Activation of Local Chorionic Villi Angiotensin II Levels But Not Angiotensin (1-7) in Preeclampsia

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Abstract—The chorionic villi in the placenta are responsible for the regulation of fetal oxygen and nutrient transport. Although the peripheral renin-angiotensin system is activated during normal pregnancy, the regulation of the local chorionic villi renin-angiotensin system remains unknown. Therefore, placental chorionic villous tissue was collected from nulliparous third-trimester normotensive or preeclamptic subjects and was analyzed for angiotensin peptide content, angiotensinogen, renin, angiotensin-converting enzyme (ACE), ACE2, neprilysin, angiotensin II type 1 (AT_1), angiotensin II type 2, *Mas* receptor mRNAs, and angiotensin receptor density and subtype. Angiotensin II in chorionic villi was significantly higher in preeclamptic subjects, whereas angiotensin (1-7) was not different. Angiotensinogen and AT_1 receptor gene expression was significantly higher in preeclamptic subjects. No differences were observed in renin, ACE, ACE2, or neprilysin gene expression. *Mas* receptor mRNA in preeclamptic subjects was decreased. The AT_1 receptor was the predominant receptor subtype in normal and preeclamptic chorionic villi. There was no difference in the density of the AT_1, angiotensin II type 2, and angiotensin (1-7) receptors. These results indicate that enhanced chorionic villous expression of angiotensin II may result from increased angiotensinogen. Elevated angiotensin II, acting through the AT_1 receptor, may favor vasoconstriction in placental chorionic villi and contribute to impaired fetal blood flow and decreased fetal nutrition observed during preeclampsia. (*Hypertension*. 2008;51:1066-1072.)

Key Words: preeclampsia ■ renin angiotensin system ■ pregnancy ■ placenta ■ angiotensin receptors
■ *Mas* receptor ■ angiotensin (1-7)

P reeclampsia, a hypertensive disorder of pregnancy, is clinically defined as maternal hypertension, proteinuria, and generalized edema occurring after the 20th week of gestation. Preeclampsia is the second leading cause of maternal mortality in the United States, affecting 7% to 10% of all pregnancies and contributing significantly to stillbirths and neonatal morbidity and mortality.[1] Thus, the prevention of preeclampsia would have a significant impact on maternal and neonatal outcome. Nevertheless, the pathophysiology of preeclampsia remains poorly understood. Although preeclampsia is often considered a multisystem disorder, one of the postulated pathological features of this disease is an impaired maternal uterine spiral artery remodeling. In preeclampsia, there is a decrease of embryonic trophoblast cell invasion of the uterine spiral arteries, which, in turn, prevents the diameter of these arteries from expanding and consequently leads to a reduction of blood flow into the placenta.[2,3] Without the remodeling of the uterine vasculature, the placenta becomes hypoxic as gestation advances, resulting in an oxygen deficiency within the tissue. The hypoxic placenta can then release factors into the maternal circulation that result in generalized endothelial dysfunction, vascular inflam-

mation, and proteinuria.[3–5] Although several factors, including soluble fms-like tyrosine kinase 1[6] and soluble endoglin,[7] were discovered recently to play a role in the pathogenesis of preeclampsia, there are still many unanswered questions in the development of this disease.

The renin-angiotensin system (RAS) is an important regulator of blood pressure, sodium, and fluid homeostasis and has been shown previously to play a role in preeclampsia. In normal pregnancy, estrogen causes an overexpression of the RAS by increasing both tissue and circulating levels of angiotensinogen[8,9] and renin.[10–13] Consequently, plasma angiotensin (Ang) II is increased in association with the rise of angiotensinogen and plasma renin activity during gestation.[14,15] Normal pregnant women are resistant to the pressor effects of Ang II,[16–18] and they remain normotensive despite a 2-fold increase in Ang II. We showed that human plasma and urinary levels of Ang-(1-7) are increased in normal pregnant subjects.[19,20] The physiological consequences of the activated RAS during normal pregnancy are unknown; even less understood is how this system may be altered in women with preeclampsia. Our previously published study showed that many of the components of the circulating RAS in

women with preeclampsia are downregulated, including plasma Ang I, Ang II, Ang-(1-7), and plasma renin activity, when compared with normal pregnant women.[19] In addition, we showed previously that Ang-(1-7) and its generating enzyme, angiotensin-converting enzyme (ACE)2, are colocalized within different cell types in the placenta, including primarily the chorionic villous syncytiotrophoblast and cytotrophoblasts, of normal and preeclamptic women.[21] This study suggested that the RAS may play an important role within the chorionic villi, an essential component of the placenta that is responsible for maternal-fetal blood flow and, thus, the transport of oxygen and nutrients to the growing fetus. Several studies investigated the local tissue-specific RAS in the placenta[22–24]; however, none have characterized the entire RAS in the chorionic villi specifically. In this study, we investigated angiotensin peptides, RAS component mRNAs, and angiotensin receptor binding in chorionic villi from normal and preeclamptic subjects.

Materials and Methods

Human Subjects
These experiments were conducted using human placental tissue collected from women with both normal pregnancy and preeclampsia. Placental tissue was collected from women who had either cesarean section or vaginal deliveries. Two separate groups of subjects were included. Group 1 (n=25) consisted of normotensive pregnant subjects who have remained normotensive throughout pregnancy (BP <140/90 mm Hg), have no history of chronic blood pressure elevation, and have an absence of proteinuria. Group 2 (n=21) consisted of preeclamptic subjects who developed new-onset hypertension (BP >140/90 mm Hg) and proteinuria (1+≅30 mg/dL≅≥300 mg in a 24-hour urine sample) after the 20th week of gestation. Blood pressure readings are reported as the highest blood pressure measured in the labor and delivery suite before delivery. Subjects in the 2 groups were matched according to gestational age and parity (all nulliparous). Patients with evidence of chorioamnionitis were excluded. Women in both groups were over age 18 years and less than age 50 years and were free of other known cardiovascular, renal, or connective tissue diseases; diabetes; cancer; or hyperplasia.

The study was approved by the institutional review boards at both Wake Forest University School of Medicine and Forsyth Medical Center. The procedures followed were in accordance with institutional guidelines. After signed, informed consent was obtained and the baby and placenta were delivered, placental samples were taken.

Experimental Procedures
For both normal pregnant and preeclamptic patients, immediately after delivery, the whole placenta was collected on ice, and tissue sections were taken from the center of the placenta, near the umbilical cord attachment site. The total amount of time from delivery of the placenta until the samples were collected did not exceed 15 minutes. For each tissue section, the maternal basal plate and fetal membranes were removed so that only the fetal villous tissue was present. When taking tissue sections from the placenta, areas of necrosis or tissue damage were avoided. Tissue sections were immediately snap frozen in liquid nitrogen and stored at −80°C for analysis of angiotensin peptides [Ang I, Ang II, and Ang-(1-7)] by radioimmunoassay or for quantification of angiotensinogen, renin, ACE, ACE2, neprilysin (NEP), and Ang II type 1 (AT_1), Ang II type 2 (AT_2) and *Mas* receptor mRNA by reverse transcription, real-time PCR. In addition, tissue sections were frozen in OCT freezing medium for AT_1, AT_2, and Ang II type 1-7 (AT_{1-7}) receptor density measurements by receptor autoradiography, as described below.

Tissue Concentration of Angiotensin Peptides
Frozen tissues were rapidly weighed and homogenized, as described previously.[25] Tissue homogenates were extracted using Sep-Pak columns, as described previously.[25–27] The eluate was divided for 3 radioimmunoassays [Ang I, Ang II, and Ang-(1-7)], and the solvent was evaporated. Ang I was measured using a modification of a commercially available Peninsula radioimmunoassay kit. Ang II was measured using an Alpco Diagnostic kit. Ang-(1-7) was measured using an antibody produced in our laboratory.[25] The minimum detectable levels of the assays were 1.0 fmol/mL for Ang I, 0.8 fmol/mL for Ang II, and 2.8 fmol/mL for Ang-(1-7). The intra-assay and interassay coefficients of variation for Ang I radioimmunoassay are 18% and 22%, for Ang II are 12% and 22%, and for Ang-(1-7) are 8% and 20%, respectively.

RNA Isolation and Reverse Transcription/Real-Time PCR
RNA was isolated from human chorionic villi from normal and preeclamptic women using TRIzol reagent (GIBCO Invitrogen) and reverse transcribed using avian myeloblastosis virus reverse transcriptase. The resultant cDNA was added to TaqMan Universal PCR Master Mix (Applied Biosystems) with gene-specific primer/probe sets, and amplification was performed on an ABI 7000 Sequence Detection System. Human primer/probe sets were purchased from Applied Biosystems except for ACE2, which was our design (forward primer 5′-CCCAGAGAACAGTGGACCAAAA-3′; reverse primer 5′-GCTCCACCACACCAACGAT-3′; and probe 5′-FAM-CTCCCGCTTCATCTCC-3′). All of the reactions were performed in triplicate, and 18S ribosomal RNA, amplified using the TaqMan Ribosomal RNA Control kit (Applied Biosystems), served as an internal control. The results were quantified as Ct values, where Ct was defined as the threshold cycle of PCR at which amplified product was first detected and expressed as relative gene expression (the ratio of target to control).

Autoradiography
Chorionic villous tissues frozen in OCT were sectioned at 14 μm, and receptor autoradiography was performed using ^{125}I-[sarcosine^1, threonine^8]-Ang II at 0.6 nM to determine the apparent maximal density of receptors.[25,28] A lower concentration of ^{125}I-[sarcosine^1, threonine^8]-Ang II (0.2 nM) was used in the presence or absence of 3 μmol/L of losartan, PD 123319, or D-Ala^7-Ang-(1-7) (A779 or D-Ala) to determine the percentage of each receptor subtype present. Sections were exposed to film and films were analyzed using a computerized densitometry system (MCID) as reported previously.[29,30] Data for binding density are expressed as the amount of total binding attributed to each receptor subtype as determined by the competition study.

Statistics and Data Analysis
Data were analyzed with a standard 1-way ANOVA followed by the Newman-Keul's posthoc test for multiple comparisons. The Student *t* test for unpaired observations was used for comparing 2 groups (GraphPad Software). A *P* value of <0.05 was considered statistically different. All of the arithmetic means are presented± SEMs.

Results

Clinical Profile of Normal and Preeclamptic Patients
The Table shows the clinical profile of the study population. A total of 25 placental chorionic villous samples were collected from normal pregnant women, and 21 chorionic villous placental samples were collected from preeclamptic women. The preeclamptic subjects had significant hypertension as shown by increases in systolic (143±4 versus 173±3 mm Hg; *P*<0.0001), diastolic (80±2 versus

Table.   Clinical Profile of the Study Population

Patient Clinical Characteristics	Normal Pregnancy	Preeclamptic Pregnancy
N	25	21
Age, y	23.5±1.0	25.0±1.3
Body mass index, kg/m^2	31.5±1.0	36.6±2.0*
Birth weight, g	3187±140	2744±173*
Gestational age, week	38.2±0.6	36.8±0.5
Systolic blood pressure, mm Hg	143±4	173±3†
Diastolic blood pressure, mm Hg	80±2	106±2†
Mean blood pressure, mm Hg	101±2	128±2†
Proteinuria	None	>1+
No. of patients receiving antihypertensive medications	None	2‡

Values are expressed as means±SEMs.

*$P<0.05$.

†$P<0.0001$.

‡Antihypertensive treatment for 2 preeclamptic patients included Methyldopa and Hydralazine.

106 ± 2 mm Hg; $P<0.0001$), and mean blood pressure (101 ± 3 versus 128 ± 2 mm Hg; $P<0.0001$). In addition, the preeclamptic subjects had proteinuria measured by >300 mg in a 24-hour urine sample, >1+, or (30 mg/dL) on a urine dipstick. The birth weight of the preeclamptic subjects was significantly lower than that of the normal pregnant subjects ($P<0.05$). In addition, the body mass index of the preeclamptic subjects before pregnancy was significantly higher than that of the normal pregnant subjects (31.5 ± 1.0 versus 36.6 ± 2.0; $P<0.05$). There was no significant difference in maternal age between normal and preeclamptic subjects. All of the subjects were matched for gestational age.

Angiotensin Peptide Levels in the Chorionic Villi of Placentas From Normal and Preeclamptic Subjects

Angiotensin peptide content was measured in the chorionic villi of both normal and preeclamptic placentas as shown in Figure 1. Compared with chorionic villi from normotensive subjects, chorionic villi from preeclamptic subjects were found to have significantly higher tissue Ang II levels (15 ± 2 versus 28 ± 6 fmol/mg of protein; $P<0.05$; Figure 1B). These results also indicate that Ang II is the predominate peptide in both the normal ($P<0.001$) and preeclamptic ($P<0.001$) chorionic

villi. In addition, Ang II levels were 30-fold higher than Ang I levels (28.5 ± 6.2 versus 0.9 ± 0.07 fmol/mg of protein; $P<0.001$) and 8-fold higher than Ang-(1-7) levels (28.5 ± 6.2 versus 3.4 ± 0.3 fmol/mg of protein; $P<0.001$) in the preeclamptic chorionic villi. Analysis of Ang II expression levels between preeclamptic women undergoing cesarean ($n=10$) versus vaginal ($n=11$) delivery revealed no significant differences (data not shown). There was no significant difference in chorionic villi Ang I or Ang-(1-7) levels between normal and preeclamptic subjects (Figure 1A and 1C).

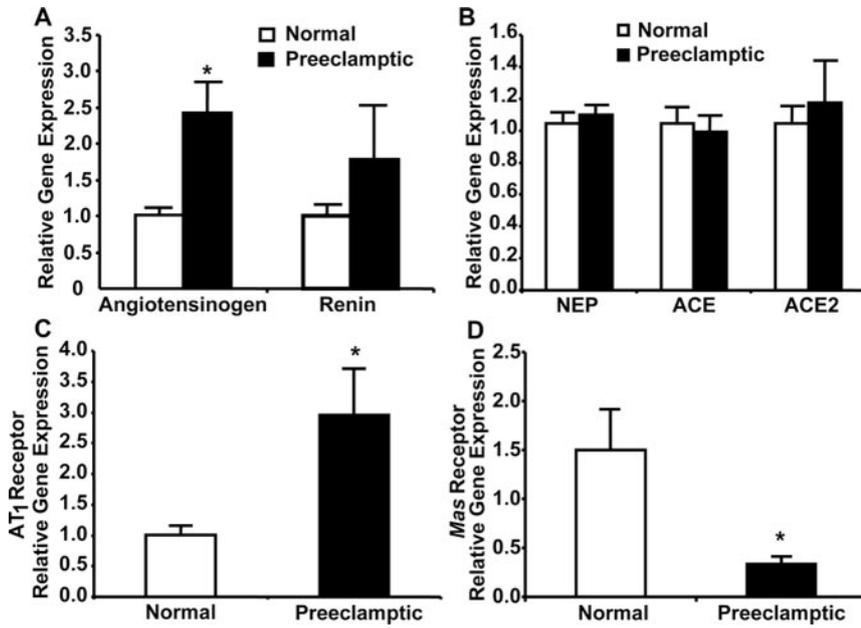## RAS Gene Expression in Normal and Preeclamptic Chorionic Villi

Gene expression of RAS components, including renin, angiotensinogen, NEP, ACE, ACE2, AT$_1$ receptors, AT$_2$ receptors, and *Mas* receptors, was measured as shown in Figure 2. Preeclamptic chorionic villi were found to have 2.5-fold higher angiotensinogen mRNA when compared with normal chorionic villi (1.0 ± 0.1 versus 2.4 ± 0.4 U; $P<0.01$). No significant differences were found in angiotensinogen mRNA levels in preeclamptic women undergoing cesarean versus vaginal delivery. In addition, there was a trend for a higher renin mRNA in preeclamptic chorionic villi versus normal; however, statistical significance was not reached (Figure 2A). In addition, there were no significant differences in NEP, ACE, or ACE2 mRNAs in preeclamptic chorionic villi when compared with normal controls (Figure 2B). However, in the preeclamptic chorionic villi, AT$_1$ receptor mRNA levels were significantly higher than the concentrations found in tissue from normal pregnant subjects (1.0 ± 0.1 versus 3.0 ± 0.7 U; $P<0.01$; Figure 2C). In addition, AT$_1$ receptor mRNA levels were significantly higher in preeclamptic women undergoing a cesarean section versus those who had a normal vaginal delivery (4.7 ± 1.5 versus 1.6 ± 0.2; $P<0.05$). An analysis of the correlation between AT$_1$ receptor mRNA and Ang II peptide levels revealed no correlation ($r=0.21$; $P>0.05$). *Mas* receptor mRNA was low but present in both the normal and preeclamptic villi. *Mas* receptor mRNA levels were lower in preeclamptic chorionic villi when compared with normal (1.5 ± 0.40 versus 0.3 ± 0.06 U; $P<0.01$; Figure 2D). AT$_2$ receptor mRNA levels were below detectable limits in both the normal and preeclamptic chorionic villi.

Angiotensin Receptor Binding in Normal and Preeclamptic Chorionic Villi

Chorionic villous tissue from both normal and preeclamptic subjects was also analyzed by in vitro receptor autoradiogra-
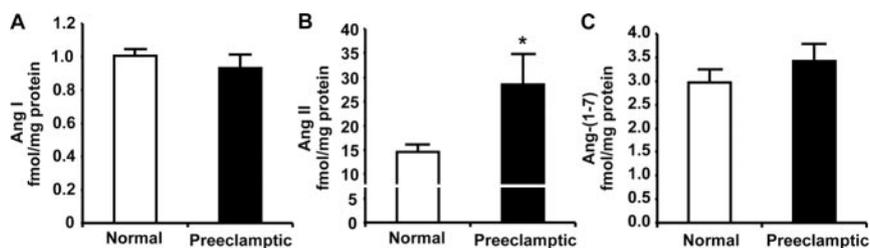
Figure 1. Angiotensin peptide levels in normal pregnant and preeclamptic chorionic villi. Angiotensin peptide levels were measured by radioimmunoassay of Ang I (A), Ang II (B), and Ang-(1-7) (C) in normal and preeclamptic placental chorionic villi. There was a significant increase in Ang II ($P<0.05$) but no change in Ang I or Ang-(1-7) peptide levels in preeclamptic vs normal chorionic villi. Ang II is the predominant peptide in the chorionic villi ($P<0.001$). Data are expressed as the means±SEMs. *$P<0.05$ preeclamptic vs normal chorionic villi.

Figure 2. Gene expression of RAS components in normal pregnant and preeclamptic chorionic villi. Relative gene expression as determined by reverse transcription, real-time PCR of angiotensinogen and renin (A); NEP, ACE, and ACE2 (B); AT_1 receptor (C); and *Mas* receptor (D) in normal and preeclamptic placental chorionic villi. Angiotensinogen ($P<0.01$) and AT_1 receptor ($P<0.01$) mRNA were upregulated in preeclamptic chorionic villi when compared with normal subjects. The *Mas* receptor was downregulated in preeclamptic chorionic villi when compared with normal subjects ($P<0.01$). There was no change in renin, NEP, ACE, or ACE2 mRNA. Data are expressed as the means±SEMs. $P<0.01$ preeclamptic vs normal chorionic villi.
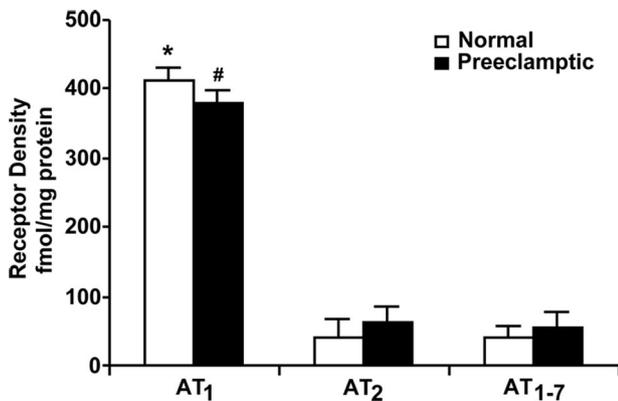
phy to determine the maximal density of binding and the percentage of each receptor subtype, AT_1, AT_2, and AT_{1-7} (Figure 3). AT_1 receptors were the predominant receptor subtype in both the normal and preeclamptic chorionic villi ($P<0.001$), with AT_2 and AT_{1-7} receptors making up <15% of the total RAS receptor binding. There was no statistical difference in AT_1 receptor density, defined by losartan competition, between normal and preeclamptic chorionic villi (412±17 versus 380±19 fmol/mg of protein). There was no difference in either AT_2, defined by PD12339 competition

Figure 3. Angiotensin receptor binding in normal pregnant and preeclamptic chorionic villi. Receptor density of AT_1, AT_2, and AT_{1-7} was measured by receptor autoradiography in normal and preeclamptic placental chorionic villi and was assessed by the displacement of ^{125}I-[sarcosine^1, threonine^8]-Ang II binding by the AT_1 receptor antagonist losartan (3 μmol/L), the AT_2 receptor antagonist PD123319 (3 μmol/L), and the AT_{1-7} receptor antagonist A779 (3 μmol/L). Quantification of receptor density showed no changes between normal and preeclamptic subjects for AT_1, AT_2, or AT_{1-7} receptors. The AT_1 receptor was found to be the predominant receptor in the chorionic villi of both normal and preeclamptic placentas ($P<0.001$). In addition, the AT_2 and AT_{1-7} receptors made up <15% of the RAS receptors in the chorionic villi of both groups. Data are expressed as the means±SEMs. *$P<0.001$ AT_1 vs AT_2 and AT_{1-7} receptor density in the normal chorionic villi, #$P<0.001$ AT_1 vs AT_2 and AT_{1-7} receptor density in the preeclamptic chorionic villi.

(41±26 versus 61±24 fmol/mg of protein), or AT_{1-7}, defined by A779 competition (41±20 versus 55±21 fmol/mg of protein), receptor binding density between normal and preeclamptic chorionic villi.

Discussion

This study is the first to demonstrate that all 3 of the key RAS peptides, Ang I, Ang II, and Ang-(1-7), are found in the chorionic villi of both normal and preeclamptic subjects. In addition, we found the presence of angiotensinogen, renin, ACE, ACE2, NEP, AT_1, and *Mas* receptors. Ang II, a potent vasoconstrictor of the RAS, is by far the most predominant peptide in the chorionic villi. Ang II was also twice as high in preeclamptic chorionic villi when compared with normal tissue. This indicates that the local placental RAS may play an important role in the regulation of the maternal-fetal interface in the chorionic villi. No changes were seen in either Ang I or Ang-(1-7) levels between normal and preeclamptic chorionic villi. In addition to increased Ang II peptide levels, we also observed an increase in angiotensinogen and AT_1 receptor mRNAs in the preeclamptic chorionic villi. The observation of no significant differences in renin, NEP, ACE, or ACE2 mRNA in preeclamptic chorionic villi indicates that the highly increased activation of the vasoconstrictor arm of the RAS in the preeclamptic chorionic villi arises at the level of the angiotensinogen substrate and AT_1 receptors of the system. Although there were no significant changes in Ang I and Ang-(1-7) peptide levels and renin, NEP, ACE, and ACE2 mRNA, the presence of these RAS components in the chorionic villi indicates that they may be playing a role in both normal and preeclamptic pregnancies.

Angiotensinogen mRNA was found in our study to be present in normal and preeclamptic chorionic villi, which is consistent with earlier reports showing the presence of angiotensinogen in the human placenta, amnion, and chorion.[31,32] Previous studies also show that angiotensinogen mRNA is present in the whole placenta throughout normal pregnancy

starting at 6 weeks of gestation[23,33] and in decidual spiral arteries in the first and second trimesters of normal pregnancy.[34] Angiotensinogen mRNA was higher in the chorionic villi obtained from preeclamptic subjects in our study. On the contrary, Herse et al[22] found no significant difference in angiotensinogen mRNA levels in the placenta of normal versus preeclamptic pregnancies. The conflicting results of our study versus Herse et al[22] may result from the fact that we investigated angiotensinogen expression exclusively in the chorionic villi, whereas the previous study used the whole placenta, including the amnion and chorion, possibly diluting the contribution of the chorionic villi. The upregulation of angiotensinogen in the chorionic villi of the preeclamptic placenta is consistent with an activation of the RAS in these tissues and may be the primary rate-limiting protein of the RAS in the chorionic villi.

Renin gene expression in the placenta of normal and preeclamptic pregnancies was previously studied by Shah et al,[35] where the placenta was microdissected into chorionic villous tissue, decidua basalis, and decidua vera. Our studies are in agreement with the results of Shah et al[35] in that we also found no significant difference in renin expression between normal and preeclamptic chorionic villous tissue. However, whereas there was a trend for higher renin mRNA in the preeclamptic chorionic villi, this did not reach statistical significance. Herse et al[22] also found a trend for elevated renin expression in preeclamptic placental tissue. Measurements of total renin concentration and active renin were significantly higher in preeclamptic placentas,[36] which corroborates our data showing a possible trend for increased renin mRNA in the preeclamptic chorionic villi.

Ang II was significantly higher in preeclamptic when compared with normal pregnant chorionic villi and is consistent with the elevated angiotensinogen mRNA. Kalenga et al[24] found no difference in Ang II levels between normal and preeclamptic placentas, but differences in placenta dissection and experimental methodologies may contribute to the results observed. Interestingly, circulating Ang II is significantly downregulated in women with preeclampsia,[19] suggesting a difference in circulating versus local chorionic villous RAS regulation in normal and preeclamptic women. The actions of elevated Ang II in the chorionic villi may contribute to vasoconstriction of the fetal blood vessels resulting in a decrease of maternal-fetal transport and, thus, contributing to the pathophysiology of preeclampsia.

There was no significant difference in Ang-(1-7) peptide levels in normal versus preeclamptic chorionic villi. A previous study done by our laboratory investigated the expression and localization of Ang-(1-7) and its generating enzyme, ACE2, by immunohistochemistry and found that both Ang-(1-7) and ACE2 were present in the cells of normal and preeclamptic chorionic villi, including the syncytiotrophoblast and cytotrophoblasts.[21] Similar to the present study, we found no significant difference in the amount of Ang-(1-7) or ACE2 staining in normal versus preeclamptic chorionic villous syncytiotrophoblasts in the third trimester. Ang-(1-7) was decreased in the circulation of normal and preeclamptic women,[19] again highlighting differences in local tissue versus circulating RAS. The fact that Ang-(1-7) did not

change in the chorionic villi of preeclamptic women, whereas Ang II was increased suggests that the balance of these 2 biologically active peptides may be skewed toward the Ang II vasoconstrictor arm of the RAS.

The gene expression of the enzymes of the RAS, including ACE, ACE2, and NEP, were similar in normal and preeclamptic chorionic villi. Previous studies using quantitative reverse transcription, real-time PCR and radioenzymatic assay indicate that ACE expression does not differ between normal and preeclamptic placentas.[22,24] However, a study by Ito et al[37] measured ACE in uncomplicated and preeclamptic villous tissue and found significant increases in ACE protein and mRNA in the preeclamptic placenta. Reasons behind the differences in the results are unclear. No change in ACE enzyme levels with a significant increase in angiotensinogen and Ang II suggests that the system is being driven by the increased substrate, although an increase in ACE activity cannot be ruled out. Consideration should also be given to other enzymes involved in the production of Ang II, such as chymase.

ACE2, an Ang-(1-7) generating enzyme, has been shown to be present in the chorionic villi of the human placenta by immunohistochemistry.[21] We show for the first time the expression of ACE2 mRNA in the human placenta. Although no differences in ACE2 mRNA were observed between normal and preeclamptic tissues, its presence in the chorionic villi of the placenta indicates that it may play a role in the generation of Ang-(1-7). Studies investigating ACE2 activity are required to understand whether this enzyme participates in the increased level of Ang II in the presence of no change in Ang-(1-7). NEP, another enzyme with the potential to convert Ang I or Ang-(1-9) to Ang-(1-7), was not different in preeclamptic chorionic villi. A previous study showed that NEP is present in the placenta, including the trophoblast cells of normal and preeclamptic women as assessed by immunohistochemistry. The authors also observed a qualitative increase in NEP staining in the preeclamptic villous trophoblasts.[38] The presence and expression of NEP in normal placental villous tissue with no differences in preeclamptic women are consistent with the lack of change in either Ang I or Ang-(1-7) peptide levels in preeclamptic chorionic villi.

Although a number of studies have measured AT_1 receptors in the placenta of women with normal and preeclamptic pregnancies few have focused specifically on the chorionic villi. Moreover, there are conflicting reports in preeclamptic women that find the AT_1 receptor upregulated,[39,40] downregulated,[41] or not different.[22] We found a 3-fold upregulation of AT_1 receptor mRNA levels in preeclamptic chorionic villi, but measurement of receptor binding by receptor autoradiography showed no differences in AT_1 receptors between normal and preeclamptic chorionic villi. Numerous studies reveal mismatches of mRNA and binding density or protein measurements; however, the presence of AT_1 receptor autoantibodies in women with preeclampsia[42,43] may offer a potential explanation for the discoordinate findings. The bound autoantibodies are agonists at the AT_1 receptor. In addition, if there is a tight association of autoantibodies with the receptor, they may block the radioactive Ang II ligand from binding to the AT_1 receptors present in the tissue. If this

were the case, then the AT_1 receptor density in the preeclamptic chorionic villi would appear to be lower than it actually is. However, more studies are needed to confirm this hypothesis. Regardless of whether there is an upregulation of AT_1 receptors, the increase in Ang II content in preeclamptic chorionic villi strongly suggests that the downstream actions of Ang II may be playing a major role in the pathophysiology of preeclampsia.

AT_2 receptor mRNA was below the detectable limits in the placental tissue, and results from receptor autoradiography show that the AT_2 receptor levels were low in the chorionic villi. There was no difference in AT_2 receptor density between normal and preeclamptic tissues consistent with several previously studies.[22,41,44] In addition, mRNA levels of the *Mas* receptor, a reported Ang-(1-7) receptor,[45] were lower in preeclamptic than normal chorionic villi. Low levels of the *Mas* receptor were also observed with the measurement of AT_{1-7} receptor density by autoradiography; however, there was no difference in the density between normal and preeclamptic chorionic villi. Taken together with the observed higher Ang II levels in preeclamptic subjects, the modest downregulation in *Mas* receptors and normal Ang-(1-7) peptide levels would shift the balance toward the vasoconstrictor arm of the RAS.

Perspectives

This study provides evidence for the presence and regulation of a local tissue-specific RAS in the chorionic villi of normal and preeclamptic women. Our results show that the chorionic villous RAS is dysregulated in preeclamptic women with a significant increase in Ang II, associated with the upregulation of angiotensinogen and AT_1 receptor mRNAs. With no increase in Ang-(1-7) and a modest decrease in the *Mas* receptor, the balance of the 2 active peptides of the RAS is tilted toward Ang II in the preeclamptic chorionic villi, the components of the placenta that contain the fetal vessels and make up the cell barrier between maternal and fetal blood. Our results indicate that the major actions of Ang II, including vasoconstriction, will predominate and may be contributing to the pathophysiology of preeclampsia by decreasing the maternal-fetal exchange of vital oxygen and nutrients. In addition, this study, along with our previous findings, demonstrates that differential regulation exists between circulating and local chorionic villous RAS. Although ACE inhibitors are contraindicated during pregnancy, clinical strategies aimed at RAS regulation in preeclamptic women might be beneficial. However, therapeutic agents that do not cross the placental barrier should be used to target the local chorionic villous RAS rather than the systemic RAS as a whole.

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