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Circulating and Vascular Bioactive Factors during Hypertension in Pregnancy

Alain F. Tanbe and Raouf A. Khalil

Division of Vascular and Endovascular Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Abstract

Normal pregnancy is associated with significant vascular remodeling in the uterine and systemic circulation in order to meet the metabolic demands of the mother and developing fetus. The pregnancy-associated vascular changes are largely due to alterations in the amount/activity of vascular mediators released from the endothelium, vascular smooth muscle and extracellular matrix. The endothelium releases vasodilator substances such as nitric oxide, prostacyclin and hyperpolarizing factor as well as vasoconstrictor factors such as endothelin, angiotensin II and thromboxane A₂. Vascular smooth muscle contraction is mediated by intracellular free Ca²⁺ concentration ([Ca²⁺]_i), and [Ca²⁺]_i sensitization pathways such as protein kinase C, Rho-kinase and mitogen-activated protein kinase. Extracellular matrix and vascular remodeling are regulated by matrix metalloproteases. Hypertension in pregnancy and preeclampsia are major complications and life threatening conditions to both the mother and fetus, precipitated by various genetic, dietary and environmental factors. The initiating mechanism of preeclampsia and hypertension in pregnancy is unclear; however, most studies have implicated inadequate invasion of cytotrophoblasts into the uterine artery, leading to reduction in the uteroplacental perfusion pressure and placental ischemia/hypoxia. This placental hypoxic state is thought to induce the release of several circulating bioactive factors such as growth factor inhibitors, anti-angiogenic proteins, inflammatory cytokines, reactive oxygen species, hypoxia-inducible factors, and vascular receptor antibodies. Increases in the plasma levels and vascular content of these factors during pregnancy could cause an imbalance in the vascular mediators released from the endothelium, smooth muscle and extracellular matrix, and lead to severe vasoconstriction and hypertension. This review will discuss the interactions between the various circulating bioactive factors and the vascular mediators released during hypertension in pregnancy, and provide an insight into the current and future approaches in the management of preeclampsia.

Keywords

cytokines; endothelium; nitric oxide; vascular smooth muscle; calcium; preeclampsia

INTRODUCTION

Normal pregnancy (Norm-Preg) is associated with increased heart rate, plasma volume and cardiac output, as well as decreased vascular resistance and late gestational decrease in blood pressure (BP). These beneficial hemodynamic changes ensure critical blood and nutrient supply to the growing fetus. In 5–10% of pregnancies women develop hypertension in pregnancy (HTN-Preg) [1]. HTN-Preg may take one of four forms: chronic HTN that predates pregnancy, preeclampsia (PE)-eclampsia, chronic HTN with superimposed PE, and

nonproteinuric gestational HTN [2]. The World Health Organization Antenatal Care Trial has shown that 9.1% of pregnant women have either PE or gestational HTN [3]. PE is characterized by a high BP 140/90 mmHg or more in two separate readings taken at least 4 hours apart, 300 mg of protein in a 24-hour urine sample, and pitting edema. Also, if untreated, PE can lead to eclampsia, and life-threatening maternal neurovascular complications including convulsions and severe HTN [4]. Although HTN-Preg represents a major cause of maternal and fetal morbidity/mortality, the exact mechanisms of this disorder have not been clearly identified. Analysis of samples of plasma, body fluids, and postpartum placenta from PE women has been useful in determining some of the underlying biochemical changes. Because of the difficulty to perform mechanistic studies in pregnant women, animal models of HTN-Preg with characteristics similar to those observed in human PE have been developed. Studies in animal models of HTN-Preg have demonstrated significant changes in the renal control mechanisms of BP, and highlighted potential glomerular injury and alteration in kidney functions as possible causes of the increased BP [5]. However, HTN is a multifactorial disorder that involves additional alterations in the neurohumoral and vascular control mechanisms of BP as well as changes in lipid metabolism and the blood coagulation system. Also, several genetic, dietary and environmental risk factors are thought to play a role in the development of HTN-Preg. These maternal risk factors may lead to poor placentation, placental ischemia/hypoxia, and increased release of biologically active circulating factors that eventually cause vascular dysfunction and HTN-Preg (Fig. 1). Understanding the mechanistic interactions between circulating bioactive factors and vascular mediators in non-pregnant conditions characterized by hypoxia could also be useful, although such interactions may differ from those occurring during pregnancy due to the influence of sex hormones. The purpose of this review is to discuss the interactions between the various biologically active circulating factors and vascular mediators, released during PE as compared to those released in other conditions characterized by hypoxia, in order to provide insight into the sequence of events that lead to PE and other forms of HTN-Preg. Throughout the review we will discuss the changes in Norm-Preg followed by those in PE, evidence in human followed by data from experimental animals, and data from large vessels followed by small vessels and isolated vascular cells.

Genetic Risk Factors of PE

Maternal Genes—Women offspring of a pregnancy complicated by PE may have a higher risk of developing PE in their own pregnancies. The increased risk may reflect the inheritance of genes that enhance maternal susceptibility to PE [6]. Genome-wide scans and linkage studies have implicated more than one gene locus, and identified loci on chromosomes 1, 2p13, 2q, 3p, 4q, 9, 10q, 11q23–24, 12q, 15q, 18 and 22q, suggesting that PE could be a multigenic disorder [7]. Also, genes encoding novel molecules such as sialic acid binding Ig-like lectin 6 (Siglec-6), a potential leptin receptor, and pappalysin-2 (PAPP-A2), a protease that cleaves insulin-like growth factor (IGF) binding proteins, are overexpressed in PE [8]. Additionally, genes encoding different components of the renin-angiotensin system (RAS) have been studied, and variations in the angiotensinogen, angiotensin type 1 receptor (AT₁R) and endothelial nitric oxide synthase (NOS) genes have been associated with HTN-Preg. Also, mutation in factor V Leiden gene, which results in resistance to activated protein C, has been found in 10% of Caucasian women with HTN-Preg compared with only 4% of Caucasian Norm-Preg women [9].

Fetal Genes—Another important factor in the development of HTN-Preg could be the fetal genes. Mouse models have been used to study the fetal genetic factors associated with HTN-Preg. The cyclin-dependent kinase inhibitor *cdkn1c* (*p57^{Kip2}*) is a major regulator of embryonic growth, and mutant embryos demonstrate altered placental architecture and histological changes indicative of reduced utero-placental blood flow. Also, pregnant mice expressing wild-

type levels of p57^{Kip2} develop a PE-like condition when carrying p57^{Kip2}-deficient pups [7], suggesting that fetal and paternal genes play a role in the development of HTN-Preg.

Ethnic Background—Ethnic background may also be a factor in the development of HTN-Preg. A study of pregnant women in New York found that African-American women had the highest rates of HTN-Preg, while Asian women had the lowest incidence rates [10]. Also, African-American and Hispanic women had much higher rates of PE compared to Caucasian and Asian women.

Dietary Risk Factors

Dyslipidemia and Hyperglycemia—Diet can also be a risk factor for the development of HTN-Preg. There has been a progressive increase in the prevalence of obesity and metabolic syndrome among the United States population. Metabolic syndrome is characterized by glucose intolerance, dyslipidemia, insulin resistance, diabetes, obesity, and HTN. Patients with metabolic syndrome also have vascular endothelial dysfunction, and their blood vessels could be more sensitive to circulating factors released during HTN-Preg. PE is also characterized by changes in lipid metabolism, and such changes may play a role in the endothelial cell (EC) damage characteristic of PE. Recent epidemiological studies have shown elevated maternal plasma concentration of triglycerides in women who develop PE. Women with PE also have higher serum levels of free fatty acids and higher concentrations of cholesterol, phospholipids, and lipid peroxides in the placenta [11]. Furthermore, increased intake of energy, sucrose, or polyunsaturated fatty acids is associated with increased risk of PE [1]. Increased sugar consumption may also lead to hyperglycemia, which in turn could inhibit endothelium-dependent vasorelaxation. Thus both dyslipidemia and hyperglycemia have been associated with EC dysfunction, an important feature of PE. Also, increased intake of polyunsaturated fat, which is easily oxidized, may enhance the oxidative environment that exists in PE.

Calcium, Vitamin D, Magnesium, Potassium—Dietary intake of micro- and macronutrients may influence the risk of HTN-Preg. Increased calcium (Ca²⁺) intake has been associated with lower incidence of HTN-Preg; however, the beneficial effect of Ca²⁺ supplementation may depend on the mother's age and socioeconomic status [12]. Experimental studies on pregnant ewes showed that restriction of Ca²⁺ intake is associated with a decrease in uterine blood flow, increased BP and elevated urine protein; all symptoms similar to those of PE [13]. Other studies suggested that increasing Ca²⁺ intake during pregnancy may not lower the risk of PE [14]. Randomized controlled clinical trials by the World Health Organization on Ca²⁺ supplementation during pregnancy showed that Ca²⁺ may not decrease the risk of PE but may help in lowering the severity of the condition [15]. Vitamin D is a major regulator of Ca²⁺ absorption and metabolism. Vitamin D deficiency in early pregnancy is a strong, independent risk factor for PE. Studies have shown that women with PE have lower levels of Vitamin D compared to Norm-Preg women [16]. Also, experimental studies examining the influence of vitamin D on the renin-angiotensin system in mice have shown that renin expression and plasma AngII production are increased in vitamin D-deficient mice, and are associated with HTN [17]. Magnesium (Mg²⁺) is an essential dietary mineral needed in a relatively large amount. Mg²⁺ is a cofactor of many enzymes that regulate temperature and protein synthesis, and maintain electrical potential in nerves and muscle membrane [18]. Observational studies based on medical records reported that Mg²⁺ supplementation during pregnancy was associated with a reduced risk of fetal growth retardation and PE. Maternal Mg²⁺ intake is also positively associated with birth weight. Also, diets high in potassium and fiber are associated with a reduced risk of HTN [19]. Thus, the maternal intake of recommended amounts of Ca²⁺, vitamin D, Mg²⁺, potassium and fiber may lead to a reduced risk of HTN-Preg.

Oxidative Stress—Oxidant-antioxidant imbalance may also play a pathogenic role in PE. Vitamin E is an important antioxidant, and its level and relation with circulating levels of lipid peroxides in women with HTN-Preg have been studied. As with other antioxidants, studies have shown lower levels of vitamin E in serum of women with HTN-Preg or PE compared to Norm-Preg women. Also, studies have shown decreased level of Vitamin C in plasma taken from women with PE, suggesting that supplemental intake of Vitamin C and E may decrease the incidence of PE in women at high risk [20]. On the other hand, the role of vitamin A in HTN-Preg and PE is unclear. Some clinical studies found markedly lower serum vitamin A levels in PE than Norm-Preg women in the third trimester. However, no clinical trials have assessed the effect of vitamin A supplementation on HTN-Preg or PE. Furthermore, the potential teratogenic effects of vitamin A should be considered.

Other Environmental Risk Factors of HTN-Preg

Lead—Environmental exposure to toxic compounds may cause undesirable health effects to the mother and fetus. Exposure to lead and high lead accumulation in the bones are associated with the development of HTN. In experimental animals, chronic exposure to low levels of lead affects the electrical and mechanical properties of the heart and alters vascular smooth muscle (VSM) function. Studies have also shown a dose-dependent relationship between lead exposure and HTN-Preg, and demonstrated a 4% increase in the risk of HTN per 0.05 mg/m³ increase in the seasonal average lead level [21]. Additionally, some lead is absorbed into the bones, where it is stored and accumulated with age. Thus women who have had past lead exposure or chronic low level exposure may have a higher risk of HTN-Preg, even after a long period of no exposure [21,22].

Medical Factors—PE and HTN-Preg are also associated with medical risk factors such as diabetes, renal or cardiac disease, PE in a previous pregnancy, urinary tract infection, extreme maternal age (<16 or >40 years old), and twin pregnancy. Risk factors associated with HTN-Preg also include a previous neonatal macrosomia, history of reproductive tract surgery, and antepartum hemorrhage. Primiparity and a history of chronic respiratory conditions may also be risk factors for PE [3].

Abnormal Placentation as an Initiating Event of HTN-Preg

During the early stages of Norm-Preg, the cytotrophoblasts invade the uterine spiral arteries, progressively replacing ECs, medial elastic tissue, VSM, and neural tissue. Consequently, the uterine spiral arteries undergo marked transformation from small muscular arterioles to low resistance large capacitance vessels. By the end of the second trimester, the spiral arteries become dilated tubes lined by cytotrophoblasts, which ensures sufficient blood supply and nutrition to the growing fetus. During PE, abnormal expression of the cell adhesion molecules integrins as well as apoptosis of cytotrophoblasts lead to limited invasion of the uterine spiral arteries to only the superficial layers of the decidua [23,24]. The shallow cytotrophoblast invasion and the inadequate vascular remodeling of the uterine spiral arteries do not meet the fetal blood flow and nutrition demands, resulting in fetal intrauterine growth retardation (IUGR) [25,26]. Placental ischemia also initiates a cascade of events leading to marked changes in the maternal circulation. The ischemic placenta may induce the release of bioactive circulating factors that cause the harmful changes in the maternal circulation and EC function observed in PE. It is also likely that the ischemic placenta may not be capable of releasing potentially beneficial biological factors that are necessary for the normal hemodynamic effects observed in Norm-Preg.

Animal models of HTN-Preg have been useful in understanding the role of placental ischemia as a possible initiating event of the elevated BP in PE. Although experimental induction of uteroplacental ischemia during pregnancy has shown variable effects in different species, it is

still considered one of the most promising animal models of HTN-Preg. Reduction of uteroplacental perfusion pressure (RUPP) in late pregnant sheep, dogs, rabbits and rats results in a hypertensive state that resembles PE [27].

Biologically Active Factors Involved in HTN-Preg

Placental hypoxia is thought to induce the release of various bioactive circulating factors. Elevated plasma and vascular tissue levels of these bioactive factors during pregnancy could cause EC and VSM dysfunction, severe vasoconstriction and PE in human (Table 1) and HTN-Preg in rats (Table 2). Studies have searched for a unique factor as a single cause of the HTN-Preg, but this concept has been challenged, and numerous factors have been suggested [27]. Understanding the effects of these biologically active factors on vascular-derived mediators is crucial in determining the specific compounds involved in the development of HTN-Preg.

The circulating biologically active factors in PE have not been fully characterized. Placental trophoblasts may be a potential source of the factors that are released into the maternal circulation as result of placental ischemia, and consequently cause the increased vascular permeability observed in PE. Fragments of syncytiotrophoblast microvillous membranes have been detected in blood of PE women and may cause EC dysfunction as a part of a more generalized intravascular inflammatory response of the maternal immune system to pregnancy [53]. Also, a complex purified from syncytiotrophoblast microvillous membranes inhibits the proliferation of cultured human EC [54]. Studies on human umbilical vein ECs co-cultured with trophoblast cells isolated from normal and PE placentas have suggested that endothelial cell-to-cell junctions are sensitive targets for trophoblast-derived factors, leading to marked increase in ECs permeability, and may translate into increased vascular permeability *in vivo* [55]. Other studies do not support the trophoblast deportation theory and have shown that intraluminal perfusion of isolated myometrial arteries of Norm-Preg women with syncytiotrophoblast microvillous membranes at concentrations up to 100 times those reported in PE does not affect bradykinin-induced dilation or cause significant damage to the endothelium [56]. The preliminary characterization of the putative biologically active factor (s) in PE plasma suggested a high-molecular-weight protein/glycoprotein, and possible contribution of a hydrophobic, lipophilic factor [57]. Plasma VEGF, antiangiogenic factors, cytokines, oxidative stress, hypoxia-inducible factors (HIFs), matrix metalloproteases (MMPs), and numerous modulators of endogenous hormones and vascular receptors have been implicated as possible biologically active factors in PE (Fig. 2).

Vascular Endothelial Growth Factor—VEGF is a glycoprotein that acts as a mitogen and mediator of EC growth and tissue neovascularization. VEGF expression is induced by hypoxia in various cell types, including ECs and VSM cells. The main mechanism of regulation of VEGF by hypoxia occurs at the level of VEGF mRNA stability [58]. Some studies suggest that serum VEGF immunoreactivity is suppressed during Norm-Preg, and may further decrease during PE [59]. Other studies have shown that elevated serum VEGF levels may be involved in the EC activation/dysfunction that occurs in PE [60]. Maternal plasma VEGF increases before the clinical onset of PE and is further elevated during the vasoconstriction phase of the disease. It is possible that the hyperdynamic circulation in the latent phase of PE may induce vascular shear stress, which in turn increases circulating VEGF. Because VEGF normally acts as a vasodilator, its increase may represent an unsuccessful vascular rescue response during PE [61]. Studies on PE women have shown that treatment with VEGF impairs bradykinin-mediated vascular relaxation, but enhances basal tone and permeability, supporting a role for VEGF in the EC dysfunction in PE [62]. The vasodilator effects of VEGF may be due to stimulation of the NO-cGMP vascular relaxation pathway. Studies have shown that VEGF increases $[Ca^{2+}]_i$, which promotes calmodulin binding to eNOS and stimulates NO production.

Also, VEGF-induced NO production has been associated with upregulation of eNOS activity in ECs [63].

sFlt-1—Soluble fms-like tyrosine kinase-1 (sFlt-1) is a VEGF and placental growth factor (PLGF) antagonist protein that binds VEGF and prevents its interaction with its endogenous receptors. Compared with Norm-Preg women, circulating levels of sFlt-1 are increased in PE women [42,64]. The excess production of sFlt-1 in PE may be secondary to placental ischemia/hypoxia [43]. Increased placental expression of sFlt-1 appears to play a central role in the pathogenesis of PE. sFlt-1 administered to pregnant rats induces albuminuria, HTN, and renal pathologic changes in the form of glomerular endotheliosis [30]. *In vitro*, removal of sFlt-1 from supernatant of PE tissue culture restores EC function and angiogenesis to normal levels [29]. Interestingly, two karyotypic anomalies, trisomy 13 and hydatiform mole, are associated with a risk for PE. Redman and coworkers suggested an association between trisomy 13 and PE [65], and predicted the involvement of fetal factors and proteins encoded by genes located on chromosome 13. In effect, the sFlt-1 gene is localized on chromosome 13 [65,66].

Endoglin—Endoglin is a transmembrane receptor for transforming growth factor- β (TGF- β) that is expressed on ECs. A novel placenta-derived soluble endoglin (sEng) is an anti-angiogenic protein that appears to be an important factor in PE [67]. sEng inhibits TGF- β 1 signaling in ECs and blocks TGF- β 1 mediated activation of eNOS and vasodilation, suggesting that dysregulated TGF- β signaling may be involved in the pathogenesis of PE [68]. sEng is elevated in sera of PE women 2 to 3 months before the onset of clinical signs of PE, correlates with the severity of the disease, and falls after delivery. An increased level of sEng accompanied by an increased ratio of sFlt-1:PLGF is predictive of developing PE [69]. In pregnant rats, sEng increases vascular permeability and induces HTN [68]. Also, in pregnant rats, sEng potentiates the vascular effects of sFlt-1 to induce a severe PE-like state, including the development of HELLP syndrome and restriction of fetal growth [69].

Cytokines—Various cytokines, including tumor necrosis factor- α (TNF- α), interleukin (IL)-6, and IL-8, are involved in cell proliferation and hypoxia-mediated cell activation. Hypoxia has been shown to induce marked increases in IL-6 and IL-8 synthesis in VSM cells [46]. RUPP during pregnancy and the ensuing placental ischemia are also thought to increase the release of cytokines such as TNF- α and IL-6 into the maternal circulation. The increased plasma cytokines could then lead to maternal EC dysfunction, generalized vascular changes and HTN [70]. This is consistent with the observation that the plasma concentrations of TNF- α and IL-6 are elevated in women with PE [70]. IL-10 may be involved in the maintenance of pregnancy by inducing corpus luteum maturation and progesterone production, and the level of IL-10 is decreased in PE women [71].

Cytokines have been shown to affect vascular mediators as well as other biologically active factors (Fig. 2A). TNF- α induces structural as well as functional alterations in ECs. TNF- α also enhances the formation of a number of EC substances such as endothelin-1 (ET-1), reduces acetylcholine-induced vasodilatation, and destabilizes the mRNA of endothelial eNOS [45]. Also, both TNF- α and IL-6 induce VEGF expression in ECs [72].

Infusion of cytokines has been used to develop animal models of HTN-Preg. Experimental studies have shown that chronic infusion of TNF- α or IL-6 in late pregnant rats to increase their plasma 2–3 fold results in marked elevation of renal vascular resistance and BP [73–75]. Also, the vascular contraction is greater in TNF- α and IL-6 infused pregnant rats compared with Norm-Preg rats [74,75]. Furthermore, endothelium-dependent vascular relaxation is reduced in TNF- α and IL-6 infused compared with Norm-Preg rats possibly due to inhibition of the endothelium-dependent NO-cGMP pathway [74,75].

Reactive Oxygen Species (ROS)—An increase in oxidative stress is another factor that could provide the link between localized RUPP and the generalized EC dysfunction in PE [76]. Hypoxia is known to cause increases in the production of ROS, including superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide in ECs and VSM cells [77]. Free radicals and placental lipid peroxides are increased in PE. Also, a deficiency in the plasma concentration of the antioxidant ascorbate is typical of PE [78]. It has been shown that brachial artery flow-mediated and endothelium-dependent vasodilation is reduced in women who previously developed PE compared with controls. Administration of ascorbic acid increases flow-mediated vasodilation in women who previously developed PE but not in controls, suggesting that the EC dysfunction in women with previous PE is possibly due to increased oxidative stress and ROS [79]. However, whether antioxidants are beneficial in preventing the impaired EC function associated with PE needs to be further examined [80].

Neutrophils are another possible source of EC damage through their ability to produce ROS. Stimulated neutrophils from women with PE produce more $O_2^{\bullet-}$ compared to neutrophils from Norm-Preg women. Neutrophils also produce NO, which can react with $O_2^{\bullet-}$ and protect cells from $O_2^{\bullet-}$ -induced damage in Norm-Preg. However, in PE $O_2^{\bullet-}$ production may dominate neutrophil and EC NO production, resulting in EC injury [34]. Furthermore, the plasma concentration of lipid peroxidation markers is increased in women with PE. This supports the hypothesis that poor uteroplacental perfusion results in an increase in placental free radical synthesis and maternal oxidative stress [81].

Oxidant species, including $O_2^{\bullet-}$ and peroxynitrite, are potent stimulators of matrix metalloproteases MMP-1, -2, and -9 in VSM [82]. Also, upregulation of ROS during hypoxia is associated with increased degradation of HIF-1 α [83]. ROS also cause increases in VSM $[Ca^{2+}]_i$ via activation of voltage-gated Ca^{2+} channels, leading to an influx of extracellular Ca^{2+} . ROS can also increase $[Ca^{2+}]_i$ by inhibiting Ca^{2+} -ATPase activity and promoting inositol triphosphate (IP_3)-induced Ca^{2+} release from intracellular stores. The increases in $[Ca^{2+}]_i$ induce VSM contraction, and activate Ca^{2+} -dependent protein kinase C (PKC) to further promote vascular contraction. ROS can also promote vasoconstriction by activating Rho-A/Rho-kinase signaling mechanisms [84].

Hypoxia Inducible Factors—Induction of HIF-1 by hypoxia requires synthesis of both the α and β -subunits, which are quickly degraded after reoxygenation. HIF-1 binds to the enhancer region of genes that encode glycolytic enzymes as well as VEGF [85]. Because placental hypoxia likely plays a role in PE, the role of HIFs in the human placenta has been investigated. The expression of HIF-1 α and HIF-2 α , but not HIF-1 β , is selectively increased in PE placenta [33]. The molecular mechanisms underlying the changes in HIF expression and the genes affected during PE are unclear. However, a HIF-1 α binding site exists in the promoter region for the ET-1 gene and acts, in combination with other transcription factors, to regulate ET-1 expression in response to hypoxia [86]. Upregulation of HIF-1 also enhances the expression of VEGF [83].

Matrix Metalloproteases—MMPs are zinc-dependent endopeptidases that are capable of degrading extracellular matrix proteins and to process a number of bioactive molecules. MMPs may play a role in the vascular dysfunction associated with HTN-preg. MMP-2 is the main collagenolytic enzyme in umbilical cord artery (UCA) wall. MMP-2 also cleaves big endothelin-1 into ET-1₁₋₃₂, which is a potent vasodilator. MMP-2 also inhibits relaxin and thrombin. The levels of MMP-2 in PE have been controversial; some studies suggest an increase [87], while other studies suggest that a reduced level of MMP-2 in UCA reduces the breakdown of collagen in the arterial wall. The accumulation of collagen and with a reduction of elastin in the UCA may decrease the blood flow in the fetus of women with PE [88]. Hypoxia has been shown to increase MMP-9 mRNA and enzymatic activity 3- to 4-fold in ECs [85].

Also, the higher net MMP-9 activity in HTN-Preg compared with Norm-Preg suggests that MMP-9 may play a role in the pathophysiology of PE. Recent studies have suggested that MMP-2 and MMP-9 inhibit increases in vascular $[Ca^{2+}]_i$, through reduction of Ca^{2+} entry from extracellular spaces, but not from the intracellular stores [89]. Interestingly, the relationship between MMP and VEGF is bi-directional. Studies have shown that VEGF upregulates MMP expression; while membrane type-1 MMP promotes VEGF expression [90].

2-Methoxy-estradiol (2-methoxy-E2)—2-Methoxy-E2, a natural metabolite of E2, is elevated during the third trimester of Norm-Preg and has recently been linked to PE. Studies have shown that pregnant mice deficient in catechol-O-methyltransferase (COMT) show a PE-like phenotype resulting from an absence of 2-methoxy-E2. 2-Methoxy-E2 ameliorated all PE-like features without toxicity in the COMT deficient pregnant mice and suppressed placental hypoxia, HIF-1 α expression and sFLT-1 elevation. The plasma levels of 2-methoxy-E2 are markedly lower in women with severe PE. These findings suggested that measurement of 2-methoxy-E2 in plasma and urine may be used as a diagnostic marker for PE. Also, 2-methoxy-E2 may serve as a therapeutic measure to prevent or treat PE [36].

Vascular Mediators in HTN-Preg

Blood vessels maintain their tone through a balance between vasodilator and vasoconstrictor mediators. The release of biologically active factors in response to placental hypoxia/ischemia could result in EC and VSM dysfunction and influence the release of various vascular mediators (Fig. 1). ECs regulate vascular tone through the release of vasodilators including NO, prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) as well as vasoconstrictor mediators such as ET-1 and AngII in human (Table 1) and in rats (Table 2).

Endothelial Mediators

Nitric Oxide—NO is a powerful vasodilator and relaxant of VSM. NO is produced from the transformation of L-arginine to L-citrulline by neuronal nNOS, inducible iNOS, and endothelial eNOS. It has been reported that low oxygen tension *in vivo* results in a substantial decrease in eNOS mRNA expression and eNOS protein and NO production [91]. The vascular changes during Norm-Preg have been attributed, in part, to the increased NO synthesis by various maternal cells including vascular ECs [92]. NOS expression and activity are increased in human uterine artery during Norm-Preg [93]. Also, the plasma concentration and urinary excretion of cGMP, a second messenger of NO and cellular mediator of VSM relaxation, are increased in Norm-Preg. Interestingly, cGMP production is markedly increased during the first trimester when the maternal circulation is rapidly vasodilating, while the whole body NO production is not proportionately elevated, suggesting additional sources of cGMP [94].

Measurements of total NO production during HTN-Preg have not produced consistent findings. Some studies have shown that the concentration of nitrite/nitrate, stable byproducts of NO, is reduced in the sera of women with PE [95], while other studies have shown increased levels [96]. The difference in the nitrite/nitrate levels during PE could be related to dietary nitrate intake. However, in a study of women with PE, during which dietary nitrate/nitrite intake was carefully controlled, unequivocal support for reduced NO production was not demonstrated [94]. The apparent dissociation between whole body NO production and the decreased vascular relaxation during HTN-Preg suggest that tissue-specific differences in NOS expression and NO bioavailability may occur during pregnancy. For instance, NOS expression may not be different in the placentas of Norm-Preg and PE women [97]. However, in late pregnant rats the amount of renal eNOS is decreased by 39% while iNOS and nNOS increase by 31% and 25%, respectively [98].

Although the total nitrate/nitrite production may be unchanged in the plasma of PE women, 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 PE plasma and might result in decreased decomposition rates of S-nitrosothiols [20]. This is supported by reports that PE plasma contains higher concentrations of total S-nitrosothiols and S-nitrosoalbumin than plasma taken during Norm-Preg, possibly due to insufficient NO release from these major NO reservoirs in PE [78].

While measurements of nitrate/nitrite have not produced consistent findings in PE, the increase in NO production and the reduction of vascular resistance during Norm-Preg suggests that a decrease in NO production during pregnancy should significantly affect the vascular resistance and BP. Studies have suggested endothelial dysfunction in PE [99]. Also, NOS blockade with N^o-nitro-L-arginine methyl ester (L-NAME) during mid to late gestation in rats has been shown to produce pathological changes similar to those observed in women with PE including renal vasoconstriction, proteinuria, thrombocytopenia and IUGR [100,101]. Also, BP is markedly increased in pregnant rats treated with L-NAME compared with virgin rats treated with equal doses of L-NAME [101].

Prostacyclin—PGI₂ is an anti-platelet aggregator and a vasodilator with significant beneficial effects in the maternal circulation. PGI₂ is produced from the metabolism of arachidonic acid by the cyclooxygenase (COX)-1 and COX-2. Hypoxia has been shown to down-regulate COX-1 and PGI₂ production [102]. Endothelium-derived PGI₂ may contribute to the hemodynamic and vascular changes during pregnancy. The plasma and urinary levels of 6-keto-prostaglandin F1 α (PGF1 α), a hydration product of PGI₂, are increased during Norm-Preg [103]. Alterations in PGI₂ production have been reported in PE [103]. The plasma and urinary concentrations of PGF1 α are lower in women with severe PE than in Norm-Preg women, suggesting that overall PGI₂ synthesis is diminished in PE. Endothelial PGI₂ production may also be decreased in PE [104]. Interestingly, in vascular strips of RUPP rats, acetylcholine-induced relaxation is not completely inhibited by L-NAME or methylene blue, suggesting changes in other endothelium-derived vasodilator substances such as PGI₂ in animal models of HTN-preg [38].

Endothelium-Derived Hyperpolarizing Factor—While the exact nature of EDHF is unclear, it is a prominent relaxing factor particularly in the small resistance vessels. Studies have shown that EDHF is the predominant relaxing factor in female mice, while NO and PGI₂ are the dominant vasodilators in male mouse vessels [105]. This suggests that an increase in EDHF production/activity may play a role in the vascular adaptation during Norm-Preg. Studies on the uterine vascular beds of pregnant rats have suggested that EDHF release is activated by a delayed rectifier type of voltage-sensitive K⁺ channel [106], and cause vasodilation by affecting myoendothelial gap junctions alone or in combination with H₂O₂ or cytochrome P-450 epoxygenase, a metabolite of arachidonic acid. Although EDHF does not appear to be reduced by chronic hypoxia [107,108], its release may be impaired during PE [109,110].

While NO, PGI₂, and EDHF act as vasodilators, the interactions between these molecules has not been clearly defined. PGI₂ analogue has been shown to upregulate eNOS and NO production in ECs [111]. Studies have also shown that PGI₂ may activate the inward rectifier potassium channels through the release of EDHF [112].

Endothelin-1—ET-1 is produced by ECs, and acts on VSM cells to cause marked vasoconstriction. Hypoxia is one of the most potent stimuli of ET-1 production. Hypoxia induces the synthesis and secretion of ET-1 from ECs by a mechanism that is antagonized by NO and carbon monoxide [86]. ET-1 production also appears to be increased in women with

PE [113], perhaps via activation of AT₁R [40]. ET-1 secretion is 4- to 8-fold higher in umbilical cord ECs of PE compared to Norm-Preg women [114]. Also, the concentration of immunoreactive ET-1 is elevated in plasma of PE women and rapidly returns to a normal value within 48 h of delivery, suggesting that ET-1 may contribute to the vasoconstriction associated with PE [115]. Typically, plasma ET-1 levels are highest during the later stage of the disease, suggesting that ET-1 may not be involved in the initiation of PE, but rather in the progression into the malignant hypertensive phase of the disease [40].

ET-1 interacts with ET_AR and ET_{B2}R in VSM, initiating a cascade of biochemical events that leads to vascular contraction. Also, chronic infusion of ET-1 results in increased plasma concentrations of AngII [116]. ET-1 also activates ET_{B1}R in ECs and causes the release of vasodilator substances such as NO, PGI₂, and EDHF that promote vascular relaxation [117]. ET-1, via activation of ET_BR, may also mediate the reduced myogenic reactivity and vasodilation of renal arteries as well as hyperfiltration during pregnancy in rats [118,119]. Under normoxic conditions, ET-1 stimulates Ca²⁺ release from the intracellular stores, PKC-dependent inhibition of voltage-gated K⁺ channels, and subsequent membrane depolarization and Ca²⁺ influx through voltage-dependent Ca²⁺ channels, leading to increased [Ca²⁺]_i and VSM contraction. In contrast, in hypoxic conditions, up to 80% of ET-1 induced vascular contraction occurs independently of [Ca²⁺]_i and PKC [120].

Angiotensin II—AngII is an important circulating hormone for BP regulation and electrolyte homeostasis. Also, up to 40% of AngII is produced locally by non-angiotensin converting enzyme (ACE) pathways in blood vessels [121]. Hypoxia has been shown to cause an increase in plasma levels of AngII in rabbits, although this increase is temporary [122]. Also, local levels of ACE, as well as AT₁R and angiotensin type 2 receptor (AT₂R) are increased during chronic hypoxia [122]. Plasma Ang-(1–7) concentration and most other components of the rennin-angiotensin system, except serum ACE, are reduced in PE compared with Norm-Preg subjects. Only plasma AngII remains elevated in PE compared to nonpregnant women. In PE the decreased plasma Ang-(1–7) in the presence of elevated AngII may contribute to the development of HTN [123]. AngII may cause vascular remodeling by activating signal transduction cascades that promote vasoconstriction, vascular growth, and inflammation. AngII may also activate the Rho/Rho-kinase system, a prominent regulator of VSM contraction, the cytoskeleton and vascular remodeling [124]. Studies have shown increased sensitivity and vascular responses to AngII in PE. Other studies have suggested that the decreased response to AngII in Norm-Preg is caused by increased inactivation of AngII by angiotensinase in serum and placenta. Angiotensinase activity increases with advancing gestation in Norm-Preg sera, but the enzyme activity is lower in sera of women with severe PE leading to decreased degradation and increased sensitivity to AngII [125]. A fraction of circulating IgG antibodies activate the AT₁R and are considered a factor in PE. The plasma level of AT₁R autoantibodies (AT-1AA) rises at the time the symptoms of PE develop and subsides within 6 weeks after delivery. AT-1AA activity may promote Ca²⁺ signaling and VSM contraction. Also, the AT-1AA induced mobilization of intracellular Ca²⁺ initiates a signaling cascade that leads to activation of NF-κB and activator protein-1 and increased tissue factor expression which in turn cause vasoconstriction and lead to HTN (126).

TNF-α stimulates AngII production in the female reproductive tract [127] and IL-6 upregulates the expression of AT₁R in VSM cells [128]. Studies have also suggested an interaction between AngII and ET-1, such that AngII induces the production of ET-1 [129]. While AngII is often thought of as a vasoconstrictor mainly by activating AT₁R in VSM, it also stimulates AT₂R in ECs and thereby promotes the release of various endothelium-derived relaxing factors. AngII causes the release of NO through activation of eNOS [121]. In vascular tissues AngII also stimulates synthesis of prostanoid compounds, including PGI₂, which act in opposition to AngII-induced vasoconstriction [130]. It has been shown that AngII infusion in mice does not

cause any changes in EDHF-mediated vascular relaxation [131]. However, administration of an ACE inhibitor or an AT₁R antagonist results in improved EDHF-mediated vascular relaxation in rats [132,133].

Thromboxane A₂—TXA₂ is a potent vasoconstrictor and platelet aggregator released from the platelets, and was thought to play a major role in the pathogenesis of PE. TXB₂ metabolites, markers of TXA₂ synthesis, were found to be higher in PE compared to Norm-Preg women [134]. Platelet TXA₂ synthesis may be particularly increased in severe PE, suggesting that TXA₂ might be used to determine the severity of PE. Experimental studies suggested that enhanced production of TXA₂ may not play a major role in mediating the HTN and renal vasoconstriction produced by chronic RUPP in pregnant rats [135]. Some clinical studies suggested the use of a low dose of aspirin as an approach to prevent PE [136]; however, a randomized controlled clinical trial on women at high risk for PE showed no benefit of low dose aspirin when used as a preventive measure of the disease [137].

Vascular Smooth Muscle Mediators

VSM Ca²⁺—[Ca²⁺]_i is a major determinant of vascular tone. During VSM activation, increased [Ca²⁺]_i is initiated by IP₃-induced Ca²⁺ release and ryanodine-sensitive Ca²⁺-induced Ca²⁺ release mechanisms. Vasoconstrictor agonists also stimulate Ca²⁺ entry through ligand-gated and voltage-gated Ca²⁺ channels. Relaxing factors released from ECs act on VSM and inhibit phospholipase C, open K⁺ channels or stimulate [Ca²⁺]_i extrusion, and thereby decrease [Ca²⁺]_i. EC dysfunction is associated with decreased release of relaxing factors, and decreased VSM Ca²⁺ extrusion mechanisms. EC dysfunction also causes an increase in VSM contracting factors, which stimulate Ca²⁺ mobilization from the intracellular stores and Ca²⁺ entry from the extracellular space. VSM contraction in response to hypoxia is also mediated by increased [Ca²⁺]_i [138]. It is generally thought that vasoconstrictors may play a role in PE. However, studies on women myometrial and subcutaneous resistance vessels have shown that microvessel reactivity to high KCl depolarizing solution, phenylephrine (PHE) and AngII were not increased in PE compared with Norm-Preg women, and suggested that this is an unlikely mechanism of the increased peripheral vascular resistance in PE [139]. Studies on arterial VSM cells of rats have shown that basal and agonist-stimulated [Ca²⁺]_i are reduced in Norm-Preg compared with virgin rats, but significantly elevated in pregnant rats treated with L-NAME (51). [Ca²⁺]_i may also play a role in the changes in VSM contraction in RUPP rats. In renal arterial VSM, enhanced mechanisms of Ca²⁺ entry, rather than Ca²⁺ release from the intracellular stores, may be a contributing factor to the increased VSM contraction and [Ca²⁺]_i in response to AngII and KCl in RUPP rats as compared to Norm-Preg rats (52).

VSM Protein Kinase C (PKC)—PKC plays a role in VSM contraction. Activation of PKC by phorbol esters causes sustained contraction of VSM with no detectable change in [Ca²⁺]_i, suggesting that PKC increases the Ca²⁺ sensitivity of the contractile proteins. Studies on uterine artery from pregnant sheep and the aorta of late pregnant rats have demonstrated that the decreased vascular contraction during Norm-Preg is associated with a decrease in vascular PKC activity [140,141]. Studies have also shown that the expression and subcellular redistribution of the Ca²⁺-dependent α-PKC and the Ca²⁺-independent δ- and ζ-PKC are reduced in aortic VSM isolated from late pregnant compared with nonpregnant rats [141, 142].

Increased VSM PKC expression/activity has been identified in HTN (143,144). Also, the Ca²⁺ sensitivity of VSM contractile elements is increased in women with PE, suggesting that VSM PKC activity may be involved in the vascular changes observed in PE (145). PKC may also play a role in the changes in AngII and AT₁R-mediated signaling associated with PE. Studies on cultured neonatal rat cardiomyocytes have shown that IgG in plasma from PE

women enhances AT₁R-mediated chronotropic response, whereas IgG from control subjects has no effect. Treatment of cardiomyocytes with the PKC inhibitor calphostin C prevented the stimulatory effect of IgG from PE rats. Also, examination of VSM cells with confocal microscopy have shown colocalization of purified IgG from PE women and AT₁R antibody. These studies concluded that PE women develop stimulatory AT-1AA, a process that appears to be mediated via PKC [146]. We have recently shown that PHE-induced contraction is increased in aortic segments from L-NAME treated compared to non-treated pregnant rats [101]. Also, the expression, subcellular redistribution and activity of α - and δ -PKC isoforms are enhanced in L-NAME treated compared with Norm-Preg rats, suggesting a role of α - and δ -PKC in the increased vasoconstriction and vascular resistance observed in HTN-Preg [141, 142].

VSM Rho/Rho-Kinase—Rho is a family of small GTP-binding proteins that are involved in many cellular functions including cell proliferation, migration, cytoskeletal reorganization and contraction. Rho-kinase is activated by GTP binding and is inactivated by hydrolyzing GTP to GDP, and this process is influenced by various stimuli including growth factors and vasoactive substances [147]. The importance of Rho-kinase in PE is supported by a study comparing subcutaneous resistance arteries from PE, Norm-Preg and nonpregnant women, and showing that Ca²⁺ sensitivity of the contractile elements is increased in women with PE [145]. Also, stimulation of AT₁R induces upregulation of RhoA/Rho-kinase activity in hypertensive rats, and an increase in AngII activity during PE may activate Rho-kinase and promote vasoconstriction [148]. Other studies have shown that the expression of Rho-kinase mRNA may be downregulated in umbilical arteries of PE women [149].

VSM MAP kinase (MAPK)—MAPKs are serine/threonine-specific protein kinases that respond to extracellular stimuli and regulate various cellular activities such as gene expression, mitosis, differentiation, and apoptosis [150]. MAPK activities have been identified in differentiated VSM, suggesting a role in VSM contraction [151]. Tyrosine phosphorylation of MAPK targets it to the contractile myofilaments, where it phosphorylates the actin-binding protein caldesmon [152,153]. The phosphorylation of caldesmon increases the actin-myosin crossbridge cycling and enhances VSM contraction [151]. MAPK promotes vascular remodeling in many pathological conditions [154] and may be involved in PE. A decrease in VSM PKC expression during Norm-Preg and potential increase in HTN-Preg likely change the MAPK-caldesmon phosphorylation pathway and thereby VSM contraction.

Effects of Vascular Mediators on Bioactive Factors

The relationship between biologically active factors and vascular mediators is complex. In many cases the increases in vascular mediators may cause stimulation or inhibition of bioactive factors (Fig. 2B). For example, AngII, through stimulation of AT₁R, stimulates the expression of TNF- α , IL-6, and IL-1 β mRNA and increases the plasma levels of IL-6 and IL-1 β [155]. Also, AngII stimulates ROS production through an ET-1 mediated pathway [116]. AngII stimulates VEGF production through HIF-1 α activation in VSM cells [156], and may also inhibit MMP-1 activity [157]. ET-1 is also an important factor in the regulation of vascular MMPs. Increases in plasma and EC ET-1 are associated with increases in expression and activity of vascular MMPs [158]. Also, ET-1, via activation of ET_AR, stimulates the synthesis and release of VEGF in VSM cells, and both ET-1 and VEGF play a synergistic role in neovascularization [159]. ET-1 also stimulates NADPH oxidase, the main vascular source of O₂^{•-} [129], while PGI₂ attenuates ROS production by reducing NADPH oxidase activity [83].

Management of HTN-Preg

Currently, the management of mild and moderate HTN-Preg consists of bed rest and anti-hypertensive drugs. Methyldopa, hydralazine, β -blockers and nifedipine are the preferred antihypertensives because they are safe and have few side effects on the mother and fetus [160]. The antihypertensive drugs mainly decrease the risk of developing acute HTN, but may not have a clear effect on fetal mortality, premature birth, or fetal growth restriction. If PE worsens into eclampsia, maintenance of airway patency and prevention of fluid aspiration should be the first measure, then anticonvulsants should be given, with magnesium sulfate infusion being the drug of choice.

The current management of HTN-Preg is mainly symptomatic, and the most effective way to reverse the manifestations is induction of prompt delivery. Future approaches for management of HTN-Preg should focus on preventing the disease rather than treating the symptoms. Some of the potential approaches include methods to minimize placental hypoxia. Other possible approaches include cytokine antagonists, antioxidants, and blockers of the release or the effects of vasoconstrictors such as ET-1. Control of dietary and environmental factors could also reduce the incidence of PE in susceptible women. Advanced gene analysis and manipulation may also provide an alternative gene therapy approach and a more specific measure to prevent PE.

In summary, genetic predisposition, dietary and environmental factors affect the normal development of the placenta and may lead to placental ischemia/hypoxia, which in turn causes an increase in the release of biologically active factors such as growth factors, cytokines, ROS, HIF-1 and antibodies to vasoconstrictor receptors. These bioactive factors in turn decrease the vasodilator mediators and increase the vasoconstrictive substances leading to severe vasoconstriction, increased systemic vascular resistance and HTN-Preg. Understanding the cellular mechanisms of PE should provide more specific and efficient measures for treatment and prevention of this disorder.

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List of Abbreviations

AngII	angiotensin II
BP	blood pressure
$[Ca^{2+}]_i$	intracellular free Ca^{2+} concentration
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase
EC	endothelial cell
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
Eng	endoglin
ET-1	endothelin-1
HIF	hypoxia-inducible transcription factor
HTN-Preg	hypertension in pregnancy

IL	interleukin
L-NAME	N ^o -nitro-L-arginine methyl ester
MMP	matrix metalloproteinase
2-methoxy-E2	2-methoxy-estradiol
MAPK	mitogen-activated protein kinase
MLC	myosin light chain
NO	nitric oxide
NOS	NO synthase
Norm-Preg	normal pregnant
O ₂ ^{-•}	superoxide anion
PE	preeclampsia
PGI ₂	prostacyclin
PHE	phenylephrine
PKC	protein kinase C
RAS	renin-angiotensin system
ROS	reactive oxygen species
RUPP	reduced uterine perfusion pressure
sFlt-1	soluble fms-like tyrosine kinase-1
TNF- α	tumor necrosis factor- α
TXA ₂	thromboxane A ₂
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle

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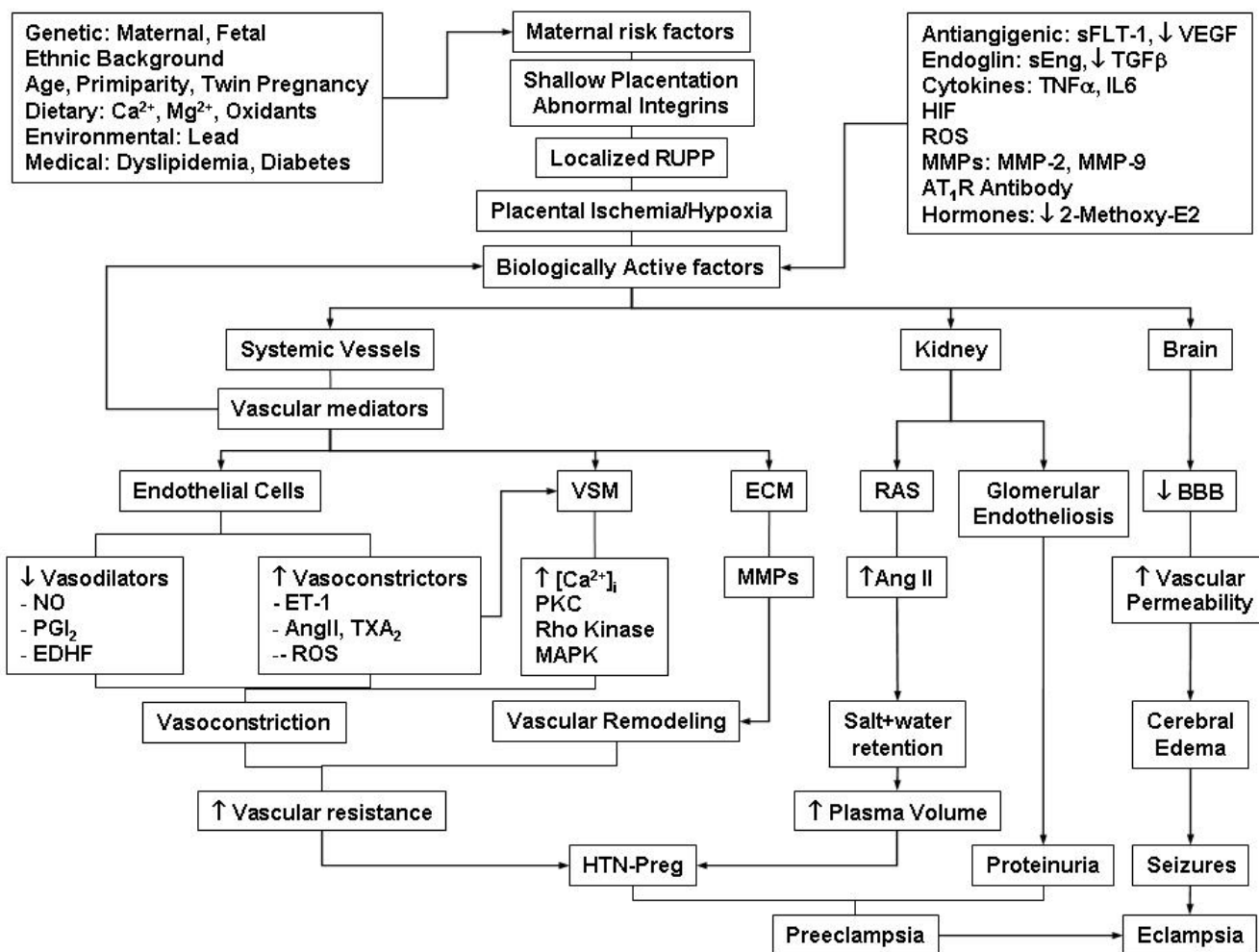


Fig. 1.

Cellular Mechanisms of HTN-Preg. Maternal genetic, dietary and environmental factors lead to shallow placentation and RUPP during late pregnancy and trigger the release of biologically active factors, which affect the systemic blood vessels and lead to generalized vasoconstriction, increased vascular resistance and HTN-Preg. The vascular mediators could in turn affect the biologically active factors and further promote their biological effects. The bioactive factors could also injure the kidney leading to increased plasma volume and severe HTN, as well as glomerular endotheliosis and proteinuria, leading to clinically manifested PE. Increases in cerebral vascular permeability and edema lead to seizures and life-threatening eclampsia.

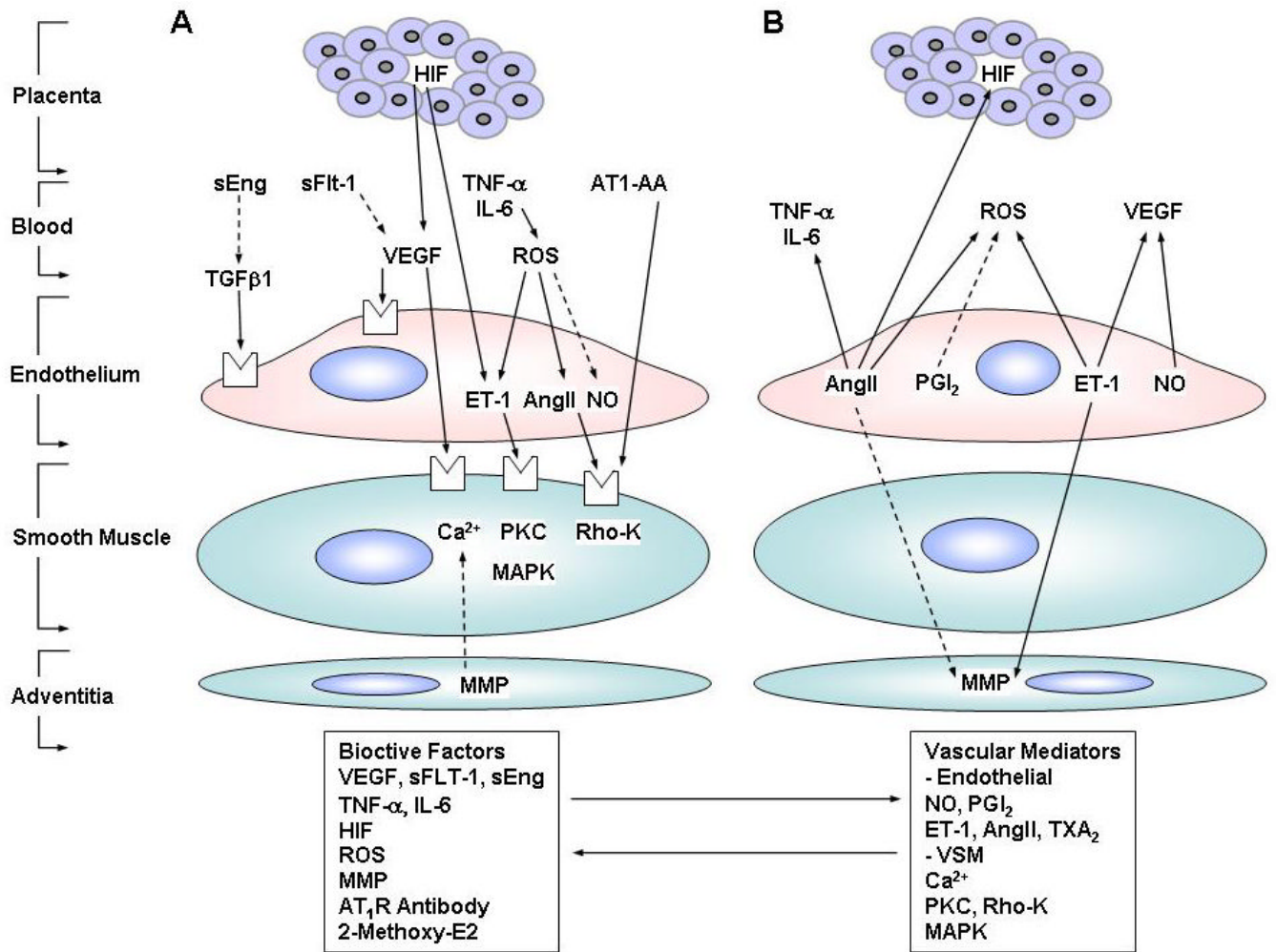


Fig. 2. Interactions between biologically active factors and vascular mediators during HTN-Preg. Increases in biologically active factors result in changes in vascular mediators and increases in other biologically active factors (A). Increases in vascular mediators causes feedback changes in biologically active factors (B). Solid arrows indicate stimulation. Interrupted arrows indicate inhibition.

Table 1

Plasma and Vascular Levels of Bioactive Factors and Vascular Mediators during Normal Pregnancy (Norm-Preg) and Preeclampsia (PE) in Humans.

Bioactive Factor	Site of Action	Effect	Norm-Preg	PE	Reference
VEGF	Endothelium	Vasorelaxation	184 pg/mL	396 pg/mL	[28]
sFlt-1	Endothelium	Anti VEGF	339.4 pg/mL	7247pg/mL	[29]
Endoglin	Endothelium	Anti TGF- β	9.8 ng/mL	46.4 ng/mL	[30]
Cytokines TNF- α IL-6	Endothelium Endothelium	EC Dysfunction EC Dysfunction	3.31 pg/ml 4.9 \pm 1.1 pg/mL	4.68 pg/mL 16.5 \pm 2.1 pg/mL	[31] [32]
HIF	DNA	Gene Transcription	↑	↑↑	[33]
ROS	Endothelium	EC Dysfunction	--	↑	[34]
MMP-2	ECM, Endothelium	Remodeling	779 \pm 194 ng/mL	783 \pm 322.3 ng/mL	[35]
MMP-9	ECM	Remodeling	121 \pm 190.5 ng/mL	262.2 \pm 398.6 ng/mL	[35]
2-Methoxy-E2	Estrogen receptor	Vasodilator	↑	↓	[36]
Vascular Mediator					
NO	VSM	Vasorelaxation	13.0 \pm 4.3 μ mol/L	18.1 \pm 6.2 μ mol/L	[37]
PGI ₂	VSM	Vasorelaxation	↑	↓	[38]
EDHF	VSM	Vasorelaxation	↑	↓	[39]
ET-1	VSM Endothelium	Vasoconstriction Vasorelaxation	12 \pm 1.0 pmol/L	22.6 \pm 2.0 pmol/L	[40]
AngII	VSM	Vasoconstriction	30.2 fmol/mL	53.3 fmol/mL	[41]

↑↑, Greatly increased;

↑, Increased;

↓, Decreased;

--, No change, relative to nonpregnant.

Table 2
Plasma and Vascular Levels of Bioactive Factors and Vascular Mediators during Norm-Preg and HTN-Preg in rats.

Bioactive Factor	Site of Action	Effect	Norm-Preg	HTN-Preg	Reference
VEGF	Endothelium	Vasorelaxation	830±33 pg/mL	594±34 pg/mL	[42]
sFlt-1	Endothelium	Anti VEGF	82±26 pg/mL	660±270 pg/mL	[43]
Endoglin	Endothelium	Anti TGF-β	8 ng/mL	17 ng/mL	[44]
Cytokines TNF-α IL-6	Endothelium Endothelium	EC Dysfunction EC Dysfunction	8±1 pg/mL 36.6±7 pg/mL	48±13 pg/mL 104.5±28.6 pg/mL	[45] [46]
HIF	DNA	Gene Transcription	↑	↑↑	[27]
ROS	Endothelium	EC Dysfunction	--	↑	[27]
Vascular Mediator					
NO (Urinary Nitrite/Nitrate)	VSM	Vasorelaxation	46.4±5.3 μmol/24 hr	49.8±6.4 μmol/24 hr	[47]
PGI ₂	VSM	Vasorelaxation	↑	↓	[48]
ET-1	VSM, Endothelium	Vasoconstriction, Vasorelaxation	1.7 pmol/L	2.2 pmol/L	[49]
AngII	VSM	Vasoconstriction	↑	↑	[50]
VSM [Ca ²⁺] _i - Basal - PHE (10 ⁻⁵ M) - AngII (10 ⁻⁷ M)	Myofilaments Myofilaments Myofilaments	Basal Tone Contraction Contraction	63 ± 5 nM 149 ± 8 nM 149 ± 8 nM	109 ± 8 nM 234 ± 1 nM 225 ± 9 nM	[51] [51] [52]

↑↑, Greatly increased;

↑, Increased;

↓, Decreased;

--, No change, relative to nonpregnant.