Vascular mechanisms of increased arterial pressure in preeclampsia: lessons from animal models

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Khalil, Raouf A., and Joey P. Granger. Vascular mechanisms of increased arterial pressure in preeclampsia: lessons from animal models. Am J Physiol Regulatory Integrative Comp Physiol 283: R29–R45, 2002; 10.1152/ajpregu.00762.2001.—Normal pregnancy is associated with reductions in total vascular resistance and arterial pressure possibly due to enhanced endothelium-dependent vascular relaxation and decreased vascular reactivity to vasoconstrictor agonists. These beneficial hemodynamic and vascular changes do not occur in women who develop preeclampsia; instead, severe increases in vascular resistance and arterial pressure are observed. Although preeclampsia represents a major cause of maternal and fetal morbidity and mortality, the vascular and cellular mechanisms underlying this disorder have not been clearly identified. Studies in hypertensive pregnant women and experimental animal models suggested that reduction in uteroplacental perfusion pressure and the ensuing placental ischemia/hypoxia during late pregnancy may trigger the release of placental factors that initiate a cascade of cellular and molecular events leading to endothelial and vascular smooth muscle cell dysfunction and thereby increased vascular resistance and arterial pressure. The reduction in uterine perfusion pressure and the ensuing placental ischemia are possibly caused by inadequate cytotrophoblast invasion of the uterine spiral arteries. Placental ischemia may promote the release of a variety of biologically active factors, including cytokines such as tumor necrosis factor-α and reactive oxygen species. Threshold increases in the plasma levels of placental factors may lead to endothelial cell dysfunction, alterations in the release of vasodilator substances such as nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor, and thereby reductions of the NO-cGMP, PGI₂-cAMP, and hyperpolarizing factor vascular relaxation pathways. The placental factors may also increase the release of or the vascular reactivity to endothelium-derived contracting factors such as endothelin, thromboxane, and ANG II. These contracting factors could increase intracellular Ca²⁺ concentrations ([Ca²⁺]ᵢ) and stimulate Ca²⁺-dependent contraction pathways in vascular smooth muscle. The contracting factors could also increase the activity of vascular protein kinases such as protein kinase C, leading to increased myofilament force sensitivity to [Ca²⁺]ᵢ and enhancement of smooth muscle contraction. The decreased endothelium-dependent mechanisms of vascular relaxation and the enhanced mechanisms of vascular smooth muscle contraction represent plausible causes of the increased vascular resistance and arterial pressure associated with preeclampsia.

endothelium; vascular smooth muscle; pregnancy; hypertension

NORMAL PREGNANCY IS ASSOCIATED with reductions in vascular resistance and arterial pressure. However, in 5–10% of pregnancies in the US and 15% of pregnancies among African-Americans, women may have hypertension as
one complication of pregnancy (101, 143). Hypertension in pregnancy is related to one of four conditions: chronic hypertension that predates pregnancy; preeclampsia; eclampsia; chronic hypertension with superimposed preeclampsia; and gestational hypertension, a nonproteinuric hypertension of pregnancy (20, 172). Preeclampsia is a serious, systemic syndrome of elevated blood pressure, proteinuria, and other clinical findings. Although preeclampsia is a major cause of maternal and fetal morbidity and mortality, the exact mechanisms of this disorder have not been clearly identified. Understanding the mechanisms of preeclampsia should help develop new strategies for prevention and treatment of this disorder. Because preeclampsia is a disease of humans, clinical studies in hypertensive pregnant women and on samples from their plasma, body fluids, and postpartum placentas have been very useful in identifying the possible mechanisms of the disease. Several excellent reviews have provided detailed information regarding the general pathophysiology and the clinical aspects of preeclampsia, and the reader is encouraged to refer to some of them (10, 53, 66, 67, 134). However, investigation of the cellular and molecular mechanisms of hypertension in pregnant women could be difficult and costly. This led investigators to perform experimental studies in animal models of hypertension in pregnancy. Although the terminology may not be completely accurate, for the sake of clarity and to avoid confusion with preeclampsia in human pregnancy, we will refer to the hypertension in pregnant animal models as pregnancy-induced hypertension (PIH). Several prior reviews highlighted the significant changes in renal control mechanisms of arterial pressure in animal models of PIH and the alterations in kidney functions as possible causes of the increased arterial pressure in preeclampsia (75, 76, 102). However, hypertension is a multifactorial disorder that could involve additional alterations in the vascular and neurohumoral control mechanisms of the arterial pressure.

The purpose of this review is to make use of data largely derived from animal models of PIH to provide insight into the possible vascular and cellular mechanisms of the increased arterial pressure in preeclampsia. In this review, some of the hemodynamic changes that occur during normal pregnancy and preeclampsia will first be outlined. The possible initiating events that could trigger the development of preeclampsia will then be briefly described. We will follow with a detailed description of the intermediary changes in the endothelium-dependent mechanisms of vascular relaxation and the mechanisms of vascular smooth muscle contraction and how these vascular changes might relate to the increases in vascular resistance and arterial pressure as observed in women with preeclampsia and in animal models of PIH. The review will end with a perspective on potential areas for future investigations to better understand the vascular mechanisms of the increased arterial pressure in preeclampsia.

HEMODYNAMIC AND VASCULAR CHANGES DURING NORMAL PREGNANCY AND PREECLAMPSIA

Normal pregnancy is associated with significant hemodynamic and cardiovascular changes to meet the metabolic needs of the mother and fetus. For example, the maternal cardiac output and plasma volume increase during pregnancy, whereas the total vascular resistance and arterial pressure tend to decrease (139). Also, normal pregnancy is associated with increased renal plasma flow, decreased renal vascular resistance, and decreased pressor response and vascular reactivity to vasoconstrictors such as α-adrenergic agonists and ANG II (26, 39, 44, 48, 56, 70, 91, 112).

Although a hyperdynamic circulation may occur before the clinical onset of preeclampsia (57), the clinical phase of the disease is associated with severe increases in vascular resistance and arterial pressure, enhanced pressor response to vasoconstrictors such as ANG II, and reduction in renal plasma flow (103). The triggering mechanisms that lead to the dramatic hemodynamic and vascular changes observed during preeclampsia have been very elusive; however, most investigations have centered on a possible role of the placenta.

PLACENTAL ISCHEMIA AS AN INITIATING EVENT OF PREECLAMPSIA

Preeclampsia develops during pregnancy and remits after delivery, implicating the placenta as a central culprit in the disease. During the early stages of normal pregnancy, the cytotrophoblasts invade the uterine spiral arteries and progressively replace the vascular endothelial cells, the medial elastic tissue, the smooth muscle layer, and the neural tissue. By the end of the second trimester, the spiral arteries are turned into dilated tubes lined by cytotrophoblast. This remodeling of the uterine spiral arteries results in the formation of a low-resistance arterial system, which ensures sufficient blood supply and nutrition to the growing fetus.

In preeclampsia, abnormal expression of the adhesion molecule integrins by the cytotrophoblasts as well as widespread apoptosis of invasive cytotrophoblasts leads to limited invasion of the uterine spiral arteries to only the superficial layers of the decidua (54, 71, 173). The shallow cytotrophoblast invasion of the decidua and the inadequate vascular remodeling of the uterine spiral arteries does not meet the fetal blood flow and nutrition demands and may lead to intrauterine growth retardation, a common observation during preeclampsia. In addition to its deleterious effects on the growing fetus, placental ischemia could also initiate a cascade of events leading to dramatic changes in the maternal circulation during preeclampsia.

Because of the difficulty of performing mechanistic studies in pregnant women, several animal models of PIH have been developed to test the role of placental ischemia as a possible initiating event of the elevated arterial pressure during preeclampsia (4, 6, 27, 38, 58, 105). Although experimental induction of chronic
uteroplacental ischemia in pregnant animals has shown variable effects in different species and preparations (127), it is considered one of the promising animal models of PIH. Studies in late pregnant sheep, dog, and rabbit showed that reduction in uteroplacental perfusion pressure induces a hypertensive state that resembles hypertension in human pregnancy and provided evidence for a possible relationship between placental ischemia and preeclampsia (27, 58, 105, 127). However, the intermediary mechanisms between placental ischemia and the increased arterial pressure in human preeclampsia and animal models of PIH are not clearly understood. Recent studies in a rat model of reduced uterine perfusion pressure (RUPP) produced by clipping the lower abdominal aorta and the main uterine branches of both the ovarian arteries during late pregnancy provided evidence for significant changes in renal functions as possible causes of the increased arterial pressure in this animal model of PIH (4, 6), and these studies have previously been discussed in prior reviews (75, 76). Other studies focused on the possible vascular and cellular mechanisms of the increased arterial pressure in the RUPP rats and the mechanisms by which a localized reduction in uteroplacental perfusion pressure during late pregnancy could cause generalized increase in vascular reactivity and thus lead to increased vascular resistance and arterial pressure (38).

ENHANCED VASCULAR REACTIVITY IN PREECLAMPSIA

During normal pregnancy the pressor response to vasoconstrictor agonists appears to be reduced (26, 56, 70, 112). Also, the vascular reactivity to vasoconstrictor agonists such as the α1-adrenergic agonist phenylephrine (Phe) and ANG II is reduced in pregnant rats compared with virgin rats (39, 44, 91, 112). In contrast, preeclampsia is characterized by generalized vasoconstriction and increased pressor response to vasoconstrictor agonists such as ANG II (103). The increased vascular reactivity to vasoconstrictors during preeclampsia could be due to decreased endothelium-dependent mechanisms of vascular relaxation and/or enhanced mechanisms of vascular smooth muscle contraction.

ENDOTHELIAL CELL DYSFUNCTION DURING PREECLAMPSIA

The decreased vasopressor responses and vascular reactivity to vasoconstrictor agonists during normal pregnancy have been attributed, in part, to increased synthesis/release of nitric oxide (NO) and perhaps other vasodilator substances such as prostacyclin (PGI2) and hyperpolarizing factor (EDHF) by various maternal cells including vascular endothelial cells (Fig. 1) (2, 16, 17, 32, 68, 72, 125, 151, 165, 170). This led to the hypothesis that preeclampsia is an endothelial cell disorder and that the increased vascular resistance and arterial pressure during preeclampsia are possibly due to endothelial cell dysfunction and alterations in endothelium-dependent vascular relaxation (66, 114, 138).

There is ample clinical and biochemical evidence of endothelial cell dysfunction during preeclampsia (137). Studies in women with overt preeclampsia showed increases in circulating levels of cellular fibronectin and factor VIII-related antigen, both of which are markers of endothelial cell injury (65, 138, 140). The increased levels of these markers precede clinically overt preeclampsia and disappear with resolution of the disease, providing evidence for a possible causal relationship between endothelial cell injury and preeclampsia (135).

We recently used the RUPP rat model of PIH to test the hypothesis that localized reduction in uterine perfusion pressure during late pregnancy is associated with enhanced systemic vascular reactivity and impaired endothelium-dependent vascular relaxation (38). We found that the reactivity of endothelium-intact vascular strips to Phe is enhanced in RUPP rats compared with normal pregnant rats. Removal of the endothelium significantly enhances the Phe contraction in pregnant rats, but to a lesser extent in RUPP rats (Fig. 2). Also, the ACh-induced relaxation is less in RUPP rats than normal pregnant rats. These studies suggested that an endothelium-dependent relaxation pathway is intact in pregnant rats but is impaired in RUPP rats (38). The impaired endothelium-dependent relaxation pathway could be related to possible abnormalities in the production and/or activity of endothelium-derived relaxing factors such as NO, PGI2, and EDHF.

NO PRODUCTION DURING PREECLAMPSIA

The vascular changes during normal pregnancy have been attributed, in part, to increased NO synthesis by various maternal cells including vascular endothelial cells (7, 17, 32, 44, 111, 112, 151). This is supported by reports that the expression and activity of NO synthase (NOS) is increased in human uterine artery during pregnancy (118). Also, the plasma level, metabolic production, and urinary excretion of cGMP, a second messenger of NO and a cellular mediator of vascular smooth muscle relaxation, are increased during pregnancy (35, 111). Interestingly, cGMP production is markedly increased during the first trimester when the maternal circulation is rapidly vasodilating, whereas the whole body NO production as estimated by the plasma level and urinary excretion of nitrite/nitrate is not proportionately elevated, suggesting additional sources of cGMP (33).

Studies in pregnant experimental animals have also suggested an increase in NO synthesis during late gestation. The endothelium-dependent NO-mediated vascular relaxation is enhanced in late pregnant rats compared with virgin rats (39, 91). Also, the expression of NOS in several tissues, particularly those of the kidney, is elevated during late gestation in rats (1, 5).

The increase in NO production and the reduction of vascular resistance and arterial pressure during nor-
mal pregnancy has led investigators to hypothesize that a reduction in NO production could be the cause of the increased vascular resistance and arterial pressure during preeclampsia. In support of this hypothesis, alterations in NO production have been reported in women with preeclampsia (40, 137, 138, 147). Also, NOS blockade with \textbf{N}G-nitro-L-arginine methyl ester (L-NAME) during mid to late gestation in rats results in pathological changes similar to those observed in women with preeclampsia, such as severe renal vasoconstriction, proteinuria, thrombocytopenia, and intrauterine growth retardation (16, 17, 41, 91, 113, 168). Furthermore, some studies have shown that the arterial pressure is significantly increased in pregnant rats treated with the NOS inhibitor L-NAME compared with virgin rats treated with equal doses of L-NAME.

Fig. 1. Vascular changes during normal pregnancy and preeclampsia. During normal pregnancy there is an increase in the activity of endothelial nitric oxide synthase (NOS) and cyclooxygenase (COX) and increased production of nitric oxide (NO), prostacyclin (PGL₂), and endothelium-derived hyperpolarizing factor (EDHF). NO increases cGMP and PGL₂ increases cAMP in smooth muscle, which decrease intracellular Ca²⁺ and the myofilament sensitivity to Ca²⁺. Also, EDHF opens K⁺ channels in smooth muscle, leading to membrane hyperpolarization. This leads to smooth muscle relaxation and decreased peripheral resistance and arterial pressure. In preeclampsia there is increased release of placental cytokines that inhibit the production of endothelium-derived relaxing factors and thereby decrease smooth muscle relaxation. Cytokines also stimulate the release of endothelium-derived contracting factors such as endothelin (ET-1) and thromboxane (TXA₂) and could activate the renin-angiotensin system (RAS) in the kidney leading to increased ANG II. ET-1, TXA₂, and ANG II stimulate specific receptors in smooth muscle leading to increased intracellular Ca²⁺, protein kinase C (PKC) activity, smooth muscle contraction, and increased peripheral resistance and arterial pressure. ER, endoplasmic reticulum; SR, sarcoplasmic reticulum.

Fig. 2. Phenylephrine (Phe)-induced contraction in endothelium-intact (+ Endo) and endothelium-denuded (– Endo) vascular strips of normal pregnant and reduced uterine perfusion pressure (RUPP) rats.
with methylene blue, which inhibits guanylate cyclase. 

It has been reported that Ach-induced relaxation is reduced in vascular strips of RUPP rats compared with normal pregnant rats (38). Pretreatment of the vascular strips with L-NAME, which blocks NO synthesis, or with methylene blue, which inhibits guanylate cyclase and decreases cGMP production in smooth muscle (83), significantly inhibits Ach-induced vascular relaxation in normal pregnant but not RUPP rats. These studies suggest that NO production or release by endothelial cells and thereby the activity of the NO-cGMP relaxation pathway is reduced in RUPP rats compared with normal pregnant rats (38).

However, whether NO production is reduced in human preeclampsia or in animal models of PIH is not clearly established. Assessment of whole body NO production by measurement of 24 h nitrate/nitrite excretion has yielded variable results. Some clinical studies showed that the nitrite/nitrate levels are reduced in the sera of preeclamptic women (116). Other studies showed that the plasma levels of nitrite/nitrate could be increased during preeclampsia (149). The discrepancy in the nitrate/nitrite levels during preeclampsia could possibly be due to the difficulty in controlling other factors such as nitrate intake. However, in a recent study in preeclamptic women in which dietary intake of nitrate and nitrite was carefully controlled, unequivocal support for reduced NO production could not be demonstrated (33). Also, studies in the RUPP rat model of PIH have shown no significant alterations in total nitrate/nitrite production or urinary excretion (4). These data are difficult to reconcile with the decreased endothelium-dependent vascular relaxation observed in the RUPP rats (38). The apparent dissociation between whole body NO production and the hemodynamic and vascular changes during human preeclampsia and in animal models of PIH can be explained by the possibility that whole body NO production may not be reflective of the relevant vascular NO. Other likely explanations include possible tissue-specific differences in the expression of the NOS isoforms and/or differences in the availability of NO to produce vascular relaxation.

Although the total urinary nitrate/nitrite excretion does not appear to be different between normal pregnant and RUPP rats (4), recent studies suggest that the basal and Ach-induced nitrate/nitrite production in endothelium-intact vascular strips is reduced in RUPP rats compared with normal pregnant rats (15). This may be related, in part, to differential expression of NOS isoforms in various tissues, particularly in the placenta, blood vessels, and the kidney. It has been reported that the expression of NOS is not different in placentas obtained from normal and preeclamptic women (30). However, studies in late pregnant rats showed that the amount of renal endothelial NOS (eNOS) decreases by 39%, whereas the renal inducible NOS (iNOS) and neuronal NOS (nNOS) increase by 31 and 25%, respectively (5). These data raise the interesting possibility that the increased NO production during normal pregnancy in rats is caused by the upregulation of iNOS and nNOS in the kidney and perhaps eNOS in blood vessels. Studies also showed that the expression of the nNOS isoform in renal tissues is reduced in RUPP rats compared with normal pregnant rats (4). Whether the amount of NOS isoforms is altered in blood vessels of RUPP rats compared with normal pregnant rats is unclear and should represent important areas for future investigations.

An emerging area of investigation is whether omitting the vasodilator NO that is derived from any of the NOS isoforms would result in hypertension during pregnancy. Recent studies suggest that NO gene knockout mice do not become hypertensive during pregnancy (150), perhaps because compensatory vasodilator substances such as prostacyclin may be recruited. However, whether genetic deficiency of any of the NOS isoforms results in PIH in other animal models remains to be investigated.

Also, although the total nitrate/nitrite production may be unchanged in preeclampsia plasma, the availability of NO to produce vascular relaxation may be reduced. Ascorbate is essential for the decomposition of S-nitrosothiols and the release of NO. Ascorbate deficiency is typical of preeclampsia plasma and might result in decreased rates of decomposition of S-nitrosothiols. This is supported by reports that preeclampsia plasma contains higher concentrations of total S-nitrosothiols and S-nitrosoalbumin than normal pregnancy plasma. The increase in the total S-nitrosothiol and S-nitrosoalbumin concentrations in preeclampsia plasma may reflect insufficient release of NO from these major reservoirs of NO in this condition (158).

PROSTACYCLIN PRODUCTION DURING PREECLAMPSIA

Although it is recognized that changes in NO production may play a role in some parts of the maternal circulation, there is considerable evidence suggesting additional NO-independent mechanisms (161, 170). Other endothelium-derived relaxing factors such as prostacyclin (PGI2) may contribute to the hemodynamic and vascular changes observed during normal pregnancy and preeclampsia (Fig. 1). PGI2 is an antiplatelet aggregator and a vasodilator compound with significant beneficial effects in the maternal circulation during pregnancy. Urinary excretion of 6-keto-prostaglandin F1α (PGF1α), a hydration product of PGI2, and 2,3-dinor-6-keto-PGF1α, generated through β-oxidation of 6-keto-PGF1α, is increased during normal preg-
nancy, reaching a maximum during the last month of pregnancy (170).

Alterations in the production of PGI₂ have been reported in women with preeclampsia, thus further suggesting abnormal endothelial cell function during this disorder (10, 62, 164). In women with severe preeclampsia, the excretion of both 6-keto-PGFₑ₉ and 2,3-dinor-6-keto-PGF₁₀ is lower than in normotensive women during late pregnancy, suggesting that renal PGI₂ synthesis is diminished in preeclampsia (170). Low endothelial generation of PGI₂ has also been suggested in women with preeclampsia (97).

However, the effects of plasma from normal pregnant and preeclamptic women on PGI₂ production by endothelial cells in vitro do not appear to reflect the plasma PGI₂ concentrations in vivo. PGI₂ production by cultured human umbilical vein endothelial cells incubated with plasma from preeclamptic women for 24 h is significantly greater than that by cells exposed to normal pregnancy plasma (45, 46, 51). The differences in endothelial PGI₂ production by plasma from pregnant and preeclamptic women could not be explained by changes in cellular cyclooxygenase and PGI₂ synthase enzyme activity or mass. Instead, the stimulatory effect of preeclampsia plasma on PGI₂ biosynthesis in human umbilical vein endothelial cells appears to be manifested at a step(s) proximal to the activation of cyclooxygenase. Possible mechanisms are increased phospholipase A₂, lipoprotein, or lipid peroxidation activities in preeclampsia (51).

The dichotomy between the in vivo reduction in intravascular PGI₂ production that occurs in preeclampsia and the in vitro stimulatory effect of plasma from preeclamptic patients on endothelial cell PGI₂ production could be due to differential effects of acute vs. chronic exposure to the plasma. Recent studies investigated the acute vs. chronic effects of 2% plasma from normal pregnant and preeclamptic women on measuring endothelial PGI₂ production at different time periods of exposure to plasma (11). After 24 h, cells exposed to plasma from preeclamptic women produced more PGI₂ than cells exposed to plasma from normal pregnant women. In contrast, a 72-h exposure to plasma from preeclamptic women resulted in less endothelial cell PGI₂ production than exposure to plasma from normal pregnant women. Thus, in contrast to acute exposure, chronic exposure to plasma from preeclamptic women alters endothelial cells and results in decreased PGI₂ production, an observation consistent with the in vivo findings (11).

Interestingly, in vascular strips of RUPP rats some relaxation to ACh is not completely inhibited by L-NAME or methylene blue (38), suggesting changes in other endothelium-derived vasodilator substances such as PGI₂ in animal models of PIH.

EDHF PRODUCTION IN ANIMAL MODELS OF PIH

In addition to enhanced endothelium-dependent NO/PGI₂ synthesis, a hyperpolarizing factor (159) may contribute to the vascular adaptation during normal pregnancy (Fig. 1). Endothelium-derived hyperpolarizing factor (EDHF) has been suggested to play an important role in the enhanced ACh-induced relaxation of small mesenteric arteries of pregnant rats (72). Also, studies on the uterine vascular beds of pregnant rats have suggested that EDHF release is activated by a delayed rectifier type of voltage-sensitive potassium channel (68). Whether EDHF release from vascular endothelial cells is impaired during human preeclampsia or in animal models of PIH remains to be investigated.

ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PREECLAMPSIA

Serum vascular endothelial growth factor (VEGF) immunoreactivity has been shown to be suppressed during normal pregnancy (106). It has also been suggested that serum VEGF may be decreased during preeclampsia (106). However, other studies have shown that the serum VEGF levels are elevated in patients with preeclampsia, suggesting that the growth factor may have a role in the endothelial cell activation/dysfunction that occurs in the disease (13). Maternal plasma VEGF increases before the clinical onset of preeclampsia and is further elevated during the vasoconstricted state observed in this disorder. It has been suggested that the hyperdynamic circulation that characterizes the latent phase of preeclampsia causes vascular shear stress, which in turn increases the levels of circulating VEGF. Because VEGF normally acts as a vasodilator, its increase may represent an unsuccessful vascular rescue response during preeclampsia (19).

EVIDENCE FOR ENDOTHELIUM-DERIVED Contracting Factors During Preeclampsia

Because an increase in NO production could, in part, explain the reduced vascular reactivity observed during normal pregnancy (17, 32, 44, 112, 151), one would predict that blocking NO synthesis during pregnancy would bring the vascular reactivity back to the level observed in virgin rats. However, the vascular reactivity to Phe in L-NAME-treated pregnant rats is greater than that in virgin rats untreated or treated with L-NAME (39, 91). These data suggest that treatment of pregnant rats with L-NAME not only blocks NO synthesis in endothelial cells, but may also increase the synthesis of vasoactive compounds that would increase vascular reactivity. Thus endothelial cell dysfunction during PIH may be manifested not only as a reduction in vascular relaxation due to decreased endothelium-derived relaxing factors, but also as an increase in vascular reactivity due to increased production of endothelium-derived contracting factors such as endothelin and thromboxane.

ROLE OF ENDOTHELIN IN PREECLAMPSIA

The production of endothelin is increased in women with preeclampsia (25, 52, 123, 157). The concentration of immunoreactive endothelin is elevated in plasma of...
women with preeclampsia and rapidly returns to a normal pregnancy value within 48 h of delivery, as predicted by the prompt clinical resolution of this disorder. These data suggest that endothelin may contribute to the vasospasm associated with preeclampsia and lend further support to the involvement of endothelial cell dysfunction in the pathophysiology of this disorder (157). Typically, however, the plasma levels of endothelin are highest during the later stage of the disease, suggesting that endothelin may not be involved in the initiation of preeclampsia but rather in the progression of the disease into the malignant hypertensive phase (25, 53, 123, 157).

Experimental studies have also suggested a role for endothelin in mediating the hypertension in animal models of PIH. It has been shown that the increased arterial pressure in animal models of PIH is associated with endothelial cell dysfunction, leading to alterations not only in the synthesis of vasodilators such as NO and PG12, but also in the production of endothelin-1 (16, 17, 113, 167). This is supported by reports that long-term inhibition of endothelin synthesis during late gestation in rats is associated with increased blood pressure and elevated plasma levels of endothelin-1 (59). Also, the expression of preproendothelin is elevated in both the renal cortex and medulla of the RUPP rat model of PIH compared with normal pregnant rats (6). Furthermore, chronic administration of the endothelin A (ETA) receptor antagonist ABT-627 markedly attenuates the increase in arterial pressure observed in the RUPP rats (6). These studies suggest that endothelin plays a major role in mediating the hypertension produced by chronic reduction of uterine perfusion pressure in pregnant rats.

However, the increased endothelin levels during human preeclampsia and in animal models of PIH may have other vascular effects in addition to promotion of vascular spasm. Endothelin is known to interact with ETA and ETB receptors. The interaction of endothelin with specific ETA and ETB receptors in smooth muscle initiates a cascade of biochemical events leading to smooth muscle contraction (99, 128, 145, 146, 156). Endothelin also interacts with specific ETB receptors in the endothelium (128, 145, 146). Basal activation of endothelial ETB receptors by endothelin and the ensuing release of relaxing factors such as NO, PG12, and EDHF have been suggested to promote vascular relaxation and reduce vascular reactivity (73, 142, 145, 146). This is supported by reports that endothelin, via activation of ETB receptors, could mediate the reduced myogenic reactivity of small renal arteries and the renal vasodilation and hyperfiltration during pregnancy in rats (31, 69). These studies suggest that during preeclampsia an increase in endothelin production and activation of ETB-mediated vascular relaxation pathways may serve as a rescue mechanism against the excessive increases in vascular resistance and arterial pressure. In relation to these studies, it was shown that a bolus injection of endothelin decreases the arterial pressure in pregnant rats chronically treated with L-NAME. Similar depressor effects are also observed with sarafotoxin S6c, a specific ETB agonist, and are blocked by the specific ETB antagonist BQ-788 (109).

ROLE OF THROMBOXANE IN PREECLAMPSIA

Another important endothelium-derived contracting factor is thromboxane A2 (TXA2). TXA2 is released not only from the endothelium, but also from the platelets. TXA2 is a potent vasoconstrictor with a strong platelet aggregation action. The urinary excretion of TXB2 metabolites as markers of TXA2 synthesis is significantly higher in women with preeclampsia than in normotensive pregnant women (63, 64, 164). Also, TXB2 metabolite excretion correlates with the changes in mean arterial pressure and platelet count, which are indexes of the severity of preeclampsia. Additionally, the excretion of TXB2 metabolites falls rapidly postpartum parallel with resolution of clinical signs (63). Thus increased TXA2 biosynthesis appears to correlate with the severity of preeclampsia and may have a pathogenetic role in the disease. These findings have provided a rationale for the use of aspirin in the treatment and prevention of preeclampsia (63). Some clinical studies suggested that low-dose aspirin may attenuate the development of preeclampsia in women at risk for the disease (37). However, other randomized placebo-controlled trials involving women at high risk for preeclampsia showed that low-dose aspirin may have no or only small to moderate benefits when used for prevention of the disease and thus raised questions regarding the validity of this practice (9, 21, 55).

Studies in animal models of PIH also suggested that reduction in placental blood flow during pregnancy and the ensuing endothelial cell dysfunction may increase the production of TXA2 (167). This is supported by reports that short-term increases in arterial pressure produced by acute reduction in uterine perfusion in pregnant dogs are prevented by TXA2 receptor antagonists (167).

ENHANCED VASCULAR SMOOTH MUSCLE REACTIVITY IN ANIMAL MODELS OF PIH

Experimental studies have shown that the Phe-induced vascular reactivity in endothelium-intact aortic strips is enhanced in the RUPP rat model of PIH compared with normal pregnant rats. Removal of the endothelium enhances the Phe contraction in pregnant rats, but to a lesser extent in RUPP rats. Also, the Phe contraction in endothelium-denuded vascular strips is still greater in RUPP rats compared with pregnant rats (Fig. 2), suggesting an endothelium-independent component of the increased vascular reactivity in RUPP rats (38).

In addition to the observed pregnancy-associated changes in vascular reactivity in large conduit arteries such as the thoracic aorta (39, 91, 141), recent studies on single smooth muscle cells isolated from resistance renal arteries showed that the Phe-induced cell contraction is reduced in pregnant rats compared with virgin rats but significantly enhanced in pregnant rats
treated with L-NAME (115). Although the pregnancy-associated alterations in smooth muscle cell contraction to Phe can be explained by changes in the sensitivity to Phe at the α-adrenergic receptor level, they could also be due to changes in the signaling mechanisms downstream from receptor activation.

CELLULAR MECHANISMS OF VASCULAR SMOOTH MUSCLE CONTRACTION

It is widely accepted that vascular smooth muscle contraction is triggered by increases in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) due to Ca\(^{2+}\) release from the intracellular stores and Ca\(^{2+}\) entry from the extracellular space (82, 95, 133). Ca\(^{2+}\) binds calmodulin to form a complex, which in turn activates myosin light chain (MLC) kinase, causes MLC phosphorylation, initiates actin-myosin interaction and produces smooth muscle contraction (133). However, several laboratories reported dissociations between [Ca\(^{2+}\)]\(_i\) and force (50, 81, 94), between MLC phosphorylation and force, and between [Ca\(^{2+}\)]\(_i\) and MLC phosphorylation and suggested additional regulatory pathways of vascular smooth muscle contraction (132, 155).

In addition to MLC kinase, other protein kinases such as Rho-kinase and mitogen-activated protein kinase (MAPK) have been suggested to contribute to smooth muscle contraction (82, 152). Also, in several cell types, including smooth muscle, the agonist-receptor interaction is coupled to increased breakdown of phosphatidylinositol 4,5-bisphosphate and production of diacylglycerol (DAG), which activates protein kinase C (PKC), an enzyme that enhances the cellular responses to Ca\(^{2+}\) (89, 122). Biochemical studies in vascular smooth muscle have shown that PKC is mainly cytosolic under resting conditions and undergoes translocation from the cytosolic to the particulate fraction when the cells are activated by DAG or phorbol esters (89, 122). Also, direct activation of PKC by phorbol esters causes sustained contraction of vascular smooth muscle (42, 96, 131) with no significant change in [Ca\(^{2+}\)]\(_i\) (84, 120). These reports suggested a role for PKC in regulating vascular smooth muscle contraction, at least in part by increasing the Ca\(^{2+}\) sensitivity of the contractile proteins. PKC is now known to be a family of Ca\(^{2+}\)-dependent (α, βI, βII, and γ) and Ca\(^{2+}\)-independent (δ, ε, ζ, η, θ, μ, and λ/ρ) isoforms. These PKC isoforms appear to have different enzyme properties, substrates, and functions and to exhibit different subcellular distributions in the same blood vessel from different species and in different vessels from the same species (85, 87, 93, 104).

VASCULAR SMOOTH MUSCLE [Ca\(^{2+}\)]\(_i\) IN ANIMAL MODELS OF PIH

We recently investigated the cellular mechanisms of the reduction in vascular smooth muscle reactivity in normal pregnant rats and its enhancement in pregnant rats chronically treated with the NOS inhibitor L-NAME. We found that the Phe- and caffeine-induced vascular smooth muscle contractions in Ca\(^{2+}\)-free solution are not significantly different between pregnant and virgin rats untreated or treated with L-NAME, suggesting that the IP\(_3\)-sensitive and the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanisms from the intracellular stores are not altered (91). On the other hand, the contractile response to membrane depolarization by high-KCl solution, which is mainly due to Ca\(^{2+}\) entry from the extracellular space (95), is reduced in pregnant rats compared with virgin rats but significantly enhanced in pregnant rats treated with L-NAME (39, 91). Measurements of 45Ca\(^{2+}\) influx also suggested that the Phe- and high KCl-induced Ca\(^{2+}\) entry from the extracellular space is reduced in pregnant rats and enhanced in pregnant rats treated with L-NAME (39). However, these data should be interpreted with caution because 45Ca\(^{2+}\) influx measurements in vascular strips do not necessarily reflect the [Ca\(^{2+}\)]\(_i\).

Studies in single interlobular renal arterial smooth muscle cells showed that the basal and agonist-stimulated [Ca\(^{2+}\)]\(_i\) are reduced in normal pregnant rats compared with virgin rats but significantly elevated in pregnant rats treated with L-NAME (Fig. 3) (115). In smooth muscle cells incubated in a Ca\(^{2+}\)-containing physiological solution, Phe causes an initial transient increase followed by a smaller but maintained increase in [Ca\(^{2+}\)]\(_i\). The Phe- and caffeine-induced cell contraction and transient increase in [Ca\(^{2+}\)]\(_i\) in Ca\(^{2+}\)-free solution are not significantly different between pregnant and virgin rats untreated or chronically treated with L-NAME, suggesting that the pregnancy-associated changes in cell contraction are not due to changes in Ca\(^{2+}\) uptake to or Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores. In contrast, the Phe-induced maintained [Ca\(^{2+}\)]\(_i\) in Ca\(^{2+}\)-containing medium, a measure of Ca\(^{2+}\) entry from the extracellular space, is reduced in pregnant rats compared with virgin rats but significantly enhanced in L-NAME-treated pregnant rats (115). Also, the KCl-induced cell contraction and [Ca\(^{2+}\)]\(_i\) are reduced in normal pregnant rats compared with virgin rats but significantly enhanced in L-NAME-treated pregnant rats, providing evidence that Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels is reduced during normal pregnancy but enhanced in L-NAME-treated pregnant rats (115). The cause of the reduced Ca\(^{2+}\) entry into arterial smooth muscle in normal pregnant rats and its enhancement in L-NAME-treated pregnant rats is unclear, but could be related to possible changes in the Ca\(^{2+}\) permeability or the number of Ca\(^{2+}\) channels.

The reduced renal arterial smooth muscle cell contraction and [Ca\(^{2+}\)]\(_i\) in normal pregnant rats may explain the decreased renal vascular resistance associated with normal pregnancy. Also, the enhanced renal arterial smooth muscle cell contraction and [Ca\(^{2+}\)]\(_i\) during inhibition of NO synthesis in late pregnant rats may explain the increased renal vascular resistance and arterial pressure in the L-NAME-treated rat model of PIH. However, whether the increases in vascular reactivity associated with reduction in uteroplacental perfusion during late pregnancy reflect changes in vas-
cular smooth muscle $[Ca^{2+}]_i$ is unclear and should represent important areas for future investigations.

**EVIDENCE FOR ALTERATIONS IN OTHER VASCULAR CONTRACTION MECHANISMS DURING PIH**

An increase in $Ca^{2+}$ entry from the extracellular space and increased $[Ca^{2+}]_i$ may not fully explain the enhanced vascular reactivity to Phe observed in the L-NAME-treated rat model of PIH. We found that the vascular reactivity to Phe in pregnant rats treated with L-NAME is enhanced to levels significantly greater than those observed in virgin rats (39, 91). Also, parallel measurements of $^{45}Ca^{2+}$ influx and force showed that the $Ca^{2+}$ influx-force relation in aortic strips of L-NAME-treated pregnant rats is enhanced compared with normal pregnant rats (39), suggesting activation of other vascular contraction mechanisms in addition to $Ca^{2+}$ entry. For example, Phe may alter the activity of smooth muscle protein kinases and phosphatases such as MLC kinase and phosphatase, Rho-kinase, and mitogen-activated protein kinase, which may in turn contribute to smooth muscle contraction (82, 152). Also, Phe may increase the myofilament force sensitivity to $Ca^{2+}$ or perhaps stimulate a completely $Ca^{2+}$-independent pathway through activation of PKC (92, 93, 104).

**PKC OF VASCULAR SMOOTH MUSCLE DURING PIH**

Although a growing body of evidence suggests a role for PKC in smooth muscle contraction, little information is available on the changes in vascular PKC activity during pregnancy. We recently reported that in vascular smooth muscle of virgin rats, the phorbol ester phorbol 12,13-dibutyrate (PDBu), a direct activator of PKC, and the $\alpha$-adrenergic agonist Phe cause significant increases in contraction and PKC activity that are inhibited by the PKC inhibitors staurosporine and calphostin C (86, 88). Also, we and others reported that the vascular PKC activity is not significantly changed during early and mid-gestation (61, 88, 107). In contrast, the basal, PDBu-, and Phe-induced vascular reactivity and PKC activity are reduced in late pregnant rats compared with virgin rats (86, 88). These data are consistent with reports that PKC activity is reduced in late pregnant ewes and gilts (61, 107) and suggest a decrease in the amount and/or activity of vascular PKC during late pregnancy.

The changes in vascular PKC activity during late pregnancy could be related, in part, to the increased NO and cGMP production (5, 35, 36). NO has been shown to directly inhibit PKC through the formation of disulfide bridges with the PKC molecule (74). Also, NO and cGMP inhibit PKC by mechanisms involving inhibition of phosphatidylinositol breakdown and decreased DAG production (14, 100, 121, 144, 153). On the basis of these premises one would predict that blocking NO synthesis during late pregnancy would bring the vascular PKC activity back to the level observed in virgin rats. However, vascular PKC activity has been shown to be greater in pregnant rats treated with L-NAME than virgin rats (86, 88). Thus treatment of pregnant rats with L-NAME not only inhibits NO synthesis, but may also increase the synthesis of vasoactive compounds that would increase the vascular PKC activity. 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 (59) and that endothelin-1 increases PKC activity in vascular smooth muscle (8, 77, 100, 154).

Although the changes in PKC isoforms and activity have been well characterized in blood vessels of normal
male rats and ferrets (92, 93, 104), little information is available on whether the changes in vascular reactivity observed in animal models of PIH reflect changes in the expression and/or activity of specific vascular PKC isoforms. Immunoblot analyses showed significant amounts of the α-PKC isoform in aortic smooth muscle of virgin rats. Both phorbol esters and Phe cause translocation of α-PKC from the cytosolic to the particulate fraction. Interestingly, the amount of α-PKC is reduced in late pregnant rats but significantly increased in L-NAME-treated pregnant rats (Fig. 4). Also, the phorbol ester- and Phe-induced translocations of α-PKC are reduced in pregnant rats but significantly enhanced in pregnant rats treated with L-NAME (88). These data suggest that the reduction in vascular reactivity in pregnant rats and its enhancement during inhibition of NO synthesis are related, in part, to underlying changes in the amount and activity of the α-PKC isoform in vascular smooth muscle. The causes of the pregnancy-associated changes in the amount and activity of α-PKC are not clear at the present time but could be related to changes in the rate of phospholipid turnover and DAG production in vascular smooth muscle (28).

PHENOTYPIC CHANGES IN VASCULAR SMOOTH MUSCLE DURING PREECLAMPSIA

In addition to changes in the mechanisms of vascular smooth muscle contraction, changes in the contractile proteins of smooth muscle have been suggested during preeclampsia. Recent studies examined renal biopsy specimens from normal pregnant and preeclamptic women and immunohistochemically stained with antibodies to the smooth muscle myosin heavy chain isoforms SM-1 and SM-2 as well as smooth muscle α-actin (117). The interlobular arteries and afferent arterioles showed significant reduction in the SM-1, SM-2, and α-actin staining in specimens of preeclamptic women compared with normotensive controls. The reduction in contractile proteins in interlobular arteries is particularly evident with the SM-2 myosin heavy chain isoform. The phenotypic changes in contractile proteins of vascular smooth muscle cells in preeclamptic women, especially the disappearance of SM-2 isoform in interlobular arteries and afferent arterioles, may reflect the stage of the underlying hypertension (117).

LINKING PLACENTAL ISCHEMIA TO ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE DYSFUNCTION

There is clearly agreement that the uteroplacental ischemia/hypoxia in late pregnancy is associated with decreased vascular relaxation and enhanced vascular reactivity of the systemic vessels and that these vascular changes could be the cause of the increased vascular resistance and arterial pressure associated with preeclampsia (27, 38, 58, 105). However, it is not clear how a localized reduction in uteroplacental perfusion pressure could lead to localized 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 factor(s) from the ischemic placenta into the systemic circulation.

Preeclampsia is believed to result from placental release of circulating factors that may damage or activate the maternal vascular endothelium or smooth muscle cells. In support of this hypothesis, it was shown that incubation of myometrial resistance vessels from healthy pregnant women with plasma of preeclamptic women results in a significant reduction in endothelin-dependent vascular relaxation to bradykinin (78). However, incubation of omental vessels from normotensive pregnant women or myometrial vessels from nonpregnant women with preeclamptic plasma has no effect on endothelium-dependent relaxation. These studies demonstrate that the changes in relaxation of resistance vessels in response to preeclamptic plasma are dependent on the tissue bed under investigation (78).

The release of putative placental factors during preeclampsia is supported by reports that exposure of cultured endothelial cells to preeclamptic plasma results in increased NOS expression and activity and enhanced NO production (12, 43). Also, PGI2 production by cultured endothelial cells incubated with plasma from preeclamptic women is altered compared with cells exposed to normal pregnancy plasma (45, 51).

In addition to its effect on endothelium-derived vasodilators, the preeclamptic plasma may also stimulate the production of endothelium-derived vasoconstrictors. It has been shown that the ANG II- and epinephrine-induced expression of endothelin-converting enzyme (ECE) and endothelin-1 release from human umbilical vein endothelial cells are significantly increased in sera from women with preeclampsia com-
pared with sera from normotensive pregnant and non-pregnant women (119). These studies suggest that endothelin-1 release from endothelial cells may contribute to the increased vascular sensitivity to vasoconstrictors observed in preeclampsia and that the vasoconstrictor-induced endothelin-1 release may be related to enhanced ECE expression (119).

Although the circulating factor(s) in preeclampsia have not been fully characterized, several factors have been suggested. It has been hypothesized that trophoblastic deportation and/or transferred trophoblastic factors into the maternal circulation could occur as a result of the ischemic conditions (23). In support of this hypothesis, significantly increased fragments of syncytiotrophoblast microvillous membranes have been detected in blood from preeclamptic women and have been suggested to contribute to the endothelial dysfunction in vivo or as one part of a more generalized intravascular inflammatory response of the maternal immune system to pregnancy (23). This is further supported by reports that a complex from syncytiotrophoblast microvillous membranes has been purified and has been shown to inhibit the proliferation of cultured human endothelial cells (90). However, other studies were not supportive of the trophoblast deportation theory as a cause of vascular dysfunction in preeclampsia and showed that intraluminal perfusion of isolated myometrial arteries of healthy pregnant women with syncytiotrophoblast microvillous membranes at concentrations up to 100 times those reported in preeclampsia did not affect bradykinin-induced dilation or cause significant damage to the endothelium (160).

The preliminary characterization of the putative vasoactive circulating factor(s) in preeclampsia plasma suggested a high molecular weight protein/glycoprotein, with possible contributions from a hydrophobic, lipophilic factor (79). Also, plasma cytokines, oxidative stress, and several other factors have been suggested as possible intermediary placental factors in preeclampsia.

ROLE OF CYTOKINES AS POSSIBLE MEDIATORS OF VASCULAR CHANGES DURING PIH

According to the “cytokine” hypothesis of hypertension in pregnancy, the reduction in uteroplacental perfusion pressure and the ensuing placental ischemia are thought to increase the release of cytokines as tumor necrosis factor-α (TNF-α) from the placenta into the maternal circulation. The increased plasma cytokines would then lead to maternal endothelial cell dysfunction, generalized vascular changes, and hypertension (29, 34, 98, 162). This is supported by reports that small concentrations of TNF-α induce functional alterations in endothelial cells leading to reduction in ACh-induced vasodilation and increased production of endothelium-derived contracting factors such as endothelin (108, 126, 163). This is also consistent with the findings that the plasma levels of TNF-α and interleukin-6 (IL-6), which is activated by TNF-α, are elevated approximately twofold in women with preeclampsia (98, 162, 166). Although the ischemic placenta is often thought to be the source of the increased TNF-α and IL-6 during preeclampsia, recent studies showed no significant differences in the amount of the cytokines protein or the TNF-α mRNA between the normal term and preeclamptic placentas (18). It has also been shown that although the peripheral and uterine venous levels of TNF-α are elevated in preeclamptic women compared with normal pregnant women, the ratio of uterine to peripheral venous TNF-α levels is not significantly different from 1.0 for either patient group. These findings suggested that sources other than the placenta may contribute to the elevated concentrations of TNF-α and IL-6 found in the circulation of preeclamptic women (18).

Although high levels of TNF-α, as observed during septic shock or after lipopolysaccharide (LPS) administration, activate gene expression of iNOS, modest levels of TNF-α have been shown to downregulate the mRNA of eNOS (171). This is consistent with a recent study by Faas and colleagues (60) that showed that intravenous infusion of a high dose of LPS, which is known to activate TNF-α, decreases blood pressure in conscious pregnant rats, whereas a very low dose infusion of the endotoxin results in long-term increase in blood pressure, platelet aggregation, and urinary albumin excretion. We recently observed that a two- to threefold elevation in plasma TNF-α in late pregnant rats results in significant elevation in renal vascular resistance and arterial pressure (3, 47). We also found that the vascular reactivity is greater in TNF-α-infused pregnant rats compared with control pregnant rats (47). Additionally, the endothelium-dependent vascular relaxation is less in TNF-α-infused pregnant rats than control pregnant rats, possibly due to reduction in the activity of the endothelium-dependent NO-cGMP pathway in TNF-α-infused pregnant rats (Fig. 5). Interestingly, the arterial pressure, vascular reactivity, and vascular relaxation are not significantly different between control virgin rats and TNF-α-infused virgin rats. 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 estradiol enhances leukocyte binding to TNF-α-stimulated endothelial cells via an increase in TNF-α-induced adhesion molecules (24).

ROLE OF OXIDATIVE STRESS DURING PREECLAMPSIA

An increase in oxidative stress secondary to reduced placental perfusion has also been suggested as a possible mediator of the endothelial cell dysfunction associated with preeclampsia (134). Preeclampsia is characterized by increased free radical formation, elevated oxidative stress, and increased placental lipid peroxides (164). Deficiency in the plasma level of the antioxidant ascorbate is also typical of preeclampsia (158).
Recent studies showed that the brachial artery flow-mediated and endothelium-dependent dilation is reduced in previously preeclamptic women compared with controls. To investigate whether the endothelial dysfunction is mediated by oxidative stress, these measurements have been repeated after administration of the antioxidant ascorbic acid. Ascorbic acid administration increased flow-mediated dilatation in previously preeclamptic women but not in controls. These studies suggest that the impaired endothelial function in women with previous preeclampsia is possibly due to increased reactive oxygen species and is reversed by administration of the antioxidant ascorbic acid (22). However, whether antioxidants will be beneficial in prevention of the impaired endothelial dysfunction associated with overt preeclampsia remains to be elucidated.

OTHER POSSIBLE PLACENTAL FACTORS DURING PREECLAMPSIA

Other factors have been shown to be increased in the plasma of preeclamptic women and have been suggested as possible mediators of the vascular changes observed in the disease. Angiotensin type-1 receptor agonistic autoantibodies have been identified in the sera of preeclamptic women and have been suggested to stimulate the angiotensin receptors and activate extracellular signal-related kinase in vascular smooth muscle cells and thereby increase the expression of tissue factor (49). Angiotensin type-1 receptor agonistic antibody could also potentially lead to peripheral vasoconstriction and activation of signal transduction pathways, many of which culminate in the production of reactive oxygen species.

The role of antiendothelial cell antibody (AECA) in systemic vasculitis has also been investigated. AECA has been detected more frequently in severe than in mild preeclampsia. The appearance of AECA is related to the severity of proteinuria and the cytotoxicity to endothelial cells by AECA-positive sera, suggesting that AECA may play a role in causing the endothelial damage in preeclampsia (169).

Because placental hypoxia likely plays an important role in both normal and abnormal placentation, the role of the hypoxia-inducible transcription factors (HIFs) in the human placenta has been investigated. It has been shown that the protein expression of HIF-1α and HIF-2α, but not HIF-1β, is selectively increased in the preeclamptic placenta (130). However, the molecular mechanism(s) of this abnormality as well as the genes affected downstream are unclear.

Total plasma homocysteine concentration is also increased in preeclampsia and is significantly correlated with cellular fibronectin concentration, suggesting that homocysteine plays a role in promoting endothelial dysfunction in preeclampsia (129). Furthermore, preeclampsia is associated with an increase in maternal plasma leptin concentrations (110). However, the relationship between the increased plasma leptin and the vascular changes during preeclampsia remains to be clarified.

Recently, neurokinin B and cytokeratin-18 and -19 have been suggested as potential placental factors that could contribute to the vascular changes during preeclampsia (80, 124); however, their role in the disease needs to be further investigated.

Perspectives

The search for the cellular and vascular mechanisms underlying the increased arterial pressure in animal models of PIH should help us to 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 increases in vascular resistance and arterial pressure during preeclampsia are associated with changes in vascular endothelial cell function and the mechanisms of smooth muscle contraction. As in most other forms of hypertension, it is not always clear whether the changes in the vascular and cellular mechanisms are the cause or the consequence of the increased arterial pressure. Establishing a causal relation between the alterations in endothelial vascular relaxation and smooth muscle reactivity and the changes in arterial pressure could be difficult and costly in preeclamptic women or in animal models of PIH in which the vascular changes and the hypertension develop.

Fig. 5. ACh-induced endothelium-dependent relaxation is reduced in vascular strips of tumor necrosis factor (TNF)-α-infused pregnant rats compared with normal pregnant rats.
over an extended period of time. The RUPP rat model of PIH is unique in that the hypertension develops over a short 5-day period in late pregnancy and the remission occurs over a 3-day postpartum period. The hypertensive RUPP rat should then be useful to perform integrated analysis of the magnitude and time course of the alterations in endothelial vascular relaxation and smooth muscle reactivity and the changes in arterial pressure as well as investigating the effect of blocking specific cellular mechanisms using specific pharmacological tools, and thereby establish a cause-and-effect relationship between these parameters.

Evidence suggests that the localized placental ischemia is associated with increased placental factor(s) in the maternal circulation. In thinking about how the hypoperfused preeclamptic placenta secretes a factor or factors in the maternal circulation, one has to bear in mind how the maternal circulation might be affected. Any released factor has to leave the uterus via the uterine veins, which then enter the iliac veins, then vena cava to the right side of the heart and the pulmonary circulation before getting into the maternal arterial circulation. One of the more provocative arguments is what is the relevance of maternal venous blood concentrations of a factor in relation to what is happening in the arterial circulation of the mother and to what was originally in the uterine vein.

TNF-α is one potential factor that may lead to endothelial cell dysfunction, decreased vascular relaxation, and thereby increased vascular resistance and arterial pressure in human preeclampsia and animal models of PIH. However, placental ischemia during late pregnancy may be associated with increased plasma levels of not only TNF-α but also other cytokines such as IL-6. Whether chronic infusion of other cytokines such as IL-6 in late pregnant rats 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 chronic effects of TNF-α infusion represent direct vascular effects of the cytokine or may be mediated by other factors or even other cytokines. 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 the vascular changes in human preeclampsia and animal models of PIH.

Finally, the susceptibility to preeclampsia may have specific genetic components; however, the relative contributions of maternal and fetal genotypes to the disease are still unclear (136). Whole genome mapping could ultimately define the causative genes involved in the hemodynamic and vascular changes and the elevation of arterial pressure associated with preeclampsia.

This work was supported by grants from the National Heart, Lung, and Blood Institute (HL-33849, HL-51971, HL-52696, and HL-65998) and the American Heart Association (grant-in-aid, Mississippi Affiliate). R. A. Khalil is an Established Investigator of the American Heart Association.

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