pressure greater than 140 mmHg systolic and 90 mmHg diastolic), edema, and proteinuria (≥1+ protein on dip stick) after 20 wk gestation in a previously normotensive woman. Placentas from multifetal pregnancies and pregnancies affected by drug abuse or with coexisting medical conditions other than insulin-dependent diabetes mellitus or preeclampsia were excluded from the study.

Placentas were transported to the laboratory immediately following delivery and processed for perfusion. Samples of placental villous tissue were flash frozen in liquid nitrogen within 15 min after delivery or immediately following perfusion experiments and stored at −80°C until further processed for immunohistochernistry.

Preeclampsia perfusion. Perfusion of the placentas was performed as previously described by Glance et al. (15). To establish perfusion of the fetal-placental circulation, a suitable third- or fourth-order artery and corresponding vein were cannulated at a site immediately before passage through the chorionic plate. For perfusion of the maternal intervillous space, two remnants of the spiral arteries were cannulated in the basal plate. The effluent from the intervillous space was collected by gravity into a Plexiglas funnel, over which the cotyledon was placed basal plate downward. The perfusion medium for both fetal and maternal circulation consisted of Hanks’ balanced salt solution containing polyvinylpyrrolidone 40 (25 mg/ml), 0.1% BSA, heparin (20 IU/ml), and gentamicin (48 µg/ml). The pH of the perfusion buffer was adjusted to 7.3–7.5 with sodium bicarbonate, and the medium was gassed with 95% O2-5% CO2 and maintained at 37°C. The perfusion rates were 4 ml/min for the fetal and 10 ml/min for the maternal circulations. Lateral pressure readings were taken in the fetal and maternal inflow lines close to the points of cannulation. Data on perfusion pressure and inflow and outflow PO2 were sampled every second and stored using a computer system with WinDAQ 200 software (Dataq Instruments; Akron, OH).

Vascular reactivity in diabetic or preeclamptic placentas. Perfusion pressure was recorded following the administration of 2-ml bolus injections into the intervillous space of vehicle followed by the vasoconstrictors U-46619 (a thromboxane mimetic, 10−9–10−7 M) or ANG II (10−9–10−7 M), or by the vasodilators glyceryl trinitrate (GTN, 10−6–10−5 M) or prostacyclin (PGI2; 10−8–10−6 M). When dose responses to the vasodilators were being studied, the placental cotyledons were first preconstricted with constant infusion of 1 × 10−8 to 5 × 10−8 M U-46619 to the same range of perfusion pressures (between 80 and 120 mmHg) in each group. Each experiment was repeated in five or six separate placentas from each group.

Immunohistochemical staining. Immunohistochemical staining was performed as previously described (28). Placental villous tissues were obtained before or following perfusion with ONOO•− vehicle, frozen in liquid nitrogen-cooled isopentane, and cryosectioned at 6–8 µm. Tissue sections were blocked with 1.5% goat serum for 30 min and immunostained with rabbit polyclonal anti-nitrotyrosine antibody (10 µg/ml; Upstate Biotechnology; Lake Placid, NY), which had been preabsorbed in human normal serum using the Vectastain Elite ABC Kit, and aminoethylcarbazole as peroxidase substrate. Sections were rinsed in PBS and coverslipped, and localization pattern and intensity of immunostaining for nitrotyrosine were assessed subjectively. Images were captured using Image Pro-Plus software (Media Cybernetics; Silver Springs, MD). Sections treated with pooled rabbit IgG instead of the primary antibody served as negative immunological controls.
Effect of peroxynitrite on placental vascular reactivity. Two protocols were used to test the reactivity of placentas to either vasoconstrictors or vasodilators before and after ONOO− treatment. To study the effect on vasoconstrictors, placentas were first equilibrated for a period of at least 30 min, after which they were treated with bolus administrations of either solvent solution or U-46619 or ANG II (10−9−10−7 M) and perfusion pressure was recorded. Then, after infusion of ONOO− [3.16 × 10−4 M, based on work by Villa et al. (40)] into the fetal-placental vasculature for 30 min, the injections of solvent solution and the two vasoconstrictors were repeated and again changes in perfusion pressure were recorded. The stock ONOO− solution was infused into the perfusion inflow line immediately proximal to the placental tissue at a dilution of 1:538 to yield a final concentration at the site of infusion of 3.16 × 10−4 M. pH was maintained between 7.3 and 7.5. A separate series of control experiments following the same protocol was performed where vehicle for the stock ONOO− solution (5.5 × 10−4 M NaOH) was infused at the appropriate dilution for 30 min. To study the effect of ONOO− on vasodilators, placentas were again equilibrated for at least 30 min. Then the fetal placental vasculature was preconstricted to between 80 and 120 mmHg with constant infusion of U-46619. In the continued presence of U-46619, dose-response curves to solvent solution and GTN (10−9−10−7 M) or PGI2 (10−8−10−6 M) were performed. After a 30-min infusion of 3.16 × 10−4 M ONOO−, administration of vasodilators was then repeated in the same placentas ensuring that they were preconstricted with U-46619 to the same perfusion pressure as prior to ONOO− treatment. Again for control purposes, a separate series of experiments following the same protocol was conducted where the vehicle for the ONOO− solution was infused for 30 min. For each protocol six or seven placentas were studied in each group.

Statistical analysis. Data were expressed as means ± SE. Statistical significance was tested using repeated measures ANOVA for studies evaluating the vasoreactivity to the different agonists in control vs. diabetic or preeclamptic placentas, in diabetic vs. preeclamptic placentas, and between ONOO−-treated and vehicle-treated placentas. P < 0.05 was considered significant.

RESULTS

Table 1 shows the maternal characteristics and pregnancy outcomes for the control, diabetic, and preeclamptic groups studied. Although mean fetal and placental weights were greater in the diabetic group, the difference from the control group did not reach statistical significance. Delivery mode, gestational age, birth weight, placental weight, ratio of placenta weight to birth weight, and Apgar scores were similar between control and preeclamptic pregnant women. As expected, blood pressures of preeclamptics were significantly higher compared with control patients. In addition, we found a statistically significant difference in maternal age between pre eclamptic and control groups, which may simply reflect increased incidence of preeclampsia in first pregnancies. All women in the preeclamptic group received MgSO4 as a routine course of treatment. Four of the five preeclamptic women had urinary protein levels of 4+ and one of 1+ on dipstick. The diabetic group consisted of two women in White classification C, two in class D, one in class R, and one in class F. The two White class D were proteinuric with levels of 2+ and 3+, and the White class R+ F patient had urinary protein of 4+. The third trimester hemoglobin A1C level of five of the six women in this group fell within the normal range of 5.8–6.4% in our population, suggesting they had good glycemic control, with the remaining woman having a hemoglobin A1C level of 6.8%. None of the women in the diabetic or control groups was treated with MgSO4. All patients in the preeclamptic and diabetic groups were gravid 1/parity 0 (G1/P0) except for one G3/P0 in each of these two groups. The normal control group consisted of patients who were G8/P6, G4/P1, G2/P1, G4/P1, G6/P3, and G4/P2.

Figure 1, A–D, illustrates fetal vascular reactivity of diabetic, preeclamptic, and control placentas. Significant concentration-dependent responses were observed in each group to increasing concentrations of the vasoconstrictors U-46619 (Fig. 1A) and ANG II (Fig. 1B) and the vasodilators GTN (Fig. 1C) and PGI2 (Fig. 1D), respectively. However, the responses to each of the four vasoactive agents were significantly attenuated in the diabetic (P < 0.01, P < 0.05, P < 0.0005, and P < 0.0001 for U-46619, ANG II, GTN, and PGI2, respectively) and preeclamptic groups (P < 0.01, P < 0.001, P < 0.05, and P < 0.01, respectively) compared with controls. The responses to the highest concentration of the vasoactive agents used were only 31, 41, 36, and 23% of control values in the diabetic placentas and 39, 43, 78, and 51% of controls in preeclamptic placentas for U-46619, ANG II, GTN, and PGI2, respectively. Differences in responses to vasodilators were not due to differences in the extent of preconstriction to U-46619 between the three groups of placentas (P > 0.05, ANOVA). The decreases in vasoactive responses to U-46619 and ANG II from controls were similar (P > 0.05, repeated

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Control</th>
<th>Diabetes</th>
<th>Preeclampsia</th>
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<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Maternal age, yr</td>
<td>33.2 ± 4.6</td>
<td>26.3 ± 8.5</td>
<td>18.8 ± 3.7*</td>
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<td>Mode of delivery</td>
<td>Vaginal</td>
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<tr>
<td>Gestational age, wk</td>
<td>38.8 ± 1.6</td>
<td>37.3 ± 0.9</td>
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<td>Birth wt, g</td>
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<td>3,838 ± 199</td>
<td>3,148 ± 345</td>
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<td>Placental wt, g</td>
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<td>887 ± 207</td>
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<td>hemoglobin A1C, %</td>
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<td>Neg (3), 2+ (1), 1+ (1), 4+ (4)</td>
<td>3+ (1), 4+ (1)</td>
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<td>BP at delivery</td>
<td>Systolic</td>
<td>123 ± 7</td>
<td>137 ± 13</td>
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<td>Diastolic</td>
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<td>69 ± 14</td>
<td>90 ± 7‡</td>
</tr>
<tr>
<td>MgSO4 treatment</td>
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Values are expressed as means ± SE; n = number of cases. Normal range of hemoglobin A1C, 5.8–6.4%. Statistically significantly different compared with controls (Student’s t-test): *P < 0.002, †P < 0.001, ‡P < 0.02.
measures ANOVA) in the diabetic and preeclamptic placentas, whereas the decrease in responses to GTN from control and PGI2 was greater in diabetic than preeclamptic placentas (P < 0.05, repeated measures ANOVA). Thus the responses to vasodilators were more affected in the placentas from diabetic individuals, whereas the responses to vasoconstrictors were equally affected in the diabetic and preeclamptic groups.

The responses of the human placentas to bolus infusions of U-46619, ANG II, GTN, and PGI2 before and after treatment with ONOO− are shown in Fig. 2, A–D, respectively. Again, the vasoconstrictor and vasodilator responses increased in a significant concentration-dependent manner with increasing amounts of vasoactive agent infused into the fetal-placental circulation both before and after ONOO− treatment. However, the responses were significantly altered for U-46619, GTN, and PGI2 after the 30-min ONOO− infusion compared with responses before ONOO− treatment, being diminished by 65% (P < 0.001), 80% (P < 0.05), and 65% (P < 0.001) at the highest concentration of vasoactive agent used. The concentration of U-46619 used was varied so that the extent of preconstriction to U-46619 in the vasodilator experiments was not different before and after ONOO− treatment (P > 0.05, paired t-test). The apparent decreased vasoconstriction of the fetal-placental vasculature to ANG II caused by ONOO− did not reach statistical significance. Experiments carried out in a separate series of placentas employing the vehicle for ONOO− to control for a potential temporal effect on vasoreactive responses to agonist showed no effect of time on responses to repeated infusion of vasoactive agents.

Representative photomicrographs of placental villous tissue immunostained for nitrotyrosine are shown in Fig. 3. No positive immunostaining for nitrotyrosine was observed in nonperfused placental villous tissue (Fig. 3A), ONOO−-perfused placental tissue stained with control preimmune IgG instead of the primary anti-nitrotyrosine antibody (Fig. 3B), or villous tissue perfused with vehicle for the ONOO− solution (Fig. 3C). In contrast, however, as is seen in Fig. 3D, villous tissue perfused with authentic peroxynitrite showed intense immunostaining for nitrotyrosine. Nitrotyrosine residues were evident in the vascular endothelium, which in some places had become detached from the underlying basement membrane, and also in the vascular smooth muscle and the surrounding mesenchyme.
DISCUSSION

The human placenta lacks local autonomic vascular control, and thus circulating autocrine-paracrine or humoral factors are essential for placental hemodynamic control. In pregnancies complicated by insulin-dependent diabetes mellitus or preeclampsia, both of which are characterized by endothelial cell injury, compromised perfusion of organ systems, including the placenta, is seen and can lead to significant morbidity and mortality in both mothers and neonates. Ample evidence has accumulated indicating that oxidative stress plays a role in the pathogenesis of diabetic and preeclamptic complications in the placenta. Oxidative stress is produced by either an increase in reactive oxygen species (ROS) formation and/or a decrease in ROS scavenging ability. The diabetic state leads to increased production of $O_2^-$ (11, 24, 35) and/or increased NO formation via increased enzymatic activity of endothelial (11) or inducible (38) isoforms of nitric oxide synthase. In addition, changes in total radical-trapping antioxidant capacity, such as the reduction in scavenger activity of superoxide dismutase and catalase, glutathione metabolism, and/or vitamin E levels as well as increases in lipid peroxides have been observed in preeclamptic and diabetic patients (23, 25, 37, 41). Preeclampsia is also characterized by an increased presence of reactive oxygen species as suggested by significantly elevated levels of fetal lipid peroxides in pregnancies complicated by preeclampsia (21, 25). Administration of antioxidative vitamins C and E has been shown to reduce placental lipid peroxidation in perfused human preeclamptic placentas (29). However, vitamin C-oxidizing activity, and thus ascorbate radical formation, is increased in preeclamptic...
plasma and may therefore contribute to vascular dysfunction in this disorder (18).

When O$_2^-$ and NO$^-$ coexist in close proximity, ONOO$^-$ is formed at a rate of $6.7 \times 10^9$ ms$^{-1}$ (19). This rate is three times faster than the interaction of O$_2^-$ with superoxide dismutase, an endogenous scavenger of O$_2^-$.

Thus at elevated concentrations of NO$^-$ or O$_2^-$, NO$^-$ outcompetes superoxide dismutase for O$_2^-$ with resultant ONOO$^-$ formation. Peroxynitrite has been shown to nitrosylate substrates such as tyrosine moieties within proteins, and although other reactive nitrogen species have the potential to nitrate tyrosine (17), the formation of nitrotyrosine in vivo is thought to be specific for ONOO$^-$ interaction with tissue. Our laboratory has recently shown increased expression of nitrotyrosine residues in the fetal vasculature and villous placental stroma of pregnancies complicated by pre-eclampsia (28) and type 1 insulin-dependent diabetes mellitus (22).

In addition, Roggensack et al. (34) have observed increased endothelial nitric oxide synthase, decreased superoxide dismutase, and increased nitrotyrosine immunostaining in the maternal vasculature of women with preeclampsia. These findings thus suggest increased ONOO$^-$ formation as a result of oxidative stress during the pathological processes of preeclamptic and diabetic injury.

Our current study provides evidence that ONOO$^-$ synthesis and action in placentas of pregnancies complicated by pre-eclampsia or diabetes mellitus may lead to dysfunction of the fetal-placental vasculature. The responses of the fetal-placental circulation to both the vasoconstrictors U-46619, a thromboxane mimetic, and ANG II, and to the vasodilator GTN and PGI$_2$ are attenuated in these conditions compared with control placentas. In addition, we found an attenuation in fetal-placental vascular reactivity to these vasoactive...

Fig. 3. Representative photomicrographs of placental villous tissue immunostained for nitrotyrosine. Nonperfused control placental villous tissue is shown in A, ONOO$^-$-treated placental tissue stained with control IgG in place of primary anti-nitrotyrosine antibody in B, and tissue perfused with vehicle for ONOO$^-$ solution is seen in C. No positive staining is observed in these 3 panels. In contrast, villous tissue perfused with authentic peroxynitrite (D) shows intense immunostaining for nitrotyrosine in vascular smooth muscle, surrounding mesenchyme, and vascular endothelium, which in some places has become detached from underlying basement membrane during ONOO$^-$ perfusion.
agents following treatment of the fetal-placental vasculature from uncomplicated pregnancies with authentic ONOO\(^{-2}\). Both ONOO\(^{-2}\)-treated placental villous tissue and placental villous tissue from preclampsic and diabetic pregnancies show nitrotyrosine residues in fetal and cord vessels, indicating ONOO\(^{-2}\)-interaction with placental tissue. Taken together, these findings suggest that ONOO\(^{-2}\) may be at least in part responsible for changes in altered vascular reactivity seen in placentas affected by diabetes or preeclampsia.

The actual concentration of ONOO\(^{-2}\) reaching the resistance vessels of the fetal-placental circulation in our preparation is unknown. Because the cotyledon vascular volume is \(\approx 4 \text{ ml}\), we estimate it takes the perfusion medium about 15 s to begin to reach the capillary bed of the placental cotyledon from the chorionic plate at an infusion rate of 4 ml/min, i.e., equivalent to 14 half-lives with a half-life of 1.08 s for ONOO\(^{-2}\). (3). The ONOO\(^{-2}\)-concentration at this point is then \(\approx 2 \times 10^{-8} \text{ M}\), which may be an overestimate because it presumes that none of the infused ONOO\(^{-2}\) reacts with any of the numerous biological targets present in the placental villous vasculature. We recognize that in CO\(_2\)-containing solutions as here ONOO\(^{-2}\) may interact with CO\(_2\) to give a ONOO\(^{-2}\)-CO\(_2\) complex, which can give rise to secondary oxidizing intermediates of different reactivities from ONOO\(^{-2}\). (13).

Our observations are supported by recent findings of other groups. Although preclampsia is principally associated with an increase in maternal vascular sensitivity to pressor agents (26), Read et al. (31) reported significant attenuation of vasoconstrictor responses to U-46619 in the fetal-placental vasculature of women with preeclampsia, although no effect was seen on vasodilator responses to PG\(_1\). Similarly, Wilkes et al. (42) found responses to U-46619 to be attenuated in the fetal-placental vasculature of diabetic placentas accompanied by a reduction in the affinity of thromboxane receptors. Gonzales et al. (16) showed that both human placental chorionic plate arteries and veins obtained from preclampsic pregnancies were significantly less sensitive to S-nitroso-N-acetyl-penicillamine, which spontaneously releases NO\(^{-}\) than the respective vessels from control placentas. Markedly reduced endothelium-dependent relaxation was found in the myometrial arteries from preclampsic women when compared with nonpregnant or normotensive pregnant women (1), and flow-induced shear stress resulted in less vasodilation in arteries of preclampsic women than in normal pregnant women (10). Thus diminished vascular responses to vasoactive agents may be observed in resistance vessels of diabetic and preclampsic patients. In addition, in diabetic placentas, vascular responses to vasoactive agents may also be affected by other pathophysiological processes, such as progressive glycation and cross-linking of connective tissue proteins of the fetal-placental vasculature, which can produce vascular stiffness as shown in other vascular beds (7).

The deleterious effects of reactive oxygen species have been studied in many systems. Peroxynitrite, which can interact with and injure the mitochondrial electron transport system, resulting in inhibition of cellular respiration (30), is also thought to contribute to endothelial dysfunction in septic shock (40) and initiates lipid peroxidation, a mechanism that contributes to the pathogenesis of atherosclerosis, where extensive nitrotyrosine residues have been localized to foamy macrophages and endothelium in the atheromatous area (4). DNA breaks have been shown to be caused by ONOO\(^{-2}\), which can lead to initiation of a futile DNA repair cycle by activation of poly(ADP-ribose)polymerase, resulting in depletion of cellular NAD\(^+\) and ATP stores (43). The ischemia and reperfusion tissue injury phenomenon may at least partially also be exacerbated by ONOO\(^{-2}\)-mediated oxidation of xanthine dehydrogenase to xanthine oxidase, a ROS-generating system (36). Other modifications of proteins include nitration of aromatic amino acids, such as tyrosine, which has been demonstrated to affect signal transduction pathways (20). The decrease in reactivity of the fetal-placental vasculature observed in pregnancies affected by insulin-dependent diabetes mellitus or preeclampsia may be the result of ONOO\(^{-2}\)-mediated alterations of signal transduction pathways, including receptors and changes in the contractile apparatus of the vascular smooth muscle. Recently, it has been shown that ONOO\(^{-2}\) may selectively inactivate the PG\(_1\) receptor (44). If it is possible that different vascular receptors show different sensitivity to ONOO\(^{-2}\), then this may explain the apparent lack of effect on responses to ANG II with in vitro treatment by ONOO\(^{-2}\).

Both preeclampsia and diabetes mellitus are associated with increased fetal morbidity and mortality and may display abnormal placental blood flow velocity waveforms, indicating increased vascular resistance. In vitro the placental vasculature of these pregnancies displays altered vascular reactivity despite apparently good fetal outcomes. However, this dysfunctional vasculature may not allow the placenta to adequately respond to increased demands for oxygen and nutrient transfer when the fetus is stressed by severe insult. This perhaps explains the increased morbidity and mortality and some of the unexpected fetal demise that occur near term in diabetic pregnancies. Also, despite the apparent good glycemic control and good fetal outcomes seen in this and our previous study (22), the placenta of diabetic pregnancies still displays strong nitrotyrosine staining indicating ONOO\(^{-2}\) formation and action. Perhaps this illustrates subclinical disease despite good glycemic control.

In conclusion, in well-controlled diabetes and preeclampsia, alterations in fetal-placental vasoreactivity occur. Our finding suggests that these alterations may be linked by increased ONOO\(^{-2}\) production from NO\(^{-}\) and O\(_2\)\(^{-}\) and its subsequent interaction with signal-transduction pathways linked to vasoactive agents. Further investigations are necessary to determine whether ONOO\(^{-2}\) is a participant in the pathogenesis of these and other abnormalities observed in diabetic or...
preeclamptic placentas or changes seen in maternal tissues affected by these conditions.

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