Endocrine, *vol. 16, no. 2, 117–122, November 2001* 0969–711X/01/16:117–122/$11.50

Urinary Vasodilator and Vasoconstrictor Angiotensins During Menstrual Cycle, Pregnancy, and Lactation

Gloria Valdés,[1,3] Alfredo M. Germain,[2,3] Jenny Corthorn,[3] Cecilia Berrios,[2] Arnaldo C. Foradori,[4] Carlos M. Ferrario,[5] and K. Bridget Brosnihan[5]

Departamentos de [1]Nefrología and [2]Obstetricia/Ginecología, [3]Centro de Investigaciones Médicas, [4]Unidad de Laboratorios Clínicos, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile; and [5]Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston-Salem, NC

Since normal human pregnancy is characterized by normotension in the face of an increased renin-angiotensin-aldosterone system (RAAS), we evaluated the temporal pattern of urinary excretion of a novel vasodilator within this system, angiotensin-(1–7) (Ang-[1–7]), during the menstrual cycle, pregnancy, and lactation. The urinary profiles of Ang I, Ang II, human chorionic gonadotropin, 17β-estradiol, and progesterone were also determined. During the menstrual cycle, urinary Ang-(1–7) and Ang II remained stable (mean cycle value: 94.6 ± 11.3 and 11.4 ± 1.1 pmol/g of creatinine, respectively) in nine females. In 10 normal pregnant women, urinary Ang-(1–7) and Ang II increased throughout gestation, averaging 1499.8 ± 310 and 224.4 ± 58 pmol/g of creatinine, respectively ($p < 0.05$) at wk 35 and falling during lactation to 394.0 ± 95 and 65.7 ± 20 pmol/g of creatinine ($p < 0.05$), respectively. The Ang-(1–7)/Ang II ratio was unchanged in the different reproductive periods. During the menstrual cycle, Ang II and Ang-(1–7) correlated with 17β-estradiol and progesterone using multivariate analysis ($r = 0.31$, $p < 0.001$) and $r = 0.28$, $p < 0.02$, respectively). During gestation, 17β-estradiol and progesterone correlated with urinary Ang-(1–7) ($r = 0.48$, $p < 0.001$ and $r = 0.47$, $p < 0.001$, respectively) and Ang II ($r = 0.24$, $p < 0.03$ and $r = 0.25$, $p < 0.03$, respectively); by multiple regression, only Ang-(1–7) correlated with both steroids ($r = 0.49$, $p < 0.001$). The progressive rise of Ang-(1–7) throughout gestation, probably modulated by estrogen and progesterone, suggests a physiologic counterregulation within the RAAS.

Key Words: Renin-angiotensin-aldosterone system; endogenous vasodilators; menstrual cycle; pregnancy.

Introduction

Pregnancy is characterized by a progressive increase in the activity of the renin-angiotensin-aldosterone system (RAAS) *(1,2)*. The activation of the accompanying prohypertensive mechanisms is associated with an adaptive vasodilator response, since almost 90% of pregnant women either maintain their blood pressure (BP) within normal limits or show a slight decrease during the second trimester *(1)*. Maintenance of normotension in pregnancy has been attributed to increases in prostacyclin *(3,4)*, nitric oxide (NO) *(5)*, and tissue kallikrein *(6)*.

Recent studies have shown that the vasoconstrictor RAAS contains a novel peptide, angiotensin-(1–7) (Ang-[1–7]), which derives from either angiotensin I (Ang I) or Ang II and opposes the pressor and trophic actions of Ang II *(7–10)* (Fig. 1). This peptide releases NO *(11,12)*, bradykinin *(11,12)*, and prostacyclin *(13,14)* and vasodilates a number of regional beds *(11,12,14)*. These effects would favor a BP-lowering effect under conditions of high Ang II activity and support a counterregulatory action exerted within the RAAS. Although pregnancy has been shown to stimulate the RAAS, Ang-(1–7) in physiologic pregnancy has not been identified. With this in mind, we characterized the angiotensin system during pregnancy and lactation by longitudinal assessment of the urinary excretion of Ang I, Ang II, and Ang-(1–7).

Results

The urinary excretion of the three angiotensin peptides remained stable over d 10, 16, 21, and 26 of the ovulatory menstrual cycle (Table 1). During the menstrual cycle, urinary estradiol values on d 10 and 21 did not attain a significant difference (157 ± 9 vs 255 ± 83 nmol/g of creatinine; $p > 0.05$), while progesterone showed a significant rise in the luteal phase (3.8 ± 0.5 vs 19.7 ± 8.7 μmol/g of creatinine, d 10 vs 21; $p < 0.005$). While no significant linear correlation during the menstrual cycle was observed between estradiol or progesterone, and Ang-(1–7) or Ang II, the multiple regression analysis showed a positive correlation for both steroids and Ang-(1–7) ($r = 0.28$, $p < 0.02$) and Ang II ($r = 0.31$, $p < 0.001$).

Received August 7, 2001; Revised November 1, 2001; Accepted November 8, 2001.

Author to whom all correspondence and reprint requests should be addressed: Dr. K. Bridget Brosnihan, Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157-1032. E-mail: bbrosnih@wfubmc.edu

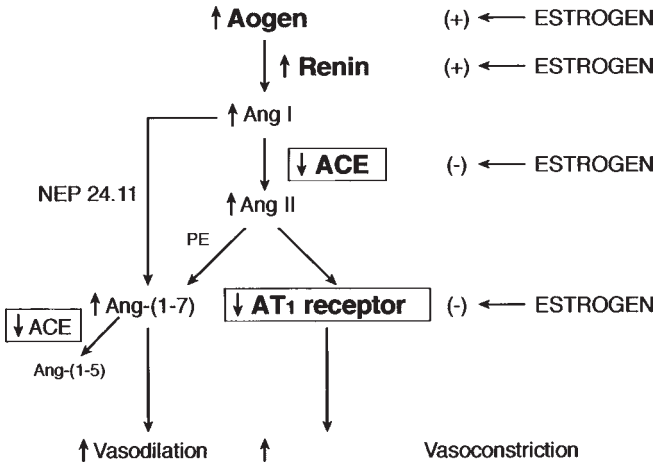

Fig. 1. Schematic of pathways of formation of Ang II and Ang-(1–7) in the renin-angiotensin system indicating potential sites of estrogen regulation. Aogen = angiotensinogen; ACE = angiotensin-converting enzyme; PE = prolyl endopeptidase; NEP 24.11 = neprilysin.

Table 1
Urinary Angiotensins Along Menstrual Cycle[a]

Cycle day	Ang I (pmol/g Cr)	Ang II (pmol/g Cr)	Ang-(1–7) (pmol/g Cr)
10	47 ± 23	12 ± 2	70 ± 5
16	55 ± 19	7 ± 2	102 ± 21
21	45 ± 12	13 ± 2	104 ± 20
26	27 ± 11[b]	15 ± 4[b]	104 ± 48[b]

[a] Expressed as mean ± SE, per gram of creatinine.
[b] Values were not significantly different for any peptide.

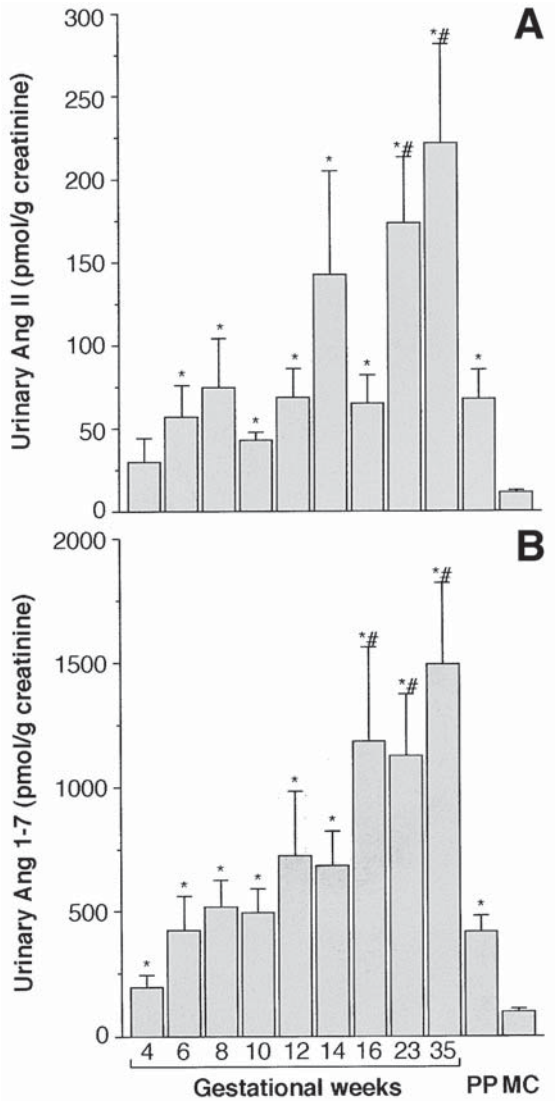

Fig. 2. (**A**) Urinary Ang II excretion during gestation, postpartum and complete breast feeding (PP), and menstrual cycle (MC). $*p < 0.05$ vs mean of values obtained in menstrual cycle. Means of wk 23 and 35 were significantly higher than wk 4 of gestation ($H = 21.5$, $^{\#}p = 0.011$ by Kruskal-Wallis ANOVA on ranks). (**B**) Urinary Ang-(1–7) excretion during gestation, postpartum and complete breast-feeding (PP), and menstrual cycle (MC). $*p < 0.05$ vs mean of values obtained in MC. Means of wk 16, 23, and 35 were significantly higher than wk 4 of gestation ($H = 31.4$, $^{\#}p = 0.001$ by Kruskal-Wallis ANOVA on ranks).

During pregnancy, urinary Ang-(1–7) and Ang II increased progressively to attain significantly higher values at wk 16, 23, and 35, respectively (Fig. 2), whereas urinary excretion of Ang I tended to increase but did not reach statistical significance ($p > 0.05$) (data not shown). Both urinary Ang II and Ang-(1–7) reached their highest levels at wk 35 of gestation ($p < 0.05$), reaching values 16- and 20-fold higher than in the menstrual cycle, respectively. At 1 mo postpartum and during complete lactation, urinary excretion of Ang-(1–7) and Ang II was reduced significantly compared with the values determined at wk 35 of gestation ($p < 0.05$) but remained higher than those found during the menstrual cycle. With the exception of an increase in the ratio of Ang-(1–7)/Ang II at wk 16, neither this ratio nor that of Ang I/Ang II showed significant differences in the reproductive phases studied.

In pregnancy, urinary human chorionic gonadotropin (hCG) showed its highest value at wk 6–8 (85,609 ± 875 and 58,358 ± 11,928 IU/g of creatinine, respectively) and declined abruptly thereafter, attaining its lowest value at postpartum (16.8 ± 24.9 IU/g of creatinine; $F = 193.98$, $p = 0.0001$ by Student-Newman-Keuls analysis of variance [ANOVA]). Urinary 17β-estradiol showed a sustained increase in gestation with respect to the menstrual cycle (400 ± 40 nmol/g of creatinine), attaining at 23 and 35 wk values significantly higher than those of previous gestational periods (1000 ± 109 and 1422 ± 243 nmol/g of creatinine, respectively; $F = 13.84$, $p = 0001$ by Student-Newman-Keuls ANOVA). Urinary pregnanediol was significantly higher at wk 35 than in previous gestational periods (67 ± 11 μmol/g of creatinine; $F = 8.07$, $p = 0.001$ by Student-Newman-Keuls ANOVA). No correlations were found between levels of urinary angiotensins and hCG. On the other hand, a positive correlation

was observed among 17β-estradiol and pregnanediol and both Ang-(1–7) ($r = 0.48$, $p < 0.001$ and $r = 0.47$, $p < 0.001$, respectively) and Ang II ($r = 0.24$, $p < 0.03$ and $r = 0.25$, $p < 0.03$, respectively). A multiple regression test showed that Ang-(1–7) presented a similar correlation to that obtained for each steroid ($r = 0.49$, $p < 0.001$), whereas no significant correlation was reached for Ang II and both steroids ($p = 0.070$).

BP during pregnancy ranged from a minimum of 91/54 ± 9/5 mmHg to a maximum of 115/75 ± 7/5 mmHg; the duration of pregnancy was 39 ± 1 wk of amenorrhea, and newborn weight was 3690 ± 488 g.

Discussion

This first characterization of urinary Ang-(1–7) excretion in normotensive pregnancy, showing an increase in mid- and late pregnancy, suggests that this hormone may act to buffer the vasoconstrictor influence of an increased production of Ang II.

To our knowledge, this is the first report of longitudinal changes in urinary angiotensin peptide excretion in different phases of human reproduction. Our data suggest that urinary Ang II excretion shows a pattern similar to that described for plasma levels, i.e., low and comparable to postpartum during the first trimester, with an increase during the second and third trimesters of normal pregnancy and with a maximal value in the third trimester that reached values five times higher than those of postpartum *(1,15)*. Urinary Ang-(1–7) showed the earliest increase (wk 16) compared with the other two angiotensin peptides. Urinary Ang-(1–7) reached levels 7- and 16-fold higher than those found during postpartum and the menstrual cycle, respectively.

Although the source of angiotensin peptides in the urine was not investigated in our study, the variations in the urinary excretion of the angiotensin peptides are substantially greater than the increase observed in the plasma of normal pregnant women compared with nonpregnant control subjects *(1,16)*. Similarly, concentrations of angiotensin peptides in urine are substantially greater than those previously characterized in the plasma of normotensive and hypertensive humans *(17)*. These findings make it unlikely that peptide levels in the urine reflect filtration in the trapped plasma and interstitial and tubular fluid within the kidneys. The differences in the magnitude of urinary and plasma variations suggest that the kidneys may be an important source for the production of urinary angiotensin peptides. This interpretation agrees with studies that implicate the kidney as a site for the production of Ang II *(18–21)* and Ang-(1–7) *(22,23)*. We have excluded the possibility that urinary Ang-(1–7) may be a hydrolysis product of Ang I or Ang II by urinary proteases present in the urine *(22,23)*. These findings suggest that peptide levels in the urine are consistent with the possibility that the concentrations of angiotensin peptides in urine reflect their production in the kidneys.

The increase in Ang II in the urine may also reflect an increase in other carboxy-terminal fragments of Ang II, including Ang III and Ang IV. Since the Ang II antibody used in our study crossreacts with Ang II, Ang III, and Ang IV (*see* Materials and Methods), the Ang II immunoreactivity in the urine may include these fragments. Ang IV has been shown to have depressor actions *(24)*; thus, an increase in Ang II immunoreactivity throughout gestation may also include an increase in this peptide. It is therefore possible that the normotension of pregnancy could be the result of conversion of Ang I to Ang II, Ang III, or Ang IV, which we were not able to ascertain. Future studies using high-performance liquid chromatography in addition to radioimmunoassay (RIA) are required to clarify the profile of angiotensin peptides comprising Ang II immunoreactivity.

The profile of urinary Ang II and Ang-(1–7) parallels that previously described for urinary aldosterone and plasma renin activity in pregnancy *(1,25)*. Most likely the changes in plasma renin activity reflect maternal renal renin secretion, rather than placental renin, because plasma renin activity in pregnant women, although much higher than in the nonpregnant state, responds appropriately to changes in posture, sodium depletion or load, prostaglandin E_2 and Ang II infusion, and captopril administration *(1,26)*. It is well known that kidney renin is upregulated by estrogen *(1, 27–29)* and thus could contribute to the elevated circulating plasma renin activity, whereas urinary aldosterone most likely results from stimulation by elevated Ang II in normal pregnancy *(1)*. Because progesterone is natriuretic and estradiol mediates vasodilation, the RAAS is probably under multiple levels of hormonal and cardiovascular control. The concurrent increase in estrogens with progesterone may augment the increase in renin, angiotensin peptides, and aldosterone. This possibility is supported by the positive correlations observed among urinary estradiol, progesterone, Ang-(1–7), and Ang II.

It is remarkable that the marked changes in the RAAS are not associated with any apparent perturbation of BP or electrolyte balance, indicating that estradiol, progesterone, and the RAAS may be part of a coordinated servo-control system that contributes to hemodynamic and fluid homeostasis. Estrogen increases the production of angiotensinogen *(30)* and renin *(1,25–29)*, two early protein components of the RAAS that when elevated could favor a vasoconstrictor state. On the other hand, estrogen supplementation downregulates the synthesis of angiotensin-converting enzyme (ACE) *(31)*, which is consistent with the observation of a decrease in serum ACE in pregnancy *(16)*. Our findings of a stable urine Ang I/Ang II ratio would suggest either that urinary peptides are not dependent on circulating or renal ACE activity or that other non-Ang II–generating proteases, such as chymase *(32)*, may be contributing to the formation of Ang II in the kidney. The lack of change in Ang I/Ang II ratio in the face of an increase in Ang II may be explained by multiple proteases contributing to the metabolism of

Ang I and Ang II. In addition, estrogen has also been shown to lower the expression of Ang II receptors *(33)*. The reduced expression of these two proteins (Ang II receptors and ACE activity) favors a shift of the renin-angiotensin cascade toward its vasodilator pathway (Fig. 1). Similar observations could be made to explain the lack of change in the Ang-(1–7)/Ang II ratio. A number of enzymes have been shown to contribute to the formation of Ang-(1–7), including prolyl endopeptidase and neprilysin (Fig. 1). Although each of these enzymes are candidates that could contribute to the formation of the elevated urinary Ang-(1–7) in pregnancy, their regulation during pregnancy has not been studied to any significant degree. Nevertheless, we suggest that the 16-fold elevated urinary Ang-(1–7), and the 2-fold elevation of this peptide in plasma during normal pregnancy *(16)*, is in keeping with a hormonally modulated counterregulation in pregnancy-associated elevation in Ang II.

During normal and preeclamptic gestation, vasoactive systems such as prostaglandins *(3,34)*, NO *(5)*, and kallikrein-kinins *(6,35)* have been initially studied in urine. More recent observations using immunohistochemistry and *in situ* hybridization have shown that apart from the renal excretion, vasoactive systems are expressed in the reproductive tract, in sites that support their role in the control of placental perfusion and growth, and therefore in fetal development *(6,36–39)*. It is for this reason that the variations in urinary Ang II and Ang-(1–7) should also be interpreted in light of their actions on local adaptations to pregnancy, especially since the uteroplacental unit represents an important site for the expression/function of the renin-angiotensin system. Prorenin/active renin, ACE, Ang II, and AT_1 and AT_2 receptors have been found in the different compartments of the female pregnant uterus, and their local synthesis has been confirmed by the presence of the respective mRNAs *(40–45)*. It is tempting to speculate that the marked increase in urinary Ang-(1–7) reflects a concurrent increase in its content in the uteroplacental structures, where it could act on the non-AT_1/non-AT_2 receptor *(10,11)*.

We propose that Ang-(1–7) represents an additional vasodilator mechanism contributing to normotension of pregnancy. A decreased level of plasma Ang-(1–7) in preeclampsia provides additional support for a physiologic role for Ang-(1–7) in gestation *(16)*. We postulate that Ang-(1–7) contributes to a multifactorial vasodilator relay system, which under the changing hormonal milieu of pregnancy, participates in the maintenance of normotension and in adequate fetoplacental perfusion in normal gestation.

Materials and Methods

The present study was performed at the Obstetrical and Gynaecological Unit of the Hospital de la Universidad Católica, Santiago, Chile. The protocol was approved by the institutional review board of the School of Medicine. Women were invited to participate after giving informed consent. The patients and urine samples included were previously used to characterize the temporal profile of tissue kallikrein excretion *(6)*.

Study Subjects

Nine normotensive (BP: 112/72 ± 14/11 mmHg) volunteer women ages 33 ± 5 yr (mean ± SD) with previous normal obstetric histories, appropriate fertility (parity: 2.6 ± 1.5), no history of abortions, and absence of proteinuria were studied during an ovulatory menstrual cycle. None of the subjects were using hormonal contraception. Ovulation was confirmed by urinary pregnanediol levels of >7.4 mol/d on cycle d 21. Urine samples were collected on d 10, 16, 21, and 26 of the menstrual cycle ($N = 28$).

Ten other women ages 27 ± 4 yr (three primiparae and seven multiparae) with no history of abortions were followed throughout a singleton pregnancy characterized by normotension, absence of proteinuria, term delivery, and appropriate-for-gestational-age newborn weight. Urine samples were obtained every 14 d from 4 to 14 wk, and at 15–17, 21–25, and 33–36 wk of gestation ($N = 80$). The counting of weeks of gestation started from the estimated date of conception. The final urine collection was obtained 1 mo postpartum when women were fully breast feeding (lactation period).

Study Protocol

BP was measured by mercury sphyngomanometry after 5 min of rest in the sitting position, once during the menstrual cycle and at every obstetric visit by duplicate determinations. Urinary protein was determined in 24-h urine samples obtained once in cycle and during the first and last trimesters of pregnancy by the Bradford reagent with a Shimadzu spectrophotometer UV 120-11. Twenty-four-hour urine was collected in a container with 2 mL of 10% thymol; samples were kept at –20ºC until assayed. Urinary creatinine was determined by the Jaffe reaction with a Beckman autoanalyzer.

Urine for determinations of Ang peptides was extracted using Sep-Pak columns activated with 5-mL sequential washes of a mixture of ethanol:water (83:13), methanol, ultrapure water, and 4% acetic acid. After the sample was applied to the column, it was washed with ultrapure water and acetone and eluted with two 1-mL washes and one 1.3-mL wash of a mixture of ethanol:water:4% acetic acid. The sample was eluted and reconstituted for RIAs. Recoveries of radiolabeled angiotensin added to the sample and followed through the extraction were 92% ($n = 23$). Samples were corrected for recoveries. Three different antisera specific for the C-terminus of Ang I, Ang II, or Ang-(1–7) were used to analyze the peptides. The Ang I antibody (New England Nuclear, RIANEN; Dupont, Billerica, MA) showed 100% crossreactivity with Ang I, Ang-(2–10), and Ang-(3–10), but <0.001% binding with Ang II and Ang-(1–7).

The Ang II antibody (Alpco, Windham, NH) showed 100% crossreactivity with Ang II and Ang III, and 96% crossreactivity with Ang-(3–8), but <0.001% binding with Ang I and Ang-(1–7). The Ang-(1–7) antibody (produced by our laboratory) showed 100% crossreactivity with Ang-(2–7), but <0.001% crossreactivity with Ang I and Ang II. The minimum detectable levels of the assays were 2.5 pg/mL for Ang I, 4 pg/mL for Ang II, and 2.5 pg/mL for Ang-(1–7). The intra- and interassay coefficients of variation (CVs) for Ang I RIA are 18 and 22%, for Ang II are 12 and 22%, and for Ang-(1–7) are 8 and 20%, respectively.

Urinary estrogens and pregnanediol glucuronides were determined by enzyme immunoassay *(46)*. Intra- and interassay CV was 10% for both steroids. Levels of estrogen and pregnanediol were expressed as nanomoles and micromoles/gram of creatinine, respectively. Urinary total hCG was determined by an immunoradiometric assay adapted from the method applied to serum *(47)*. Intra- and interassay CVs were 12 and 16%. Values were expressed as international units/grams of creatinine. During the first 14 wk of gestation, determinations performed within a 2-wk span were pooled; weeks 16, 23, and 35 included samples of 15–17, 21–25, and 33–36 wk, respectively.

Statistical Analyses

Results are expressed as mean ± SE. Statistical analysis was done with a Sigma Stat (Sigma, St. Louis, MO); comparisons of means were performed by a one-way Kruskal-Wallis ANOVA on ranks for repeated measurements analysis and by Student-Newman-Keuls and Dunnett tests when more than two periods in the same group were compared. The nonpaired *t* and the Mann-Whitney rank sum tests were used to compare the menstrual cycle with each of the gestational periods. The significance level was set at $p < 5\%$.

Acknowledgments

We are indebted to Centro de Estudios Biológicos de la Reproducción, Facultad Ciencias Biológicas, Pontificia Universidad Católica for urinary estrogens and pregnanediol determinations; to Elena Poggi, Irene Guerra, and Ana María Guzmán, MD for urinary hCG measurements; and to Margaret King and the Hypertension Core Laboratory for angiotensin peptide measurements. This study was supported by Fondecyt 1980958 and by a grant from the National Heart, Lung and Blood Institute (P10-HL-51952).

References

1. August, P., Lenz, T., Ales, K. L., Druzin, M. L., Edersheim, T. G., Hutson, J. M., Muller, F. B., Laragh, J. H., and Sealey, J. E. (1990). *Am. J. Obstet. Gynecol.* **163,** 1612–1621.
2. Alhenc-Gelas, F., Tache, A., Saint-Andre, J. P., Milliez, J., Sureau, C., Corvol, P., and Menard, J. (1986). *Adv. Nephrol. Necker Hosp.* **15,** 25–33.
3. Wang, Y., Walsh, S. W., Guo, J., and Zhang, J. **(**1991). *Am. J. Obstet. Gynecol.* **165,** 1690–1694.
4. Mills, J. L., DerSimonian, R., Raymond, E., Morrow, J. D., Roberts, J. L., Clemens, J. D., Hauth, J. C., Catalano, P., Sibai, B., Curet, L. B., and Levine, R. J. (1999). *JAMA* **282,** 356–362.
5. Boccardo, P., Soregaroli, M., Aiello, S., Noris, M., Donadelli, R., Lojacono, A., and Benigni, A. (1996). *Br. J. Obstet. Gynaecol.* **103,** 879–886.
6. Valdés, G., Corthorn, J., Oyarzún, E., Berrios, C., Foradori, A., Germain, A. M., and Villarroel, L. (1998). *Prenat. Neonat. Med.* **3,** 474–481.
7. Iyer, S. N., Ferrario, C. M., and Chappell, M. C. (1998). *Hypertension* **31,** 356–361.
8. Britto, R. R., Santos, R. A. S., Fagundes-Moura, C. R., Khosla, M. C., and Campagnole-Santos, M. J. (1997). *Hypertension* **30,** 549–556.
9. Freeman, E. J., Chisolm, G. M., Ferrario, C. M., and Tallant, E. A. (1996). *Hypertension* **28,** 104–108.
10. Ferrario, C. M., Chappell, M. C., Tallant, E. A., Brosnihan, K. B., and Diz, D. I. (1997). *Hypertension* **30,** 535–541.
11. Brosnihan, K. B., Li, P., and Ferrario, C. M. (1996). *Hypertension* **27,** 523–528.
12. Porsti, I., Bara, A. T., Busse, R., and Hecker, M. (1994). *Br. Pharmacol.* **111,** 652–654.
13. Jaiswal, N., Tallant, E. A., Jaiswal, R. K., Diz, D. I., and Ferrario, C. M. (1993). *J. Pharmacol. Exp. Ther.* **265,** 664–673.
14. Osei, S. Y., Ahima, R. S., Minkes, R. K., Weaver, J. P., Khosla, M. C., and Kadowitz, P. J. (1993). *Eur. J. Pharmacol.* **234,** 35–42.
15. Langer, B., Grima, M., Coquard, C., Bader, A. M., Schlaeder, G., and Imbs, J. L. (1998). *Obstet. Gynecol.* **91,** 196–202.
16. Brosnihan, K. B., Karoly, M., and Merril, D. C. (2000). *Hypertension* **36,** 696, (abstract).
17. Ferrario, C. M., Martell, N., Yunis, C., Flack, J. M., Chappell, M. C., Brosnihan, K. B., Dean, R. H., Fernandez, A., Novikov, S., Pinillas, C., and Luque, M. (1998). *Am. J. Hypertens.* **11,** 137–146.
18. Vos, P. F., Boer, P., Braam, B., and Koomans, H. A. (1994). *J. Am. Soc. Nephrol.* **5,** 215–223.
19. Zou, L.-X., Imig, J. D., Hymel, A., and Navar L. G. (1998). *Am. J. Hypertens.* **11,** 570–578.
20. Bunting, M. W. and Widdop, R. E. (1995). *Br. J. Pharmacol.* **116,** 3181–3190.
21. Navar, L. G., Imig, J. D., Zou, L., and Wang, C. T. (1997). *Semin. Nephrol.* **17,** 412–422.
22. Chappell, M. C., Diz, D. I., Li, P., Callahan, M. F., Morris, M., Ganten, D., and Ferrario, C. M. (1996). *Hypertension* **28,** 523 (abstract).
23. Chappell, M. C., Diz, D. I., Yunis, C., and Ferrario, C. M. (1995). *Kidney Intl.* **54,** 53–56.
24. Loufrani, L., Henrion, D., Chansel, D., Ardaillou, R., Levy, B. I. (1999). *J. Pharm. Exp. Ther.* **291,** 583–588.
25. August, P. and Sealey, J. E. (1990). In: *Hypertension pathophysiology, diagnosis, and management*. Laragh, J. H. and Brenner, B. M. (eds.). Raven: New York.
26. Brown, M. A., Zammit, V. C., and Adsett, D. (1990). *Clin. Sci.* **79,** 505–511.
27. Chen, Y.-F. and Meng, Q.-C. (1991). *Life Sci.* **48,** 85-96.
28. Glorioso, N., Atlas, S. A., Laragh, J. H., Jewelewicz, R., and Sealey, J. E. (1986). *Science* **233,** 1422–1424.
29. Howard, R. B., Pucell, A. G., Bumpus, F. M., and Husain, A. (1998). *Endocrinology* **123,** 2331–2340.
30. Tewksbury, D. A. (1990). In: *Hypertension: pathophysiology, diagnosis and management*. Laragh, J. H. and Brenner, B. M. (eds.). Raven: New York.
31. Gallagher, P. E., Li, P., Lenhart, J. R., Chappell, M. C., and Brosnihan, K. B. (1999). *Hypertension* **33,** 323–328.
32. Urata, H., Kinoshita, A., Misono, K. S., Bumpus, F. M., and Husain, A. (1990). *J. Biol. Chem.* **265(36),** 22,348–22,357.

33. Nickenig, G., Baumer, A. T., Grohe, C., Kahlert, S., Strehlow, K., Rosenkranz, S., Stablein, A., Beckers, F., Smits, J. F., Daemen, M. J., Vetter, H., and Bohm, M. (1998). *Circulation* **97,** 2197–2201.
34. Ylikorkala, O., Pekonen, F., and Vinikka, L. (1986). *J. Clin. Endocrinol. Metab.* **63,** 1307–1312.
35. Elebute, O. A. and Mills, H. I**.** (1976). In: *Hypertension in pregnancy*. Lindheimer, K. Z. (ed.). Wiley: New York.
36. Walsh, S. W. (1985). *Am. J. Obstet. Gynecol.* **152,** 335–340.
37. Telfer, J. F., Irvine, G. A., Kohnen, G., Campbell, S., and Cameron, I. T. (1997). *Mol. Hum. Reprod.* **3,** 69–75.
38. Yoshihi, N., Kubota, T., and Aso, T. (2000). *Mol. Hum. Reprod.* **6,** 283–287.
39. Valdés, G., Chacón, C., Corthorn, J., Figueroa, C. D., and Germain, A. M. (2001). *Endocrine* **14,** 197–204.
40. Szilagyi, A., Mukhopadhyay, A. K., Werling, J., and Szabo, I. (1995). *Early Pregnancy* **1,** 119–123.
41. Morgan, T., Craven, C., and Ward, K. (1996). *Br. J. Obstet. Gynecol.* **103,** 879–886.
42. Li, C., Ansari, R., Yu, Z., and Shah, D. (2000). *Hypertension* **36,** 159–164.
43. Metsarinne, K. P., Pekonen, F., and Fyhrquist, F. Y. (1990). *Acta Endocrinol.* **123,** 113–117.
44. Kalenga, M. K., Thomas, K., de Gasparo, M., and De Hertogh, H. (1996). *Clin. Endocrinol.* **44,** 429–433.
45. Cooper, A. C., Robinson, G., Vinson, K. B., Cheung, W. T., and Broughton, P. F. (1998). *Hypertension* **32,** 687.
46. Brown, J. B., Blackwell, L. F., Cox, R. I., Holmes, J. H., and Smith, M. A. (1998). *Prog. Clin. Biol. Res.* **285,** 119–138.
47. Alfhan, H., Haglund, C., Dabek, J., and Stenman, U. (1992). *Clin. Chem.* **38,** 1981–1987.