Pathophysiology of Hypertension During Preeclampsia Linking Placental Ischemia With Endothelial Dysfunction

Joey P. Granger, Barbara T. Alexander, Maria T. Llinas, William A. Bennett, Raouf A. Khalil

Abstract—Studies over the past decade have provided a better understanding of the potential mechanisms responsible for the pathogenesis of preeclampsia. The initiating event in preeclampsia has been postulated to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles. Placental ischemia is thought to lead to widespread activation/dysfunction of the maternal vascular endothelium that results in enhanced formation of endothelin and thromboxane, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as NO and prostacyclin. These endothelial abnormalities, in turn, cause hypertension by impairing renal-pressure natriuresis and increasing total peripheral resistance. The quantitative importance of the various endothelial and humoral factors in mediating the reduction in renal hemodynamic and excretory function and elevation in arterial pressure during preeclampsia are still unclear. Results from ongoing basic and clinical studies, however, should provide new and important information regarding the physiological mechanisms responsible for the elevation in arterial pressure in women with preeclampsia.

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Key Words: kidney □ pregnancy □ nitric oxide □ endothelin □ cytokines

Preeclampsia is estimated to affect 7% to 10% of all pregnancies in the United States.\(^1\) Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of preeclampsia are unclear. Hypertension associated with preeclampsia develops during pregnancy and remits after delivery, implicating the placenta as a central culprit in the disease. An initiating event in preeclampsia has been postulated to be reduced placental perfusion that leads to widespread dysfunction of the maternal vascular endothelium by mechanisms that remain to be defined (see Figure).

Placental Ischemia Is an Important Initiating Event in Preeclampsia

Although the pathophysiology of preeclampsia remains undefined, placental ischemia is widely cited as a key factor.\(^1\)\(^–\)\(^6\) During early human pregnancy, cytotrophoblast cells invade the uterine spiral arteries, replacing the endothelial layers of these vessels with the subsequent destruction of the medial elastic, muscular, and neural tissue. By the end of the second trimester of pregnancy, the uterine spiral arteries are lined exclusively by cytotrophoblast, and endothelial cells are no longer present in the endometrial or superficial myometrial regions. This remodeling of the uterine spiral arteries results in the formation of a low resistance arteriolar system with a dramatic increase in blood supply to the growing fetus. In preeclampsia, invasion of the uterine spiral arteries is limited to the proximal decidua, with 30% to 50% of the spiral arteries of the placental bed escaping endovascular trophoblast remodeling.\(^1\)\(^,\)\(^4\) Myometrial segments of these arteries remain anatomically intact and undilated, and adrenergic nerve supply to the spiral arteries is not affected. The mean external diameters of the uterine spiral arteries in women with preeclampsia are less than one half of the diameters of similar vessels from uncomplicated pregnancies.\(^2\) This failure of vascular remodeling prevents an adequate response to increased fetal demands for blood flow that occur as gestation progresses. Inappropriate integrin expression by the extravillous cytotrophoblast may explain the shallow pattern of invasion and lack of arterial remodelling that occurs in preeclampsia.\(^4\)

This failure of trophoblast invasion in preeclampsia results in a reduction in uteroplacental perfusion, with the placenta becoming increasingly ischemic as gestation progresses. This concept of placental hypoxia in preeclampsia is supported by reports of decreased clearance rates of various radioactive compounds and steroids by the preeclamptic placenta.\(^3\) In addition, placentas from women with preeclampsia display an increased frequency of placental infarcts\(^1\)\(^–\)\(^4\) and altered morphology evidenced by abnormal cytotrophoblast proliferation and increased formation of syncytial knots. Further empirical evidence for a key role of the placenta in the etiology of preeclampsia is the generally rapid recovery that patients experience following delivery.\(^1\)\(^,\)\(^4\)\(^–\)\(^6\)
Circulating levels of fibronectin are significantly increased in women with preeclampsia, with elevations detected as early as 24 weeks into the pregnancy.5–7 Von Willebrand factor, circulating levels of fibronectin and thrombomodulin increase significantly elevated in women with preeclampsia, with another coagulation cascade factor, is also elevated in women with preeclampsia.5–7 Platelets also appear to play an important role in the etiology of preeclampsia. Enhanced platelet activation, as determined by whole blood flow cytometry, and increased levels of platelet endothelial cell adhesion molecule-1 (PCAM-1) also occur in women who develop preeclampsia.5–7 Plasma levels of ICAM-1 and VCAM-1 have been reported to be significantly elevated at 3 to 15 weeks before onset of clinical manifestations. Elevations in ICAM-1 were evidenced at 18 weeks gestation, thus suggesting that markers of endothelial dysfunction may serve as predictors of preeclampsia during pregnancy. In summary, endothelial dysfunction may serve as a causative factor in preeclampsia and is not just a result of the disorder. Many markers of endothelial dysfunction may function as predictors of the syndrome in women who develop preeclampsia as many are significantly elevated at weeks before observance of clinical manifestations.

**Chronic Reductions in Uteroplacental Perfusion Pressure in Pregnant Animals as a Model to Study Preeclampsia**

Although the physiological mechanisms that mediate the alterations in cardiovascular and renal function have been extensively studied during normal pregnancy, information regarding the mediators of the reduction in renal and cardiovascular function during preeclampsia has been limited because of the difficulty in performing mechanistic studies in pregnant women.8,9 Although several animal models have been developed to study preeclampsia, information on the mechanisms involved in mediating the long-term reduction in kidney function and increase in arterial pressure is lacking.9 Experimental induction of chronic uteroplacental ischemia appears to be the most promising animal model for studying potential mechanisms of preeclampsia because reductions in uteroplacental blood flow in a variety of animal models lead to a hypertensive state that closely resembles preeclampsia in women.8

Chronic reductions in uteroplacental perfusion pressure in gravid rats after day 14 of gestation, as reported by Eder and McDonald,10 lead to significant increases in arterial pressure and proteinuria. We have recently begun to work with this model to examine potential pathophysiological mechanisms that mediate the hypertension during chronic reductions in uteroplacental perfusion pressure.11 We reduced uterine perfusion pressure (RUPP) in the gravid rat by \( \approx 40\% \) by placing a silver clip around the aorta below the renal arteries. Because this procedure has been shown to cause an adaptive increase in uterine blood flow via the ovarian artery, we also placed a silver clip on both the right and left uterine arteries at the ovarian end just before the first segmental artery. We found that reducing uteroplacental perfusion with this technique causes significant and consistent elevations in arterial pressure of 20 to 30 mm Hg compared with that of control pregnant rats at day 19 of gestation. Our data also indicate that this hypertension is associated with proteinuria, reductions in renal plasma flow and glomerular filtration rate, and a hypertensive shift in the pressure natriuresis relationship. Our data also suggest that endothelial function is significantly altered in response to chronic reductions in uteroplacental perfusion pressure in the pregnant rat.13 Finally, we have found intrauterine growth restriction in response to chronic reductions in uteroplacental perfusion pressure in the pregnant rat because the average pup size is smaller in this group than in normal pregnant rat.12 Thus, a chronic reduction in...
Vascular Mediators of Hypertension During Reductions in Uterine Perfusion

Nitric Oxide

One potential mechanism for the reduction in pressure natriuresis and elevation in arterial pressure in response to a chronic reduction in uteroplacental perfusion pressure in the pregnant rat is a reduction in renal NO synthesis. Studies from our laboratory and others have indicated that NO plays an important role in the regulation of renal function and arterial pressure under various physiological and pathophysiological conditions. Of possible relevance to preeclampsia is the finding that reduced NO synthesis results in a hypertensive shift in the pressure natriuresis relationship.

Substantial evidence indicates that NO production is elevated in normal pregnancy. Plasma and urinary levels of cGMP, the second messenger of NO, increase during pregnancy in rats. Marked increases in 24-hour urinary nitrate/nitrite excretion have also been reported during pregnancy in the rat. Increases in NO production appear to play an important role in the renal vasodilatation of pregnancy. Recent studies by Conrad and others clearly demonstrated that the renal vasodilatation in the pregnant rat is due to an increased NO production. Because NO appears to be an important physiological vasodilator in normal pregnancy, NO deficiency during preeclampsia might be involved in the disease process. Studies from several laboratories have found that chronic NO synthase inhibition in pregnant rats produces a hypertension associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth retardation, and increased fetal morbidity, a pattern that closely resembles the symptoms of human pregnancy-induced hypertension. However, whether there is a reduction in NO production during pregnancy-induced hypertension is unclear. Much of the uncertainty originates from the difficulty in directly assessing the activity of the NO system in a clinical setting.

Assessment of whole body NO production via measurement of 24-hour nitrate/nitrite excretion has yielded variable results because of difficulties in controlling for factors such as nitrate intake. We have recently reported that normal pregnancy in the rat is associated with significant increases in whole body NO production and renal protein expression of neuronal and inducible NO synthase. We also recently determined whether whole body and renal NO production is reduced in a rat model of preeclampsia produced by chronically reducing uterine perfusion pressure. Chronic reductions in uterine perfusion pressure in pregnant rats resulted in increases in arterial pressure and decreases in renal plasma flow and glomerular filtration rate, but no difference in urinary nitrate/nitrite excretion relative to control pregnant rats. The results of this study indicate that the increase in arterial pressure observed in response to chronic decreases in uterine perfusion pressure in pregnant rats is associated with no change in whole body NO production and a decrease in renal protein expression of neuronal NO synthase. Whether the reduction in renal protein expression of neuronal NO synthase occurs as a result of the hypertension or the reduction in renal protein expression of neuronal NO synthase plays a role in mediating the reduction in renal hemodynamics and elevation in arterial pressure remains to be determined.

Endothelin

Another endothelial-derived factor that may play a role in preeclampsia is the vasoconstrictor endothelin. Because endothelial damage is a known stimulus for endothelin synthesis, increases in the production of endothelin may participate in preeclampsia. Plasma concentration of endothelin has been measured in a number of studies involving normal pregnant women and women with preeclampsia. Most investigators have found higher plasma concentrations of endothelin of 2- to 3-fold in women with preeclampsia. Typically, plasma levels of endothelin are highest during the latter stage of the disease, suggesting that endothelin may not be involved in the initiation of preeclampsia but rather in the progression of disease into a malignant phase. Although the elevation in plasma levels of endothelin is only 2- or 3-fold above normal during preeclampsia, we found that this level of plasma endothelin can have significant long-term effects on systemic hemodynamics and arterial pressure regulation. Thus, long-term elevations in plasma levels of endothelin comparable to those measured in patients with preeclampsia could play a role in mediating the reductions in renal function and elevations in arterial pressure observed in women with preeclampsia.

Although some studies have reported no significant changes in circulating levels of endothelin during pregnancy-induced hypertension, a role for endothelin as a paracrine or autocrine agent in preeclampsia remains worthy of consideration. We recently examined the role of endothelin in mediating hypertension in response to chronic reductions in uterine perfusion pressure in conscious, chronically instrumented pregnant rats. Renal expression of preproendothelin was significantly elevated in both the medulla and in the cortex of the pregnant rats with chronic reductions in uterine perfusion pressure compared with those of control pregnant rats. Chronic administration of the selective endothelin type A receptor antagonist (ABT-627, 5 mg · kg⁻¹ · d⁻¹ for 10 days) markedly attenuated the increase in mean arterial pressure (MAP) observed in the pregnant rats with chronic reductions in uterine perfusion pressure. However, endothelin type A receptor blockade had no significant effect on blood pressure in the normal pregnant animals. These findings suggest that endothelin plays a major role in mediating the hypertension produced by chronic reductions in uterine perfusion pressure in pregnant rats.

Prostaglandins

Several lines of evidence suggest that changes in the prostaglandin system may play a role in mediating the renal dysfunction and increase in arterial pressure during pre-
eclampsia. Significant alterations in prostacyclin and thromboxane production occur in women with preeclampsia. plasma and urine levels of thromboxane are elevated in women with preeclampsia, whereas synthesis of prostaglandins, such as prostacyclin, is reduced. Additional evidence for a potential role of thromboxane in preeclampsia derives from a study demonstrating that short-term increases in systemic arterial pressure produced by acute reductions in uterine perfusion in pregnant dogs can be prevented by thromboxane receptor antagonism. Further evidence of a potential role for thromboxane is supported by studies in humans indicating that low-dose aspirin attenuates the development of preeclampsia in women at risk for the disease. Although some studies suggest a potential role for thromboxane in preeclampsia, the quantitative importance of this substance in mediating the long-term reduction in renal hemodynamics and elevation in arterial pressure produced by chronic reductions in uterine perfusion pressure in pregnant rats is still uncertain. In preliminary experiments, however, we found that urinary excretion of thromboxane B2 was higher in the hypertensive pregnant rats with chronic reductions in uterine perfusion pressure than in normal pregnant rats at day 19 of gestation.

**Renin-Angiotensin System**

The renin-angiotensin system (RAS) plays an important role in the long-term regulation of renal function and arterial pressure during a variety of physiological and pathophysiological conditions. During normal pregnancy, plasma renin concentration, renin activity, and angiotensin II (Ang II) levels are all elevated; however, the vascular responsiveness to Ang II appears to be reduced. The importance of the renin-angiotensin in the regulation of renal function and arterial pressure during preeclampsia has not yet been fully elucidated. Although circulating levels of Ang II may be normal during preeclampsia, it is possible that reducing uteroplacental perfusion pressure could increase the renal sensitivity to Ang II through reductions in NO or prostacyclin synthesis or by enhanced formation of thromboxane. Consistent with this suggestion are studies indicating enhanced vascular responsiveness to Ang II in vessels from animals or humans with preeclampsia. Furthermore, previous studies from our laboratory and others have found that, unlike normal conditions, the preglomerular vessels of the renal circulation become extremely sensitive to the vasoconstrictor actions of Ang II when the renal synthesis of NO or prostacyclin is reduced or when thromboxane synthesis is elevated. Increased vascular Ang II responsiveness during preeclampsia, however, does not prove Ang II as an important endogenous mediator of the vasoconstriction or hypertension in experimental models of preeclampsia because increased responsiveness may only reflect low endogenous Ang II formation. Thus, the importance of increased Ang II to the control of renal function and blood pressure during preeclampsia remains unclear. We recently determined the importance of Ang II in mediating the long-term reduction in renal hemodynamic and the hypertension produced by chronic reductions in uterine perfusion pressure in pregnant rats. Chronic oral administration of a converting enzyme inhibitor (enalapril, 250 mg/L for 6 days) decreased MAP to a similar extent in pregnant rats with RUPP and normal pregnant rats. Blockade of the RAS, however, had no significant effect on the blood pressure response to chronic reductions in uterine perfusion pressure because the differences in blood pressures between the normal pregnant and RUPP rats were similar in control and converting enzyme inhibitor–treated groups. These findings suggest that the RAS does not play a major role in mediating the hypertension produced by chronic reductions in uterine perfusion pressure in pregnant rats.

**Inflammatory Cytokines and Lipids as Potential Mediators of Endothelial Dysfunction**

**Cytokines**

Although reductions in blood flow to the uteroplacental unit are known to result in cardiovascular and renal abnormalities consistent with the pathophysiological features of human preeclampsia, the physiological mechanisms linking placental ischemia with the abnormalities in the maternal circulation are unclear. Several lines of evidence support the hypothesis that the ischemic placenta contributes to endothelial cell activation/dysfunction of the maternal circulation by enhancing the synthesis of cytokines such as tumor necrosis factor-α (TNF-α). TNFα is an inflammatory cytokine that has been shown to induce structural and functional alterations in endothelial cells. This inflammatory cytokine also enhances the formation of a number of endothelial cell substances such as endothelin and reduces acetylcholine-induced vasodilatation. TNF-α has been shown to directly induce oxidative damage as TNF-α destabilizes electron flow in mitochondria, resulting in release of oxidizing free radicals and formation of lipid peroxides. Lipid peroxides and oxygen radicals can damage endothelial cells as they are highly reactive compounds. Also supporting a potential role of TNF-α in preeclampsia are findings that plasma levels of TNF-α are significantly elevated, by ∼2-fold, in women with preeclampsia. Furthermore, IL-6, which is activated by TNF-α, is also elevated in preeclamptic women. Although high levels of TNF-α, as observed during septic shock or after lipopolysaccharide administration, activate gene expression of iNOS, modest levels of TNF-α have been shown to destabilize the mRNA of eNOS.

Whether chronic and modest increases in plasma TNF-α can activate the endothelium during pregnancy and lead to reduced kidney function, high blood pressure, and other features of preeclampsia is unknown. Consistent with a potential role of cytokine activation in preeclampsia is a recent study demonstrating that an intravenous infusion of a very low dose of the endotoxin lipopolysaccharide (LPS) resulted in significant and long-term increases in blood pressure and urinary albumin excretion and significant platelet aggregation in conscious pregnant rats. Although LPS is known to activate TNF-α, it is unclear whether the effects of low-dose LPS on cardiovascular and kidney function were mediated via TNF-α and/or interleukin-1 because these cytokines were not measured in that study.
Although plasma levels of TNF-α are elevated by 2- to 3-fold in women with preeclampsia, the importance of TNF-α in mediating the systemic and renal hemodynamic changes associated with this disease is unclear. To determine the long-term effects of a 2- to 3-fold elevation in plasma TNF-α on renal and systemic hemodynamics in pregnant rats, we recently infused TNF-α for 5 days at a rate of 50 ng/d during days 14 to 19 of gestation in pregnant rats. Plasma levels doubled in the TNF-α-treated pregnant rats. Arterial pressure was significantly higher in the TNF-α-treated pregnant rats compared with pregnant controls at day 19 of gestation. A 2-fold elevation in plasma TNF-α in pregnant rats also caused a significant reduction in renal hemodynamics. These data suggest that elevated plasma levels of TNF-α observed in preeclamptic women may play an important role in the pathogenesis of preeclampsia.

Lipid Peroxides and Reactive Oxygen Species

Lipids have also been implicated in mediating endothelial dysfunction in preeclampsia. Although plasma levels of lipids are increased during normal pregnancy, plasma concentrations of both triglyceride-rich lipoproteins and nonesterified fatty acids (NEFA) are significantly increased in women who develop preeclampsia relative to normal pregnant women. In women who develop preeclampsia, plasma triglyceride levels are significantly elevated as early as 10 weeks gestation compared with those in normal pregnant women. This significant increase in plasma triglycerides in women with preeclampsia also correlates to an increase in the plasma concentration of small dense LDLs. Fatty acids contribute to endothelial dysfunction by serving as substrates for lipid peroxidation and lipid peroxides are also significantly increased in plasma from women with preeclampsia. Therefore, the generation of free radicals, lipid peroxides, and reactive oxygen species may be an important mechanism for endothelial dysfunction in preeclampsia. As increases in the plasma concentration of small dense LDLs and triglycerides are characteristic of other lipid-mediated diseases such as atherosclerosis, and as abnormalities in lipid metabolism may have a genetic basis, alterations in lipid peroxidation may be a causative factor in the pathophysiology of preeclampsia.

Although recent studies support a role for cytokines and other factors such as lipid peroxides and reactive oxygen intermediates as potential mediators of endothelial dysfunction, finding the link between placental ischemia and maternal endothelial and vascular abnormalities remains to be an important area of investigation. Microarray analysis of genes within the ischemic placenta of women with preeclampsia and in animal models of chronic reductions in uterine perfusion pressure should provide new insights into novel factors that may provide additional links between placental ischemia and hypertension. More effective strategies for the prevention of preeclampsia should be forthcoming once the underlying pathophysiological mechanisms that are involved in preeclampsia are completely understood.

References


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