The renal circulation in normal pregnancy and preeclampsia: is there a place for relaxin?

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Submitted 17 January 2014; accepted in final form 7 March 2014

Conrad KP, Davison JM. The renal circulation in normal pregnancy and preeclampsia: is there a place for relaxin?. Am J Physiol Renal Physiol 306: F1121–F1135, 2014. First published March 19, 2014; doi:10.1152/ajprenal.00042.2014.—During the first trimester of human pregnancy, the maternal systemic circulation undergoes remarkable vasodilation. The kidneys participate in this vasodilatory response resulting in marked increases in renal plasma flow (RPF) and glomerular filtration rate (GFR). Comparable circulatory adaptations are observed in conscious gravid rats. Administration of the corpus luteal hormone relaxin (RLN) to nonpregnant rats and humans elicits vasodilatory changes like those of pregnancy. Systemic and renal vasodilation are compromised in midterm pregnant rats by neutralization or elimination of circulating RLN and in women conceiving with donor eggs who lack a corpus luteum and circulating RLN. Although RLN exerts both rapid (minutes) and sustained (hours to days) vasodilatory actions through different molecular mechanisms, a final common pathway is endothelial nitric oxide. In preeclampsia (PE), maternal systemic and renal vasoconstriction leads to hypertension and modest reduction in GFR exceeding that of RPF. Elevated level of circulating soluble vascular endothelial growth factor receptor-1 arising from the placenta is implicated in the hypertension and disruption of glomerular fenestrae and barrier function, the former causing reduced Kf and the latter proteinuria. Additional pathogenic factors are discussed. Last, potential clinical ramifications include RLN replacement in women conceiving with donor eggs and its therapeutic use in PE. Another goal has been to apply knowledge gained from investigating circulatory adaptations in pregnancy toward identifying and developing novel therapeutic strategies for renal and cardiovascular disease in the nonpregnant population. So far, one candidate to emerge is RLN and its potential therapeutic use in heart failure.

renal hemodynamics; glomerular filtration; osmoregulation; relaxin; nitric oxide; assisted reproductive technology; glomerular endotheliosis; soluble vascular endothelial growth factor receptor-1; heart failure

Renal Plasma Flow and Glomerular Filtration in Normal Pregnancy

MARKED VASODILATION OF THE MATERNAL CIRCULATION OCCURS IN the first trimester of human pregnancy. Consequently, there is a precipitous and profound decline in systemic vascular resistance (SVR) that in turn abets a reciprocal increase in cardiac output (CO) of ~40% or 2 l/min lasting throughout pregnancy (23, 163). Vasodilation of nonreproductive organs like the kidneys accounts mostly for the early gestational fall in SVR and rise in CO. The fall in SVR is nearly offset by the rise in CO; hence, mean arterial pressure (MAP) declines, but only modestly, by 5–10 mmHg (23, 38, 163). Although counterintuitive, this metamorphosis of the maternal circulation is virtually complete by the end of the first or beginning of the second trimester, when fetal crown rump length and placental diameter are only ~6 cm each (15) and uterine blood flow is ~0.25 l/min (cf. late pregnancy, ~0.75 l/min) (60). Thus, these circulatory changes are apparently preparatory events in anticipation of the rapid growth phase of the fetus and placenta in the second half of gestation, when oxygen and nutrient demands accelerate, rising exponentially. The anticipatory nature of these gestational adaptations in the maternal circulation is underscored by the difference in arterial-mixed venous oxygen content, which narrows in early pregnancy, indicating that oxygen supply exceeds demands (10, 84, 156). As shown in Fig. 1, the systemic circulatory adaptations of pregnancy in women have been modeled in the conscious gravid rat, including reduction in SVR and increases in CO and global arterial compliance (84, 176); thus affording the opportunity to explore potential hormonal and molecular mechanisms, as described below.

Renal vasodilation underlies the massive increase in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) during early gestation, as measured by the renal clear-

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Fig. 1. Maternal circulatory and osmoregulatory adaptations to pregnancy in women are recapitulated in gravid rats. SVR, systemic vascular resistance; CO, cardiac output; AC, arterial compliance; RVR, renal vascular resistance; RPF, renal plasma flow; GFR, glomerular filtration rate; Posm, plasma osmolality. Myogenic Constriction, inhibition of myogenic constriction of small renal and mesenteric arteries harvested from gravid rats. See text for details and citations.

ances of para-aminohippurate and inulin, respectively. RPF and GFR increase by 40–65 and 50–85%, respectively, during human pregnancy (reviewed in Refs. 35 and 38). This dramatic rise in RPF and GFR during early pregnancy is exemplified by the meticulous investigation of Chapman et al. (23) illustrated in Fig. 2, which corroborates one of the earliest reports of GFR measured in the first trimester by the 24-h endogenous creatinine clearance, as shown in Fig. 3 (54). As further shown in Fig. 1, the renal hemodynamic changes of human pregnancy have also been observed in the conscious gravid rat, including the reduction in RVR and elevations in RPF and GFR (29), again affording the opportunity to explore potential hormonal and molecular mechanisms. Interestingly, plasma osmolality is similarly reduced during pregnancy in both species, too (Fig. 1) (118). Investigations in gravid rats and women show that the rise in GFR is due largely to increases in RPF without elevation in glomerular hydrostatic pressure as a consequence of parallel decreases in both afferent and efferent arteriolar resistances (14, 132, 134, 161). In mothers past their child-bearing years (>52 yr of age), estimated GFR as calculated by the Modification of Diet in Renal Disease formula was not reduced by multiparity, nor was there an elevation in urinary protein excretion (87). These observations are consistent with the concept that there is glomerular hyperfiltration but not glomerular hypertension during normal pregnancy. The decrease in plasma oncotic pressure and increase in Kf also contribute to the rise in GFR (108, 132, 134, 161). As a consequence of the elevation in GFR, both serum creatinine and urea concentrations reset to lower values of ~0.5 and 9.0 mg/dl, respectively (38).

Hormonal Mechanisms of Renal and Systemic Vasodilation in Pregnancy

Whatever the dilatory mechanism(s) of pregnancy, it must be potent, because despite compensatory structural and functional hypertrophy, the renal allograft and single kidney can adapt even further, displaying gestational hyperfiltration (51, 52). Of note, both the renal and systemic hemodynamic changes of pregnancy are anticipated in the luteal phase of the menstrual cycle when RPF, GFR, and CO increase, albeit to a lesser degree (24), thus implicating a role for corpus luteal (CL) hormone(s). Although estrogen has little or no effect on the renal circulation, progesterone can increase RPF and GFR, but probably not to the same extent as observed in pregnancy (reviewed in Refs. 35 and 38).

The 6-KDa protein, relaxin (RLN) is secreted by the CL, circulates during the late luteal phase, markedly increases after conception, reaching a peak at the end of the first trimester, and falls to intermediate levels thereafter for the duration of pregnancy (172). Taking a pharmacological approach, as shown in Fig. 4A, both short- (within 1–2 h) and long-term (hours to days) RLN administration elicited renal vasodilation, increasing RPF and GFR in conscious nonpregnant, intact, or ovariectomized female and male rats analogous to the gravid state (29, 46–48, 130). Long-term RLN administration also mimicked the gestational changes in systemic hemodynamics, decreasing SVR and increasing CO and global arterial compliance in normotensive and hypertensive female and male conscious rats (34, 55, 56). However, RLN vasodilation of the systemic circulation in normotensive rats may be of slower
with the absence of a circulating renal vasodilator like RLN, B(179). The former is consistent
cline in plasma osmolality was attenuated (later time points
dued first-trimester increase in GFR, and the gestational de-
luteal support). These women demonstrated a markedly sub-
reduction in SVR during midterm pregnancy were also inhib-
illustrated further in Fig. 4, the rise in CO and global AC and
production of cyclic guanosine-3,5'-monophosphate (cGMP) rises during pregnancy in rats. Insofar
as cGMP is a major second messenger of EDRF or NO, this
onset than in the renal circulation (46, 56). Analogous to
pregnancy (Fig. 1) (75), RLN administration also inhibited
myogenic constriction of rat small renal (and mesenteric)
arteries ex vivo (Fig. 4A) (147).

As further illustrated in Fig. 4A, comparable circulatory
tional changes in response to RLN appear to occur in men and
women, although the number of studies is limited. In one
study, short-term RLN infusion (30 min to 5 h) in healthy
female and male volunteers led to a 60% increase in RPF but,
surprisingly, no rise in GFR (178). In contrast, long-term
administration for weeks in patients with mild scleroderma
produced a 15–20% increase in predicted creatinine clearance
(65). In a phase 1 trial of RLN in stable congestive heart
failure, the hormone reduced SVR, pulmonary capillary wedge
pressure, serum creatinine, and BUN and increased CO (62). In
a recently conducted evaluation of RLN administration in acute
heart failure (158), the hormone reduced SVR, arterial pres-
ure, pulmonary vascular resistance, pulmonary capillary wedge
pressure, pulmonary artery pressure, and right atrial pressure
and increased 24-h endogenous creatinine clearance. There
was no significant increase in CO. On balance and
perhaps of little surprise, the circulatory effects of RLN in
human heart failure appear to be less pronounced than in
healthy conscious rats.

Taking a physiological approach, as shown in Fig. 4B, RLN
neutralization with specific antibodies prevented the gesta-
tional changes in the renal circulation of conscious, midterm
pregnant rats and abrogated the inhibition of myogenic con-
striction in small renal arteries ex vivo. Comparable results
were observed in ovariecetomized, midterm pregnant rats,
which lacked circulating RLN. Parenthetically, the gestational
reduction in plasma osmolality was also inhibited by RLN-
neutralizing antibodies or ovariectomy (Fig. 4B) (144). As
illustrated further in Fig. 4B, the rise in CO and global AC and
reduction in SVR during midterm pregnancy were also inhib-
ited by RLN-neutralizing antibodies (58). In women with
ovarian failure who conceive using donor eggs, in vitro fertili-
ization, and embryo transfer, circulating CL factors, including
RLN, are absent (estradiol and progesterone are given for
luteal support). These women demonstrated a markedly sub-
duced first-trimester increase in GFR, and the gestational de-
cline in plasma osmolality was attenuated (later time points
were not investigated; Fig. 4B) (179). The former is consistent
with the absence of a circulating renal vasodilator like RLN,
thereby attenuating the increase in RPF and hence, of GFR. A
logical extension is that deficient renal vasodilation (and per-
haps vasodilation of other maternal organs as well) would
compromise the gestational decline in SVR and thus rise in CO
(30). Indeed, preliminary findings from an ongoing investiga-
tion of infertile women conceiving with donor eggs support
this concept although further study is needed (Fig. 4B) (41). In
contrast, Lafayette et al. (107) recently reported no significant
 correlation between serum RLN concentration and renal func-
tion in normal pregnant or preeclamptic women in the early
postpartum period. One possible explanation for this apparent
discrepancy is that RLN may be critical to the development of
renal and systemic vasodilation in the first half of pregnancy,
but as the placenta matures, placental vasodilators contribute to
the maintenance of the vasodilatory state thereafter (30). How-
ever, there may be methodological concerns with the investi-
gation of Lafayette et al., as described elsewhere (133, 134),
and the timing of the study was not ideal being post- rather than
prepartum.

Another classic phenotype of normal pregnancy in animals
and humans is the attenuation of angiotensin II (AlI) vasocon-
striction. This phenomenon is manifested in both the systemic
(33, 76) and renal (33, 44, 148) circulations. It can be duplic-
cated in conscious, nonpregnant rats by administrating RLN
(48, 56). Vascular refractoriness to AlI during pregnancy may
also be shared by other vasoconstrictors, such as /3-adrenergic
agonists (33, 142) as well as arginine vasopressin (151), and
likely abets the gestational decline in RVR and SVR.

**Molecular Mechanisms of Renal Vasodilation in Pregnancy**

Early investigations tested the hypothesis that prostaglan-
dins (PG) are the vasodilators of pregnancy; however, on
balance, a major role for these autacoids was not revealed
either in women or animal models (reviewed in Ref. 38). In
contrast, endothhelium-derived hyperpolarizing factor (83, 86,
114, 123, 124, 126, 164, 175), KATP channel activation (100)
and endothelium-derived relaxing factor (EDRF) or nitric ox-
ide (NO) (175) may be more important players. The first clue
about a possible role for EDRF (subsequently identified as NO;
Ref. 152) emerged with the finding that plasma level, urinary
excretion, and metabolic production of cyclic guanosine-3',5'-

*AJP-Renal Physiol* • doi:10.1152/ajprenal.00042.2014 • www.ajprenal.org
autocoid was implicated in the vasodilatory phenomena of pregnancy (28, 43). Later, evidence for increased NO biosynthesis was reported in gravid rats (37). Although comparable findings for cGMP were observed in pregnant women (39, 103, 165), whether NO biosynthesis increases remains unclear at least when estimated by major metabolites in the circulation or urine (39, 175).

To test directly for a functional role of NO in the renal vasodilation and hyperfiltration of pregnancy, L-arginine analogs that inhibit NO synthase were employed as illustrated in Fig. 5. Acute infusion of nitro-L-arginine methyl ester (L-NAME) or N\textsubscript{G}-monomethyl-L-arginine (L-NMMA) in conscious, midterm pregnant rats abolished the renal hemodynamic changes of pregnancy (44). Likewise, inhibition of myogenic constriction in small renal arteries isolated from midterm pregnant rats was restored to the robust phenotype of the nonpregnant condition by endothelial removal or addition of L-arginine analogs to the vessel bath (75). Chronic administration of L-arginine analogs to gravid rats also inhibited renal vasodilation and hyperfiltration in some (13, 20) but not all studies (45); in the latter, vasodilatory PG were recruited in the setting of chronic NO synthase blockade, serving a compensatory role (45). Discrepancies between these studies might stem from the different doses of NOS inhibitor used or differences in the comparison (control) group of animals employed. An essential role for NO in renal vasodilation, hyperfiltration, and inhibited myogenic constriction of small renal arteries ex vivo was also revealed for RLN-treated nonpregnant rats again by employing L-arginine analogs (48, 147). Whether expression of endothelial NO synthase or inducible NO synthase increases in maternal kidneys or arteries during pregnancy is controversial (6, 85, 146, 177, 205). Recent evidence suggests that neuronal NO synthase (nNOS) is elevated in the kidneys of gravid rats (177); to our knowledge, however, whether neuronal nNOS mediates the gestational increase in GFR and RPF has not been tested. Another group of investigators reported that short-term infusion of 7-nitroindazole inhibited gestational renal vasodilation and hyperfiltration, thereby implicating a role for nNOS (2). However, the selectivity of the inhibitor for nNOS was not verified in vivo.

Fig. 4. A: administration of relaxin to nonpregnant rats reproduces maternal circulatory and osmoregulatory changes of pregnancy (39, 103, 165). Although fewer investigations to date in humans, similar, but not identical, responses to relaxin administration have been observed. B: neutralization or elimination of circulating relaxin in midterm pregnant rats precludes the gestational changes in the maternal circulation and in osmoregulation. Emerging evidence suggests that women conceiving with donor eggs who lack a corpus luteum and circulating relaxin may have attenuated gestational circulatory changes and decreases in plasma osmolality. AII, angiotensin II; C\textsubscript{cr}, creatinine clearance; PCWP, pulmonary capillary wedge pressure. See text for details and citations.
As shown further in Fig. 5, subsequent studies revealed a critical role for the endothelial (endothelin) ET$_B$ receptor. In the endothelium, signaling by the ET$_B$ receptor involves NO synthase and other vasodilatory pathways (88, 89, 192). Previous work revealed an important role for the endothelial ET$_B$ receptor in maintaining the physiologically low vascular resistance of the renal circulation in conscious male rats (77, 78). Short-term administration of an ET$_B$ or mixed ET$_B$/ET$_A$ receptor antagonist in conscious midterm pregnant rats or in nonpregnant rats chronically treated with RLN inhibited renal vasodilation and hyperfiltration (36, 47) (comparable with NO synthase blockade, vide supra). Furthermore, addition of an ET$_B$ or mixed ET$_B$/ET$_A$ but not ET$_A$ receptor antagonist to the vessel bath reversed the inhibition of myogenic constriction in small renal arteries isolated from midterm pregnant or RLN-treated nonpregnant rats (75, 147). Consistent with these findings using ET$_B$ receptor antagonists, inhibited myogenic constriction was not observed in small renal arteries isolated from gravid ET$_B$ receptor-deficient rats or from nonpregnant ET$_B$ receptor-deficient rats administered RLN (96, 143). Although an appealing hypothesis, neither RLN administration nor pregnancy increased endothelial ET$_B$ receptor expression (99), but not all agree (61).

The enzymatic source of ET was explored next. Unexpectedly, short-term administration of phosphoramidon, a classic big ET-converting enzyme inhibitor, at rates sufficient to block the slow rise of MAP in response to a large bolus of big ET-1 did not inhibit renal vasodilation and hyperfiltration elicited by long-term RLN treatment in conscious female rats (96). Nor was the inhibited myogenic constriction of small renal arteries isolated from RLN-treated nonpregnant or midterm pregnant rats restored to the nonpregnant phenotype of robust myogenic constriction by addition of phosphoramidon to the vessel bath (70, 96). Contemporaneously, Fernandez-Patron et al. (70, 71) reported that matrix metalloproteinase (MMP)-2 can hydrolyze big ET-1 at a Gly-Leu bond, producing a novel ET, ET$_{1–32}$, that is fully active on ET receptors. Exploiting this new knowledge, short-term infusion of either a general MMP inhibitor or a specific gelatinase inhibitor blocked renal vasodilation and hyperfiltration produced by chronic (several days) RLN administration in conscious female rats (96). Consistent with these in vivo observations, a variety of MMP inhibitors added either to the artery bath or lumen restored robust myogenic constriction in small renal arteries isolated from rats administered RLN or from midterm pregnant rats (96). Small renal and mesenteric arteries, as well as aorta isolated from pregnant or RLN-treated nonpregnant rats showed increased activity, protein, and mRNA for MMP-2 and -9 (49, 95–98, 130, 194). Small renal arteries harvested from RLN-treated, ET$_B$ receptor-deficient rats showed upregulation of vascular MMP-2 activity but failed to show inhibited myogenic constriction (96). This dissociation of the biochemical and functional responses to RLN administration in the ET$_B$ receptor-deficient rat suggests that vascular gelatinase is in series with and proximal to the endothelial ET$_B$ receptor/NO signaling pathway. In summary, the endothelial ET$_B$ receptor/NO vasodilatory pathway is activated by RLN administration or pregnancy through upregulation of vascular gelatinase(s) expression and activity. Figure 5 depicts the role of vascular gelatinase in the so-called “sustained” vasodilatory actions of the hormone (i.e., after hours to days of RLN exposure).

Interestingly, MMP-9, but not MMP-2, was upregulated in small renal arteries isolated from nonpregnant rats treated with RLN for only 4–6 h, and by using specific MMP-2- and MMP-9-neutralizing antibodies, MMP-9 was shown to specifically mediate the inhibition of myogenic constriction (97). In contrast, both MMP-2 and -9 were increased in small renal arteries isolated from nonpregnant rats administered RLN for several days, although MMP-2 activity greatly exceeded that of MMP-9. In this case, again by using a specific neutralizing antibody, MMP-2 was shown to mediate the inhibition of myogenic constriction (96). Thus, the vascular gelatinase of functional relevance to vasodilation is determined by the duration of exposure to RLN (Fig. 5).

Obviously, because of ethical reasons, the molecular mechanisms of renal vasodilation and hyperfiltration in pregnancy or by chronic RLN administration in humans cannot be tested in

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**Fig. 5. Working model for the sustained vasodilatory actions of relaxin.** Not depicted in this schema are the essential roles of vascular endothelial and placental growth factors in mediating the sustained vasodilatory actions of relaxin. Inhibitors of relaxin-induced vasodilation are shown in the boxes. ET, endothelin; MMP, matrix metalloproteinase; RBF, renal blood flow; GFR, glomerular filtration rate; GM6001, a general MMP inhibitor; cyclic CTT, a specific peptide inhibitor of MMP-2; TIMP-2, tissue inhibitor of matrix metalloproteinase; RES-701-1, a specific ET$_A$ receptor antagonist; SB209670, a mixed ET$_A$ and ET$_B$ receptor antagonist; L-NAME, nitro-L-arginine methyl ester; L-NMMA, N$^\omega$-monomethyl-L-arginine. Note that phosphoramidon (an inhibitor of the classical big endothelin-converting enzyme), STT (control peptide for cyclic CTT), heat-inactivated TIMP-2, BQ-123 (a specific ET$_A$ receptor antagonist), D-NAME, and IgGs (control antibodies for MMP-neutralizing antibodies) did not affect the slow vasodilatory responses of relaxin. See text for further details and citations.

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vivo. However, a bioassay of the in vivo vasodilatory phenotype produced by pregnancy or RLN administration is inhibition of myogenic constriction of small arteries ex vivo (vide supra). Thus, we sought to determine whether incubation of rat and mouse small arteries in vitro with RLN would inhibit myogenic constriction, and if so, then this experimental approach could be applied to human arteries. Indeed, incubation of rat and mouse small renal as well as human subcutaneous (sc) arteries with RLN in vitro for 3 h inhibited myogenic constriction (a shorter incubation time has not been evaluated for myogenic constriction) (130). The inhibition of myogenic constriction was abolished by subsequently adding to the bath or artery lumen l- but not n-arginine analogs, ETB but not ETA receptor antagonists, or general and specific inhibitors of MMPs, including neutralizing antibodies and TIMP-2, but not by phosphoramidon (130). In addition, vascular endothelial growth factor (VEGF) receptor tyrosine kinase activity was implicated in the sustained RLN vasodilatory pathway, insofar as daily subcutaneous (sc) injections of the VEGF RTK inhibitor SU5416 before and during 5-day RLN administration prevented the reduction in renal vascular resistance and increases in RPF and GFR in conscious rats (130). Moreover, pretreatment of small renal arteries from rats and mice and human sc arteries with SU5416 and placental growth factor (PIGF)- or VEGF-neutralizing antibodies, followed by a 3-h incubation with RLN, prevented the inhibition of myogenic constriction by the hormone (130). Small sc arteries isolated from late pregnant women also show inhibited myogenic constriction (Debrah JE, Novak J, and Conrad KP, unpublished observations); however, whether the vasodilatory pathway outlined above is involved has not yet been tested. Investigation of accessible arteries isolated from humans is one way to translate the discoveries of the molecular pathways of vasodilation by pregnancy and RLN in animals to humans.

RLN also elicited “rapid” relaxation (within minutes) of preconstricted rat and mouse small renal and human sc arteries in vitro (131). The signaling pathway involves Goαo, protein coupled to phosphoinositide 3-kinase, protein kinase B, and endothelial NO synthase but not VEGF or increases in endothelial cytosolic calcium (131). Because arteries express a local RLN ligand receptor system (57, 145), this signaling pathway may be activated on a minute-to-minute basis, thus fine-tuning arterial relaxation. It is possible that this signaling pathway is also engaged immediately upon RLN administration, contributing to the rapid increases of RPF and GFR in conscious rats (within 1–2 h) and the rapid rise of RPF in humans (within 30 min). On the other hand, tonic activation of this arterial RLN ligand receptor system opposes myogenic constriction (sustained vasodilation, vide supra) and arterial stiffness (57, 145). Further investigation is needed to determine which factors regulate the arterial expression of RLN and its receptor RXFP1 and to define the degree of overlap and interaction among the molecular players underlying rapid and sustained vasodilation by RLN.

The molecular mechanisms underlying the refractory vasoconstrictor responses in pregnancy or during RLN administration are not entirely clear but may involve both endothelium-dependent and -independent factors. Whether vasodilatory PG play a role is disputed (33, 67, 94, 175), although NO is likely to contribute (3, 175). Renal mesangial cells prepared from gravid rats demonstrated an attenuated rise in intracellular calcium in response to AII (72). A comparable attenuation of increases in cytosolic calcium by AII was observed in mesangial cells prepared from nonpregnant rats and incubated with RLN for 24 h (22). These results implicate a direct effect of pregnancy and RLN on contractile cells independent of the endothelium, an effect that is perhaps mediated through attenuation of phosphoinositide turnover (31).

In addition to RLN, other factors likely participate in the gestational increases in GFR and RPF. PIGF may play a role especially after the first trimester, when serum concentration begins to rise as the placenta grows and matures (115). Another candidate is calcitonin gene-related peptide (CGRP), which increases in the blood during early pregnancy in women (183). CGRP is a potent vasodilator (187), and therefore, it may contribute to systemic and renal vasodilation of pregnancy. To our knowledge, however, a potential role for CGRP has not been tested, e.g., by administration of CGRP antagonists in gravid animal models. Recent evidence supports an important contribution of the AT2 receptor in mediating the midterm decline in systolic blood pressure in mice (21, 25) and in attenuating constrictor responses to phenylephrine in the aorta from gravid rats (182), thus suggesting a potential role for AT2 receptor activation in the renal vasodilation of pregnancy. Intriguingly, both histidine decarboxylase and its enzymatic product histamine, a potent vasodilator, were reported to be increased in the superficial cortex of gravid mice (135). Finally, renal production of epoxyeicosatrienoic acid may also contribute to renal vasodilation and hyperfiltration of pregnancy (91). To what degree, if any, these vasodilatory mechanisms may be activated by RLN in pregnancy is unknown.

Renal Plasma Flow and Glomerular Filtration in Preeclampsia

Depending on severity, preeclampsia (PE) can be a volatile and dangerous life-threatening disease. PE occurs in 3–5% of pregnancies, typically arising during the third trimester, and is a leading cause of maternal, fetal, and neonatal morbidity and mortality (66, 80, 101, 104, 141, 174, 199). In developed countries, PE can lead to iatrogenic premature delivery, which is frequently associated with major morbidities for the neonate immediately after delivery and long term. In the developing world, where (timely) access to health care is frequently lacking, PE can result in maternal, fetal, and neonatal death. Of note is that specific therapies for PE, which counteract pathogenic mechanisms, are currently lacking. Rather, “palliative” measures are implemented to control blood pressure and prevent seizures, and delivery (of the placenta) remains the only known cure.

In brief, preeclampsia has been defined as onset of sustained hypertension ≥140 mmHg systolic or ≥90 mmHg diastolic, with development of proteinuria of at least 1+ on dipstick or ≥300 mg/24 h after 20 wk of gestation. Severe disease is defined as hypertension ≥160 mmHg systolic or ≥110 mmHg diastolic, proteinuria >5 g/24 h, neurologic symptoms such as seizures, pulmonary edema, hepatic or renal dysfunction, thrombocytopenia, or fetal growth restriction.1 Although differential diagnosis is beyond the scope of this review and has

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1 Finally, it should be noted that the diagnosis of preeclampsia was recently revised to exclude the requirement for proteinuria. Thus, hypertension in the absence of proteinuria but accompanied by thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, and cerebral or visual symptoms fulfills the diagnosis (1).
been detailed previously (160), it should be pointed out that many patients diagnosed as preeclamptic by clinical criteria alone are often misclassified. Such misclassification may pertain particularly to multiparous women diagnosed with PE (73). In the future, a redefining or reclassification of PE will most likely include pathologically relevant biomarkers, in addition to clinical criteria, maternal history, demographic information, and standard biochemical analyses, to guide diagnosis, management, prediction, and ultimately prevention (181).

During active disease, PE is characterized by a low-cardiac output/high-systemic vascular resistance state with compromised organ perfusion, including the kidneys (40). An exhaustive review of 23 reports in the older literature on renal function in PE shows that GFR and ERPF are modestly reduced, on average by 32 and 24%, respectively, from normal, late pregnant values (Ref. 35 and citations therein). GFR and ERPF may also be decreased in PE compared with nonpregnant levels, but to a lesser degree (35). Although the criteria used for the diagnosis of PE in these studies were often not presented or failed to meet current standards, the work of Assali et al. (9) is exceptional because of rigorous diagnosis. In this report, GFR and ERPF were decreased by 29% and 20%, respectively, relative to normal pregnancy. In the studies by McCartney et al. (129) and Sarles et al. (168) the clinical diagnosis of PE was corroborated by renal histology, i.e., the presence of glomerular endotheliosis (discussed below). In these reports, GFR and/or ERPF were also modestly reduced in women with PE compared with normal pregnant women. Several investigators calculated renal segmental vascular resistances, and only preglomerular arteriolar resistance was increased in the disease; no alterations in post-glomerular arteriolar resistances were noted (Table 1) (35). The combination of findings that reduction in GFR generally exceeded that of ERPF accompanied by an increase in pre-, but not post-glomerular arteriolar resistance suggests a concomitant reduction in the glomerular ultrafiltration coefficient during PE (35, 134). Interestingly, GFR appears to rapidly recover during the first week or so after delivery, more or less coinciding with recovery of the glomerular structural abnormalities observed on renal histology (discussed later). On the other hand, ERPF may recover more slowly (35).

More recently, Irons et al. (93) reported an ERPF and GFR of 766 ± 52 and 153 ± 13 ml/min, respectively, in normal pregnant women. In primiparous preeclamptic women, ERPF and GFR were 609 ± 24 and 97 ± 7 ml/min, respectively (92). Thus, in agreement with the earlier studies, both ERPF and GFR were reduced in PE, the latter to a greater degree. The same group reported comparable findings in a subsequent study, but simultaneous analyses of the renal clearances of inulin, para-aminomhippurate, and dextrans allowed for estimation of Kf, which was ~50% lower in PE compared with normal pregnancy (133). Using different methodologies, Lafayette et al. (106) and Hladunewich et al. (90) also concluded that there was ~50% reduction of Kf in women with PE compared with control pregnant subjects 1 day after delivery, which fully accounted for the comparable percentage decrease in GFR in their study because, in contrast to the majority of other investigations, they did not observe impairment of RPF in PE. In the aforementioned studies, impaired Kf in PE women returned to the normal range by 5 wk postpartum (90) or by ≥5 mo after delivery (133).

Clinical studies reveal that proteinuria and hypertension may take ≤2 yr to disappear, and the time interval between diagnosis and delivery is associated with postpartum time to resolution of proteinuria and hypertension, implying that duration of exposure to endothelial injury may be important (18). Thus, although renal changes have been assumed to resolve fairly quickly and completely after delivery (“delivery cures the disease”), there is the distinct possibility that PE may inflict permanent renal impairment or add further to the deficit of already damaged kidneys in some women (136, 196). This damage may be indirect or direct via hypertension and/or widespread endothelial dysfunction. After PE, women have three- to eightfold increased risk of cardiovascular disease (including ischemic heart disease, hypertension, and stroke), obesity, dyslipidemia, and end-stage renal disease (196, 203). Because PE and cardiovascular disease share risk factors such as hypertension, obesity, diabetes, and hypercholesterolemia, PE is certainly a marker for cardiovascular risk, but it is not yet definitely known whether PE per se adds to the risk (17, 63, 116). If so, then PE would be an independent risk factor and not just a marker. The remote risks are apparently greatest in those who also had preterm birth, fetal growth restriction, and/or recurrent PE (140). Furthermore, the offspring of PE mothers are more likely to have a higher blood pressure and BMI from childhood and cardiovascular disease in later life (8, 50). Low birth weight is not an uncommon neonatal outcome of PE (139) that is independently associated with increased risk of hypertension and ischemic heart disease (among other pathologies) in adulthood (11, 12). Thus there is a need to elucidate the underlying biological factors that underpin the association between PE and disease later in life.

Possible Mechanisms of Renal Impairment in Preeclampsia

The factor(s) underlying compromised GFR and ERPF in PE is unknown but may relate to the generalized “endothelial dysfunction” believed to account for widespread vasospasm and organ hypoperfusion in the disease (27, 159). Specific mechanisms have been interrogated in animal models of PE, including the RUPP (reduced uterine perfusion pressure) model in gravid rats, which produces modest hypertension relative to late pregnant control rats with increased SVR and decreased CO, proteinuria (in some but not all reports), and reduced renal function (5, 173). The hypertension and/or renal dysfunction in this animal model were not improved by angiotensin-converting enzyme (4) or thromboxane synthase (120) inhibition. In contrast, inhibition of cytochrome P450 with

Table 1. Segmental renal vascular resistances in normal pregnancy and preeclampsia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Subjects</th>
<th>Segmental Renal Vascular Resistances (dyne·s/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>97</td>
<td>6,534</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>65</td>
<td>15,994</td>
</tr>
</tbody>
</table>

*Grand means for renal vascular resistances are shown. They were calculated from mean values provided in 5 studies involving a total of 97 normal and 65 preeclamptic subjects. Segmental renal vascular resistances were estimated in these publications according to the calculations of Gomez DM (86a).
1-aminobenzotriazol, which reduced renal cortical 20-HETE and CYP4A but not epoxygenase activity, normalized MAP and renal function, thus implicating overproduction of 20-HETE in the hypertension and renal dysfunction in RUPP rats (119).

Taking the lead from emerging literature on the pathogenesis of PE in women (32, 128, 195, 200, 204), potential pathogenic roles for the angiogenic factors soluble vascular endothelial growth factor receptor-1 (also known as sFlt1) and soluble endoglin (81, 82, 137, 138, 154), the cytokines IL-6 and TNFα (74, 111–113, 138, 154), and AT1 receptor autoantibodies (111, 154, 155) were explored in the RUPP model of PE. Administration of VEGF121 ameliorated hypertension and renal dysfunction (111, 154, 155) were explored in the RUPP model of PE. Although endothelial dysfunction may figure prominently in disease pathophysiology. The lesion was definitively described when the endothelial swelling can be so severe that the glomerular tuft herniates into the proximal tubule, so-called “bloodless glomeruli.” In addition, endothelial fenestrae disappear, and the endothelial swelling can be so severe that the glomerular tuft herniates into the proximal tubule, so-called “pouting.” In 1924, using light microscopy of that era, Mayer perhaps surprisingly, there are no consistent microscopic abnormalities noted in the other glomerular structures [how- ever, some have claimed that glomerular sclerosis can occur, whereas others have suggested that these are unrecognized lesions present before conception (35)]. That is, the basement membrane, podocyte, and foot processes usually appear unaffected and intact, although some investigators have reported swollen mesangial cells and expanded mesangial matrix. Moreover, interstitial and tubular structural abnormalities are absent. Glomerular subendothelial fibrin deposition can be seen particularly in intrapartum biopsies resolving quickly postpartum. Immunoglobulin deposits (IgM and IgG) are in-
frequent and of low intensity, suggesting nonspecific trapping rather than primary autoimmune disorder. Although glomerular endotheliosis is believed to be a renal lesion characteristic of preeclampsia, it has also been reported in women with placental abruption (121, 162). A recent publication suggested that near term, mild glomerular endotheliosis may be observed in normal pregnancy, but the cases described did not have associated enlarged glomeruli (185, 186, 202). The significance of this claim has been disputed (35). (A discussion of the ethics of these studies by Stevens and colleagues, specifically renal biopsy of normal pregnant volunteers, is discussed in a correspondence exchange. Br J Obstet Gynaecol 111: 191–195, 2004.) However, if true, it may be related to the physiological elevation of sFlt1 during late pregnancy, which is highest at term (115).

In rats and mice with elevated circulating sFlt1 achieved by infection with recombinant adenovirus, hypertension, proteinuria, and glomerular endotheliosis were observed (105, 117, 122, 128). Comparable glomerular lesions and proteinuria were reported in mutant mice lacking podocyte expression of VEGF (64). In patients receiving antiangiogenic therapy for cancer, hypertension, and proteinuria have been reported (184). On balance, these findings support the concept that in PE high circulating levels of sFlt1 neutralize podocyte VEGF, thereby depriving the glomerular endothelium of this essential growth factor. In addition to disrupting the glomerular barrier function leading to proteinuria, elevated circulating sFlt1 may ultimately be one factor contributing to the reduced Kf in PE by compromising the density and size of endothelial fenestrae and hence, glomerular hydraulic conductivity (106). Another factor may be circulating AT1-AA that could conceivably cause mesangial cell contraction, thereby reducing glomerular capillary surface area for filtration. The overall consensus is that these lesions of preeclampsia resolve completely after delivery, with regression seen as early as the first week postpartum (68, 102, 150, 157). Nevertheless, as discussed above, hypertension and proteinuria may persist ≤2 yr postpartum.

Perspectives

The maternal systemic and renal circulations undergo a truly remarkable transformation during pregnancy that is epitomized by massive vasodilation. Over the past decade, the corpus luteal hormone RLN has emerged as an important player in the renal vasodilation and hyperfiltration of pregnancy, as first revealed using the conscious gravid rat model. In the renal circulation of conscious rats and small renal arteries isolated therefrom, the molecular mechanisms of vasodilation vary according to the duration of exposure to RLN, but the final common pathway is endothelial production of nitric oxide. These discoveries derived from rats appear to be translating to humans, insofar as women conceiving with donor eggs who lack a corpus luteum and circulating RLN show markedly subdued maternal circulatory adaptations, especially during early pregnancy (41, 179). Moreover, subcutaneous arteries isolated from humans manifest both the rapid and sustained vasodilatory responses to RLN, as originally described in small renal arteries from rats and mice, and they are also mediated through the same molecular mechanisms, culminating ultimately in nitric oxide production.

A reclassification of preeclampsia based in part on pathologically relevant biomarkers would improve the likelihood of making the correct diagnosis (181). This eventuality might have profound implications for healthcare, because it could lead to the early identification of a group of women at risk for adverse health outcomes later in life, and may further allow for early preventative measures to be instituted. Tremendous advances in understanding the pathophysiology of preeclampsia have transpired over the last decade. Many circulating injurious agents, including antiangiogenic factors, syncytiotrophoblast microparticles, proinflammatory cytokines, and all type 1 receptor agonist autoantibodies, were identified in preeclamptic women, and the ability of many of these factors to produce preeclamptic disease manifestations, including compromised renal function, has been verified in animal models. Taken altogether, it seems an opportune time to capitalize upon and synthesize our newly gained knowledge of the physiological and pathophysiologic mechanisms in normal pregnancy and preeclampsia, respectively, to develop specific therapeutic approaches for preeclampsia, which are presently lacking.

The investigation of the hormonal and molecular mechanisms of circulatory changes in gestation could potentially have far-reaching clinical implications in both the pregnant and nonpregnant populations. First, incorporation of RLN into the medical regimen of women conceiving with donor eggs might be considered to restore and/or augment maternal circulatory adaptations, which may be deficient, particularly if these pregnancies are at increased risk for adverse outcomes, including preeclampsia, as suggested by recent reports in the literature (reviewed in Ref. 30). Second, there are a number of pathologic mechanisms in preeclampsia, many of which might be particularly amenable to treatment by RLN administration, including maternal vasoconstriction and reduced organ perfusion (27, 130). Finally, a long-term goal of investigating circulatory adaptations in pregnancy has been to reveal possible novel therapeutic opportunities for cardiovascular and renal disease in the nonpregnant population. Fortuitously and unexpectedly, in the case of RLN, this long-term goal may apply to both sexes, because administration of RLN is equally efficacious in the circulation of males most likely because of the existence of a local, arterial-derived RLN ligand-receptor system (57, 145) (lacking a corpus luteum, endogenous RLN is unlikely to circulate in males; moreover, RLN is not feminizing). To this end, the vasodilatory attributes of RLN in the renal circulation (48), as well as its matrix-degrading attributes (193), have inspired numerous investigations of its potential therapeutic efficacy in various animal models of renal disease with remarkable success (e.g., see Refs. 26, 49, and 169). Moreover, the vasodilatory attributes of RLN, particularly in the renal circulation, as detailed in this review, as well as other salutary properties, including the potential to improve arterial stiffness (34, 42), suggested the therapeutic use of RLN in heart failure (34, 191). In further support of this idea are more recent findings that RLN improves cardiac fibrosis (153, 166) and mobilizes and increases the activity of bone marrow angiogenic progenitor cells (170). Indeed, a recent phase III study of RLN in acute heart failure appears promising (190).

ACKNOWLEDGMENTS

K. P. Conrad gratefully acknowledges the invaluable contributions of many colleagues over the years, particularly Drs. Lee A. Danielson, Arun Jeyabal,
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