Effects of Circulating and Local Uteroplacental Angiotensin II in Rat Pregnancy

Lydia Hering, Florian Herse, Nele Geusens, Stefan Verlohren, Katrin Wenzel, Anne C. Staff, K. Bridget Brosnihan, Berthold Huppertz, Friedrich C. Luft, Dominik N. Muller, Robert Pijnenborg, Judith E. Cartwright, Ralf Dechend

Abstract—The renin-angiotensin (Ang) system is important during placental development. Dysregulation of the renin-Ang system is important in preeclampsia (PE). Female rats transgenic for the human angiotensinogen gene crossed with males transgenic for the human renin gene develop the PE syndrome, whereas those of the opposite cross do not. We used this model to study the role of Ang II in trophoblast invasion, which is shallow in human PE but deeper in this model. We investigated the following groups: PE rats, opposite-cross rats, Ang II–infused rats (1000 ng/kg per day), and control rats. Ang II infusion increased only circulating Ang II levels (267.82 pg/mL), opposite cross influenced only uteroplacental Ang II (13.52 fmol/mg of protein), and PE increased both circulating (251.09 pg/mL) and uteroplacental (19.24 fmol/mg of protein) Ang II. Blood pressure and albuminuria occurred in the models with high circulating Ang II but not in the other models. Trophoblast invasion increased in PE and opposite-cross rats but not in Ang II–infused rats. Correspondingly, uterine artery resistance index increased in Ang II–infused rats but decreased in PE rats. We then studied human trophoblasts and villous explants from first-trimester pregnancies with time-lapse microscopy. Local Ang II dose-dependently increased migration by 75%, invasion by 58%, and motility by 282%. The data suggest that local tissue Ang II stimulates trophoblast invasion in vivo in the rat and in vitro in human cells, a hitherto fore unrecognized function. Conceivably, upregulation of tissue Ang II in the maternal part of the placenta represents an important growth factor for trophoblast invasion and migration. (Hypertension. 2010;56:311-318.)

Key Words: preeclampsia ■ angiotensin II ■ transgenic rats ■ trophoblasts ■ decidua ■ placenta ■ vascular remodeling

Various studies have implicated the renin-angiotensin (Ang) system in preeclampsia (PE).1–3 Female rats harboring the human angiotensinogen gene develop hypertension and proteinuria late in pregnancy when mated with male rats harboring the human renin gene; the result is a PE-like syndrome.4 However, instead of showing shallow trophoblast invasion associated with PE in humans, trophoblast invasion and spiral artery remodeling are actually increased in this animal model.4,5 Trophoblast invasion is highly relevant in pregnancy. These fetal cells invade the maternal decidua and remodel the spiral arteries, a process that includes replacing the smooth muscle cell layer with fibrinoid material. The remodeling of the uterine spiral arteries causes the vessels to increase in diameter, allowing for an increase in blood flow to the intervillous space of the placenta.6 The role of Ang II in this process is unclear.

The hallmark of PE is a shallow trophoblast invasion accompanied by a reduced percentage of remodeled spiral arteries in the decidua and no remodeling in the inner myometrium.6 Preliminary observations in our transgenic rat model indicated that the opposite cross (OC), namely dams harboring a human renin gene mated with males harboring human angiotensinogen, did not develop hypertension or a PE-like syndrome.4 In addition to the circulating renin-Ang system, several authors have described a local, uteroplacental renin-Ang system in pregnancy.7–10 However, the function of the uteroplacental renin-Ang system is unknown. Consistent with the observations in rat pregnancy, we showed earlier that components of the tissue renin-Ang system are substantially higher in the maternal part of the human placenta (the decidua), than in the fetal part (placenta), suggesting a role for local Ang II in the trophoblast-decidua interaction.11

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We investigated the effects of uteroplacental Ang II and circulating Ang II in rat pregnancy in more detail. We studied our conventional PE-syndrome cross, the OC, and chronic Ang II–infused pregnant rats and appropriate control rats. We also extended our observations to in vitro Ang II–related effects on cells derived from human trophoblasts and villous explants from first-trimester pregnancies. Our data show that uteroplacental Ang II has opposite in vivo effects on trophoblast invasion, migration, uteroplacental vascular remodeling, and maternal endothelial dysfunction compared with circulating Ang II. Local Ang II could serve as a potent regulator promoting trophoblast migration and invasion early in pregnancy.

### Methods

#### Animal Studies
Details on the TGR models (PE and OC) have been published previously. On day 9 of gestation, Sprague-Dawley (SD) dams were randomly assigned to 1 of 2 experimental groups: SD plus vehicle, which served as the control group (n=9) or SD plus Ang II (n=8), at a dose of 1000 ng/kg per min (Ang II). The animals were allowed to term (day 21), anesthetized, and then euthanized. Doppler studies were performed as described earlier. Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured from 3 cardiac cycles. The resistance index (RI=[PSV−EDV]/PSV) was calculated when EDV was >0 to quantify the pulsatile arterial blood velocity waveforms.

Tissue sections were immediately snap frozen in liquid nitrogen and homogenized as described previously. Tissue Ang II and circulating Ang II were measured by radioimmunoassay. Tissue preparations and immunostaining protocols were performed as described earlier. For the quantitative analysis of depth of invasion (invasion experiments were conducted with collagen-coated chambers after 72-hour Ang II exposure. More detailed information is given in the online Data Supplement (please see http://hyper.ahajournals.org). More detailed information is given in the online Data Supplement.

#### Cell Culture
SGHPL-4 cells (derived from primary human first-trimester extravillous trophoblasts transfected with the early region of SV40, known previously as MC418) were cultured as described earlier. The effect of 48-hour Ang II stimulation in matrigel on the invasive trophoblast outgrowth was investigated as described earlier. Human placental villous tissue explants from first-trimester pregnancies were established and characterized as described previously. Migration experiments were conducted with collagen-coated chambers after 72-hour Ang II exposure. More detailed information is given in the online Data Supplement.

#### Statistics
Data are presented as mean±SEM. We tested Kolmogorov-Smirnov for distribution. For group differences we tested 1-way ANOVA with Bonferroni post hoc test, Dunnnett T3, or Kruskal-Wallis test and Mann-Whitney U test, as appropriate. A value of \( P<0.05 \) was considered statistically significant.

### Results

#### Circulating and Local Uteroplacental Ang II
Circulating Ang II was increased in PE rats and in rats after Ang II infusion (Figure 1A). OC rats had similar plasma Ang II levels as SD. In contrast to the circulating plasma, local uteroplacental tissue concentration of Ang II was increased in both PE and OC rats (Figure 1B). Ang II infusion did not lead to an increase in uteroplacental Ang II. Thus, PE is a rat model with high uteroplacental and circulating Ang II levels. OC results in solely high uteroplacental Ang II values at term and normal circulating values. Human renin and angiotensinogen (hAogen) mRNA expressions were significantly elevated in the OC and PE uteroplacental units and were undetectable in SD and Ang II (Figure S1, available in the online Data Supplement). hAogen expression was significantly higher in PE compared with OC, especially in the mesometrial triangle. Ang II infusion raised the circulating Ang II values but not the uteroplacental Ang II concentrations.

#### Blood Pressure, Albuminuria, and Intrauterine Growth Retardation
Telemetric blood pressure measurements showed that mean arterial blood pressure significantly increased PE and Ang II and decreased in OC in the last third of pregnancy. Blood pressure was significantly increased in PE and Ang II compared with SD. Mean arterial blood pressure was significantly decreased in OC versus control (Figure 2A). Albuminuria was present in PE and Ang II–infused rats, but not in OC or SD rats, in the last third of pregnancy (Figure 2B). Placental weight was lower in PE and Ang II–infused rats. OC rats had placental weights no different from SD rats (Figure 2C, left). Fetal body weight was reduced in PE, OC, and Ang II–infused rats (Figure 2C, middle). To further
characterize intrauterine growth retardation (IUGR), we investigated the brain:liver ratio in the fetuses. In PE and Ang II–infused rats, we found a reduced liver and preserved brain weight, leading to an increased brain:liver ratio consistent with IUGR (Figure 2C, right). The OC rats had a normal brain:liver weight ratio and a normal placenta weight, so that the criteria for IUGR were not fulfilled, although these rats had pups small for gestational age compared with SD rats. These data indicate that PE-like features and IUGR were only present in the groups with high circulating Ang II levels.

However, slow fetal growth without signs of IUGR took place when uteroplacental Ang II was elevated, even with normal circulating Ang II levels.

**Trophoblast Invasion and Spiral Artery Vascular Remodeling**

Endovascular trophoblast invasion was evaluated in the mesometrial triangle in 3 zones according to the immunohistograph picture, which was stained with cytokeratin 7 to identify trophoblasts (Figure 3A, left) and as described by guest on October 30, 2017 http://hyper.ahajournals.org/ Downloaded from

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**Figure 2.** A, Telemetrically measured mean arterial blood pressures. PE rats and Ang II–infused rats showed a significantly increased blood pressure over time, whereas mean arterial pressure within OC rats significantly decreased toward the end of pregnancy (*P<0.05). Analyzing the different groups among each other, mean arterial blood pressure was significant for PE, Ang II, and OC vs SD controls (†P<0.05). The arrow with plug indicates the observation of plug in the vagina, indicating day 1 of pregnancy. B, Albuminuria, which occurred in PE and Ang II–infused rats (*P<0.01 vs SD; †P<0.01 vs OC). C, Placenta weight (left), fetal weight (middle), and brain:liver weight ratio (right). Placental weight was reduced in PE and Ang II rats. Fetal weight was decreased in PE, Ang II, and OC rats. Brain:liver ratio showed the difference in PE and Ang II rats, indicating IUGR in these 2 groups but not in OC rats. Values are mean±SEM (*P<0.001 vs SD; †P<0.001 vs OC).
earlier.\textsuperscript{5,13,18} We showed earlier that deep endovascular trophoblast invasion was significantly increased in PE and OC rats, resulting in altered vascular remodeling of spiral arteries (Figure 3A, right). In contrast, Ang II infusion resulted in a reduced deep-trophoblast invasion in the mesometrial triangle compared with SD controls (Figure 3B, left). Examples of reduced trophoblast invasion by Ang II infusion compared with SD are shown (Figure 3B, right). In accord with alterations in trophoblast invasion, persistence of vascular smooth muscle cells (VSMC) was increased in Ang II–infused compared with SD placenta, confirming this result. C, Increased persistence of VSMCs in the spiral arteries in Ang II–infused rats, indicating reduced vascular remodeling, confirming B. D, Doppler ultrasound of the uterine arteries. Resistance index was increased in Ang II–infused rats on day 21 of gestation compared with controls. OC and PE rats show reduced resistance index. Values are mean\textpm SEM (\textit{\textasteriskcentered} \textit{\textless} 0.05 vs SD).

### Invasion, Migration, and Motility of Trophoblasts

We next investigated human trophoblasts from first-trimester pregnancies to determine the effect of uteroplacental Ang II. We stimulated SGHPL-4 cells, which were derived from primary human first-trimester extravillous trophoblasts. The cells were treated with 2 concentrations of Ang II and were studied with time-lapse microscopy (Figure 4A). Ang II dose-dependently increased the various aspects of invasion (showing more and deeper invasion) and motility. The effects of Ang II were blocked completely with losartan. To determine whether Ang II has an effect on the migration, villous
explants from first-trimester normal pregnancies were used for in vitro invasion assay experiments in collagen-coated chambers (Figure 4B). Ang II for 72 hours resulted in a strong increase in the migration of extravillous trophoblast cells compared with control cells. These results were reproducible in 3 separate experiments.

**Discussion**

The novel finding in our study was that circulating Ang II reduced trophoblast invasion and vascular remodeling in the uteroplacental unit. We found opposite effects of increased circulating and uteroplacental Ang II in pregnant rats. Ang II–infused rats exhibited an increased blood pressure in the third trimester, albuminuria, and IUGR. The OC model, in which only uteroplacental tissue Ang II is increased, showed increased trophoblast remodeling. Blood pressure was lower than in controls, no albuminuria was present, and pup weights were reduced. In the PE cross, in which circulating and tissue Ang II are increased, we observed hypertension in the last trimester, albuminuria, IUGR, increased trophoblast invasion, and uteroplacental vascular remodeling. Finally, by applying Ang II directly to human first-trimester trophoblasts and villous explants, we found that invasion, migration, and motility were increased in response to local Ang II. We believe that our results show a different and unique function for uteroplacental Ang II in pregnancy. Uteroplacental Ang II but not circulating Ang II increased trophoblast invasion and vascular remodeling.

Several studies have shown the presence of a local tissue-specific renin-Ang system in the uteroplacental unit of normal pregnancies. Estrogen increases both tissue and circulating levels of angiotensinogen and renin. Plasma Ang II, together with angiotensinogen and plasma renin activity, is increased during normal pregnancy. However, the physiological function of a stimulated uteroplacental renin-Ang system during pregnancy is unknown. Our results of an increased trophoblast invasion by tissue Ang II are in line with the human results that we reported earlier in that increased renin, angiotensinogen, Ang-converting enzyme (ACE), and Ang II are substantially increased in the maternal part of the uteroplacental unit, the decidua, compared with the fetal part of the placenta. We now suggest that the maternal-fetal Ang II gradient contributes to trophoblast invasion and migration. Rosenfeld and Gant reported that Ang II infusion increased blood pressure in sheep but reduced uteroplacental
perfusion, which was measured invasively with chroni-
cally implanted flow probes around both major uterine
arteries. Their experiments suggested an autoregulation of
uterine blood flow. Earlier, Ferris et al.\textsuperscript{21} showed that an
increase of uterine renin production resulted in an increase
in uterine perfusion in rabbits. Uterine perfusion was
determined with radioactive microspheres lodged in the
uterus and placenta after injection in the left ventricle in
that study. Li et al.\textsuperscript{22} were the first to show that endocrine-
active decidual cells express both renin and angioten-
sinogen in the human uteroplacental unit. They suggested
that decidual cells regulate a tissue renin-Ang system
expression in the endometrium. Anton and Brosnihan\textsuperscript{7}
were the first to suggest a different regulation for circulat-
ing and uteroplacental Ang II in PE. They observed
contrasting changes for Ang II levels in the circulation and
chorionic villi in human preeclamptic pregnancy. Al-
though circulating Ang II was significantly decreased, the
expression of local tissue Ang II was augmented in the
placenta of the preeclamptic women. Uteroplacental Ang II
in the OC and PE groups was elevated because the
expression of both human renin gene and hAogen mRNA
in the uteroplacental unit was increased. hAogen concen-
trations were responsible for the different Ang II levels in
OC and PE rats. Morgan et al.\textsuperscript{23} were able to show
increased expression of an angiotensinogen variant in
human decidual spiral arteries that are involved in the
generation of atherotic (a histopathologic term applied to
human decidual spiral arteries that are involved in the
generation of atherotic) lesions overrepresented in PE.

Trophoblast cells of the human placenta proliferate, mi-
grate, and invade the pregnant uterus and remodel its vascula-
ture. Trophoblast-associated spiral artery remodeling is an
important uterine adaptation mechanism of human preg-
nancy, allowing an adequate maternal blood supply to the
placenta. Few factors have been shown to promote tropho-
blast invasion and survival of these fetus-derived cells in the
maternal surroundings, including corticotropin-releasing hor-
more, insulin-like growth factor binding protein 1, and
various cytokines, such as interleukin 1.\textsuperscript{25} The role of many
growth factors and proto-oncogenes has not yet been thor-
oughly studied in the decidua.

We found that fetal weight was decreased in the Ang
II–infused group compared with controls. Similar to patients
with PE, we observed a shallow trophoblast invasion, as well
as persistence of VSMCs and reduced spiral artery remodel-
ing in the group with chronic Ang II infusion. The increased
resistance indicates a disturbed placental function with re-
duced transformation of the arterioles into a highly dilated
vessel. In accordance with the human pathology in PE, the
increased vascular resistance led to IUGR and decreased birth
weight in this group. The pathophysiological processes un-
derlying IUGR are highly complex and incompletely under-
stood. The finding that the fetal weight was decreased in the
OC group appears surprising, because trophoblast invasion is
increased and vascular resistance decreased. The pups exhib-
it lower birth weight, but not IUGR, suggesting a different
mechanism compared with the Ang II–infused group that did
exhibit IUGR. In earlier studies, we showed that atherosis is
present in OC, which also might contribute to impaired fetal
growth.\textsuperscript{3,5} We noted focal necrosis in the arterial wall in 25%
of spiral arteries in that study. The atherosis in these models
is similar to the acute atherosis associated with placental
infarcts observed in uteroplacental spiral arteries of pre-
eclamptic women.\textsuperscript{26} The impact of this vasculopathy on fetal
growth is unclear.\textsuperscript{5,27} Shibata et al.\textsuperscript{28} showed that Ang II
suppresses Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in human placental villi, consistent
with possible adverse effects of enhanced placental Ang II
on fetal growth. These findings are consistent with those of
our OC.

We showed that local Ang II caused human trophoblasts
to migrate and invade, similar to the trophoblast behavior
in the PE and OC models, where uteroplacental Ang II is
elevated. These data could suggest similar effects and
functional roles of local uteroplacental Ang II in our rat
model and in the decidua of pregnant women. Furthermore,
our findings could suggest that Ang II plays a central role
in signaling trophoblast invasion and, ultimately, spiral
artery remodeling. Ang II induces migration of various
cells, including dendritic cells, VSMCs, and glomerular
mesangial cells.\textsuperscript{29} The results in trophoblasts are contro-
versial. Xia et al.\textsuperscript{30} found that Ang II inhibits migration of the
trophoblast cell line, HTR-8, via plasminogen activator
inhibitor 1 activation. Ishimatsu et al.\textsuperscript{31} found the opposite.
They showed that direct-application Ang II induces migra-
tion in human cytotrophoblast-like choriocarcinoma cell
lines. Local Ang II also influences endothelial cadherin,
platelet-endothelial adhesion molecule 1, vascular endo-
thelial adhesion molecule 1, and α4 integrins. These
factors are important in facilitating the adhesion-phenotype
switch in trophoblasts, which is required for successful
endovascular invasion and normal placentation.\textsuperscript{32} Taken
together, these data suggest that local Ang II is an
important uteroplacental factor regulating the temporal and
spatial regulation of trophoblast invasion in a paracrine
way.

Circulating Ang II levels in the PE cross increased be-
tween day 9 and day 15, exactly when we started the
Ang II infusion in the Ang II–infused group. However, a
shorter time of exposure to Ang II in the Ang II–infused
group compared with the transgenic group cannot be ex-
cluded as a variable contributing to the observed phe-
notypes. We also excluded the possibility that proteases are
dysregulated in the Ang II–infused group. Aminopeptidase A,
Prolyl-carboxypeptidase, Nephrilysin, ACE-1, and ACE-2
were not induced in the Ang II–infused group compared with
controls (data not shown). Aminopeptidase A, ACE-1, and
ACE-2 were upregulated in OC and PE compared with
control and Ang II (data not shown).

Ours and other laboratories have described activating
autoantibodies directed at the Ang II type 1 receptor in
women with human PE.\textsuperscript{3,33} We made similar observations in
PE rats.\textsuperscript{3,34} The role of these antibodies is still unclear.
However, upregulation of the decidual Ang II type 1 receptor
is also present in PE.\textsuperscript{11,35} Both events could represent a
possible physiological adaptation, suggesting the necessity of
increased Ang II–like activity in promoting trophoblast inva-
sion for spiral artery remodeling. The antibodies could represent malfunction of this mechanism resulting in PE in some women.

The rodent transgenic renin/angiotensinogen model was originally described in mice by Takimoto et al. Hypertension, proteinuria, and IUGR described in that study were very similar to the rat PE model that we report. However, placental abnormalities were different in the 2 species. Takimoto et al detected necrosis in spongitrophoblasts and decidual cells, edematous enlargement, and congestion in chorian cells. In a follow-up study, the group showed that the interaction between fetal vasculature and maternal blood canal in the labyrinth was distorted and the expression patterns of key molecules in neovascularization were dysregulated. The maternal plasma level of sFlt-1 was significantly increased after midterm in that model. In contrast to the mouse, the rat develops deep endovascular and interstitial trophoblast invasion beyond the decidua into the mesometrial triangle, showing a deep endovascular trophoblast-associated spiral artery remodeling. Thus, we can investigate the labyrinth placenta and the mesometrial triangle separately. sFlt-1 is not dysregulated in the rat placenta or in the maternal serum in PE rats.

In spite of the species differences, both models underscore the notion that cell-specific expression of renin and angiotensinogen in the fetomaternal interface strongly influences IUGR and placenta development.

A limitation in our study is that an ACE inhibitor and/or Ang II type I receptor blocker was not tested in our models. Saito et al showed that genetic deletion of the maternal Ang II type 1a receptor, and cross-breeding with the transgenic mouse model significantly ameliorated the phenotype in their mice. The role of Ang II inhibition on trophoblast function, vascular remodeling in the uteroplacental unit, and IUGR requires substantial further study in animal models, particularly because human studies along these lines cannot be performed.

Perspectives

Uteroplacental Ang II promoted trophoblast migration and invasion in rats and humans. This effect could mediate a cross-talk between invasive trophoblasts and the maternal endometrium. Such a paracrine Ang II function in normal pregnancy could also be further activated in pregnancies with abnormal placentation that exhibit shallow trophoblast invasion, as in PE. The concept of a fetal-maternal Ang II gradient warrants further investigations, such as the determination of Ang II in the amniotic fluid.

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Disclosures

None.

References


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Online supplement

Effects of circulating and local uteroplacental angiotensin II in rat pregnancy

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Methods
Animal studies
Local authorities approved the studies along American Physiological Society guidelines. Mean blood pressure, heart rate, and ambulatory activity were continuously recorded by radiotelemetry (Data Sciences International). Sprague-Dawley females were mated with Sprague-Dawley males as controls, namely pregnant Sprague-Dawley (SD). For Doppler studies, the animals were anesthetized with 1.5% isoflurane via an oxygen mask. The pregnant rats were imaged at embryonic days 9.0, 15.0, 18.0, and 21.0 with an ultrasound bio-microscope and a 30-MHz or 40-MHz transducer at 30 frames per second (Model Vevo 660, VisualSonics). The Doppler waveforms were obtained from the proximal uterine artery, arcuate artery, and distal of the main branch of the uterine artery between 2 embryonic implantation sites.

Blood for hormone analysis was drawn by aortic puncture into pre-chilled tubes containing EDTA (6.25 mmol/L) and phenanthroline (26 mmol/L) as anticoagulant and inhibitor of Ang II breakdown in vitro, respectively. The direct renin inhibitor, aliskiren (10^{-6} M), was added to plasma samples for Ang II measurement to prevent Ang II formation in vitro. For tissue immunostaining protocols all implantation sites (placenta with associated mesometrial triangle) were removed, fixed in JB-Fix fixative, and embedded into Paraplast Plus (Sherwood Medical Co) from each rat. Parallel sections were cut step-serially from each implantation site parallel to the mesometrial-fetal axis. A set containing a central maternal arterial channel was selected for further analysis. Parallel, serial cross-sections were obtained and stained for a[alpha]-actin (clone 1A4, Dako, diluted 1/200), anti cytokeratin (clone MNF116, Dako), and anti CD31 (clone TLD-3A12, BDPharmingen). For the quantitative analysis of depth of invasion and spiral artery remodeling, the KS-400 image analysis system (Carl Zeiss) connected to a Zeiss microscope (Axioskop 50) fitted with a color camera (Axiocam MRc5) was used. Briefly, the whole mesometrial triangle was photographed and the images were shade-corrected. The border of the triangle was manually delineated on the a[alpha]-actin immunostained section, and the triangle was divided into three concentric depth levels of equal thickness for the evaluation of the depth of invasion. The lumen of each spiral artery (SA) cross-section was manually delineated and stretches of endovascular trophoblast, fibrinoid, vascular smooth muscle and CD31 positive endothelium were traced separately over the lumen contour tracing. For every cross-section, the length of each traced line was measured and percentages of all lengths were calculated in relation to the total length of the spiral artery lumen contour.

Cell culture
SGHPL-4 cells were derived from primary human first trimester extravillous trophoblasts (EVT) and transfected with the early region of SV40. The cells were cultured in Ham's F10 medium supplemented with 10% FBS, L-glutamine (2 mM), penicillin (100 IU/ml) and streptomycin (100 µg/ml). These cells show similar invasive capabilities to primary EVT and retain features of normal extravillous trophoblast, including expression of HLA-G, cytokeratin-7, BC-1, CD9 and human placental lactogen. Ang II stimulation (10^{-6} M, 10^{-8} M, Calbiochem) and 10^{-6} M losartan (Calbiochem) were applied for 48h in 0.5% serum-reduced medium in matrigel. The effect on the invasive trophoblast outgrowth (number of invasive processes, length of invasion) was assessed by phase contrast microscopy (Olympus) and Image Pro Plus Software (Media Cybernetics). For this purpose, the cells were grown on beads. Motility of SGHPL-4 cells was monitored by time-lapse microscopy (TLM) using an Olympus IX70 inverted fluorescence microscope with a motorized stage and cooled CCD camera (Hamamatsu C4742-95) enclosed in a humidified chamber at 37°C with 5% CO2 in
air at 15 min intervals for 6 hours as described previously. Forty cells were scored in each field of view.

Human placental villous tissue explants from first trimester pregnancies were established and characterized as previously described. Four placentas were obtained from healthy women after legal termination of pregnancy (5-10 weeks of gestation). Informed, written consent was obtained after due approval from the local institutional ethical review board. Briefly, the placentas were washed in sterile phosphate-buffered saline (PBS) until the supernatant was nearly free of blood. Areas rich in chorionic villi were selected and were grown on collagen-coated (Sigma) plates in DMEM/Hams F12 supplemented with 1% P/S/Amphotericin B and 10% human serum. Migration experiments were conducted with collagen coated chambers (24-well plates; BD Biosciences) according to the manufacturer’s instructions. The effects of Ang II images were taken after 72 h and the area of trophoblast outgrowth was analyzed with ImageJ software.

Figure S1. Expression levels of the transgenes human renin and human angiotensinogen (AGT) in the uteroplacental unit of the SD-, OC-, PE- and AngII-group at days 15 and 21 of pregnancy. (nd = not detectable)