Endocrine, *vol. 18, no. 3, 239–245, August 2002* 0969–711X/02/18:239–245/$20.00

Angiotensin-(1–7) in Normal and Preeclamptic Pregnancy

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Angiotensin-(1–7) (Ang-[1–7]) is a bioactive component of the renin-angiotensin system, which has depressor, vasodilatory, and antihypertensive actions. In normal pregnancy, we questioned whether the known rise in plasma angiotensin II (Ang II) is counterbalanced by an increase in plasma Ang-(1–7) and whether Ang-(1–7) levels are decreased in preeclampsia and may thus be a factor involved in the development of hypertension. Nulliparous preeclamptic subjects, third-trimester normotensive pregnant subjects, and a nonpregnant group were enrolled (n = 15/group). Preeclamptic subjects had no previous history of hypertension or renal, connective-tissue, or metabolic disease, but at the time of delivery had significant hypertension (159 ± 3/98 ± 3 mmHg) and ≥3+ proteinuria. Plasma Ang-(1–7) was increased by 51% in normal pregnancy ($p < 0.05$). Plasma Ang I, Ang II, and renin activity were also significantly elevated in normal pregnancy. In preeclamptic subjects, Ang-(1–7) was significantly decreased ($p < 0.01$) compared with normal pregnant subjects. All other components of the renin-angiotensin-aldosterone system, except serum angiotensin-converting enzyme, were reduced in preeclamptic subjects compared with normal pregnant subjects; only plasma Ang II remained elevated in preeclamptic compared with nonpregnant subjects. These studies demonstrate, for the first time, increased plasma Ang-(1–7) in normal pregnant subjects compared with nonpregnant subjects and decreased Ang-(1–7) in preeclamptic subjects compared with normal pregnant subjects. In preeclampsia the decreased plasma Ang-(1–7) in the presence of elevated Ang II is consistent with the development of hypertension.

Key Words: Angiotensin peptides; angiotensin-(1–7); angiotensin II; pregnancy; preeclampsia; vasoactive peptides.

Introduction

Preeclampsia is defined clinically as elevated blood pressure (BP) and proteinuria occurring after 20 wk of gestation. It is one of the leading causes of maternal and fetal morbidity and mortality *(1)*. It is estimated to affect 6–10% of all pregnancies in the United States, and despite extensive research, the pathophysiology of preeclampsia is still poorly understood. Angiotensinogen (Aogen), prorenin, active renin, angiotensin II (Ang II) and aldosterone are all significantly elevated in normal human pregnancy *(2,3)*. The physiologic consequences of the stimulated renin-angiotensin-aldosterone system (RAAS) in normal pregnancy are incompletely understood; and even less understood is the question of how this system may be altered and contribute to the hypertensive disorders of pregnancy.

The cardiovascular consequences of normal pregnancy include an increase in cardiac output by 30–40% and plasma volume by 50%, which are associated with a decrease in total peripheral resistance (TPR). Thus, BP is normal or reduced by the end of the first trimester and reaches its nadir by the second trimester. BP then increases to normal levels by the end of pregnancy. The reasons for the reduced TPR are not established, but a number of vasodilator substances such as prostaglandins *(4,5)*, kallikrein-kinins *(6–9)*, and atrial natriuretic peptides *(10)* have been studied. In preeclampsia, the cardiovascular consequences associated with hypertension include increased TPR, failure to develop the hypervolemia of pregnancy, and reduced renal blood flow and glomerular filtration rate. It has been suggested that preeclampsia may arise not only because of stimulation of vasopressor substances, but because of a defect in the contribution of vasodilator systems. In normal human pregnancy, it is well known that there is a decrease in pressor responsiveness to Ang II *(11)*. By contrast, in women who develop preeclampsia, an increase in sensitivity to Ang II is noted, even prior to the onset of the clinically recognized syndrome *(11)*.

Angiotensin-(1–7) (Ang-[1–7]) is a novel component of the RAAS. It has been shown to be a counterregulator of the actions of Ang II in that it lacks the vasoconstrictor actions of Ang II, has no central pressor effects, and does not stimulate aldosterone *(12)*. This peptide releases nitric oxide (NO) *(13,14)*, bradykinin *(13)*, and prostacyclin *(15)* and vasodilates a number of regional beds *(13–15)*. In animal models, its effects are linked to a decrease in BP and an increase in sodium excretion *(12)*, mechanisms favoring a counterreg-

Received May 31, 2002; Revised July 2, 2002; Accepted July 8, 2002.

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Table 1
Characteristics of Individual Preeclamptic Subjects

Subject no.	Race	Gestational age	BP	Urine protein
1	Hispanic	40	147/100	4+
2	Caucasian	37	175/90	4+
3	Hispanic	41	176/97	4+
4	Caucasian	37	150/90	4+
5	African American	34	140/90	3+
6	Hispanic	38	160/90	3+
7	African American	32	150/110	4+
8	Caucasian	29	180/110	4+
9	Hispanic	27	167/92	4+
10	African American	28	167/91	4+
11	African American	27	160/101	4+
12	Caucasian	30	160/110	4+
13	Caucasian	35	146/103	3+
14	Hispanic	37	152/100	4+
15	Hispanic	32	155/92	3+

ulatory effect exerted within the RAAS. In humans, plasma levels of Ang-(1–7) show a negative correlation to BP *(16)*. Recent findings have demonstrated that it is elevated in hypertensive subjects taking angiotensin-converting enzyme (ACE) inhibitors *(16)*, and in experimental models it contributes to the antihypertensive actions of ACE inhibitors *(17)*.

Secondary to these counterregulatory effects of Ang-(1–7) and the diminished vascular reactivity to Ang II in normal pregnancy, a strong argument can be made for a potential role for Ang-(1–7) in both the vasodilation of normal pregnancy and the pathologic vasoconstriction observed in preeclamptic women. Because circulatory Ang-(1–7) has not previously been measured in human pregnancy, the objective of the present study was to determine whether plasma Ang-(1–7) levels are increased in normal pregnancy and significantly decreased in gestational age–matched preeclamptic subjects.

Results

A total of 15 subjects in each of the three groups was studied. As depicted in Tables 1 and 2, preeclamptic subjects had significant hypertension and proteinuria (all had ≥3+ on catheterized specimen). The results for the angiotensin peptides measured are shown in Fig. 1. Compared with nonpregnant control subjects, normotensive third-trimester pregnant subjects were found to have significant elevation of plasma Ang I, Ang II, and Ang-(1–7). Plasma Ang-(1–7) increased approximately 51% from a nonpregnant level of 15.6 ± 2.1 to 23.6 ± 3.1 pg/mL ($p < 0.05$). In preeclamptic subjects, all three peptides were reduced compared with normotensive third-trimester pregnant subjects. As opposed to Ang I and Ang-(1–7), plasma Ang II levels in

Table 2
Basal Characterization of Pregnant and Preeclamptic Subjects[a]

	Third-trimester pregnant subjects	Preeclamptic subjects
Age (yr)	24.5 ± 0.9	23.9 ± 1.0
BP (mmHg) systolic/diastolic	113 ± 3/67 ± 2	159 ± 3/98 ± 3[b]
Gestational age (wk)	33.7 ± 1.2	33.9 ± 1.2
Proteinuria (0–4+)	None	>3+[b]

[a]Values are expressed as mean ± SEM for BP and gestational age.
[b]$p < 0.05$.

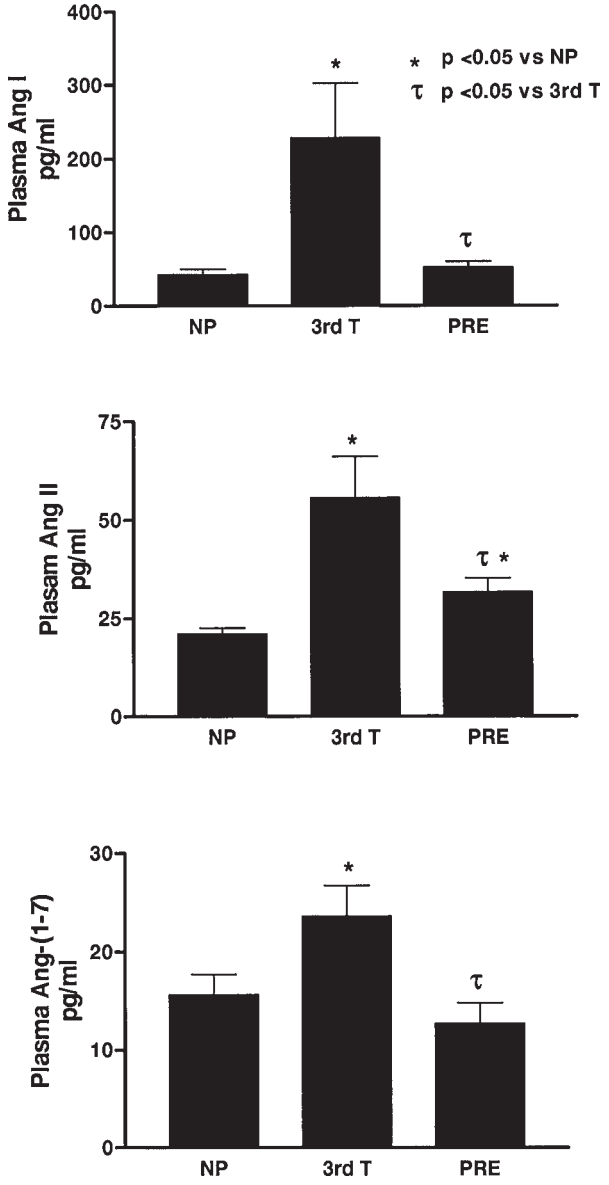

Fig. 1. Plasma levels of angiotensin peptides (Ang I, Ang II, and Ang-[1–7]) in three studied groups. NP, nonpregnant; 3rd T, third-trimester normotensive pregnant subjects; PRE, preeclamptic subjects. $n = 15$ per group. *$p < 0.05$ vs NP; τ, $p < 0.05$ vs 3rd T.

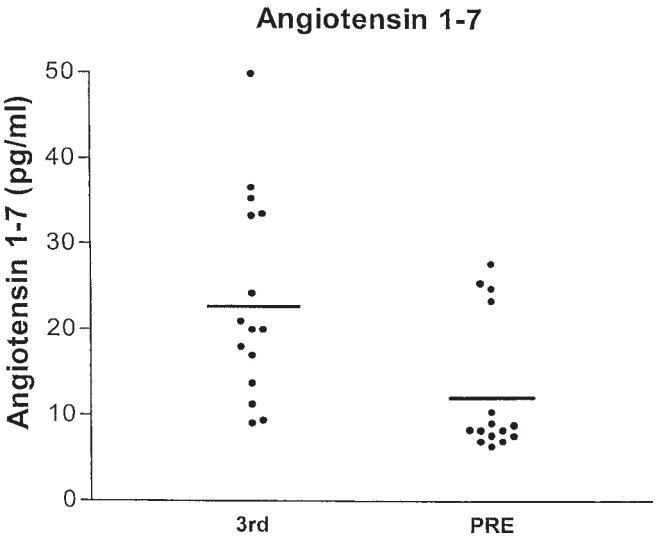

Fig. 2. Scatter gram demonstrating individual values for plasma Ang-(1–7) in normotensive third-trimester pregnant subjects (3rd), and matched for parity, gestational age, and race, preeclamptic subjects (PRE). The mean value is also demonstrated for each group.

preeclamptic subjects remained elevated compared with those of nonpregnant control subjects. A scatter gram depicting individual values for plasma Ang-(1–7) in each of the normotensive third-trimester pregnant subjects and preeclamptic subjects is shown in Fig. 2. Of note is that 11 of the 15 preeclamptic subjects had clearly suppressed levels of Ang-(1–7). Figure 3 illustrates the ratios of Ang-(1–7)/Ang II, Ang-(1–7)/Ang I, and Ang II/Ang I. The median values of the ratios showed significant decreases in all ratios of nonpregnant vs normal pregnant subjects, whereas only the Ang-(1–7)/Ang II ratio was significantly decreased in preeclamptic vs nonpregnant subjects. A comparison between third-trimester normal pregnant vs preeclamptic subjects showed that Ang-(1–7)/Ang I and Ang II/Ang I ratios were different. Figure 4 illustrates the correlations of Ang-(1–7) with systolic and diastolic BP in normal pregnant and preeclamptic subjects. Both systolic ($r = -0.51$; $p < 0.004$) and diastolic ($r = -0.44$; $p < 0.02$) BP showed significant, negative correlations with plasma Ang-(1–7).

Values for plasma renin activity (PRA) and serum ACE activity are depicted in Fig. 5. PRA increased nearly ninefold in third-trimester pregnant subjects vs nonpregnant control subjects. In preeclamptic pregnant subjects, PRA was significantly suppressed compared with normal pregnant subjects, reaching levels not different from nonpregnant subjects. By contrast, serum ACE activity was decreased in third-trimester normotensive pregnant subjects compared with control subjects and returned to nonpregnant control values in preeclamptic subjects.

Both progesterone (27.0 ±1.1 vs 160.5 ± 18.1 ng/mL; nonpregnant vs third-trimester pregnancy, $p < 0.05$) and 17β-estradiol (50.5 ± 6.0 vs 10,304 ± 1225.6 pg/mL; non-

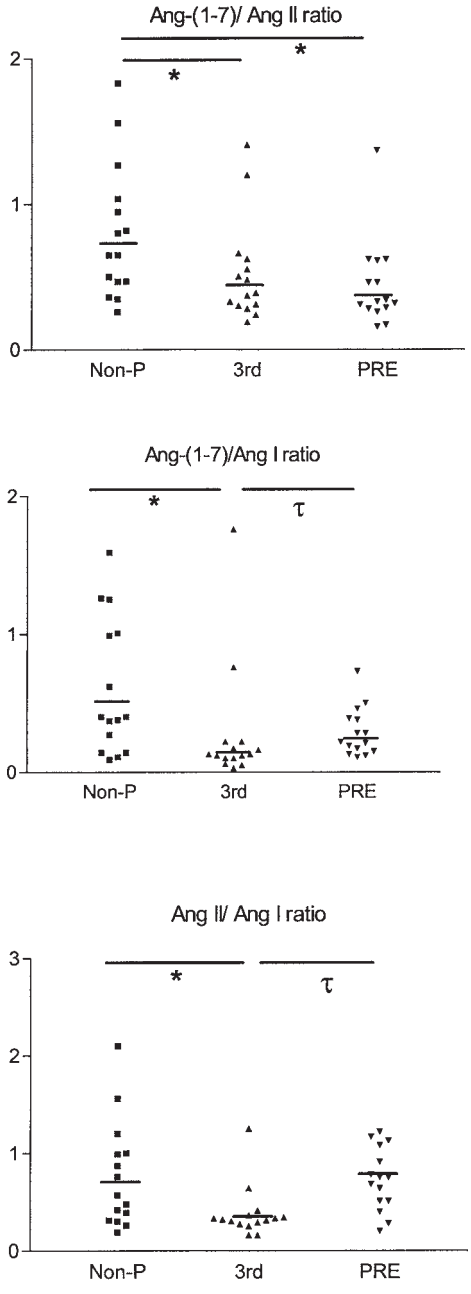

Fig. 3. Scatter gram of ratios of Ang-(1–7)/Ang II, Ang-(1–7)/Ang I, and Ang II/Ang I. The median values of the ratios were 0.652, 0.390*, and 0.327* for Ang-(1–7)/Ang II ($H = 8.784$ with 2 df; $p = 0.012$); 0.398, 0.126*, and 0.221$^{\tau}$ for Ang-(1–7)/Ang I ($H = 8.496$ with 2 df; $p = 0.014$); and 0.573, 0.323*, and 0.753$^{\tau}$ for Ang II/Ang I ($H = 9.655$ with 2 df; $p = 0.008$) for nonpregnant, 3rd and PRE, respectively. *$p < 0.05$ vs nonpregnant and $^{\tau}p < 0.05$ vs 3rd.

pregnant vs third-trimester pregnancy, $p < 0.05$) were significantly increased in pregnant subjects. There was no significant difference, however, for either when comparing normotensive pregnant subjects with preeclamptic subjects (progesterone: 178.7 ± 22.5 ng/mL; 17β-estradiol: 9463.8 ± 2301.0 pg/mL; not significant from third-trimester preg-

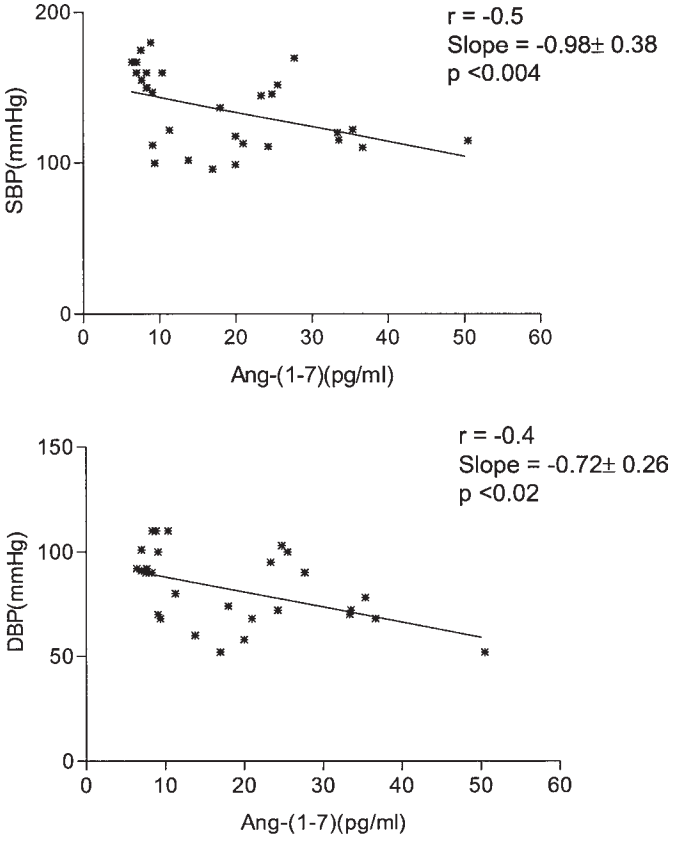

Fig. 4. Scatter gram depicting relationship between systolic BP (**A**) and diastolic BP (**B**) and plasma levels of Ang-(1–7) in 15 normal pregnant and 15 preeclamptic subjects.

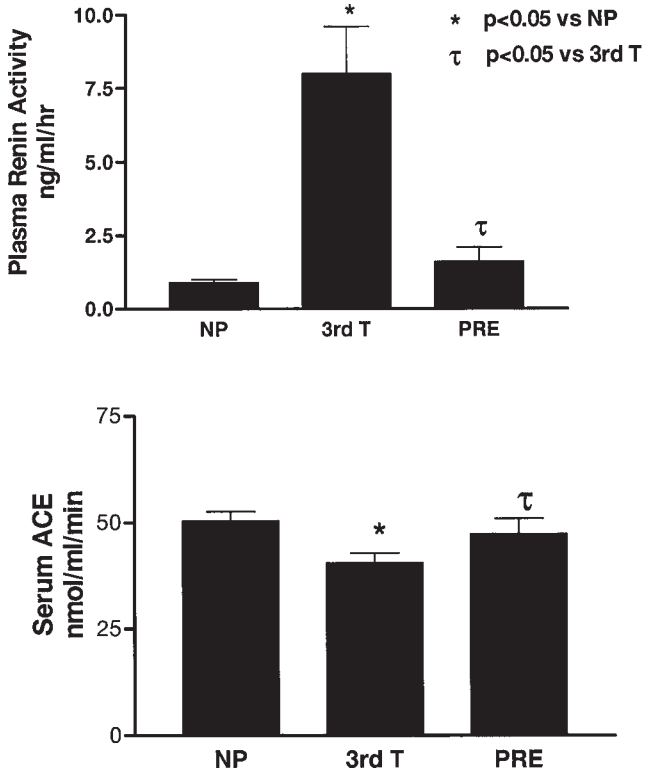

Fig. 5. Values for PRA and serum ACE in three studied groups. Abbreviations are as stated in Fig. 1. $n = 15$ per group. $^{*}p < 0.05$ vs NP; $^{\tau}p < 0.05$ vs 3rd T.

nancy). Plasma cortisol increased approximately fourfold in normotensive pregnant subjects (6.6 ± 0.7 μg/dL vs 28.2 ± 3.2 μg/dL; $p < 0.05$). Compared with third-trimester subjects, preeclamptic subjects were found to have significantly reduced cortisol levels (28.2 ± 3.2 vs 19.5 ± 3.5 μg/dL; $p < 0.05$).

Discussion

We have demonstrated, for the first time, that plasma Ang-(1–7), a novel vasodilator of the RAAS, is increased in women in normal human pregnancy as compared to nonpregnant subjects and is reduced in preeclampsia as compared to normal pregnancy. The simultaneous measurement of other components of the RAAS showed an increase in PRA, plasma Ang I, and plasma Ang II in normal pregnancy. In preeclamptic women, PRA, Ang I, and Ang II were decreased as compared to normal pregnancy. However, plasma Ang II remained significantly elevated above the levels found in nonpregnant women.

Although we currently do not know the physiologic or pathophysiologic importance of the observed changes in plasma concentration of Ang-(1–7), numerous lines of evidence support the concept of a role for this novel peptide in the regulation of arterial pressure. Ang-(1–7) has been shown to release NO, kinins, and prostaglandins *(13–15)*. The antihypertensive properties of this peptide have previously been demonstrated in transgenic and spontaneously hypertensive experimental rat models treated with ACE inhibitors and Ang II antagonists. Blockade of the actions or formation of the heptapeptide reversed the reduction in BP of animals chronically treated with ACE inhibitors *(17)*. Consistent with this effect has been the demonstration of increased circulating Ang-(1–7) during ACE inhibitor treatment *(16,18,19)*. Furthermore, in salt-sensitive hypertensive patients treated with the vasopeptidase inhibitor omapatrilate, BP was effectively controlled by a mechanism associated with increases in urinary Ang-(1–7), suggesting a contribution of Ang-(1–7) to the antihypertensive renal response of this inhibitor *(20)*.

The observation in our study that there was an inverse association between circulating Ang-(1–7) and both systolic and diastolic BP provides support for a role of Ang-(1–7) in the control of BP. In preeclampsia, the data suggest a potential role for reduced production of Ang-(1–7) contributing to the elevated BP. Furthermore, these data are consistent with negative correlations of urinary Ang-(1–7) with BP found in normotensive and hypertensive subjects *(21)*.

A recent study by Valdes et al. *(22)* showed that urinary Ang-(1–7) increases throughout gestation, reaching levels by 35 wk of gestation that were 20-fold elevated over the levels found during the normal menstrual cycle or lactation. Although the direction of change of Ang-(1–7) in plasma and urine in normal pregnancy is the same, the greater fold increase found in the urine suggests a local kidney production of Ang-(1–7). Indirect evidence for a potential role of Ang-(1–7) in cardiovascular regulation during pregnancy has previously been reported, however. Pipkin and Baker *(23)* reported that acute infusions of Ang II have pressor as well as depressor effects in both nonpregnant and pregnant subjects. They further noted that the depressor response observed after cessation of the infusion deepened as pregnancy progressed. Although this depressor response may be secondary to the release of other vasodilators or actions of Ang II at the AT_2 receptor subtype *(24)*, increases in Ang-(1–7) (formed by metabolism of Ang I or Ang II) could also be a mediator of this response.

The ratios of the angiotensin peptides provide evidence that pregnancy and preeclampsia have significant effects on the balance of the peptides and offer insights into changes in the metabolism of the peptides. Both pregnant and preeclamptic subjects had a significant decrease in the ratio of Ang-(1–7)/Ang II compared with nonpregnant subjects; however, there was no difference in the ratio between the pregnant and preeclamptic groups. This lack of change in the vasodilator and vasoconstrictor ratio of the two pregnant groups is different from that described previously for the ratio of prostacyclin/thromboxane within the prostaglandin casade by Walsh *(4)* and suggests that major changes for angiotensin peptide processing occur when comparing preeclampsia and pregnancy to nonpregnancy. Another possible reason for the lack of change in our study as compared to the observation by Walsh *(4)* is that measurements in that study were made by assessing the production rate of prostaglandins from uterine tissue. Similar studies characterizing local tissue production need to be conducted for the angiotensin peptides.

From previous studies and our study, we know that the enzyme contribution to Ang metabolism is complicated in pregnancy and preeclampsia; that is, PRA was increased in normal pregnancy and decreased in preeclamptic subjects compared with normal pregnant subjects, whereas ACE activity was reduced in normal pregnancy and returned to normal levels in preeclamptic subjects. The change in the ratios of Ang II/Ang I between pregnant and preeclamptic subjects, an index of ACE activity, reflects the change in serum ACE activity. The change in ratio between normal pregnant and preeclamptic subjects may also indicate that there is an abnormality in Ang II formation that may be contributing to the hypertension of pregnancy. It is possible that the hypertension of the preeclamptic subjects may be inhibiting renin release, yet the amount of Ang II being formed is more than would normally occur in the face of such low renin levels. The increased ACE activity in preeclampsia vs normal pregnant subjects would also contribute to the increased Ang II.

Other enzymes contribute to the formation of Ang-(1–7), including prolyl endopeptidase and neprilysin *(25)*, and, thus, these enzymes could contribute to the difference in the ratio of Ang-(1–7)/Ang I found between preeclamptic and pregnant subjects. Their regulation during pregnancy and preeclampsia has not been studied to any significant degree. However, the predominant factor contributing to the decrease in the ratio of Ang-(1–7)/Ang I in normal pregnancy may be the increase in Ang I concentration following both the increase in PRA and decrease in serum ACE.

In summary, the present studies have demonstrated, for the first time, that plasma Ang-(1–7) is increased in women in normal human pregnancy compared with nonpregnant subjects, and in carefully defined preeclamptic women, plasma Ang-(1–7) is reduced compared with normal pregnant subjects. We speculate that normal pregnancy is a balance of the RAAS comprising both vasocontrictor (Ang II) and vasodilator (Ang-[1–7]) pathways. In preeclampsia, the balance of the RAAS is shifted to an elevated vasoconstrictor component in the face of a reduced vasodilator pathway by mechanisms that deserve further study. In normal pregnancy, the counterregulating contribution of Ang-(1–7) favors a normal or a reduced BP. In preeclampsia, the persistence of an activated vasoconstrictor (Ang II) in the presence of a diminished vasodilator (Ang-[1–7]) may contribute to an elevation in BP and increased total peripheral resistance Balancing the activated RAAS by a vasodilator component within the same system is a new hypothesis that may explain the overall vasodilator state in normal pregnancy and the disrupted BP regulation in preeclampsia. Future research will be aimed at elucidating the physiologic significance of the changes in plasma concentration observed in the present study.

Materials and Methods

Subjects and Experimental Protocol

Three separate groups of subjects were enrolled: nonpregnant women, third-trimester normotensive pregnant women, and women diagnosed with preeclampsia. For the nonpregnant group, individuals were normotensive, did not currently use hormonal contraception, and were younger than 40 yr (mean ± SEM: 28.1 ± 1.5 yr). For the normotensive third-trimester subjects, all women were normotensive and had an absence of proteinuria. For the preeclamptic group, inclusion criteria were the following: subjects were nulliparous, had a BP >140/90 mmHg, had a proteinuria of ≥2+, and had a normal BP at the beginning of prenatal care and throughout pregnancy prior to the onset of preeclampsia. Pregnant women (third-trimester normotensive and preeclamptic) were matched for gestational age and parity (all

nulliparous). All three groups were matched for race, and none of the subjects had a history of cardiovascular, renal, connective-tissue, or metabolic disease.

The study was approved by the institutional review committee at both Wake Forest University School of Medicine (WFUSM) and Forsyth Medical Center. The procedures followed were in accordance with institutional guidelines. After signed informed consent was obtained, venous blood sampling was performed. Samples were analyzed for components of the RAAS, estradiol, progesterone, and cortisol as described next.

Assays

Angiotensin Peptides

Blood was collected in a cocktail of protease inhibitors as previously described *(26)*. Plasma was extracted using Sep-Pak columns activated with 5-mL sequential washes of a mixture of ethanol:water:4% acetic acid and methanol: ultrapure water:4% acetic acid. After the sample was applied to the column, it was washed with ultrapure water and acetone and eluted with a 3.3-mL wash of a mixture of ethanol: water:4% acetic acid. The eluted samples were divided for the three radioimmunoassays (RIAs) and the solvent was evaporated. For Ang II, samples were reconstituted in assay buffer, and for Ang I and Ang-(1–7), a Tris buffer with 0.1% bovine serum albumin was used. Recovery of radiolabeled angiotensin added to the sample and followed through the extraction averaged 90.1% ($n = 81$). Samples were corrected for recovery. Ang I was measured using a modification of the commercially available New England Nuclear RIA kit (Rianen®; Dupont, Billerica, MA). Ang II was measured using the Quest/Nichols Institute RIA (San Juan Capistrana, CA), and Ang-(1–7) was measured using the antibody previously described *(26)*. The minimum detectable levels of the assays were 2.5 pg/tube for Ang-(1–7), 4 pg/tube for Ang II, and 1.25 pg/tube for Ang I. Values at or below the minimum detectable level of the assay were arbitrarily assigned that value for statistical analysis. The intraassay coefficient of variation (CV) was 18% for Ang I, 12% for Ang II, and 8% for Ang-(1–7).

Plasma Renin Activity

PRA, defined as the rate of Ang I generation from endogenous substrate, was measured in incubated plasma treated with EDTA and phenylmethylsulfonyl fluoride to prevent the degradation of the generated peptide. Ang I was quantitated by RIA using a clinical human renin kit (DiaSorin, Stillwater, MN). The interassay precision is 7.8% for values with a mean of 6.6 ng/(mL·h).

Angiotensin-Converting Enzyme

ACE was measured using the radioactive tripeptide ^{3}H-Hip-Gly-Gly and incubating the serum at pH 8.0 for 60 min at 37ºC (Alpco, Windham, NH). The interassay precision of the assay is 5.9% for a mean of 39.9 nmol/(mL·min).

Estradiol and Progesterone

Serum estradiol and progesterone were measured by RIAs using commercially available kits (Diagnostic Systems, Webster, TX). The separation of free and bound estradiol and progesterone was achieved using a double antibody system. The sensitivity of the estradiol kit is 4.7 pg/mL. The crossreactivity of the estradiol antibody with estrone is 3% and with estradiol is <1%, and <0.0001% with testosterone, DHEA, and diethystilbestrol. The sensitivity of the progesterone assay is 0.12 ng/mL. The crossreactivity of the progesterone assay is 6% for 5-α-pregnandiene-3,20-dione and not detectable for cortisol, cortisone, estradiol, and testosterone. The intra- and interassay CVs were 8 and 10%, respectively, for each assay.

Cortisol

Plasma cortisol was measured by RIA using a commercially available kit from Incstar (Stillwater, MN).

Statistical Analyses

All values are expressed as mean ± SEM. Groups were compared by standard one-way analysis of variance (ANOVA) and Student-Neuman-Keuls post-hoc test. For the ratios, one-way ANOVA was used to test normality. The Kruskal-Wallis one-way ANOVA on ranks was used to test the difference in median values, followed by the multiple comparison procedure of Student-Newman-Keuls methods. The relationship between plasma Ang-(1–7) and arterial blood pressure was investigated using linear regression. Values were considered significantly different for $p < 0.05$.

Acknowledgments

We acknowledge the contributions of the Hypertension Core Laboratory of WFUSM; and of Cheryl Moorefield, RN, for her efforts in patient recruitment and sample collection. This work was supported in part by an intramural grant from WFUSM and by a grant from NIH/NHLBI (HL51952).

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