DISEASE OF THE MONTH

EBERHARD RITZ, FEATURE EDITOR

J Am Soc Nephrol 15: 2440–2448, 2004

New Aspects in the Pathophysiology of Preeclampsia

JOHN M. DAVISON,* VOLKER HOMUTH,† ARUN JEYABALAN,‡ KIRK P. CONRAD,‡ S. ANANTH KARUMANCHI,§ SUSAN QUAGGIN,‖ RALF DECHEND,† and FRIEDRICH C. LUFT†

**School of Surgical and Reproductive Sciences, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle, United Kingdom; †Medical Faculty of the Charité, Franz Volhard Clinic HELIOS Klinikum-Berlin and Max Delbrück Center for Molecular Medicine, Berlin, Germany; ‡Departments of Ob/Gyn and Reproductive Sciences and Cell Biology and Physiology, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, Pittsburgh, Pennsylvania; §Departments of Medicine and Obstetrics and Gynecology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts; and ‖The Samuel Lunenfeld Research Institute and Mount Sinai Hospital, Toronto, Ontario, Canada*

Abstract. Preeclampsia, the *de novo* occurrence of hypertension and proteinuria after the 20th week of gestation, continues to exert an inordinate toll on mothers and children alike. Recent clinical trials, new physiologic insights, and novel observations on pathogenesis have altered the thinking about preeclampsia. The mechanisms surrounding relaxin and its effects on the circulation and on matrix metalloproteinases have been elucidated. The growth factor's receptor, fms-like tyrosine kinase 1, has been shown to exist in a soluble form that is able to inactivate vascular endothelial-derived growth factor and human placental growth factor. Compelling evidence has been brought forth suggesting that fms-like tyrosine kinase 1 is a circulating factor that can cause preeclampsia. Preeclamptic women have high circulating levels of asymmetric dimethyl arginine that could account for the generalized endothelial dysfunction observed in preeclampsia. Preeclamptic women also produce novel autoantibodies that may serve to activate angiotensin receptors. These new observations raise the possibility that the treatment of preeclamptic women will soon be improved.

The term *preeclampsia* refers to the new onset of hypertension (>140/90 mmHg) and proteinuria after 20 wk of gestation in previously normotensive, nonproteinuric women (1). The condition is common and occurs in ~5% of pregnancies in the United States and Europe. Eclampsia is a life-threatening complication and is characterized by grand mal seizures. The term comes from the Greek word for lightning. A severe variant of preeclampsia also features hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome). This condition occurs in ~1 per 1000 pregnancies. Predisposing factors are a positive family history, hypertension, diabetes, preexisting renal disease, multiple pregnancies, and a poor obstetric history. Nephrologists are often called on to see preeclamptic women because of the severe BP elevation and renal disease. Thus, new clinical or experimental information on this condition is important information for nephrologists.

Correspondence to Dr. Friedrich C. Luft, Franz Volhard Clinic, Wiltberg Strasse 50, 13125 Berlin, Germany. Phone: +49-30-9417-2202; Fax: +49-30-9417-2206; E-mail: luft@fvk-berlin.de

1046-6673/1509-2440

Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000135975.90889.60

Preeclampsia and Subsequent Cardiovascular Risk

The relevance of preeclampsia to the offspring is well recognized. Children who are born to preeclamptic mothers commonly have low birth weight, and their subsequent cardiovascular risk has been a vast investigational field. The mother's outcome has attracted less interest. Chesley, the father of modern preeclampsia research, was of the opinion that once the condition was over, the mothers had no greater risk of adverse long-term outcomes than women without preeclampsia from the general population (2). This issue may prove to be the only matter in which Chesley's opinion was erroneous. Several recent studies suggest that the converse is the case. Smith *et al.* (3) studied the pregnancy complications and the maternal risk of ischemic cardiac death in 129,290 births. They found that delivering an infant with low birth weight for gestational age increased the hazard ratio for ischemic heart disease or death to 1.9. Preterm delivery was associated with a risk of 1.8, compared with women who had no such delivery. The associations were additive. Preeclamptic women who delivered a small infant early, generally the rule, had a risk of hospital admission for ischemic heart disease or death seven times higher than the control women did.

A second cohort study was presented shortly thereafter. Irgens *et al.* (4) published the results of 626,272 live births in Norway between 1967 and 1992. They found that women who

had had preeclampsia had a 1.2-fold higher long-term risk of all-cause death than women who did not have this condition. In women with preeclampsia and a preterm birth, the risk was 2.7-fold higher. The risk of death from cardiovascular causes was increased eightfold in preeclamptic women with a child of low birth weight. Strangely, preeclampsia seemed to protect women from death by cancer by 0.36-fold. This state of affairs resulted in the finding that women with preeclampsia had only a 1.2-fold higher long-term risk of death from any cause. Perhaps expected, the fathers did not enter into the equation. This huge cohort study provides very strong evidence that cardiovascular risk is increased in women with preeclampsia compared with control subjects, particularly when the child is born preterm and has low birth weight, both of which are generally the rule in women with preeclampsia. Wilson *et al.* (5) examined the relationship between preeclampsia and the risk of hypertension and stroke later in life. They selected women from a cohort who had delivered between 1951 and 1970. They found that any hypertensive disorder of pregnancy increased the later risk for hypertension and stroke. The stroke relative risk was increased in women who had been preeclamptic and was 3.59.

Women with preeclampsia develop pronounced swelling of glomerular endothelial cells and feature various deposits under these cells (6). Moreover, they display fibrin within endothelial cells and mesangial cells. The basement membrane thickens. These changes are reversible; however, the functional changes are not necessarily totally reversible. Bar *et al.* (7) followed a group of women who had had preeclampsia and compared these women with women who had normal spontaneous deliveries. They found that at 2 to 4 mo after delivery, two thirds of preeclamptic women had microalbuminuria. More disturbing is that 3 to 5 yr later, half of the women with the preeclampsia history still had microalbuminuria. The protein excretion at this time point was approximately fourfold higher than that of women who had not had preeclampsia. Whether the microalbuminuria persists indefinitely is uncertain. Data from Roest *et al.* (8) suggest that microalbuminuria in postmenopausal women is a substantial cardiovascular risk factor. We have little reason to believe that microalbuminuria in premenopausal women is any less of a risk. Microalbuminuria is a manifestation of endothelial dysfunction. Endothelial function in preeclamptic women is impaired. The impairment was still present in a recent postpartum follow-up study (9). Preeclampsia is associated with insulin resistance and elevated homocysteine levels. Evidence exists that these conditions continue after delivery and represent a long-term risk.

Sattar and Greer (10) focused on pregnancy complications and maternal cardiovascular risk and pointed out the opportunities for screening and risk reduction. Women with a history of adverse pregnancy outcome are at increased risk for cardiovascular disease later in life. Pregnancy complications and coronary heart disease may have common mechanisms. Clearly, women with gestational diabetes, a preeclampsia risk factor, must be screened for diabetes later in life. Women with low birth weight infants should be screened for cardiovascular disease in their 30s. Maternal vascular risk factors, such as higher BP, insulin resistance, obesity, excessive weight gain in pregnancy, and hyperlipidemia, correlate with increased risk of preterm delivery and low birth weight. In conclusion, asking about preeclampsia, low birth weight infants, or any adverse pregnancy outcome provides valuable information in terms of assessing cardiovascular risk in women.

Abnormal Placentation and Placental Ischemia

Preeclampsia occurs only in the presence of the placenta or a hydatidiform mole and remits dramatically postpartum after the delivery of the placenta (11). The pathogenesis of preeclampsia may involve abnormal cytotrophoblast invasion of spiral arterioles, decreased uteroplacental hypoperfusion, an imbalance between increased synthesis of thromboxane and decreased production of prostaglandin I_2, increased oxidative stress, disordered endothelin metabolism, or endothelial dysfunction.

During normal placental development, cytotrophoblasts invade the maternal spiral arterioles and completely remodel the maternal spiral arterioles into large capacitance vessels with low resistance (12). This endovascular cytotrophoblast invasion involves replacement of not only the endothelium but also the highly muscular tunica media. Furthermore, during normal differentiation, invasive trophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells (integrin α_6/β_3, $\alpha\omega/\beta_5$ and E-cadherin) to those of endothelial cells (integrin α_1/β_1, $\alpha\omega/\beta_3$, Platelet Endothelial Cell Adhesion Molecule and Vascular Endothelial-Cadherin), a process referred to as "pseudovasculogenesis" (13,14). In preeclampsia, there is shallow placental cytotrophoblast invasion of uterine spiral arterioles, leading to reduced placental perfusion and consequently placental insufficiency. Both *in vitro* and *in vivo* studies show that trophoblasts obtained from patients with preeclampsia fail to undergo these alterations of adhesion molecules and pseudovasculogenesis (15,16). The molecular pathways that regulate pseudovasculogenesis may involve a vast array of transcription factors, growth factors, and cytokines (17). Considerable attention has recently been focused on angiogenesis-related gene products such as vascular endothelial growth factor (VEGF), angiopoietin/tie, and ephrin family proteins and their role in regulating pseudovasculogenesis and invasiveness. Invasive trophoblasts express VEGF, placental growth factor (PlGF), and VEGF-C and their receptors. Furthermore, blocking their signaling pathways decreases the expression of marker of pseudovasculogenesis *in vitro* (18).

Generalized endothelial dysfunction may be responsible for all of the clinical aspects of the maternal syndrome in preeclampsia. The identification of circulating factors that mediate endothelial dysfunction has been the source of great research interest for decades. Several groups have reported alterations in cytokines/growth factors/chemicals such as TNF-α, IL-6, IL-1α, IL-1β, Fas ligand, oxidized lipid products, neurokinin-B, and asymmetric dimethyl arginine (ADMA) that are released by the placenta and/or other maternal sources in preeclampsia (18–22). Recently, a lower-than-normal L-arginine concentration that is caused by arginase II overexpression and that may redirect placental endothelial nitric oxide (NO) synthase toward peroxynitrite was described in preeclampsia (23).

Newer Aspects on Relaxin in Pregnancy and Preeclampsia

Relaxin is produced by the corpus luteum of the ovary and circulates in the luteal phase of the menstrual cycle and rises early in gestation. Human chorionic gonadotrophin produced by the placenta is a major stimulus for relaxin secretion during pregnancy in women. Chronic administration of relaxin to conscious female rats increases GFR and effective renal plasma flow, thereby mimicking the changes in the renal circulation during pregnancy (24,25). This renal vasodilatory effect does not require the presence of the ovaries (25) and is also observed during relaxin administration to male rats (26). Relaxin administration to nonpregnant female rats also diminishes the renal vasoconstrictor response to angiotensin II, similar to the dampening influence of rat gestation (25,27,28). Moreover, reduced myogenic reactivity of small renal arteries is observed after relaxin administration, analogous to vessels isolated from midterm pregnant rats (29,30). Finally, relaxin-neutralizing antibodies or removal of circulating relaxin by ovariectomy completely abolishes the renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries, as well as the osmoregulatory changes in midterm pregnant rats (31).

Jeyabalan *et al.* (32) (Figure 1) recently proposed that relaxin upregulates vascular gelatinase activity during pregnancy, thereby contributing to renal vasodilation, hyperfiltration, and reduced myogenic reactivity of small renal arteries through activation of the endothelial endothelin B (ET_B) receptor–NO pathway. The notion that metalloproteinase 2 (MMP-2) plays a pivotal role in the relaxin-gelatinase pathway is based on the confluence of several observations. First, relaxin, the endothelial ET_B receptor, and NO have an essential role in pregnancy-mediated renal vasodilation. Second, relaxin serves to upregulate MMP expression at least in fibroblasts (33). Third, vascular MMP, such as MMP-2, are able to process big ET at the gly-leu bond to $ET_{1–32}$ with subsequent activation of endothelin receptors (34).

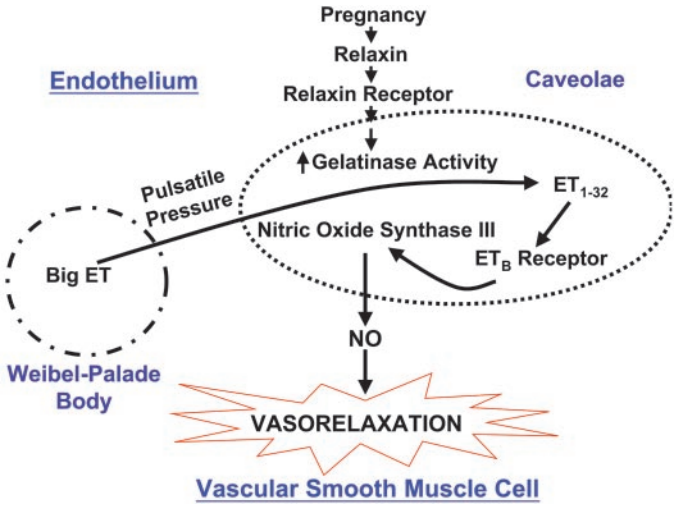

Figure 1. Proposed scheme of cellular mechanisms underlying pregnancy and relaxin-induced renal vasodilation and hyperfiltration and reduced myogenic reactivity of small renal arteries. Big ET is released from Weibel-Palade bodies in response to pulsatile pressure in vivo or increases of intraluminal pressure in vitro. MMP-2, eNOS, and possibly the ETb receptor are localized in caveolae of endothelial cells.

By inhibiting gelatinase activity in chronically instrumented rats *in vivo* and in isolated small renal arteries *in vitro*, Jeyabalan *et al.* (32) demonstrated an essential role for vascular gelatinase in the relaxin-mediated, renal circulatory changes of pregnancy. In contrast, the traditional endothelium-converting enzyme pathway that processes big ET to $ET_{1–21}$ is not involved on the basis of the lack of any hemodynamic effect of the traditional endothelium-converting enzyme inhibitor phosphoramidon. In small renal arteries harvested from relaxin-treated nonpregnant or midterm pregnant rats, vascular MMP-2 activity is upregulated by ~50%. Thus, vascular gelatinase activity not only is part of the endothelial ET_B-NO vasodilatory pathway but also is a major locus of regulation by relaxin. Finally, relaxin was administered to ET_B receptor–deficient rats. Although the small renal arteries harvested from these rats showed upregulation of vascular MMP-2 activity, they failed to elicit the typical reduction in myogenic reactivity. This observation when taken in the context of the other results (*supra vide*) indicates that vascular gelatinase is in series with and upstream of the endothelial ET_B-NO signaling pathway in the renal vasodilatory response to pregnancy mediated by relaxin (32).

Circulating levels of immunoreactive H2 relaxin have been reported to be similar in women with preeclampsia and normal pregnancy at comparable gestational ages (35). However, whether circulating relaxin *bioactivity* may be deficient during the disease is uncertain. The LGR7 and LGR8 relaxin receptors were only recently identified (36). Little is known about their expression on blood vessels. By analogy to other receptor systems, fewer receptors, increased expression of inactive receptors, or soluble receptors could undermine relaxin signaling to the vasculature, thereby compromising renal vasodilation in preeclampsia. Because increased vascular gelatinase activity by relaxin is a proximal step in the vasodilatory pathway of pregnancy, inappropriate MMP-2 activity may contribute to reduced renal function in preeclampsia. Excessive expression of ET or of the ET_A or ET_B receptor on the vascular smooth muscle of renal arterioles may overwhelm the vasodilatory pathway initiated by relaxin. Mutations or polymorphisms of the ET_B receptor or of endothelial NO synthase that reduce activity may predispose a woman to preeclampsia by impairing trophoblast invasion on the one hand (37,38) and by compromising maternal endothelial behavior on the other. Much earlier work indirectly alluded to such possibilities (11).

Circulating Proangiogenic Factors and Their Inhibitors

An increased placental expression and secretion of soluble fms–like tyrosine kinase 1 (sFlt1), a naturally occurring circulating VEGF antagonist in patients with preeclampsia, has recently been demonstrated (39–41). Importantly, when administered exogenously to rats, sFlt1 alone has been shown to

be sufficient to induce preeclampsia-like phenotype (39). Finally, data using VEGF conditional knockout mice gave us definitive genetic evidence that interference with VEGF signaling in the kidneys leads to clinical preeclampsia, namely proteinuria and glomerular endotheliosis (42).

VEGF is an endothelial-specific mitogen that plays a key role in promoting angiogenesis. VEGF's activities are mediated primarily by its interaction with two high-affinity receptor tyrosine kinases—kinase-insert domain region (KDR) and Flt1—that are selectively expressed on vascular endothelial cell surface (43). Alternative splicing of Flt1 results in the production of an endogenously secreted protein referred to as sFlt1, which lacks the cytoplasmic and transmembrane domain but retains the ligand-binding domain (44,45) (Figure 2). Thus, sFlt1 can antagonize circulating VEGF by binding to it and preventing VEGF's interaction with its endogenous receptors. sFlt1 also binds and antagonizes PlGF, another member of the VEGF family that is made in the placenta predominantly.

In vitro studies confirm that excess placental sFlt1 production induces an antiangiogenic state in the serum of preeclamptic women that can be rescued by exogenous VEGF and PlGF (39). Excess sFlt1 alone, when administered to pregnant rats, induces albuminuria, hypertension, and renal pathologic changes of glomerular endotheliosis by antagonizing circulating VEGF and PlGF and inducing endothelial dysfunction. In addition, circulating levels of free VEGF and free PlGF were decreased in conjunction with elevated sFlt1 in the bloodstream at the time of disease presentation (39,40). More recently, when free PlGF and free VEGF were measured throughout pregnancy, the growth factors were decreased in preeclamptic women well before the onset of clinical disease (46–48).

Recently, Levine *et al.* (49) presented exciting evidence suggesting that sFlt1 is a—or perhaps the—circulating factor responsible for preeclampsia. They performed a nested, case-control study within the Calcium for Preeclampsia Prevention Trial that involved healthy nulliparous women. Each woman with preeclampsia was matched to one normotensive control subject. A total of 120 pairs of women were randomly chosen. Serum concentrations of angiogenic factors (total sFlt1, free PlGF, and free VEGF) were measured throughout pregnancy. The sFlt1 level increased beginning ~5 wk before the onset of preeclampsia. At the onset of clinical disease, the mean serum level in the women with preeclampsia was 4382 pg/ml, compared with 1643 pg/ml in control subjects with fetuses of similar gestational age. The PlGF levels were significantly lower in the women who later had preeclampsia than in the control subjects beginning at 13 to 16 wk of gestation (mean, 90 *versus* 142 pg/ml), with the greatest difference occurring during the weeks before the onset of preeclampsia, coincident with the increase in the sFlt1 level. Alterations in the levels of sFlt1 and free PlGF were greater in women with an earlier onset of preeclampsia and in women in whom preeclampsia was associated with a small-for-gestational-age infant. The authors concluded that increased levels of sFlt1 and reduced levels of PlGF predict the subsequent development of preeclampsia.

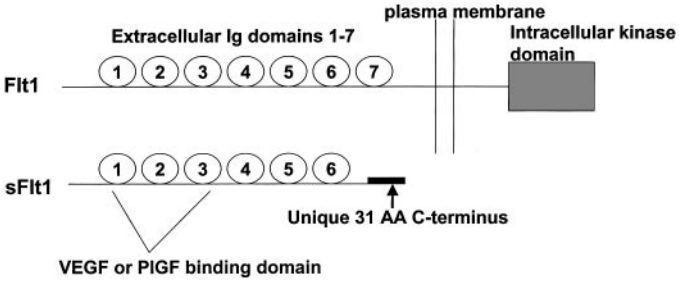

Figure 2. Flt1 and sFlt1 protein structures are shown. Flt1 has 7 Ig domains (IgG) that are thought to mediate ligand binding to vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). sFlt1 protein has a unique 31–amino acid C-terminus region derived from alternative splicing and lacks the transmembrane and cytoplasmic domains.

These data support the hypothesis that elevated sFlt1 may induce the maternal syndrome and that the elevated sFlt1 is not a consequence of the maternal syndrome. VEGF is known to stimulate angiogenesis, as well as promote vasodilation by stimulating NO and prostacyclin formation, signaling molecules that are decreased in preeclampsia (50). Furthermore, a significant percentage of cancer patients who receive VEGF signaling antagonists develop hypertension and proteinuria (51,52). Even the loss of a single VEGF allele from the glomerulus in genetically modified mice resulted in glomerular endotheliosis and proteinuria (42). It is interesting that dramatic endothelial defects were observed even though the circulating levels of VEGF were unaffected, which emphasizes that tight local regulation of VEGF signaling seems to be critical for endothelial function. These observations suggest that excess sFlt1 may play a causal role in the pathogenesis of the maternal syndrome in preeclampsia by neutralizing VEGF and PlGF (Figure 3). However, there are limitations and several unanswered questions to the sFlt1 story. The precise mechanisms of excess sFlt1 production by the placenta are not known and, importantly, the role of sFlt1 in normal placental development and in placental pseudovasculogenesis is not clear. No coagulation or liver function abnormalities or brain abnormalities (eclampsia) were reported in sFlt1-treated animals. The mechanisms of proteinuria during VEGF-deficient states are still unclear. Additional synergistic factors that are elaborated by the placenta may yet be identified to play a role in the pathogenesis of the generalized endothelial dysfunction noted in preeclampsia.

Endothelium-Derived Relaxing Factors and Their Inhibitors

Endothelial factors such as prostaglandins (PG) and NO have been proposed as mediators of gestational renal vasodilation and hyperfiltration. However, studies in gravid humans and animal models have not shown a compelling role for vasodilatory PG in the pregnancy-induced increases in GFR and effective renal plasma flow (27,53–55) or in the decrease of total peripheral vascular resistance (56,57). The first indi-

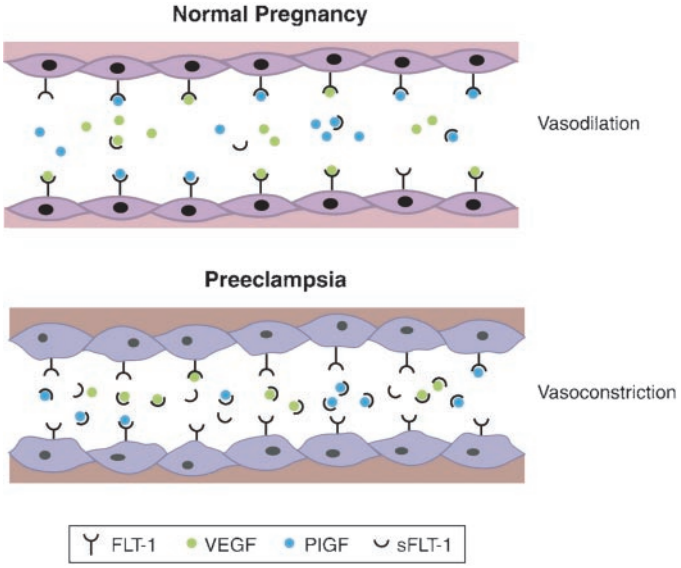

Figure 3. (Top) Flt1 on endothelial cells with few sFlt1 in the circulation. Agonists VEGF and PlGF can occupy Flt1. (Bottom) Vessel from preeclamptic patient with abundant sFlt1 that are able to catch VEGF and PlGF so that the growth factors cannot occupy Flt1 on the cell surface.

cation of a potential role for NO came from the evaluation of guanosine 3',5'-cyclic monophosphate (cGMP), an important second messenger of NO. Plasma concentration, urinary excretion, and metabolic production of cGMP are increased in rat pregnancy and pseudopregnancy (58,59), as well as in human gestation (60–62). Urinary excretion of nitrate and nitrite, stable metabolites of NO, are increased in pregnant and pseudopregnant rats that consume a diet low in nitrite and nitrate corresponding to the rise in cGMP excretion (59). Furthermore, NO metabolites are increased in the plasma of pregnant rats, and NO-hemoglobin in red blood cells is detected in pregnant but not in nonpregnant rats (61). These data suggest that endogenous NO production is increased in gravid rats, although the tissue source(s) of the increased NO metabolites and cGMP remain unknown (63,64).

Specific defects in resistance artery endothelium from women with preeclampsia have been shown (65). Supportive evidence for NO deficiency in preeclampsia has recently been obtained from the reduced uterine perfusion pressure rat model (66). The investigators found that supplementation with L-arginine decreased BP by 19 mmHg in pregnant rats with reduced uterine perfusion pressure (untreated *versus* treated) as compared with 12 mmHg in pregnant (untreated *versus* treated) rats. Thus, these results suggest that L-arginine supplementation may be beneficial in attenuating the hypertension in preeclampsia.

An attractive preeclampsia factor that could directly interfere with NO and induce endothelial dysfunction in pregnant women is ADMA. Savvidou *et al.* (22) tested the hypothesis that ADMA, an endogenous endothelial NO synthase inhibitor, contributes to the development of preeclampsia. A role for NO—or its absence—has been established in earlier studies of preeclampsia. The authors measured forearm ischemia-reperfusion as a marker of endothelial function. They also monitored uterine blood flow by means of Doppler techniques. They searched for the occurrence of intrauterine growth retardation, and they obviously measured ADMA and its symmetrical analog. The authors found that women with evidence for impaired placental perfusion had >30% prevalence of children with intrauterine growth retardation and >20% prevalence of preeclampsia. Women with preeclampsia clearly had significantly lower flow-mediated vasodilation than women with normal uterine perfusion. In women with preeclampsia, there was a remarkably tight correlation between ADMA levels and flow-mediated vasodilation. Taken together, the authors found that endothelial dysfunction develops before preeclampsia, women with higher uterine flow resistances are at risk for intrauterine growth retardation and preeclampsia, and ADMA may be a potentially contributing factor to endothelial dysfunction in these women.

Circulating Autoantibodies

Haller *et al.* (65,66) observed that a circulating factor in preeclamptic women caused endothelial cells to express surface adhesion molecules and made endothelial cell layers more permeable. The latter process involved the activation of protein kinase C. Wallukat *et al.* (67) subsequently identified circulating autoantibodies that are capable of activating the angiotensin II (Ang II) AT1 receptor. The autoantibodies (AT-1AA) arise approximately at the time that symptoms develop, namely after the 20th week of gestation, and subside within 6 wk after delivery. AT-1AA were purified and identified as belonging to a fraction of IgG antibodies. Wallukat *et al.* (67) showed that AT1-AA bound to a certain seven amino acid sequence on the second extracellular loop of AT1-AA. They documented specificity by means of Western blotting and co-localization studies. Indeed, AT-1AA functioned in a Western blot at least as well as currently available commercial antibodies to the AT-1 receptor. Dechend *et al.* (68,69) confirmed these findings by relying on co-immunoprecipitation studies. They could not find evidence for calcium signaling or smooth muscle cell contraction resulting from the autoantibodies. However, they were able to show that the autoantibodies initiated a signaling cascade that culminated in transcription factor activation (NF-κB and activator protein-1) and subsequently tissue factor expression.

Independently, Zia *et al.* (70) studied 38 pregnant patients, 20 of whom had severe preeclampsia and 18 of whom were normotensive. IgG was purified from these individuals, and the presence of AT1-AA was determined. Immortalized human trophoblasts were chosen to study plasminogen activator inhibitor 1 production and secretion after treatment with IgG from normotensive and preeclamptic women. Their findings suggest that maternal autoantibody with the ability to activate AT1 receptors may account for two features of preeclampsia: increased plasminogen activator inhibitor 1 production and shallow trophoblast invasion.

Recently, Dechend *et al.* (69) showed that AT-1AA can cause human trophoblasts or vascular smooth muscle cells to

produce reactive oxygen species (ROS) by activating the NADPH oxidase. Vascular smooth muscle cells from p47 phox gene-disrupted −/− and control +/+ mice were subjected to AT1-AA. By means of DCF fluorescence, both Ang II and AT1-AA produced a strong response. The ROS production was greatly ameliorated by the antioxidant tiron or by using vascular smooth muscle cells lacking p47 phox. In these cells, the NADPH oxidase was not operative. In other studies, the investigators used human trophoblasts and electromobility shift assays with supershifts and showed that the NF-kB units p50 and p65 were activated by either Ang II or AT-AA. Human trophoblasts, as shown by the anticytokeratin 7 marker, also exhibited p22 phox.

AT1-AA have also been found in patients with malignant hypertension (71) and in patients with humoral renal transplant rejection (unpublished observations). Thus, they are not a specific preeclampsia phenomenon. The existence of these antibodies is exciting. However, the field is hampered because detection still relies on a bioassay. Attempts to establish an ELISA have not yet been successful. Thus, confirmatory studies in large populations of women with preeclampsia, as with sFlt1, have not yet been conducted.

It is interesting that AT1 receptor heterodimers are increased in preeclampsia. An AT1 receptor-bradykinin-2 receptor heterodimer with increased Ang II signaling has been described (72). A promising transgenic rat model that relies on human renin and angiotensinogen transgenes has been developed (73). This model features the development of AT1-AA (unpublished observations). AT1-AA could be an epiphenomenon. Nevertheless, there are precedents for the notion that activating antibodies can cause disease. The argument that activating antibodies to the β1 adrenergic receptor can cause cardiomyopathy is compelling (74) (Figure 4).

Conclusions

Exciting news has emanated from the preeclampsia research arena recently on all fronts. Compelling basic research is in progress. Exciting clinical trials have been conducted, and more are in progress. Is it possible that these divergent mechanisms interact in some way? Ang II plays some role in angiogenesis. Angiotensin-converting enzyme inhibitors and AT1 receptor blockers seem to be antiangiogenic and decrease microvessel formation (75). VEGF-mediated angiogenesis can be decreased with AT1 receptor blockers (76). ADMA generation can be diminished by angiotensin-converting enzyme inhibitors and by AT1 receptor blockers in patients who receive these drugs (77). How Ang II might stimulate ADMA formation is not clear. ADMA is synthesized from methylated arginine residues in proteins by protein arginine methyltransferases. The compound is metabolized to citrulline by the actions of dimethylarginine dimethyldiaminohydrolases I and II. The enzymes may be impaired in preeclampsia. Could Ang II act to influence the expression of TGF-β3? This mediator has been implicated in regulating trophoblast outgrowth, invasion, or both and must be downregulated at a crucial point (78).

Could Ang II possibly play a role in upregulating sFlt1? The signal presumably has to do with hypoxia and therefore may involve the hypoxia-inducible factor (HIF) (79). It is interesting that Ang II may increase HIF-1α induction. According to Page *et al.* (80), Ang II relies on ongoing translation to maintain elevated HIF-1α protein levels. Ang II increases HIF-1α translation by an ROS-dependent activation of the phosphatidylinositol 3-kinase pathway, which acts on the 5'-untranslated region of HIF-1α mRNA. Their results suggest that the nonhypoxic induction of the HIF-1α transcription factor *via* vasoactive hormones such as Ang II might be important to vascular

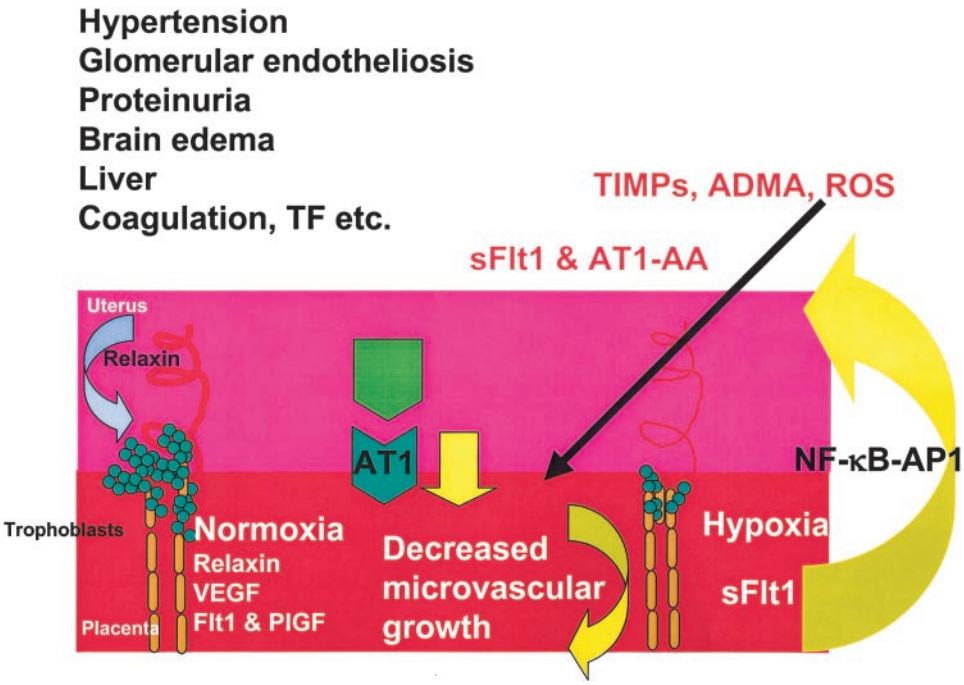

Figure 4. Left shows normal placentation and vessel formation. PlGF signals *via* Flt1. Relaxin signals *via* its receptor to increase gelatinase activity and to generate NO *via* the ET_B receptors. Preeclamptic, relatively ischemic placenta generates sFlt1, perhaps *via* hypoxia-inducible factor signaling. Endothelial dysfunction and injury, in part involving asymmetric dimethylarginine (ADMA), may result in autoantibody production capable of activating the AT1 receptor. Resultant signaling *via* NAD(P)H oxidase can generate reactive oxygen species (ROS) and stimulate NF-κB, which increases expression of various genes including those for coagulation factors such as tissue factor (TF). The mechanisms that are directly responsible for sFlt1 production and for autoantibody generation are unknown.

biology. Finally, evidence has recently been presented that HIF transcription factors are overexpressed in preeclamptic placentas (81). *In vitro* DNA binding activity for HIF-1α was demonstrated in these studies. Flt1 and tyrosine hydroxylase, both of which are equipped with hypoxia response elements, were expressed to a greater degree in preeclamptic compared with normal placentas. Mechanisms that contribute to alternative Flt1 splicing would be elucidative.

Acknowledgment

A.J. and K.P.C. were supported by the National Institutes of Health (K11 HD00662, RO1 HL38076, RO1 HD30325, RO1 DK63321, RO1 HL67937), the American Heart Association, and the 8th Mallinckrodt Scholar Award. S.A.K. was supported by National Institutes of Health Grants KO8 and RO3 from the National Institute of Diabetes and Digestive and Kidney Diseases and the American Society of Nephrology Carl W. Gottschalk Research Scholarship. F.C.L. received support from the Deutsche Forschungsgemeinschaft.

Authors are listed in order of appearance. This review resulted from a symposium held at the American Society of Nephrology annual meeting, San Diego, CA, 2003.

We thank Lee Danielson, Jackie Novak, David Sherwood, Laurie Kerchner, Hermann Haller and Gerd Wallukat. We profited from the mentorship of James M. Roberts, Benjamin P. Sachs, Vikas P. Sukhatme, Franklin Epstein, Ravi Thadhani, Myron H. Weinberger, and Marshall D. Lindheimer.

References

1. Conrad KP, Lindheimer MD: Renal and cardiovascular alterations. In: *Chesley's Hypertensive Disorders in Pregnancy*, 2nd Ed., edited by Lindheimer MD, Roberts JM, Cunningham FG, Stamford, CT, Appleton & Lange, 1999, pp 263–326
2. Chesley LC, Annitto JE, Cosgrove RA: The remote prognosis of eclamptic women: Sixth periodic report. *Am J Obstet Gynecol* 124: 446–459, 1976
3. Smith GC, Pell JP, Walsh D: Pregnancy complications and maternal risk of ischaemic heart disease: A retrospective cohort study of 129,290 births. *Lancet* 357: 2002–2006, 2001
4. Irgens HU, Reisaeter L, Irgens LM, Lie RT: Long term mortality of mothers and fathers after pre-eclampsia: Population based cohort study. *BMJ* 323: 1213–1217, 2001
5. Wilson BJ, Watson MS, Prescott GJ, Sunderland S, Campbell DM, Hannaford P, Smith WC: Hypertensive diseases of pregnancy and risk of hypertension and stroke in later life: Results from cohort study. *BMJ* 326: 845, 2003
6. Suzuki S, Gejyo F, Ogino S, Maruyama Y, Ueno M, Nishi S, Kimura H, Arakawa M: Postpartum renal lesions in women with pre-eclampsia. *Nephrol Dial Transplant* 12: 2488–2493, 1997
7. Bar J, Kaplan B, Wittenberg C, Erman A, Boner G, Ben-Rafael Z, Hod M: Microalbuminuria after pregnancy complicated by preeclampsia. *Nephrol Dial Transplant* 14: 1129–1132, 1999
8. Roest M, Banga JD, Janssen WM, Grobbee DE, Sixma JJ, de Jong PE, de Zeeuw D, van Der Schouw YT: Excessive urinary albumin levels are associated with future cardiovascular mortality in postmenopausal women. *Circulation* 103: 3057–3061, 2001
9. Agatisa PK, Ness RB, Roberts JM, Costantino JP, Kuller LH, McLaughlin MK: Impairment of endothelial function in women with a history of preeclampsia: An indicator of cardiovascular risk. *Am J Physiol* 286: H1389–H1393, 2004
10. Sattar N, Greer IA: Pregnancy complications and maternal cardiovascular risk: Opportunities for intervention and screening? *BMJ* 325: 157–160, 2002
11. Page EW: The relation between hydatid moles, relative ischemia of the gravid uterus and the placental origin of eclampsia. *Am J Obstet Gynecol* 37: 291–300, 1939
12. Gerretsen G, Huisjes HJ, Elema JD: Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *Br J Obstet Gynaecol* 88: 876–881, 1981
13. Zhou Y, Damsky CH, Chiu K, Roberts JM, Fisher SJ: Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J Clin Invest* 91: 950–960, 1993
14. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, Damsky CH: Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *J Clin Invest* 99: 2139–2151, 1997
15. Zhou Y, Damsky CH, Fisher SJ: Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 99: 2152–2164, 1997
16. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, Fisher SJ: Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. *Am J Pathol* 151: 1809–1818, 1997
17. Zhou Y, Genbacev O, Fisher SJ: The human placenta remodels the uterus by using a combination of molecules that govern vasculogenesis or leukocyte extravasation. *Ann N Y Acad Sci* 995: 73–83, 2003
18. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C, Fisher SJ: Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol* 160: 1405–1423, 2002
19. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP: Expression of inflammatory cytokines in placentas from women with preeclampsia. *J Clin Endocrinol Metab* 86: 2505–2512, 2001
20. Conrad KP, Miles TM, Benyo DF: Circulating levels of immunoreactive cytokines in women with preeclampsia. *Am J Reprod Immunol* 40: 102–111, 1998
21. Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, Manyonda IT, Lowry PJ: Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. *Nature* 405: 797–800, 2000
22. Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH: Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet* 361: 1511–1517, 2003
23. Noris M, Todeschini M, Cassis P, Pasta F, Cappellini A, Bonazzola S, Macconi D, Maucci R, Porrati F, Benigni A, Picciolo C, Remuzzi G: L-arginine depletion in preeclampsia orients nitric oxide synthase toward oxidant species. *Hypertension* 43: 614–22, 2004
24. Conrad KP: Renal hemodynamics during pregnancy in chronically catheterized, conscious rats. *Kidney Int* 26: 24–29, 1984
25. Danielson LA, Sherwood OD, Conrad KP: Relaxin is a potent renal vasodilator in conscious rats. *J Clin Invest* 103: 525–533, 1999

26. Danielson LA, Kercher LJ, Conrad KP: Impact of gender and endothelin on renal vasodilation and hyperfiltration induced by relaxin in conscious rats. *Am J Physiol* 279: R1298–R1304, 2000
27. Conrad KP, Colpoys MC: Evidence against the hypothesis that prostaglandins are the vasodepressor agents of pregnancy. Serial studies in chronically instrumented, conscious rats. *J Clin Invest* 77: 236–245, 1986
28. Danielson LA, Conrad KP: Acute blockade of nitric oxide synthase inhibits renal vasodilation and hyperfiltration during pregnancy in chronically instrumented conscious rats. *J Clin Invest* 96: 482–490, 1995
29. Novak J, Ramirez RJ, Gandley RE, Sherwood OD, Conrad KP: Myogenic reactivity is reduced in small renal arteries isolated from relaxin-treated rats. *Am J Physiol* 283: R349–R355, 2002
30. Gandley RE, Conrad KP, McLaughlin MK: Endothelin and nitric oxide mediate reduced myogenic reactivity of small renal arteries from pregnant rats. *Am J Physiol* 280: R1–R7, 2001
31. Novak J, Danielson LA, Kerchner LJ, Sherwood OD, Ramirez RJ, Moalli PA, Conrad KP: Relaxin is essential for renal vasodilation during pregnancy in conscious rats. *J Clin Invest* 107: 1469–1475, 2001
32. Jeyabalan A, Novak J, Danielson LA, Kerchner LJ, Opett SL, Conrad KP: Essential role for vascular gelatinase activity in relaxin-induced renal vasodilation, hyperfiltration and reduced myogenic reactivity of small arteries. *Circ Res* 93: 1249–1257, 2003
33. Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME, Amento EP: Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. *J Clin Invest* 98: 2739–2745, 1996
34. Fernandez-Patron C, Radomski MW, Davidge ST: Role of matrix metalloproteinase-2 in thrombin-induced vasorelaxation of rat mesenteric arteries. *Am J Physiol* 278: H1473–H1479, 2000
35. Szlachter BN, Ouagliarello J, Jewelewicz R, Osathanondh R, Spellacy WN, Weiss G: Relaxin in normal and pathogenic pregnancies. *Obstet Gynecol* 59: 167–170, 1982
36. Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, Hsueh AJ: Activation of orphan receptors by the hormone relaxin. *Science* 295: 671–674, 2002
37. Martin D, Conrad KP: Expression of endothelial nitric oxide synthase by extravillous trophoblast cells in the human placenta. *Placenta* 21: 23–31, 2000
38. Goligorsky MS, Budzikowski AS, Tsukahara H, Noiri E: Cooperation between endothelin and nitric oxide in promoting endothelial cell migration and angiogenesis. *Clin Exp Pharmacol Physiol* 26: 269–271, 1999
39. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111: 649–658, 2003
40. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, Takeda S, Yano T, Tsutsumi O, Taketani Y: Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. *J Clin Endocrinol Metab* 88: 2348–2351, 2003
41. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A, Schaaps JP, Cabrol D, Frankenne F, Foidart JM: Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: Pathophysiological consequences. *J Clin Endocrinol Metab* 88: 5555–5563, 2003
42. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111: 707–716, 2003
43. Dvorak HF: Vascular permeability factor/vascular endothelial growth factor: A critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol* 20: 4368–4380, 2002
44. Kendall RL, Thomas KA: Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A* 90: 10705–10709, 1993
45. He Y, Smith SK, Day KA, Clark DE, Licence DR, Charnock-Jones DS: Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT1) pre-mRNA is important for the regulation of VEGF activity. *Mol Endocrinol* 13: 537–545, 1999
46. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA: Longitudinal serum concentrations of placental growth factor: Evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol* 188: 177–182, 2003
47. Polliotti BM, Fry AG, Saller DN, Mooney RA, Cox C, Miller RK: Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia. *Obstet Gynecol* 101: 1266–1274, 2003
48. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, Ecker J, Karumanchi SA: First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab* 89: 770–775, 2004
49. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 350: 672–683, 2004
50. He H, Venema VJ, Gu X, Venema RC, Marrero MB, Caldwell RB: Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through flk-1/KDR activation of c-Src. *J Biol Chem* 274: 25130–25135, 1999
51. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 349: 427–434, 2003
52. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E: Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 21: 60–65, 2003
53. Baylis C: Renal effects of cyclooxygenase inhibition in the pregnant rat. *Am J Physiol* 253: F158–F163, 1987
54. Venuto RC, Donker AJ: Prostaglandin E2, plasma renin activity, and renal function throughout rabbit pregnancy. *J Lab Clin Med* 99: 239–246, 1982
55. Gallery ED, Ross M, Grigg R, Bean C: Are the renal functional changes of human pregnancy caused by prostacyclin? *Prostaglandins* 30: 1019–1029, 1985
56. Sorensen TK, Easterling TR, Carlson KL, Brateng DA, Benedetti TJ: The maternal hemodynamic effect of indomethacin in normal pregnancy. *Obstet Gynecol* 79: 661–663, 1992

57. Harrison GL, Moore LG: Blunted vasoreactivity in pregnant guinea pigs is not restored by meclofenamate. *Am J Obstet Gynecol* 160: 258–264, 1989
58. Conrad KP, Vernier KA: Plasma level, urinary excretion, and metabolic production of cGMP during gestation in rats. *Am J Physiol* 257: R847–R853, 1989
59. Conrad KP, Joffe GM, Kruszyna H, Kruszyna R, Rochelle LG, Smith RP, Chavez JE, Mosher MD: Identification of increased nitric oxide biosynthesis during pregnancy in rats. *FASEB J* 7: 566–571, 1993
60. Kopp L, Paradiz G, Tucci JR: Urinary excretion of cyclic 3',5'-adenosine monophosphate and cyclic 3',5'-guanosine monophosphate during and after pregnancy. *J Clin Endocrinol Metab* 44: 590–594, 1977
61. Conrad KP, Kerchner LJ, Mosher MD: Plasma and 24-h NO(x) and cGMP during normal pregnancy and preeclampsia in women on a reduced NO(x) diet. *Am J Physiol* 277: F48–F57, 1999
62. Sala C, Campise M, Ambroso G, Motta T, Zanchetti A, Morganti A: Atrial natriuretic peptide and hemodynamic changes during normal human pregnancy. *Hypertension* 25: 631–636, 1995
63. Pascoal IF, Lindheimer MD, Nalbantian-Brandt C, Umans JG: Preeclampsia selectively impairs endothelium-dependent relaxation and leads to oscillatory activity in small omental vessels. *J Clin Invest* 101: 464–470, 1998
64. Alexander BT, Llinas MT, Kruckeberg WC, Granger JP: L-arginine attenuates hypertension in pregnant rats with reduced uterine perfusion pressure. *Hypertension* 43: 832–836, 2004
65. Haller H, Ziegler E-M, Homuth V, Eichhorn J, Nagy Z, Luft FC: Endothelial adhesion molecules and leukocyte integrins in preeclamptic patients. *Hypertension* 29: 291–296, 1997
66. Haller H, Hempel A, Homuth V, Mandelkow A, Maasch C, Drab M, Lindschau C, Vetter K, Dudenhausen J, Luft FC: Endothelial cell permeability and protein kinase C in preeclampsia. *Lancet* 351: 945–949, 1998
67. Wallukat G, Homuth V, Fischer T, Horstkamp B, Jüpner A, Baur E, Nissen E, Vetter K, Dudenhausen JW, Haller H, Luft FC: Patients with preeclampsia develop agonistic antibodies against the angiotensin AT_1 receptor. *J Clin Invest* 103: 945–952, 1999
68. Dechend R, Homuth V, Wallukat G, Kreuzer J, Park JK, Theuer J, Juepner A, Gulba DC, Mackman N, Haller H, Luft FC: AT(1) receptor agonistic antibodies from preeclamptic patients cause vascular cells to express tissue factor. *Circulation* 101: 2382–2387, 2000
69. Dechend R, Viedt C, Muller DN, Ugele B, Brandes RP, Wallukat G, Park JK, Janke J, Barta P, Theuer J, Fiebeler A, Homuth V, Dietz R, Haller H, Kreuzer J, Luft FC: AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. *Circulation* 107: 1632–1639, 2003
70. Xia Y, Wen H, Bobst S, Day MC, Kellems RE: Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells. *J Soc Gynecol Investig* 10: 82–93, 2003
71. Fu ML, Herlitz H, Schulze W, Wallukat G, Micke P, Eftekhari P, Sjogren KG, Hjalmarson A, Muller-Esterl W, Hoebeke J: Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension. *J Hypertens* 18: 945–953, 2000
72. AbdAlla S, Lother H, el Massiery A, Quitterer U: Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat Med* 7: 1003–1009, 2001
73. Bohlender J, Ganten D, Luft FC: Rats transgenic for human renin and human angiotensinogen as a model for gestational hypertension. *J Am Soc Nephrol* 11: 2056–2061, 2000
74. Staudt A, Bohm M, Knebel F, Grosse Y, Bischoff C, Hummel A, Dahm JB, Borges A, Jochmann N, Wernecke KD, Wallukat G, Baumann G, Felix SB: Potential role of autoantibodies belonging to the immunoglobulin G-3 subclass in cardiac dysfunction among patients with dilated cardiomyopathy. *Circulation* 106: 2448–2453, 2002
75. de Boer RA, Pinto YM, Suurmeijer AJ, Pokharel S, Scholtens E, Humler M, Saavedra JM, Boomsma F, van Gilst WH, van Veldhuisen DJ: Increased expression of cardiac angiotensin II type 1 (AT(1)) receptors decreases myocardial microvessel density after experimental myocardial infarction. *Cardiovasc Res* 57: 434–442, 2003
76. Shimizu T, Okamoto H, Chiba S, Matsui Y, Sugawara T, Akino M, Nan J, Kumamoto H, Onozuka H, Mikami T, Kitabatake A: VEGF-mediated angiogenesis is impaired by angiotensin type 1 receptor blockade in cardiomyopathic hamster hearts. *Cardiovasc Res* 58: 203–212, 2003
77. Delles C, Schneider MP, John S, Gekle M, Schmieder RE: Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical N(G), N(G)-dimethylarginine in human essential hypertension. *Am J Hypertens* 15: 590–593, 2002
78. Cannigia I, Grisaru-Gravnosky S, Kuliszewsky M, Post M, Lye SJ: Inhibition of TGF-beta 3 restores the invasive capability of extravillous trophoblasts in preeclamptic pregnancies. *J Clin Invest* 103: 1641–1650, 1999
79. Luttun A, Carmeliet P: Soluble VEGF receptor Flt1: The elusive preeclampsia factor discovered? *J Clin Invest* 111: 600–602, 2003
80. Page EL, Robitaille GA, Pouyssegur J, Richard DE: Induction of hypoxia-inducible factor-1α by transcriptional and translational mechanisms. *J Biol Chem* 277: 48403–48409, 2000
81. Rajakumar A, Doty K, Daftary A, Harger G, Conrad KP: Impaired oxygen-dependent reduction of HIF-1alpha and -2alpha proteins in pre-eclamptic placentae. *Placenta* 24: 199–208, 2003