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# Roles of the circulating renin-angiotensin-aldosterone system in human pregnancy

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**Lumbers ER, Pringle KG.** The roles of the circulating renin-angiotensin-aldosterone system in pregnancy. *Am J Physiol Regul Integr Comp Physiol* 306: R91–R101, 2014. First published October 2, 2013; doi:10.1152/ajpregu.00034.2013.—This review describes the changes that occur in circulating renin-angiotensin-aldosterone system (RAAS) components in human pregnancy. These changes depend on endocrine secretions from the ovary and possibly the placenta and decidua. Not only do these hormonal secretions directly contribute to the increase in RAAS levels, they also cause physiological changes within the cardiovascular system and the kidney, which, in turn, induce reflex release of renal renin. High levels of ANG II play a critical role in maintaining circulating blood volume, blood pressure, and uteroplacental blood flow through interactions with the ANG II type I receptor and through increased production of downstream peptides acting on a changing ANG receptor phenotype. The increase in ANG II early in gestation is driven by estrogen-induced increments in angiotensinogen (AGT) levels, so there cannot be negative feedback leading to reduced ANG II production. AGT can exist in various forms in terms of redox state or complexed with other proteins as polymers; these affect the ability of renin to cleave ANG I from AGT. Thus, during pregnancy the rate of ANG I production varies not only because levels of renin change in response to homeostatic demand but also because AGT changes not only in concentration but in form. Activation of the circulating and intrarenal RAASs is essential for normal pregnancy outcome subserving the increased demand for salt and, hence, water during pregnancy. Thus, the complex integration of the secretions and actions of the circulating maternal renin-angiotensin system in pregnancy plays a key role in pregnancy outcome.

renin-angiotensin-aldosterone system; pregnancy; fluid and electrolyte balance; renal function; circulation

IN HUMAN PREGNANCY, THE MATERNAL and fetal circulating renin-angiotensin-aldosterone systems (RAAS) and various tissue renin-angiotensin systems (RAS) interact to ensure a satisfactory pregnancy outcome. Tissue RASs critically involved in normal pregnancy are the ovarian, intrauterine (placental and decidual), and the intrarenal RASs. The nonrenal RASs not only play key roles in ovulation, implantation, placentation, and development of the uteroplacental and umbilicoplacental circulations, but they also contribute to the activity of the circulating maternal RAAS, so influencing maternal cardiovascular and renal function. The role of the maternal circulating RAAS is the topic of this review. It should be appreciated that the fetus also has a circulating functional renin-angiotensin system, and this system together with its intrarenal RAS is essential for normal renal development and function (33, 81).

The activity of the maternal circulating RAAS in pregnancy does not solely depend on release of active renin from the kidney. Although this source of renin is important, increased production of angiotensinogen by the liver is also a major influence. Its activity is also influenced at various stages of gestation by contributions from the ovary and uteroplacental unit. The actions of the maternal circulating RAAS are mediated through various ANG peptides and receptors (see Fig. 1). ANG II acting via the ANG II type 1 receptor ($AT_1R$) is predominantly vasoconstrictor; it is also a major regulator of aldosterone secretion. There are additional actions of ANG II mediated via an angiotensin type 2 receptor ($AT_2R$) and of other ANG peptides, in particular, ANG 1–7 via the Mas receptor and ANG IV via $AT_4R$. ANG IV can, however, also act via $AT1_aR$ in the mouse kidney (88). These other ANG peptides, which are also part of the circulating RAAS, and their actions are also likely to contribute to pregnancy outcome.

This review describes our current understanding of the circulating RAAS, how it changes during normal pregnancy, and how it contributes to changes in cardiovascular and renal function to maintain fluid and electrolyte balance and tissue perfusion.

### Circulating RAAS

The circulating RAAS is defined by the action of renin, a 36-kDa aspartyl protease, which cleaves a Val-Leu bond in a large 62-kDa $\alpha$2-globulin substrate, angiotensinogen (AGT), to form ANG I. ANG I is converted to ANG II by angiotensin-converting enzyme (ACE). ANG II is the major ANG peptide;

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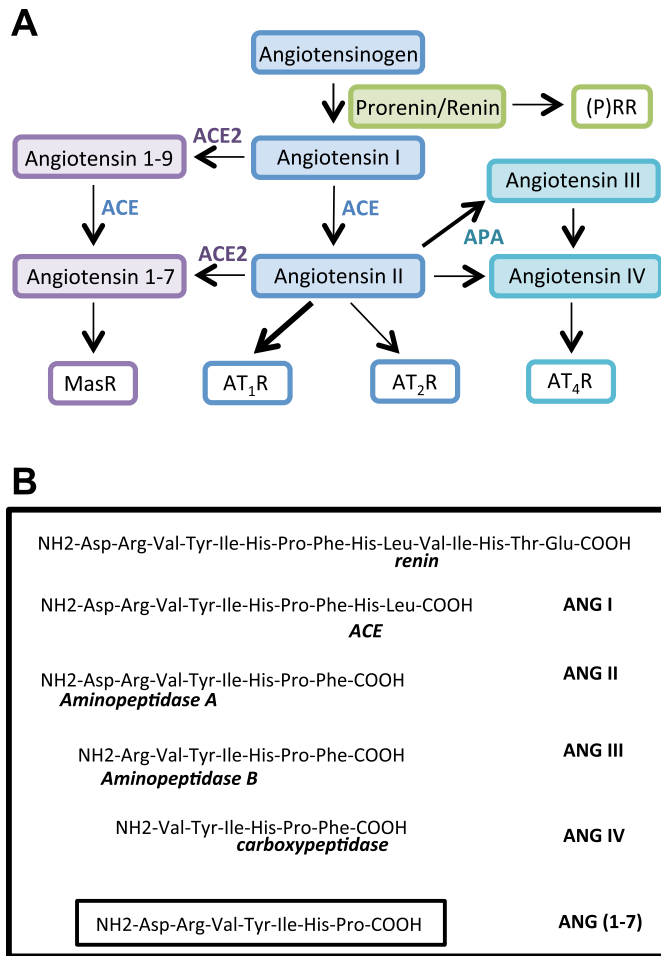
**A**



**B**



Fig. 1. *A*: renin-angiotensin system. (P)RR, prorenin receptor; ACE, angiotensin-converting enzyme; ACE2, a homolog of angiotensin-converting enzyme; APA, aminopeptidase A. AT$_2$R and AT$_1$R are ANG II receptors; AT$_4$R or IRAP is a receptor for ANG IV and MasR a receptor for ANG (1–7). *B*: angiotensin peptides. ANG (1–7) can be produced by ACE2 actions on ANG II or ANG I.

its most well-described biological actions are mediated via the AT$_1$R, (Fig. 1*A*). Other ANG peptides in the circulation include ANG (1–7), which is formed at the fastest rate from ANG II (15, 64), ANG III, and ANG IV (Fig. 1*B*) (3, 15).

ANG II, as well as acting via its AT$_1$R, can also bind to the type 2 receptor, AT$_2$R. Many actions of the ANG II/AT$_2$R interaction oppose the actions of ANG II/AT$_1$R. Briefly, ANG II/AT$_1$R interactions cause vasoconstriction, aldosterone synthesis, secretion, angiogenesis, and cell proliferation. Those mediated by ANG II/AT$_2$R include vasodilatation and apoptosis (15). ANG (1–7) acts via the MasR, a G protein-coupled receptor (64), and many of its actions oppose the ANG II/AT$_1$R-induced effects, as do the actions of ANG IV mediated via the AT$_4$R, also known as IRAP [insulin regulated aminopeptidase (3)]. ANG IV/IRAP-induced effects include hypertrophy, vascularization, inflammation, and vasodilation (10), as well as actions mediated via AT$_1$Rs (88). IRAP has been shown to be identical to placental oxytocinase (45).

The discovery of a precursor of active renin (now called prorenin) many years ago (32) was initially regarded as of little biological significance because prorenin, which has a 28-amino

acid prosequence that covers its catalytic site, was thought to be biologically inactive. It could be shown to be activated in vitro by low pH (32), cold, and proteases such as trypsin (44) and cathepsin D (43). Even today, the biological significance of "in vivo" proteolytic activation of prorenin, remains obscure except for the specific proteolysis that occurs within the juxtaglomerular cells lining the afferent arterioles of the kidney that results in storage and release of active renin from these cells into the blood.

In 2002, the biological significance of prorenin was realized following the discovery of a prorenin receptor, (P)RR. The 45-kDa protein cloned by Nguyen et al. (49) is, in part, identical to a 8.9-kDa truncated protein "M8–9" that is copurified with a vacuolar proton-ATPase, or V-ATPase. (P)RR binds prorenin so that its catalytic site is exposed and ANG I can be cleaved from AGT (49). There are three pathways via which prorenin bound to (P)RR can have biological effects: first by cleavage of ANG I from AGT, second by activation of intracellular signaling [phosphorylation of ERK1/ERK2 or by activation p27/HSP pathway (49, 66)] and third, through the interaction of (P)RR with Wnt signaling pathways (48). The fact that (P)RR knockouts are embryo-lethal indicates that (P)RR plays an essential role in normal development.

A soluble form of (P)RR, s(P)RR, has also been described. It is the 28-kDa portion of the receptor that is cleaved from the M8–9 component by the enzyme furin (48) and is found in the circulation. Thus, circulating prorenin, which is much more abundant in the blood than active renin and which increases to very high levels early in pregnancy (Fig. 2*A*), is no longer confined to the role of an inactive precursor of active renin. It has its own biological activity, possibly as a circulating hormone. s(P)RR may also be important in influencing the rate of formation of ANG I from AGT in plasma and other bodily fluids (89).

### Changes in Components of the Circulating RAAS in Normal Pregnancy

*Angiotensinogen.* As stated above, the activity of the circulating renin-angiotensin system depends upon both the amount of renin capable of interacting with AGT and the amount of AGT. Plasma renin activity is a measure of the angiotensin-forming capacity of plasma. AGT production parallels that of estrogen; the correlation coefficient for AGT and estradiol-17β is 0.60 and for AGT and estriol, it is 0.68 (25). Thus, both AGT (Fig. 2*B*) and ANG II levels rise progressively throughout pregnancy (4). The significance of the increase in AGT in human pregnancy (70) has been underestimated despite the claim by Skinner in 1993 that "at all stages of pregnancy, angiotensinogen is the most important factor determining plasma renin activity and presumably ANG II production" (69).

Native AGT is a 62-kDa protein, although it can exist in high-molecular-weight forms (see below). It is a serpin with the cleavage site for renin (a Val-Leu bond) in a relatively inaccessible site. Accessibility by renin to this site is improved by a redox-induced conformational change. Oxidation of the Cys 18−Cys 138 bond in AGT (89) significantly increases its renin binding affinity in the presence of the (P)RR. Oxidized AGT reacting with renin has a $K_m$ that is about 30% of reduced AGT, while in the presence of the (P)RR, oxidized AGT has a $K_m$ only 9% that of reduced AGT. That is, oxidized AGT has
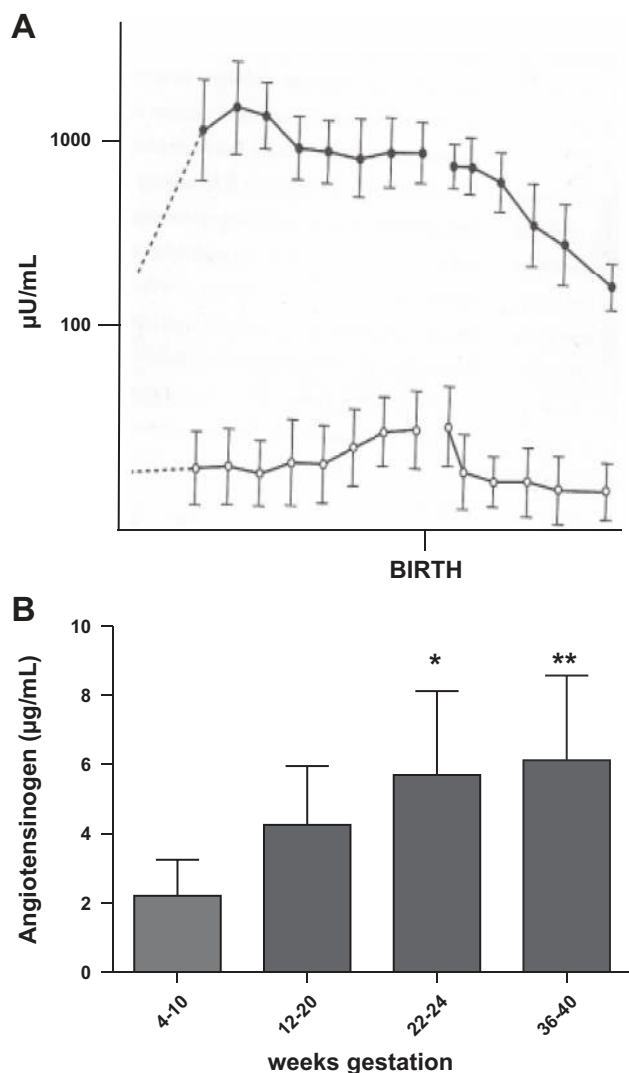
Fig. 2. *A*: plasma prorenin (●) and active renin (○) levels throughout pregnancy [redrawn with permisson from the Endocrine Society from the *Journal of Clinical Endocrinology*, Derkx FH, Alberda AT, de Jonng FH, Zeilmaker FH, Makovitz JW, and Schalekamp MA, vol. 65, 1987, permission conveyed through Copyright Clearance Center; from Derkx et al. (16)]. *B*: angiotensinogen levels (μg/ml) in maternal plasma throughout pregnancy. Levels were measured by incubation of maternal plasma with an excess of human renal renin. Angiotensinogen levels in early pregnancy were less than those measured after 20 wk gestation, *$P < 0.035$ and **$P < 0.001$ compared with values obtained in first 10 wk gestation. [Drawn from raw data presented by Skinner et al. (70)].

a higher affinity for renin than reduced AGT, and the presence of the (P)RR further enhances AGT's affinity for renin caused by oxidation of AGT. (P)RR has no effect, however, on the $K_m$ of the reduced AGT-renin reaction [$K_m$ is 86% of that measured in the absence of (P)RR]. Therefore, with increasing levels of AGT in pregnancy, and the fact that the oxidized-to-reduced ratio of AGT has been shown to be increased in preeclampsia (89), the role of AGT has, as Skinner pointed out, been underestimated, in terms of its ability to affect ANG II production. Clearly, the roles of oxidized AGT and (P)RR in the etiology of abnormal pregnancy outcomes, such as preeclampsia, intrauterine growth restriction, and preterm birth deserve detailed investigation. Other conformational

changes in the molecular structure of AGT may also alter the kinetics of the renin-AGT reaction. For example, the Met 235 polymorphism in the AGT gene is associated with a conformational change that leads to an increased rate of formation of ANG II (89).

The molecular weight of circulating AGT can vary because it forms polymers. Monomeric AGT is a protein of 61.5 kDa or 65.5 kDa (depending on glycosylation) produced by the liver (80). Polymeric forms of AGT alter the rate of the renin-AGT reaction.

High-molecular-weight AGTs, which are different from those in plasma from nonpregnant women, have been found in plasma from pregnant women. Levels of high-molecular-weight AGT rise throughout pregnancy and are about 16% of total AGT; they increase further in pregnancy-induced hypertension and hypertension that is exacerbated in pregnancy (79). The reaction of high-molecular-weight AGT with renin is slow (86).

High-molecular-weight AGTs were first described by Gordon and Sachin (20) and quantified by Tewksbury and Dart (79). Five distinct forms exist in extra fetal tissues, i.e., amnion, chorion, and placenta, while only three forms exist in plasma (78). In plasma from pregnant women, high-molecular-weight AGTs are polymers complexed with other proteins, such as the proform of eosinophil major basic protein (proMBP), which is highly expressed in the placenta. Low levels of proMBP occur in Down's syndrome and are also associated with poor pregnancy outcome (86). It is produced by the trophoblast placental X cells (extravillous trophoblast) (54). 2:2 polymers of proMBP/AGT and 2:2:2 of proMBP/AGT/Cd3g occur in plasma from pregnant women (55). Cd3g is complement. ProMBP and Cd3g complexes with AGT only occur in plasma from pregnant women, although there are high-molecular-weight forms of AGT (140 and 100 kDa), which account for about 3–5% of total AGT in plasma from nonpregnant subjects (46).

Therefore, not only are levels of plasma AGT increased in pregnancy, but AGT also influences the rate of production of ANG II, depending on its redox state and the amount complexed with other proteins.

*Prorenin and active renin.* In the luteal phase of the menstrual cycle, prorenin levels peak shortly after ovulation, while active renin levels rise in the mid-luteal phase (68). This suggests that ovarian prorenin is secreted at ovulation. The ovarian follicle contains renin, 99% of which is in the form of prorenin, although ANG I and ANG II are also both present, suggesting that either prorenin is nonproteolytically activated by binding to (P)RR or the very small amount of active renin, perhaps formed spontaneously, is sufficient to generate ANG I and ANG II (34).

Maternal plasma prorenin levels are at a maximum at 8–12 wk gestation (16, 70), being about 10 times nonpregnant levels at their peak (16), while active renin levels do not rise until ~20 wk of pregnancy (Fig. 2*A*).

A strong correlation between serum renin levels (total) and the number of ovarian follicles was found in women in whom cycling was managed by LH, FSH, and human chorionic gonadotrophin (hCG) (27). Ovarian prorenin is a major contributor to circulating prorenin levels in early pregnancy. Derkx et al. (16) demonstrated that in a woman with primary ovarian failure in whom embryo transfer was performed, plasma prorenin levels were only about 17% of those normally

seen in early pregnancy while levels of active renin, which are secreted only by the kidney, were similar to those found in normal pregnant women. The contribution of the uteroplacental unit to the maternal circulating prorenin levels has not been demonstrated as convincingly.

Very high levels of prorenin might contribute to an increase in active renin levels, perhaps after 20 wk gestation when the materno-placental interface is fully established. This could occur through spontaneous conversion to active renin, as it is thought that there is an equilibrium between the two molecules (67). Alternatively, prorenin could interact with the 28-kDa soluble prorenin receptor, thus increasing the biological activity of the circulating RAAS through two actions, exposure of the renin catalytic site, as well as affecting the rate of reaction of renin with AGT.

It is surprising that despite the increased demand for retention of salt and water to compensate for the very significant increase in cardiovascular volume that occurs in pregnancy (so that it is effectively "underfilled") and the salt-losing effects of changes in renal function (discussed below), there is not a marked increase in active renin until later in pregnancy. This emphasizes the critical role of AGT in regulating plasma ANG II levels early in gestation. This tightly controlled activity of the RAAS through the action of estrogens on AGT provides a "fail-safe" mechanism that offsets the natriuretic effects of the high glomerular filtration rate (GFR) and high levels of progesterone, both of which are characteristic of normal pregnancy (1, 51). The "locking" of AGT synthesis to estrogen production means that its regulation is freed from the control by complex integratory pathways and/or maternal behavior, e.g., salt intake.

Since active renin is only secreted by the kidney, one has to conclude that the influence of AGT on plasma ANG II levels results in a negative feedback suppression to midluteal levels of active renin in early gestation, and only after 20 wk do those mechanisms that normally balance renin activity to homeostatic demand (i.e., renal sympathetic nerve activity, renal perfusion pressure, and tubular flow dynamics) become significantly more influential in the control of maternal plasma renin levels.

*ACE.* In normal pregnancy ACE activity does not change throughout gestation (56). This may not be the case in pathological pregnancies. Although we have not found any changes in ACE levels at 15 wk gestation in women who go on to develop preeclampsia or gestational hypertension (76), pregnant diabetic rats have higher serum ACE and lung ACE than nondiabetic control animals; as well, ANG II levels are higher (84).

*ACE2.* ACE2 has 40% homology with ACE. It removes a single amino acid from either ANG II to form ANG (1–7) or from ANG I to form ANG (1–9), which can subsequently be cleaved to ANG (1–7) by ACE. ACE2 is primarily localized to endothelial cells. It is upregulated in disease states such as myocardial infarction and may be shed into plasma (31). ACE2 is expressed in high amounts in early gestational placentae on the syncytiotrophoblast (59); levels of expression are lower at term. In this location, placental ACE2 may cleave maternal circulating ANG II to form ANG (1–7), a vasodilator peptide, which acts via the Mas receptor (see below). As far as we can tell, ACE2 has not been measured in human plasma from pregnant women. This is because it is difficult to measure. Its

catalytic activity in human plasma is inhibited. The inhibitor is a small-molecular-weight molecule; it is not a protein, nor is it a divalent cation. Removal of the inhibitor by anion-exchange, yielded plasma ACE2 activity of $4.44 \pm 0.56$ pmol·ml$^{-1}$·min$^{-1}$ in plasma from nonpregnant women (31). Be that as it may, the perfusion of maternal blood through the placenta and exposure to ACE2 in syncytiotrophoblast may reflect an important physiological site of production of ANG (1–7).

*ANG peptides.* ANG I (the decapeptide) has no known biological activity. ANG II, the octapeptide, is the most potent of the ANG peptides (Fig. 1B) having 2 receptors, ANG II type 1 (AT$_1$R) and type 2 receptors (AT$_2$R). Additional ANG peptides resulting from the removal of N-terminal amino acids also exist in the circulation. Because biological activity depends on the phenylalanine grouping at the carboxy end, the heptapeptide, ANG III (des-aspartyl$^1$-ANG II) is almost as potent as ANG II. As well, ANG III appears to be the preferred agonist for the AT$_2$R in certain organs (e.g., the kidney), where its actions via the tubular AT$_2$R, release cGMP and cause a profound natriuresis (30). The hexapeptide, ANG 3–8, (also known as ANG IV) and pentapeptide, ANG 4–8, have a similar efficacy but are weak agonists of the AT$_1$R because of their poor affinity (15). ANG IV, however, does have a specific receptor, IRAP, identified by Albiston et al. (3) that is involved in cognition and memory. ANG (1–7), formed by the action of a carboxypeptidase, such as ACE2 acting on ANG II (Fig. 1A) or from ANG I via other pathways acts on a very different receptor, the MasR (64).

Of these peptides, ANG II and ANG (1–7) have been most studied in human pregnancy. At 15 wk gestation, ANG II levels are lower and the ANG (1–7)/ANG II ratio higher in women carrying male fetuses than in women carrying female fetuses (75). Baker et al. (4) found that plasma ANG II levels were elevated by the second trimester. By late gestation, ANG I levels were $176.4 \pm 57.1$ fmol/ml compared with nonpregnant levels of $32.4 \pm 5.6$ fmol/ml, and ANG II levels were about 50% above nonpregnant levels while ANG (1–7) levels were increased by about 34% [Fig. 3 (5)]. As explained above, these high levels of ANG II and ANG (1–7) are predominantly due, at least in early gestation, to the rising levels of AGT. Since ACE2 activity has not been clearly determined in normal human plasma, it is possible that the rise in ANG (1–7) represents conversion from ANG II by ACE2 at the placental
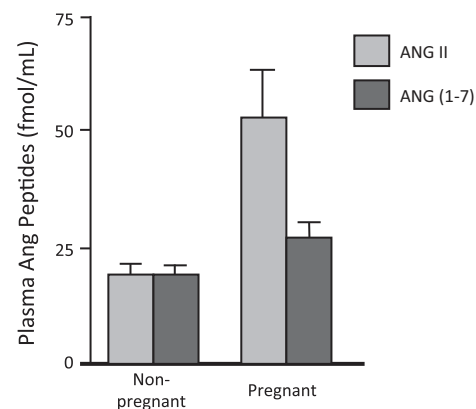
Fig. 3. ANG II and ANG (1–7) levels in plasma from nonpregnant (NP) and pregnant women in the third trimester. Data are reported as means ± SE values. [Redrawn from Brosnihan et al. (5)].

interface (82, 83), but it is also possible that increased conversion of ANG II to ANG (1–7) occurs in the pregnant kidney. In Sprague-Dawley rats, Joyner et al. (28) showed that renal levels of both ACE2 and ANG 1–7 were increased in the middle to late stages of pregnancy. Coupled with the higher renal blood flow of pregnancy, it is probable that renal ACE2 makes a significant contribution to circulating levels of ANG (1–7) in human pregnancy (28).

*Angiotensin Receptors*

The roles of the circulating RAAS in human pregnancy cannot be considered in isolation from changes in the density of ANG receptors located in all those sites at which ANG peptides have biological actions. The ANG II receptors ($AT_1R$, $AT_2R$) are G protein-coupled receptors (15).

*$AT_1R$.* ANG II/$AT_1R$ interactions acting via phospholipase C increase cytosolic calcium, inhibit adenyl cyclase, and activate tyrosine kinases, causing vasoconstriction, aldosterone synthesis and secretion, and cardiac hypertrophy. Significantly, ANG II acts within the central nervous system to stimulate thirst (39), vasopressin secretion (38), and sympathetic nerve activity (60), as well as inhibit cardiac vagal efferent nerve traffic (36). These actions potentiate its peripheral vasoconstrictor actions on vascular smooth muscle, thus leading to increased arterial pressure. Through actions in the zona glomerulosa of the adrenal cortex, ANG II/$AT_1R$ stimulates aldosterone synthesis and secretion (72). Within the kidney, ANG II/$AT_1R$ interactions stimulate tubular sodium reabsorption (13). As well, ANG II acting via $AT_1R$ partially mimics the actions of growth factors using Ras pathways to activate MAPK; this action is enhanced by ANG II transactivation of growth factor signaling. Transactivation of EGF by ANG II stimulates MAPK and calcium-dependent phosphorylation leading to activation of growth factor proto-oncogenes (15).

$AT_1R$ density in vascular smooth muscle taken from pregnant rats is suppressed by 1 $\mu$M of estradiol (15). The changes in $AT_1R$ density in the maternal vasculature in pregnancy are unknown, but ANG II binding to platelet $AT_1Rs$ from pregnant women has been described. Baker et al. (4) found that platelet ANG II receptors were low throughout pregnancy, rising at 6 wk postpartum. Pawlak and MacDonald (57) showed that in nonpregnant subjects there was a negative relationship between ANG II levels and ANG II receptors. In early pregnancy this relationship was lost because at this time, there was a "significant reduction or nil receptor capacity but only a slight elevation in mean plasma angiotensin II concentration" and "this phenomenon of reduced or absent binding persisted into the third trimester when plasma angiotensin II was significantly elevated compared with all other groups" (57).

It is well known that vascular reactivity to ANG II is decreased both in terms of a reduction in pressor responses to ANG II (19) and a reduction in the reactivity of the maternal peripheral vasculature (35). Whether this is due to the counteracting effects of other vasodilator influences in pregnancy or to a reduction in $AT_1R$ is unknown, but the fact that hand vascular reactivity of pregnant women to another vasoconstrictor, namely noradrenaline, was not altered, but the response to ANG II was reduced (35), suggests that there is a reduction in $AT_1R$ vascular receptor density, as found by others in platelets (4, 57).

*$AT_2R$.* ANG II acting via the $AT_2R$ receptor causes vasodilation and apoptosis. In pregnancy, myometrial $AT_2R$ are downregulated, but in the uterine arteries of pregnant sheep, a different picture is seen. Vascular $AT_2R$ are upregulated by estrogens (62).

In uterine arteries from pregnant sheep, there are $AT_2R$, but these are not present in uterine arteries from nonpregnant sheep (7, 41). Because ANG II/$AT_2R$ interactions mediate vasodilation via nitric oxide (NO) and bradykinin, the presence of $AT_2R$ in the uterine arteries could be important in offsetting the vasoconstrictor action of ANG II, so maintaining a high uteroplacental blood flow. This proposition would seem to be supported by the finding in sheep that uteroplacental flow did not change during short-term intravenous infusions of ANG II of <60 ng·kg$^{-1}$·min$^{-1}$ (46) or 4-h infusions of 20–30 ng·kg$^{-1}$·min$^{-1}$ (74). However, when 20–30 ng·kg$^{-1}$·min$^{-1}$ intravenous infusions of ANG II were continued for 16–24 h, uteroplacental flow did decrease, and the fetuses became hypoxemic (74). Infusions (30 ng·kg$^{-1}$·min$^{-1}$) of ANG II for 24 h cause uterine arteries from pregnant sheep (studied in vitro) to contract more vigorously in response to ANG II. This is due, in part, to downregulation of $AT_2R$. Thus $AT_2R$ in the pregnant uterine vasculature protects against the vasoconstrictor actions of ANG II unless high circulating levels of ANG II are sustained over many hours, resulting in their downregulation (40). In addition, in female rats, low doses of ANG II cause a fall in blood pressure (BP) not seen in male rats (63). In genetically modified mice, $AT_2R$-null mice develop high blood pressure in the third trimester (77), and $AT_2R$ receptor antagonism abolishes the midgestation decline in BP in $AT_{1a}^{-/-}$ and C57BL/6J mice (73). Thus, $AT_2R$ plays a role in regulation of maternal blood pressure and uteroplacental flow in animal models, and it is likely that $AT_2Rs$ are upregulated in the systemic, as well as the uteroplacental vasculature of the pregnant human.

*MasR.* In 2003, Santos et al. (65) showed that ANG (1–7) acted via a G protein orphan receptor, Mas. ANG (1–7) acting via this pathway is also a vasodilator via endothelium-dependent mechanisms, in particular, via NO. ANG (1–7) is also antiaquaretic and promotes thirst (29, 37), important actions of the RAAS regulation of fluid and electrolyte homeostasis in pregnancy.

*Insulin-regulated aminopeptidase.* IRAP, also known as the $AT_4$ receptor, is the receptor for ANG IV (3). It is the same as placental oxytocinase (45). ANG IV bound to IRAP inhibits it (2). Estrogen treatment of ovariectomized ewes results in downregulation of IRAP in the outer myometrial layer (45). Whether or not the inhibitory effect of ANG IV on IRAP plays a role in parturition is unknown at this time.

*Role(s) of the Circulating RAAS in Pregnancy*

*Underfilled hypotensive cardiovascular system of pregnancy.* The RAAS is activated in the 2nd half of the menstrual cycle, following ovulation. At this time, mean arterial pressure falls (from 81.7 ± 0.2 during the follicular phase to 75.4 ± 0.2 mmHg in the proliferative phase), and systemic vascular resistance is decreased (declines from 1,224 ± 82 to 959 ± 59 dynes·s$^{-1}$·cm$^{-5}$, Fig. 4A). These changes in cardiovascular function could stimulate renin release via the renal baroreceptor or increased renal sympathetic nerve activity.
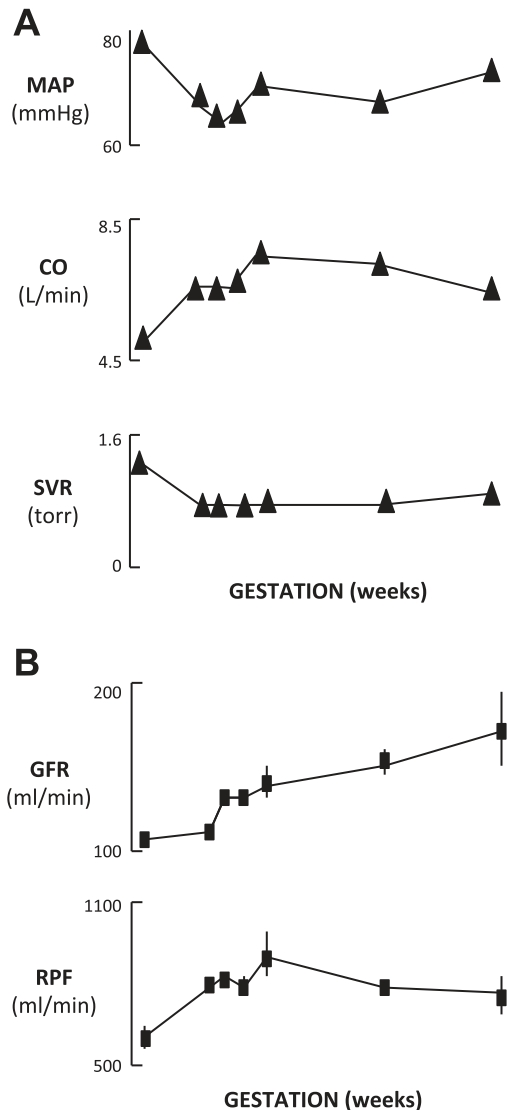
Fig. 4. Changes in mean arterial pressure (MAP, mmHg), cardiac output (CO, l/min), and systemic vascular resistance (SVR, torr; *A*); as well as glomerular filtration rate (GFR, ml/min) and renal plasma flow (RPF, ml/min) (*B*) in pregnancy. Measurements were made in nonpregnant women and throughout pregnancy up to 36 wk. [Redrawn from Chapman et al. (8) with correction to *x*-axes; Macmillan Publishers Ltd., *Kidney International*, 54, 2056–2063, 1998].

Also, glomerular filtration rate is increased from $109 \pm 6$ to $116 \pm 7$ ml·min$^{-1}$·1.73 m$^2$ [Fig. 4*B* (9)] and progesterone levels increase from $0.22 \pm 0.02$ to $8.13 \pm 1.55$ ng/ml. The rise in renin activity ($0.36 \pm 0.05$ to $1.21 \pm 0.18$ ng·ml$^{-1}$·h$^{-1}$) and aldosterone levels [from $3.1 \pm 0.3$ to $8.5 \pm 1.0$ ng/ml (9), Fig. 5] should be seen as a compensatory response to the changes in GFR, the increased capacity of the cardiovascular compartment and to the salt-losing effects of progesterone (9).

Thus, late in the menstrual cycle, the circulating RAAS is activated, because the fall in maternal blood pressure acting via the renal baroreceptor and sympathetic nervous system, the salt-losing effects of a high GFR stimulating the macula densa and inhibition of aldosterone by progesterone, all stimulate release of renin from the kidney (in particular, active renin). No further increase in active renin levels then occurs until 20 wk

gestation. The progressive rise in renin activity and ANG II prior to this time (i.e., 20 wk) is the result of estrogen-induced stimulation of AGT production (see above). The integrated pattern of changes leading to the observed alterations in the circulating RAAS in pregnancy is summarized schematically in Figs. 6 and 7.

Maternal cardiovascular and renal function are profoundly altered by the presence of a conceptus. There is marked vasodilation and an increase in blood flow, particularly to the uterus, breasts, skin, and kidneys, presumably in response to metabolic demand and to eliminate the waste products of metabolism. There must also be an increase in coronary blood flow to cope with the increase in cardiac output. An increase in vascular compliance occurs very early in pregnancy and threatens maintenance of blood pressure, even though cardiac output is increased. The increased capacitance of the maternal circulation is due to both a reduction in systemic vascular resistance and an increase in global arterial compliance.

What causes this increase in capacitance of the maternal circulation? The following may be involved: angiogenesis and vascular remodeling; relaxin; vasodilator peptides of the RAS [e.g., ANG (1–7)]; downregulation of the AT$_1$R and upregulation of AT$_2$R; vascular endothelial growth factor (VEGF); and NO, kallikrein-kinin, and prostanoids.

This topic has been reviewed by Valdes et al. (83). The role of the RAS in creating the proangiogenic state of pregnancy is discussed below. Evidence for downregulation of AT$_1$R in pregnancy has been cited above as has the vasodilator roles of ANG II or ANG III/AT$_2$R interactions, ANG IV, and ANG (1–7). These all also involve other dilator systems (e.g., NO, kallikrein-kinin system, and prostacyclin) but of particular interest is the role of relaxin.

As stated above, cardiac output rises in the first trimester, mainly due to an increase in stroke volume, but systemic
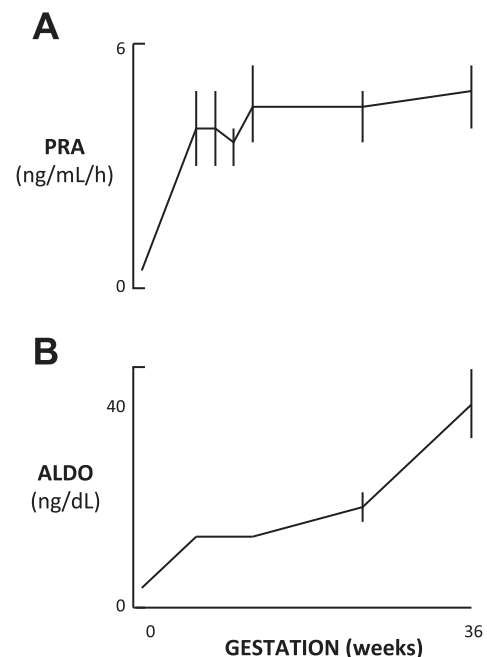


Fig. 5. Changes in plasma renin activity (PRA; ng·ml$^{-1}$·h$^{-1}$) (*A*) and plasma aldosterone levels (ALDO; ng/dl) (*B*) in human pregnancy. [Redrawn from Chapman et al. (8); Macmillan Publishers Ltd., *Kidney International*, 54, 2056–2063, 1998].
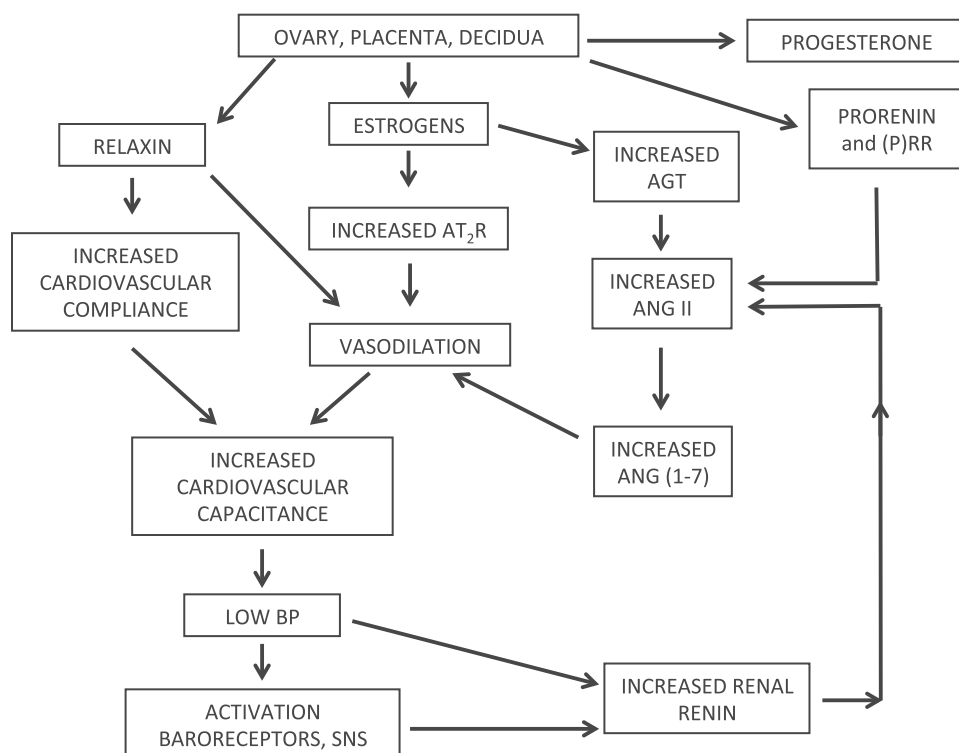
Fig. 6. The actions of ovarian, placental, and possibly decidual hormones directly and indirectly via their effects on maternal physiology on the circulating maternal renin-angiotensin system in human pregnancy.

vascular resistance (SVR) falls to a greater extent, so that by the beginning of the second trimester arterial pressure is reduced, effective renal plasma flow has increased by about 80%, and GFR is 50% above nonpregnant values (Fig. 4) (1). At the beginning of the second trimester, the decrease in systemic vascular load reaches its nadir and the increase in global arterial compliance (AC) peaks. This synergism has been proposed by Conrad to depend on the actions of the ovarian hormone, relaxin (Figs. 6 and 7) (11). Animal studies have shown that relaxin plays a major role in pregnancy as a

vasodilator, in osmoregulation, and renal vasodilation (11, 12, 50). Relaxin-induced increases in renal blood flow might be thought to account for pregnancy-induced increases in GFR, and this seems to be the case in rodents (14). However, in nonpregnant human volunteers recombinant human relaxin caused a rise in renal blood flow, but this was not accompanied by an increase in GFR; thus the situation in human pregnancy seems to be more complex (71).

Conrad and Novak (12) postulates that not only is relaxin a vasodilator, but acting via a relaxin receptor induces matrix
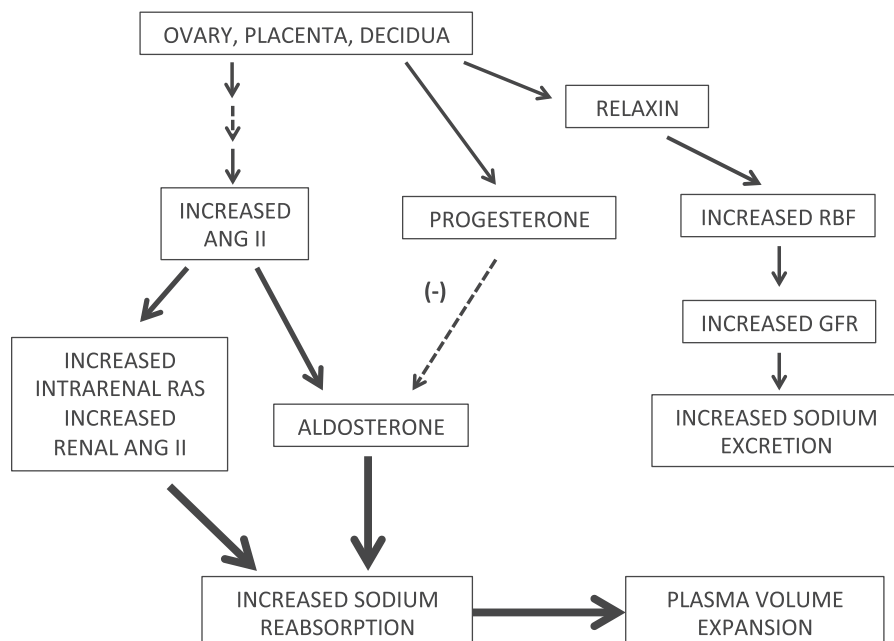


Fig. 7. Effects and pathways of ovarian, placental, and possibly decidual hormones on renal handling of salt in human pregnancy.

metalloproteinase-2 (MMP2), which has the ability to cleave endothelin$_{1-32}$ (ET$_{1-32}$) from big endothelin. ET$_{1-32}$ acting via ET$_B$ in endothelial cell caveolae induces nitric oxide synthase and so increases NO and vascular relaxation. This adds to the fall in renal vascular resistance and systemic vascular resistance. Also, MMP-2, a gelatinase, affects the vascular extracellular matrix of small vessels leading to increases in global arterial compliance (which is derived from the cardiac output and the diastolic decay of the aortic waveform) (11, 12). This increase in global AC prevents excessive falls in diastolic pressure resulting from the decrease in SVR, decreases the loss of pulsatile work by the heart, and contributes to the underfilling of the vascular system, which together with the reduction in SVR, results in a massive demand for salt and water to fill the cardiovascular system in the face of a high GFR, causing salt and water loss. The RAAS plays a pivotal role in maintaining BP and retaining salt and water under these circumstances, possibly aided by increased renal sympathetic nerve activity, and early in pregnancy, a lack of a rise in plasma atrial natriuretic peptide levels (Fig. 8) (8), which do not increase until 12 wk gestation, although, thereafter, they are increased relative to the nonpregnant state (26, 61).

*Hunger for salt to maintain pregnancy and renal function.* No other physiological state is characterized by such an intense activation of the renin-angiotensin-aldosterone system as occurs in human pregnancy (Figs. 6 and 7). A pregnant woman requires about 500 mEq of sodium in normal pregnancy, about 20 mmol/wk (21). Adequate levels of sodium are required to maintain the extracellular volume, which includes an expanded circulating blood volume and the demands of the conceptus for salt and water. For changes in these values during pregnancy, see Ref. 1. The RAAS is the mechanism that fulfills this

demand for extra salt and water. The massive increase in sodium retention is partly masked by increased water retention because in pregnancy, the threshold to vasopressin-induced hyperosmotic stimulation is reduced, leading to the characteristic hyponatremic hypervolemia of pregnancy (6).

The profound need for salt in pregnancy and the role of the RAAS system in fulfilling that role is emphasized by studies in the Yanamamo Indians, who at the time of the study lived on a no-salt diet (52). Their daily loss of sodium was about 1 mEq/day (assuming a urinary output of about 1 l/day), and a measured urinary sodium concentration of 1 mEq/l. When urinary aldosterone levels from pregnant Yanamamo women were compared with their sodium excretion, they were extraordinarily high (585 $\pm$ 46 ng/ml) compared with nonpregnant (38.8 $\pm$ 31.4 ng/ml) and lactating pregnant Yanamamo women (46.2 $\pm$ 62.5 ng/ml), resulting in only a slightly lower sodium excretion (0.7 compared with 1.4 mEq/l and 0.8 mEq/l, respectively). Presumably, gastrointestinal and sweat losses of sodium were also reduced below nonpregnant levels. Levels of aldosterone were far in excess of pregnant Guyami women who had a high-salt intake (urinary sodium; 77.8 $\pm$ 30 mEq/l) and lower urinary aldosterone (92.1 $\pm$ 119 ng/ml) (53), even though pregnant Guyami women also had higher urinary aldosterone levels and lower urinary sodium levels than nonpregnant tribal women. Like urinary aldosterone, plasma renin activity in pregnant Yanamamo women was very much greater (25.6 $\pm$ 6.4 ng·ml$^{-1}$·h$^{-1}$) compared with lactating and nonpregnant Yanamamo women (5.0 $\pm$ 2.6 and 6.2 $\pm$ 4.1 ng·ml$^{-1}$·h$^{-1}$, respectively) (53). Thus, in Yanamamo women, one can only assume that much of the increase in renin activity is, in fact, due to very high rates of secretion of renal (i.e., active renin). These data emphasize the interaction between the demand for salt in pregnancy and the activity of the RAAS. This demand for sodium is much greater than that of lactating women from the same tribes, because of the large capacitance of the circulation and the high GFR characteristic of pregnancy but not lactation. After birth, with reversion to a smaller blood volume and lower GFR, the demand for salt is reduced; also, the concentration of sodium in breast milk is low.

*Renal function in pregnancy: the intrarenal RAS and the circulating RAAS.* GFR used to be described as the "first factor" involved in control of sodium excretion and aldosterone as the "second factor". This was based on the fact that GFR is high (about 170 l/day), so the amount of sodium filtered by the kidney is high (about 24,000 mmol/day). Of that amount, 99% is reabsorbed by the renal tubules. This reabsorptive process accounts for about 90% of renal oxygen consumption, which increases in proportion to the filtered load (i.e., the amount reabsorbed) (23). Aldosterone can regulate the excretion of up to about 2% of this filtered sodium load. Progesterone is natriuretic because it is a natural antagonist of aldosterone (51).

The renal tubules also balance the amount of sodium reabsorbed to keep it in proportion to the GFR, a phenomenon known as glomerulotubular balance. Glomerulotubular balance only maintains the fraction of glomerular filtrate reabsorbed constant. Thus, a rise in GFR, such as that occurs in pregnancy would result in an increase in the amount of sodium excreted, unless there was a compensatory increase in aldosterone and ANG II-mediated sodium reabsorption.

ANG II has a multiplicity of actions important for maintaining tissue perfusion. Most of these are mediated via the AT$_1$R.
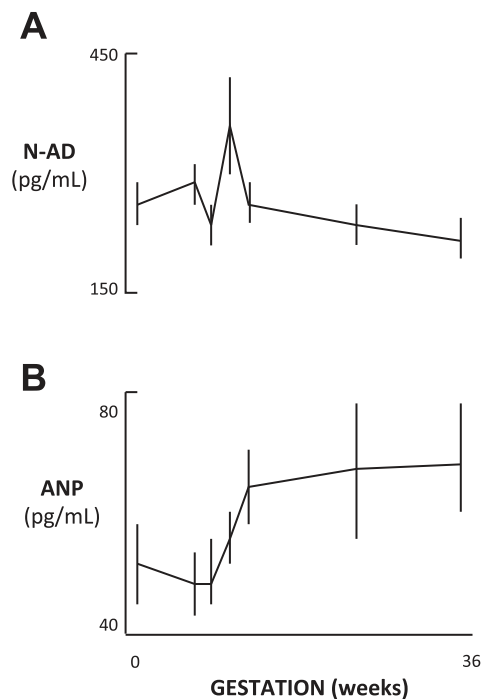


Fig. 8. Changes in plasma noradrenaline (N-AD, pg/ml) (*A*) and atrial natriuretic peptide (ANP, pg/ml) (*B*) in human pregnancy. [Redrawn from Chapman et al. (8); Macmillan Publishers Ltd., *Kidney International*, 54, 2056–2063, 1998].

In the 1950s and 1960s, ANG II's role as a key regulator of aldosterone, a salt-retaining hormone, which acts in the distal segments of the nephron, was firmly established. Its role in controlling renal salt reabsorption is, however, not confined to this indirect pathway. The proximal convoluted tubule reabsorbs about 65% of the filtered sodium load, and blockade of the proximal convoluted tubule ANG II/AT$_1$R interaction reduces this amount (13, 22), demonstrating the key role of the intrarenal RAS described by Navar et al. (47) in sodium homeostasis. ANG II/AT$_1$R interactions also influence the activity of other sodium transporters located along the renal tubule (see Ref. 47).

*Pregnancy is a proangiogenic state—role of the RAS.* It is likely that there are other actions of the maternal circulating RAAS in pregnancy. One is its role in angiogenesis. Pregnancy is a "proangiogenic state". The increases in renal blood flow, the growth of the uteroplacental circulation, the marked increase in the vascularity of the mammary vascular bed, and possibly changes in vascularity of other tissues, including arterial remodeling mentioned above, may involve the RAS.

In certain tissues, the RAS is clearly involved in angiogenesis, such as in renal development (81) and in the eye (42). Angiotensin is known to induce angiogenesis via an ANG II/AT$_1$R interaction, and we postulate it may be a key factor in placental angiogenesis (58). Angiogenesis is stimulated in human umbilical vein cells by ANG II (24). In early pregnancy, VEGF levels are increased in parallel with the increase in β-hCG levels (18). β-hCG also stimulates prorenin secretion by the ovary (27) and by placental trophoblast (17). Because the (P)RR is associated with increased VEGF levels in the eye and this effect is abolished by (P)RR blockade and by blockade of ERK1/2 signaling (87), it is tempting to suggest that the (P)RR-prorenin/renin system, which is activated in early pregnancy by hCG could be involved in this early high level of production of VEGF and, hence, in stimulation of angiogenesis. Also, aldosterone has been shown in vitro to stimulate VEGF production (85). So the rapid onset of increased tissue perfusion of the uterus, breasts, skin, and kidney, which must involve increased vascularity, may, in part, be determined by the early activation of the RAAS.

*Perspectives and Significance*

The activity of the circulating RAAS in pregnancy is influenced by contributions from tissue RASs (in particular, the ovary), as well as the secretion of active renin by the kidney. Angiotensinogen also plays a major role. Since its production is strongly influenced by estrogens, it would seem that maintenance of fluid and electrolyte homeostasis in normal pregnancy is assured. Even so, when dietary salt intake is limited, the circulating RAAS is activated further through secretion of active renin.

Although the renin-angiotensin system is usually thought of as "hypertensinogenic" in normal pregnancy, the balance between the production of the vasodilator ANG peptide (ANG 1–7) and the vasoconstrictor peptide, ANG II, is altered so that the vasopressor actions of the circulating RAAS are, to some extent, offset (Fig. 3). Furthermore, under the influence of estrogens, the vasodilator AT$_2$R is induced. As described above, this receptor has been shown to be important within the uteroplacental vasculature in maintaining uteroplacental blood flow. Thus, through changing activity of the various components of the circulating RAAS and its receptors, the obligatory activation of the RAAS in pregnancy, which, in terms of human evolution, has been critical for maintenance of salt and water balance, does not cause hypertension.

Despite the fact that AGT levels are linked to estrogen production, overall, the activity of the circulating RAAS in pregnancy is driven mainly by maternal physiological demand, so it is not surprising that its activity does not reflect the role(s) of placental and possibly other intrauterine RASs in pathological pregnancies, e.g., intrauterine growth restriction or preeclampsia.

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