0021-972X/95/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 80, No. 4
Printed in U.S.A.

Angiotensin-II Stimulates Estradiol Secretion from Human Placental Explants through AT_1 Receptor Activation*

MUENZE KAYAMBA KALENGA, MARC DE GASPARO, KARL THOMAS, AND RENÉ DE HERTOGH

Physiology of Human Reproduction Research Unit, Department of Obstetrics and Gynecology (M.K.K., K.T., R.D.H.), University of Louvain, Brussels, Belgium; and the Cardiovascular Research Department, Pharmaceuticals Division, Ciba (M.G.), Basel, Switzerland

ABSTRACT

A complete renin-angiotensin system has been shown to be present in human placenta, but its physiological role is poorly known. To investigate the implication of this system in the regulation of steroid hormone secretion, we studied the effect of angiotensin-II on the release of estradiol and progesterone from human placental explants. Our experiments showed that angiotensin-II stimulated estradiol secretion from term placental explants in a dose- and time-dependent fashion, although progesterone release was unaffected. Estradiol release induced by angiotensin-II (0.2 μmol/L) was blocked by angiotensin AT_1 receptor antagonist losartan in a dose-dependent manner, suggesting the involvement of the AT_1 receptor subtype in the process. On the contrary, the angiotensin AT_2 receptor antagonist PD123319 (1 μmol/L) or the angiotensin AT_2 receptor agonist CGP42112A (1 μmol/L) had no effect. Analysis of the amount of steroid hormones in the placental tissues incubated for 12 h showed that angiotensin-II increased estradiol production by 34% compared with the unstimulated explants, whereas the total levels of the estrogen precursor androstenedione and testosterone were decreased by 30–45% in the presence of the peptide, suggesting a stimulatory effect on the aromatization step. This hypothesis was reinforced by the absence of effect of angiotensin-II on both estradiol and testosterone concentrations in the placental explants pretreated with the aromatase inhibitor 4-hydroxyandrostenedione (25 μmol/L). Progesterone synthesis was not affected by angiotensin-II. The present study indicates that angiotensin-II induces the secretion of estradiol from human placenta through the angiotensin AT_1 receptor subtype activation, and this effect seems to be linked to the stimulation of local androgen aromatization. (*J Clin Endocrinol Metab* **80:** 1233–1237, 1995)

THE HUMAN placenta is an important site of synthesis of estrogens during pregnancy (1). On the other hand, it produces abundantly diverse components of the renin-angiotensin system. Angiotensinogen as well as renin and angiotensin-converting enzyme have been found in high concentrations in human placenta. In this structure, the conversion of angiotensinogen leads to the formation of angiotensin-I, -II, and -III. However, the octapeptide angiotensin-II is the most biologically active hormone of the system. In addition, human placenta contains angiotensin-II receptors, mostly expressed as the AT_1 subtype (2–10).

Our previous experiments performed *in vivo* showed that in term human placenta, angiotensin-II was positively correlated to the estradiol/progesterone ratio (11), but it was not known how these steroid hormones are modulated by angiotensin-II. Bumpus *et al.* (12) previously reported that in rat ovarian slices, angiotensin-II stimulated estradiol and 17α-hydroxyprogesterone, but not progesterone, secretion.

The aim of this work was to study the effect of angiotensin-II on the production and/or secretion of estradiol from term human placental explants and to determine which angiotensin-II receptor subtype was involved in the estradiol regulation. The release of progesterone, another sexual steroid abundantly produced within human placenta, was also analyzed in the presence of angiotensin-II. On the other hand, it is of interest to know whether angiotensin-II affects the concentrations of placental androstenedione and testosterone, which are the local estrogen precursors produced by human trophoblast cells.

Materials and Methods

Chemicals

BSA and angiotensin-II were purchased from Boehringer Mannheim GmH (Mannheim, Germany). Ringer solution was obtained from Baxter (Cambridge, MA). Losartan (Dup753), PD123319, and CGP42112A were kindly provided by Ciba Geigy (Basel, Switzerland). 4-Hydroxyandrostenedione was purchased from Sigma Chemical Co. (St. Louis, MO). 17β-Estradiol, androstenedione, and testosterone RIA kits were obtained from Sorin (Saluggia, Italy). The progesterone RIA kit was purchased from Amersham (Amersham, United Kingdom).

Experiments

Permission to use human placental tissues was granted by the medical ethics committee of the Catholic University of Louvain. The placentas were obtained from full-term normal pregnancies (37–40 weeks gestation) within 1 h after spontaneous vaginal delivery and processed as previously described (13, 14). Briefly, the placenta was separated from the amnion and chorion, cut into quarters with a scalpel, and thoroughly

Received May 31, 1994. Revision received November 29, 1994. Accepted December 1, 1994.

Address all correspondence and requests for reprints to: Dr. Muenze K. Kalenga, Ph.D., Physiology of Human Reproduction Research Unit, Department of Obstetrics and Gynecology, University of Louvain School of Medicine, 5330 Avenue Emmanuel Mounier, 1200 Brussels, Belgium.

* This work was supported by a grant from the Fonds National de la Recherche Scientifique (Brussels, Belgium).

washed four or five times through a strainer with sterile 0,9% NaCl solution. One gram of minced tissue was suspended in 5 mL Ringer solution (pH 7.4) containing 10 mmol/L HEPES, 1 mg/mL glucose, 1 mmol/L EDTA, and 0.1 mg/mL BSA; 100 μL of the mixture were collected to determine the initial values. Some explants were pretreated separately for 10 min with the AT_1 receptor antagonist losartan (10 μmol/L), the AT_2 receptor antagonist PD123319 (1 μmol/L), the AT_2 agonist CGP42112A (1 μmol/L), or 4-hydroxyandrostenedione (25 μmol/L) (15–18) before the addition of angiotensin-II (0.2 μmol/L). The doses of losartan, PD123319, and CGP42112A were chosen from their affinity for the angiotensin-II receptors in human placenta and were used in large excess to totally saturate the receptor sites (7). The control and treated samples were incubated with gentle shaking at 37 C for different time periods.

At the end of the indicated incubation time, the tubes were immersed in ice and thereafter centrifuged at 1900 × *g* at 4 C for 5 min. Three hundred microliters of resulting supernatant were removed and stored at −20 C until assayed. To obtain the placental extract, the villous tissue was homogenized in Ringer solution (1:5, wt/vol) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and centrifuged at 3000 × *g* for 30 min. The supernatant from each sample was collected and frozen at −20 C until assayed. 17β-Estradiol, progesterone, androstenedione, and testosterone levels in the incubation medium or in the extract of placental tissues were measured using specific commercial RIA kits. Samples from each experiment were analyzed in the same assay. Statistical evaluation was performed using Student's *t* test, and $P < 0.05$ was considered statistically significant.

Results

Dose-response effect of angiotensin-II on estradiol and progesterone release

Angiotensin-II added to human placental explants incubated for 12 h was shown to stimulate estradiol release in a dose-dependent manner (Fig. 1). At concentrations of 50 and 100 nmol/L, angiotensin-II increased estradiol levels by 135% and 170% of the control value, respectively. The maximal response was obtained with 200 nmol/L angiotensin-II, reaching 210% of the control value. On the contrary, progesterone release was not affected by the addition of angiotensin-II to the placental explants.

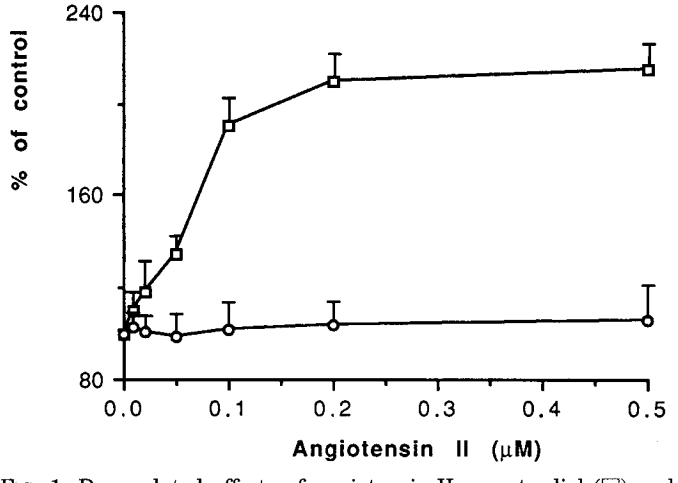

FIG. 1. Dose-related effects of angiotensin-II on estradiol (□) and progesterone (○) release from term human placental explants. The minced villous tissue of placenta were incubated in HEPES-Ringer solution at 37 C for 12 h with increasing concentrations of angiotensin-II. Each *point* represents the mean ± SEM of five experiments.

Time course of estradiol and progesterone release in the presence of angiotensin-II

Estradiol release from human placental explants was shown to increase with incubation time in both angiotensin-II-treated and untreated samples (Fig. 2). Treatment of the placental slices with angiotensin-II for 2 h led to a significant increase in estradiol release compared to that in the control ($P < 0.05$). The maximal hormonal secretion was observed after 4 h of stimulation, reaching 10.56 ± 1.20 ng/g (mean ± SEM), whereas the control value was 4.90 ± 0.81. Progesterone release also increased with time of incubation, but it was not affected by the addition of angiotensin-II.

Synthesis of estradiol and progesterone in human placenta

To study the effect of angiotensin-II on the synthesis of estradiol and progesterone, we compared the amount of each hormone in the nonincubated placental tissue with the sum

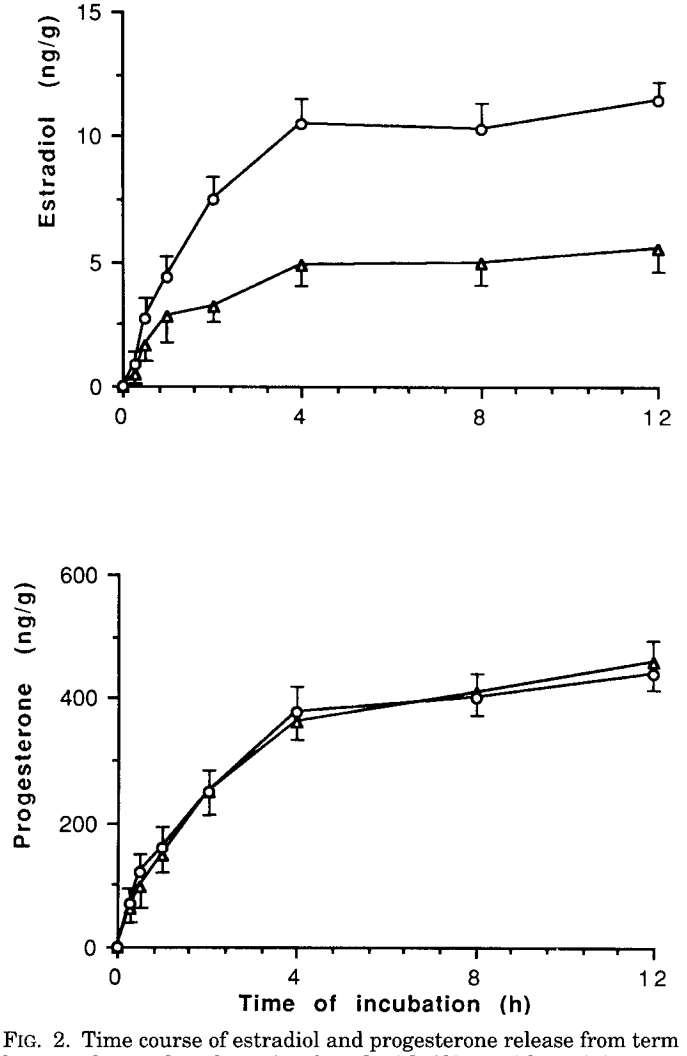

FIG. 2. Time course of estradiol and progesterone release from term human placental explants incubated with (○) or without (△) angiotensin-II (0.2 μmol/L). The results are expressed as the mean ± SEM (n = 5) after subtraction of the initial values. At time zero, the initial values were 2.34 ± 0.60 ng/g for estradiol and 192 ± 35 ng/g for progesterone.

of the hormone levels in the incubation medium and in the tissue extract observed at the end of a 12-h incubation in the presence or absence of angiotensin-II. The levels of the estrogen precursor androstenedione and testosterone were similarly measured.

In the placental explants incubated for 12 h without angiotensin-II (n = 6), the total levels of estradiol, androstenedione, and testosterone reached 26.63 ± 2.04, 47.69 ± 4.05, and 21.12 ± 1.23 ng/g (mean ± SEM), respectively (Fig. 3). Such hormone values were, respectively, 30% ($P < 0.05$), 54% ($P < 0.01$), and 31% ($P < 0.05$) higher than those observed in the nonincubated control placental tissue, suggesting that the three hormones continued to be synthesized during the incubation. Progesterone levels also increased significantly in the placental tissues incubated without angiotensin-II, reaching 130% of the control value ($P < 0.01$).

When the placental fragments were incubated for 12 h in the presence of angiotensin-II (0.2 μmol/L), the total levels of estradiol in the stimulated explants were 34% and 75% higher than those in the unstimulated incubated tissues ($P < 0.01$) and nonincubated controls ($P < 0.001$), respectively. In contrast, the levels of androstenedione and testosterone were significantly lower in the angiotensin-II-stimulated explants than in the unstimulated ones ($P < 0.001$; n = 6). These observations suggested that angiotensin-II induced estradiol synthesis by enhancing the aromatization of androstenedione and testosterone. Analysis of the progesterone levels under the same experimental conditions indicated that angiotensin-II had no effect on the synthesis of this hormone (Fig. 3).

To determine whether angiotensin-II increased placental estradiol levels by stimulating aromatase activity, we used 4-hydroxyandrostenedione, an irreversible specific inhibitor of aromatase, which was found in this study to inhibit in a dose-dependent manner the increase in estradiol concentrations in the placental explants incubated for 12 h (data not shown). In the presence of 25 μmol/L 4-hydroxyandrostenedione (maximal inhibitory concentration), angiotensin-II failed to increase the amount of estradiol or decrease that of testosterone in the placental explants. The estradiol and testosterone levels in the aromatase inhibitor-pretreated explants were not significantly different regardless of whether angiotensin-II was added to the incubation medium (Fig. 4). This strongly suggests that the effect of angiotensin-II on testosterone and estradiol concentrations in the placental tissues was linked to aromatase activity enhancement. Androstenedione was not measured in the presence of 4-hydroxyandrostenedione because of the cross-immunoreaction between the two steroid products.

Mediating role of angiotensin-II receptor subtypes

To determine which of the AT_1 and AT_2 receptor subtypes was implicated in angiotensin-II-induced estradiol release from human placenta, we used three compounds, losartan (Dup753), PD123319, and CGP421112A, which bind to angiotensin-II receptors with a selective affinity. Various concentrations of these compounds were tested in an attempt to determine their maximal effect on estradiol secretion. In the placental explants incubated for 12 h, losartan decreased the angiotensin-II-stimulated estradiol release in a dose-dependent fashion (Fig. 5). A complete inhibition of the angiotensin-II-induced estradiol secretion was observed with 7.5 and 10 μmol/L losartan, showing the involvement of the AT_1 receptor in the process. The angiotensin AT_2 receptor antagonist PD123319 (1–10 μmol/L) or the angiotensin AT_2 agonist CGP421112A (1–5 μmol/L) had no effect on the secretion of estradiol. However, at concentrations of 7.5 and 10 μmol/L, CGP421112A decreased the secretion of estradiol from the angiotensin-II-treated placental explants by 10%

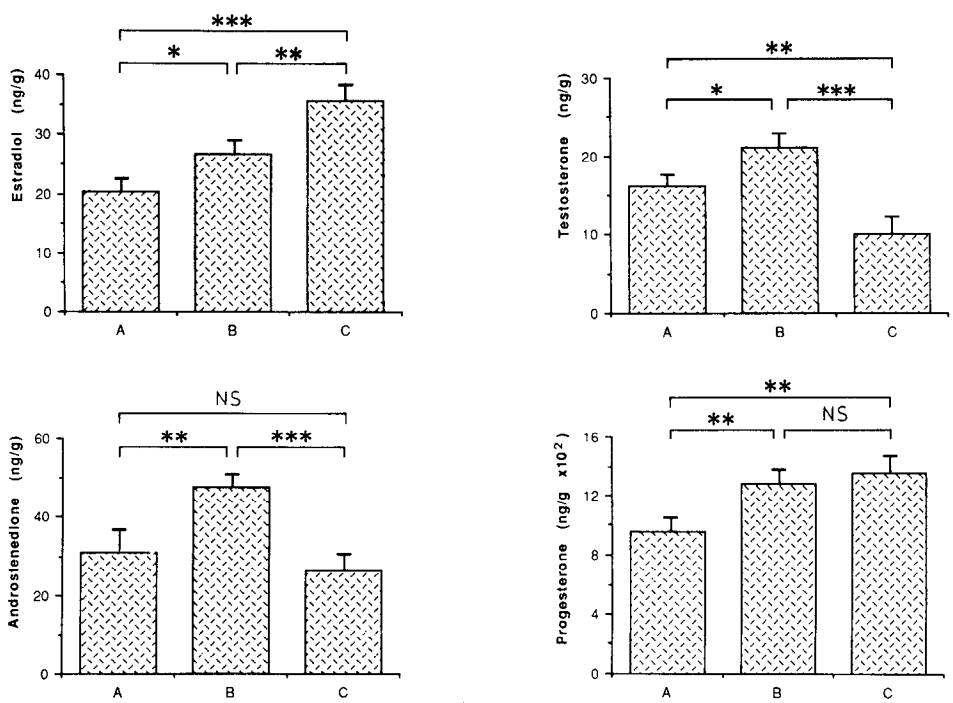

FIG. 3. Total levels of estradiol, androstenedione, testosterone, and progesterone in the villous tissue of term human placenta (sum of the hormone levels in the incubation medium and in the tissue extract). A, Nonincubated control placental tissue. B, Placental tissue incubated for 12 h in HEPES-Ringer solution without angiotensin-II. C, Placental tissue incubated for 12 h in the presence of angiotensin-II (0.2 μmol/L). The results are expressed as the mean ± SEM (n = 6). NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

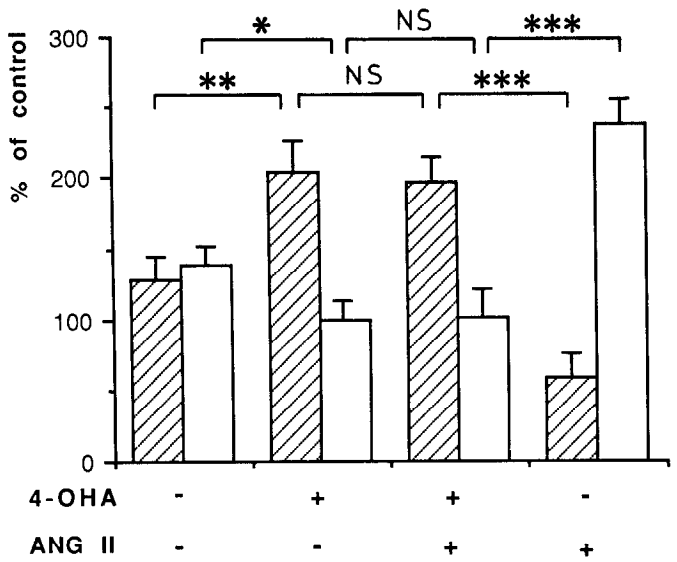

FIG. 4. Total amounts of testosterone (▨) and estradiol (□) in term human placental explants pretreated for 10 min with or without the aromatase inhibitor 4-hydroxyandrostenedione (4-OHA; 25 μmol/L) and incubated for 12 h with or without angiotensin-II (ANG II; 0.2 μmol/L). The results are expressed as a percentage of the nonincubated tissue control value (mean ± SEM; n = 6). NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

and 30%, respectively. Such a phenomenon is due to a partial inhibition of the AT_1 receptor by high concentrations of CGP421112A (7). This compound is known to be selective for AT_2 receptor only at low concentrations (≤1 μmol/L). The levels of estradiol secreted by the placental explants treated with losartan, CGP421112A, or PD123319 alone were not significantly different from the control value (Fig. 6).

Discussion

In the present study, angiotensin-II was shown to stimulate estradiol secretion from human placental explants in a dose- and time-dependent manner. Moreover, angiotensin-II appeared to increase the production of placental estradiol at the end of a 12-h incubation. These results are in agreement with those of other studies, which demonstrated the stimulating effect of angiotensin-II on the synthesis and/or release of steroid hormones. Angiotensin-II was reported to stimulate estradiol and 17α-hydroxyprogesterone secretion from rat ovarian slices (12, 13). On the other hand, it is well known that angiotensin-II induces aldosterone production from adrenal cells by enhancement of both early and late steps of the synthesis; the early step consists of the formation of pregnenolone from cholesterol, and the late step leads to the conversion of corticosterone to aldosterone (19, 20).

For the synthesis of estradiol in human placenta, the early steps of steroidogenesis are performed outside of the placental tissue. It is well known that the human placenta is unable to synthesize dehydroepiandrosterone from pregnenolone because it is deficient in 17α-hydroxylase. In trophoblast cells, dehydroepiandrosterone sulfate (originating from the maternal or fetal compartment) is transformed by sulfatase activity into dehydroepiandrosterone, which is,

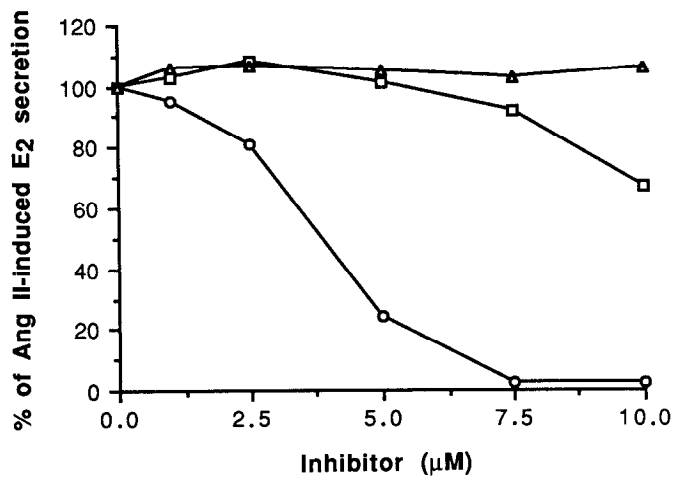

FIG. 5. Dose-related effect of the angiotensin AT_1 receptor antagonist losartan (○), the AT_2 receptor antagonist PD123319 (△), and the AT_2 receptor agonist CGP42112A (□) on angiotensin-II-induced estradiol (E_2) secretion from term human placental explants. The minced villous tissue of placenta was pretreated for 10 min with various concentrations of losartan, PD123319, or CGP42112A and incubated for 12 h with angiotensin-II (Ang II; 0.2 μmol/L). Each *point* represents the mean of three values.

in turn, converted by the action of 3β-hydroxysteroid dehydrogenase isomerase into androstenedione and testosterone; both of the latter will then be aromatized into estrone and estradiol (1). We observed in the present study that in the angiotensin-II-treated human placental tissues, the total amount of estradiol was significantly increased, whereas the levels of androstenedione and testosterone were markedly decreased. Such observations strongly

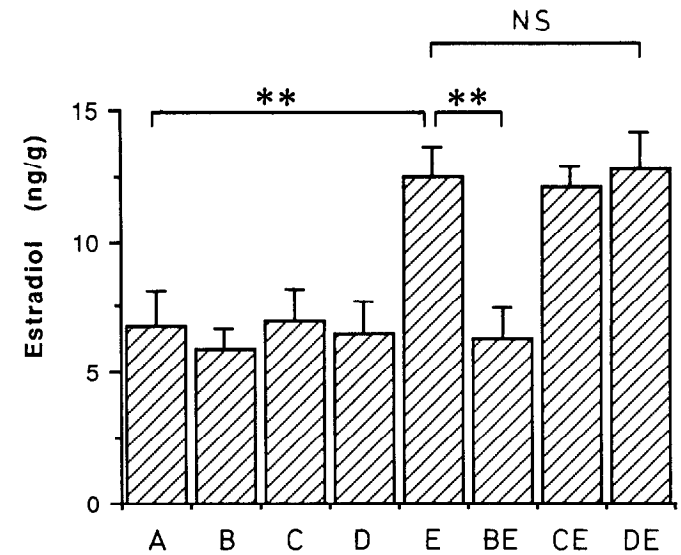

FIG. 6. Estradiol release from term human placental explants. The minced villous tissue of placenta was incubated for 12 h in the presence of the agent studied. A, Control; B, the angiotensin AT_1 receptor antagonist losartan (10 μmol/L); C, PD123319 (1 μmol/L), antagonist of the AT_2 subtype; D, CGP42112A (1 μmol/L), agonist of the AT_2 subtype; E, angiotensin-II (0.2 μmol/L); BE, losartan plus angiotensin-II; CE, PD123319 plus angiotensin-II; DE, CGP42112A plus angiotensin-II. The results are expressed as the mean ± SEM (n = 6). NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

suggest that angiotensin-II stimulated estradiol production by enhancing androstenedione and testosterone aromatization. The absence of effect of angiotensin-II on both estradiol and testosterone concentrations in placental explants pretreated with the aromatase inhibitor 4-hydroxyandrostenedione provides an additional argument that in human placenta, angiotensin-II increases estradiol levels, essentially by enhancement of aromatase activity, rather than by blocking estradiol metabolism. These results are concordant with those of Pucell *et al.* (21), who postulated from the experiments on rat ovarian slices that the angiotensin-II-linked increase in estrogens could be caused by conversion of the local androgens.

Progesterone synthesis is performed in human placenta, following the same steps as those observed in adrenal or ovary. All of the enzymatic machinery necessary for progesterone production is present within human trophoblast cells. These use maternal or fetal cholesterol to produce pregnenolone, which will be converted by the action of 3β-hydroxysteroid dehydrogenase isomerase to progesterone. In the angiotensin-II-treated placental tissues, the levels of progesterone were not found in this study to be significantly different from the control values, indicating that angiotensin-II had no effect on the enzymatic system involved in progesterone synthesis. This observation is in agreement with the results of previous studies, showing that in the presence of angiotensin-II, progesterone release from rat ovarian explants was not affected, whereas the levels of estradiol were significantly increased (12, 13).

The angiotensin-II-induced estradiol release from human placental explants was totally blocked by the AT_1 receptor antagonist losartan, whereas CGP421112A and PD123319, specific ligands of the AT_2 subtype (either agonist or antagonist, respectively), were ineffective. We have previously shown that the human placenta contained high concentrations of angiotensin-II receptors, expressed mostly as the AT_1 subtype (7). These findings strongly suggest that the AT_1 receptor could be the main mediator of angiotensin-II in the stimulation of estradiol secretion in human placenta, as previously reported for human placental lactogen and SP_1 secretion (14).

Interestingly, the human placental villous tissue, composed of a large population of syncytiotrophoblastic cells able to aromatize estrogen precursors (1), was found to abundantly contain not only the angiotensin AT_1 receptor, but also renin, angiotensin-converting enzyme, and angiotensin-II (2–10). The coexistence of these diverse components in human placenta coupled to the angiotensin-II-stimulating and AT_1 antagonist losartan-inhibiting effects on estradiol release provide evidence that the placental renin-angiotensin system plays a role in the regulation of steroid hormones during pregnancy.

In conclusion, this study shows the ability of angiotensin-II to stimulate estradiol secretion from human placenta through the angiotensin AT_1 receptor subtype activation. The stimulating effect of angiotensin-II on estradiol release appears to be linked to the enhancement of androstenedione and testosterone aromatization.

Acknowledgments

We would like to thank Mr. L. Vankrieken and I. Vanderheyden for their technical assistance. We wish also to express our thanks to Mrs. De Clercq and A. Kalenga for their assistance in the preparation of the manuscript.

References

1. **Solomon S.** 1988 The placenta as an endocrine organ: steroids. In: Knobil E, Neill J-D, Erving L-L, et al, eds. The physiology of reproduction. New York: Raven Press; 2093–2144.
2. **August P, Sealey J-E.** 1990 The renin-angiotensin system in normal and hypertensive pregnancy and in ovarian function. In: Laragh J-H, Brenner B-M, eds. Hypertension: pathophysiology, diagnosis, and management. New York: Raven Press; 1761–1778.
3. **Alhenc-Gelas F, Yasui T, Allegrini J, et al.** 1984 Angiotensin I-converting enzyme in fetal membranes and chorionic cells in culture. J Hypertens. 2(Suppl 3):247–249.
4. **Alhenc-Gelas F, Tache A, Saint-André J-P, et al.** 1986 The renin-angiotensin system in pregnancy and parturition, Adv Nephrol. 15:25–33.
5. **Cooke S-F, Craven D-J, Symonds E-M.** 1981 A study of angiotensin II binding sites in human placenta, chorion, and amnion. Am J Obstet Gynecol. 140:689–692.
6. **Ihara Y, Taii S, Mori K.** 1987 Expression of renin and angiotensinogen genes in the human placental tissues. Endocrinol Jpn. 34:887–896.
7. **Kalenga M-K, de Gasparo M, De Hertogh R, Whitebread S, Vankrieken L, Thomas K.** 1991 Human placenta contains only AT_1 angiotensin II receptor subtype. Reprod Nutr Dev. 31:257–267.
8. **Lenz T, Sealey J-E, August P, James G-D, Laragh J-H.** 1989 Tissue levels of inactive and total renin, angiotensinogen, human chorionic gonadotropin, estradiol, and progesterone in human placentas from different methods of delivery. J Clin Endocrinol Metab. 69:31–37.
9. **Tencé M, Petit A.** 1989 Characterization of angiotensin II binding sites in the human term placenta. Mol Cell Endocrinol. 63:111–119.
10. **Wilkes B-M, Krim E, Mento P-F.** 1985 Evidence for a functional renin-angiotensin system in full term fetoplacental unit. Am J Physiol. 249:366–373.
11. **Kalenga M-K, De Hertogh R, Whitebread S, Vankrieken L, Thomas K, de Gasparo M.** 1991 Distribution of the concentrations of angiotensin II, angiotensin II receptors, hPL, prolactin and steroids in human placenta, amnion and chorion. Rev Fr Gynecol Obstet. 86:585–591.
12. **Bumpus F-M, Pucell A-G, Daud A-I, Husain A.** 1988 Angiotensin II: an intraovarian regulatory peptide. Am J Med Sci. 295:406–408.
13. **Pucell A-G, Bumpus F-M, Husain A.** 1987 Rat ovarian angiotensin II receptors characterization and coupling to estrogen secretion. J Biol Chem. 262:7076–7080.
14. **Kalenga M-K, de Gasparo M, Thomas K, De Hertogh R.** 1994 Angiotensin II induces human placental lactogen and pregnancy-specific β1-glycoprotein secretion via an angiotensin AT_1 receptor. Eur J Pharmacol. 268:231–236.
15. **de Gasparo M, Whitebread S, Mele M, et al.** 1990 Biochemical characterization of two angiotensin II receptor subtypes in the rat. J Cardiovasc Pharmacol. 16:31–35.
16. **Kambayashi Y, Bardhan S, Takahashi K, et al.** 1993 Molecular cloning of a novel angiotensin II receptor isoforms involved in phosphotyrosine phosphatase inhibition. J Biol Chem. 268: 24543–24546.
17. **Brechler V, Jones P-W, Levens N-R, de Gasparo M, Bottari S-P.** 1993 Agonistic and antagonistic properties of angiotensin analogs at the AT_2 receptor in PC12W cells. Regul Pept. 44:207–213.
18. **Di Salle E, Giudici D, Briatico G, Ornati G.** 1990 Novel irreversible aromatase inhibitors. Ann NY Acad Sci. 595:357–367.
19. **Fraser R, Brown J-J, Lever A-F, Mason P-A, Robertson J-I-S.** 1979 Control of aldosterone secretion. Clin Sci. 56:389–399.
20. **Campbell W-B, Brady M-T, Gomez-Sanchez C-E.** 1986 Effects of Angiotensin, prostaglandin E_2 and Indomethacin on the early and late steps of aldosterone biosynthesis in isolated adrenal cells. J Steroid Biochem. 24:865–870.
21. **Pucell A-G, Bumpus F-M, Husain A.** 1988 Regulation of angiotensin II receptors in cultured rat ovarian granulosa cells by follicle-stimulating hormone and angiotensin II. J Biol Chem. 263:11954–11961.