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Renal function during normal pregnancy and preeclampsia

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1. ABSTRACT

Glomerular filtration rate and renal plasma flow increase by 40 to 65 and 50 to 85 %, respectively, during normal pregnancy in women. Studies using the gravid rat as a model have greatly enhanced our understanding of mechanisms underlying these remarkable changes in the renal circulation during gestation. Hyperfiltration is largely due to increased renal plasma flow, the latter attributable to profound reductions in both the renal afferent and efferent arteriolar resistances. The ovarian hormone, relaxin, mediates renal vasodilation during pregnancy.. Relaxin increases vascular gelatinase activity, thereby converting big ET to ET_{1-32}, which leads to renal vasodilation, hyperfiltration and reduced myogenic reactivity of small renal arteries via the endothelial ET_B receptor and nitric oxide. Serum concentration of uric acid falls during normal pregnancy as a consequence of increased GFR and/or reduced proximal tubular reabsorption. The elevated urinary excretion of protein during pregnancy is secondary to increased GFR, reduced proximal tubular reabsorption, and perhaps alteration in the electrostatic charge of the glomerular filter. Whether the tubular secretion of Tamm-Horsfall protein increases during normal pregnancy is uncertain. In most women with preeclampsia, renal plasma flow and glomerular filtration rate are at most only modestly decreased as a consequence of increased afferent arteriolar resistance and/or reduced ultrafiltration coefficient. Serum uric acid concentrations are increased mainly as a consequence of reduced renal clearance. Reduced GFR leads to decreased filtered load of uric acid, and plasma volume contraction contributes to increased proximal tubular reabsorption coupled to sodium. The increase in urinary protein excretion in preeclampsia occurs secondary to alterations in the size and/or charge selectivity of the glomerular filter, possible increases in glomerular capillary pressure, and compromise of proximal tubular reabsorption. The renal histologic lesion characteristic of preeclampsia is termed "glomerular endotheliosis". Recent evidence suggests that anti-angiogenic factors emanating from the placenta in preeclampsia contribute to glomerular endotheliosis, proteinuria, and hypertension during disease.

2. INTRODUCTION

Knowledge of the changes in maternal renal and cardiovascular function, as well as volume homeostasis during normal pregnancy is critical to complete understanding, proper diagnosis and management of preeclampsia. In this chapter, we focus on the alterations in renal hemodynamics and glomerular filtration of normal pregnancy as well as the underlying mechanisms. In addition, we consider two additional aspects of renal function during normal pregnancy that are typically deranged in preeclampsia; namely, the renal handling of uric acid and proteins. Finally, renal hemodynamics and glomerular filtration, as well as the renal handling of uric acid and proteins are discussed in the context of preeclampsia. Because these topics have been recently and comprehensively reviewed (1-3), we provide succinct summaries and refer the reader to the previous reviews for complete presentations. Thus, in this chapter, we highlight the recent advances. At the end, we provide a section on "Perspectives" in which we discuss overall biological relevance and therapeutic implications.

3. RENAL HEMODYNAMICS AND GLOMERULAR FILTRATION DURING NORMAL PREGNANCY

In several longitudinal studies throughout gestation in women, renal plasma flow (RPF) and glomerular filtration rate (GFR) were measured by the renal clearances of para-aminohippurate and inulin, respectively (the "gold standards" for accurate measurement of renal function, see reviews 1-3, and citations therein). On average, these studies demonstrated an increase in GFR and RPF of 40-65% and 50-85%, respectively, during the first half of gestation compared to pre-pregnant or postpartum values. Because the rise in RPF exceeded that of GFR, the renal filtration fraction fell. During the latter stages of pregnancy, a modest decline in RPF towards non-pregnant levels was observed, while GFR was maintained.

The 24-h endogenous creatinine clearance is a reliable estimate of GFR in the setting of pregnancy (reviewed in 1). Using this methodology, Davison and Noble (4) demonstrated that GFR rises 25% by the fourth gestational week compared to week 1, and attains a 45% increase by week 9 (post LMP). Thus, the alterations in renal hemodynamics are among the earliest and most dramatic maternal adaptations to pregnancy.

4. MECHANISMS OF RENAL VASODILATION AND HYPERFILTRATION DURING NORMAL PREGNANCY

Animal models have been used to investigate underlying mechanisms. The Munich-Wistar rat manifests glomeruli belonging to the superficial cortical nephrons on the surface of the kidney that are accessible to micropuncture. Using this methodological approach, Baylis demonstrated that the gestational increase in single nephron glomerular filtration rate was secondary to a rise in glomerular plasma flow with no increase in glomerular hydrostatic pressure (5). This constellation of events was made possible by parallel and comparable decreases in both the afferent and efferent renal arteriolar resistances. Although such an invasive approach cannot be applied to human pregnancy, indirect evidence suggested similar mechanisms for the gestational increase in GFR (6). Finally, because the alterations of renal hemodynamics and GFR in the chronically instrumented, conscious gravid rat are comparable to human gestation, this animal model has been extensively used to investigate underlying hormonal and molecular mechanisms (7).

4.1. Plasma volume expansion

Plasma volume expands markedly during pregnancy, although the greatest increase occurs in the second half of gestation, and as such, does not correlate well with the rise in RPF and GFR that occurs in the first half of pregnancy. Nevertheless, *acute* expansion of plasma volume by 10-15% was investigated in virgin female Munich-Wistar rats, and it failed to increase GFR, single nephron glomerular filtration rate (sngfr), or glomerular plasma flow (8). Moreover, volume expansion has been shown to suppress tubuloglomerular feedback activity that, in turn, could mediate the gestational rise in glomerular plasma flow and sngfr. However, tubuloglomerular feedback was not suppressed in gravid Munich-Wistar rats, thus eliminating this mechanism as an explanation for gestational renal vasodilation and hyperfiltration (9). Yet to be tested is whether *chronic* plasma volume expansion abets the increase in RPF and GFR during early gestation, or the maintenance of RPF and GFR during late gestation by some means other than through the tubuloglomerular feedback mechanism (reviewed in 1,2).

4.2. Pseudo-pregnancy

By mating a female rat with a vasectomized male, pseudo-pregnancy--a condition that physiologically mimics the first half of gestation in rats, but lacks fetal-placental development--is produced. This condition mimics the increases in RPF and GFR that are observed during early pregnancy in rats (10,11). Thus, maternal influences alone may be sufficient to initiate the changes in the renal circulation during pregnancy. On the other hand, the mechanisms recruited in pseudo-pregnancy are not necessarily the same as those utilized during early pregnancy.

4.3. Menstrual cycle

The gestational increases in RPF and GFR are observed, albeit on a smaller scale, in the luteal phase of the menstrual cycle (reviewed in 1-3). This observation may provide insight into the hormones responsible, because several that rise during early gestation also increase, but to a lesser degree, during the luteal phase of the menstrual cycle (e.g., the ovarian hormones, progesterone and relaxin, see below).

4.4. Hormonal regulation: sex steroids

Based on both acute and chronic administration of ***estrogens*** to humans and laboratory animals, this hormone has little or no influence on RPF or GFR, although it can clearly increase blood flow to other non-reproductive and reproductive organs (reviewed in 1-3). On the other hand, ***progesterone*** (reviewed in 1-3) (or possibly progesterone

metabolite(s) (12,13)) is a reasonable candidate according to the studies of exogenous administration to humans and laboratory animals. However, in most of these studies, only modest increases in RPF and GFR were achieved, falling well short of those observed during pregnancy.

4.5. Hormonal regulation: peptide hormones

Peptide hormones of maternal origin may contribute to the early gestational increases in RPF and GFR. Thus, ***prolactin*** has been investigated, because circulating concentrations increase in both pseudo-pregnant and early pregnant rats coinciding with the increases in RPF and GFR. Unfortunately, whether prolactin can increase RPF and GFR remains controversial (reviewed in ref. 14), and as such, further study is needed. ***Placental lactogens*** which activate the same receptor as prolactin have not been investigated as possible mediators of renal vasodilation and hyperfiltration during the last half of pregnancy in rats when these hormones circulate.

Relaxin may contribute to the alterations in renal and possibly other organ circulations, as well as osmoregulation during pregnancy. In gravid rats and women circulating relaxin arises from the corpus luteum of the ovary (reviewed in ref. 15). Human chorionic gonadotrophin (hCG) is a major stimulus for relaxin secretion during pregnancy in women (15). Thus, serum relaxin concentrations are highest during the first trimester (~ 1 ng/ml), and fall to lower levels in late gestation (~ 0.4 ng/ml; 15). There were several compelling, albeit circumstantial reasons to consider relaxin as the pivotal reproductive signal for initiation of the circulatory and osmoregulatory changes of pregnancy as recently reviewed (1-3).

Although renal vasodilation and hyperfiltration are detectable in gravid rats before gestational day 8, when serum relaxin is undetectable, there is a marked jump in renal function between gestational days 8 and 12, when ovarian and circulating relaxin levels surge (7,14). The increases in GFR and RPF that occur during rat gestation before gestational 8 or during pseudo-pregnancy when circulating relaxin is undetectable may be mediated by other, as yet, undefined mechanisms. Alternatively, circulating relaxin concentrations below the level of detection may be responsible.

Chronic administration of porcine relaxin or of recombinant human relaxin (rhRLX) to chronically instrumented, conscious female rats over 2-5 days increased both RPF and GFR (and reduced serum osmolality) to levels observed during midterm pregnancy when renal function peaks in this species (7,16). This response was independent of ovaries (16), and observed in male rats (17). Chronic relaxin administration also blunted the renal vasoconstrictor response to angiotensin II infusion (16) – a phenomenon observed during rat gestation (18-20). Furthermore, the myogenic reactivity of small renal arteries isolated from the relaxin-treated rats was markedly inhibited (21) and comparable to the inhibition previously demonstrated in small renal arteries isolated from midterm pregnant rats (22). Short-term administration of rhRLX to conscious rats for hour(s) also produced renal vasodilation and hyperfiltration (23). By administering relaxin neutralizing antibodies or removing circulating relaxin by ovariectomy and maintaining pregnancy with exogenous sex steroids, gestational renal hyperfiltration, vasodilation and reduced myogenic reactivity of small renal arteries were completely abolished in midterm pregnant rats (24). The osmoregulatory adaptations of pregnancy were also prevented (24). Thus, relaxin is essential for the renal circulatory and osmoregulatory changes in midterm pregnant rats.

In normal human volunteers, short term intravenous infusion of rhRLX over 6 hours increased RPF by 60%, but surprisingly, not GFR (25). This renal vasodilatory response was observed in men and women, and as soon as 30 minutes after initiating the rhRLX infusion. There were no significant changes in blood pressure or serum osmolality. After 26 weeks of rhRLX administration to patients with mild scleroderma, the predicted creatinine clearance increased by 15-20%, and serum osmolality and blood pressure declined slightly, but significantly throughout the study in a dose dependent fashion (26). Finally, in women who lacked ovarian function and became pregnant through egg donation, IVF and embryo transfer, the gestational increase in GFR and decrease in serum osmolality were significantly subdued (27). Because these women lacked ovarian function, serum relaxin was undetectable. Thus, like gravid rats, endogenous circulating relaxin has a role in establishing the renal and osmoregulatory responses to pregnancy in women. However, unlike the gravid rat, partial responses may remain despite the absence of circulating relaxin.

Chronic administration of rhRLX for days to either normotensive or hypertensive, male and female rats mimicked the changes in systemic hemodynamic and arterial properties of pregnancy and increased the passive compliance of isolated small renal arteries (28-30). In contrast, short-term rhRLX administration over hours was only active in the angiotensin-II model of hypertension, and not in spontaneously hypertensive or normotensive rats (29,30). Thus, relaxin generally acted more rapidly in the renal circulation of rats than in the systemic vasculature (23). An essential role for relaxin in the alterations of systemic hemodynamics and arterial properties during midterm pregnancy in conscious rats was recently identified (31). Specifically, by administering relaxin neutralizing antibodies, the gestational increases in cardiac output and global arterial compliance, as well as the reduction in systemic vascular resistance were inhibited. Whether neutralization of circulating relaxin has similar inhibitory effects during late gestation is currently under investigation. It is possible that other hormones possibly emanating from the placenta rather than the ovary assume control of systemic vasodilation and osmoregulatory changes during late pregnancy.

4.6. Cellular and molecular mechanisms

As reviewed recently, ***nitric oxide*** (NO) mediates renal vasodilation and hyperfiltration in the gravid rat model (1-3). In contrast, the vasodilatory prostaglandins have little or no role (1-3). In addition, NO mediates the inhibition of myogenic reactivity in small renal arteries isolated from midterm pregnant rats (22). This dependency on NO was also demonstrated for identical changes elicited

in the renal circulation by chronic relaxin administration to non-pregnant rats (16,21). The mechanism for increased NO in the renal circulation of midterm pregnant rats or of relaxin-infused non-pregnant rats is not a consequence of increased expression of endothelial nitric oxide synthase (32).

In midterm pregnant rats or during chronic administration of rhRLX to non-pregnant rats, ***endothelin*** mediates the renal vasodilatory changes through stimulating the endothelial ET_B receptor subtype which is coupled to NO production (reviewed in 1-3). Whether expression of the endothelial ET_B receptor increases, thereby representing a primary alteration that mediates renal vasodilation, hyperfiltration and reduced myogenic artery of small renal arteries during pregnancy or after chronic administration of rhRLX to non-pregnant rats is controversial (33,34).

Typically, the traditional endothelin converting enzymes that are inhibited by phosphoramidon mediate the processing of big ET, an inactive precursor, to ET_{1-21}. However, phosphoramidon did not affect renal vasodilation, hyperfiltration or reduced myogenic reactivity of small renal arteries isolated from midterm pregnant rats or from non-pregnant rats chronically administered rhRLX (35). Based on the confluence of several of our own experimental findings as well as those of others, we deduced that relaxin may up-regulate vascular ***matrix metalloproteinase-2*** (MMP-2) activity in the renal vasculature during pregnancy, that in turn, cleaves big ET to ET_{1-32} at a gly-leu bond, thereby stimulating the endothelial ET_B receptor-NO vasodilatory pathway (35 and citations therein).

Briefly, after acute infusion of a specific inhibitor of MMP-2 and -9 or of a general inhibitor of MMP to conscious rats during chronic administration of rhRLX, renal vasodilation and hyperfiltration were abolished. Moreover, the reduced myogenic reactivity of small renal arteries isolated from midterm pregnant rats or from non-pregnant rats chronically administered rhRLX was reversed by incubating the arteries with the specific MMP-2 and -9 inhibitor, the general MMP inhibitor, TIMP-2 (tissue inhibitor of metalloproteinase), or a specific MMP-2 neutralizing antibody, but not by phosphoramidon. Moreover, MMP-2 activity was increased in small renal (and mesenteric) arteries isolated from midterm pregnant rats or non-pregnant rats chronically administered rhRLX. An increase in MMP-2 activity in response to chronic rhRLX administration was also observed in small renal arteries from rats that were genetically deficient in the ET_B receptor. However, myogenic reactivity remained robust and did not become attenuated in these arteries. The latter finding reinforced the essential role of the endothelial ET_B receptor in mediating the inhibition of myogenic reactivity by rhRLX and corroborated our previous work using pharmacological inhibitors of the receptor. Of greater significance, however, is that the dissociation of increased vascular MMP-2 activity from inhibition of myogenic reactivity indicates that MMP-2 is in series with and upstream of the endothelial ET_B receptor-NO vasodilatory pathway (35).

The mechanism for the increase in vascular MMP-2 by rhRLX or pregnancy is incompletely understood. Both pro and active MMP-2 activities are increased to a similar extent, MMP-2 protein and mRNA are also increased, and there is no change in TIMP-1 or 2 (36). MMP-2 protein is localized to both endothelium and vascular smooth muscle, but further investigation is needed to determine in which of these two compartment(s) it increases in response to pregnancy or chronic rhRLX administration. Also unknown is which relaxin receptor mediates the vascular responses to pregnancy or chronic relaxin administration, if it is located on the endothelium, vascular smooth muscle or both, and whether there are intermediary molecules linking relaxin receptor activation to increased MMP-2 expression.

An emerging view is that the vasodilatory mechanisms of relaxin vary according to the time of exposure to the hormone. The mechanisms involved after chronic administration of hormone to non-pregnant rats or during pregnancy when endogenous relaxin circulates were detailed above. Relaxin can also rapidly relax within minutes some, but not all pre-constricted arteries from humans and rats (37, and Conrad et. al., unpublished). This rapid response ultimately involves nitric oxide, but the proximal mechanisms have yet to be completely elucidated. Recently, we demonstrated that ***matrix metalloproteinase-9*** (MMP-9) rather than MMP-2 activity is increased in small renal and mesenteric arteries isolated from rats after short-term administration of rhRLX for 4-6 hours (38). Small renal arteries demonstrated loss of myogenic reactivity, but robust myogenic reactivity was restored by incubation with a specific MMP-9 antibody, rather than a specific MMP-2 antibody as observed following chronic relaxin administration (supra vide). Like MMP-2, MMP-9 can process big ET at a gly-leu bond in the vasculature; the inhibition of myogenic reactivity after short-term rhRLX administration was also mediated by the endothelial ET_B receptor-nitric oxide vasodilatory pathway.

5. RENAL HANDLING OF URIC ACID DURING NORMAL PREGNANCY

The reader is referred to a recent and comprehensive review of this topic (1). Briefly, uric acid is the end product of purine metabolism in humans (39). Purines originate from the diet and are endogenously produced, the latter being the major source. Most circulating uric acid is produced by the liver, and in humans approximately 66% is excreted by the kidney. The remaining 33% is excreted by the gastrointestinal tract. Five percent of circulating uric acid is bound to plasma proteins, thus the majority of this circulating solute is available for glomerular filtration. Once filtered, it undergoes both reabsorption and secretion, mainly in the proximal tubule. In humans, however, net reabsorption occurs: the bulk of filtered uric acid--88 to 93%--is reabsorbed by the renal tubules back into the blood, and only 7 to 12% of the filtered load is excreted in the urine (39).

Serum concentrations of uric acid are significantly decreased from non-pregnant values by approximately 25 to 35 percent throughout most of normal human pregnancy (40-43). During late gestation, they rise toward non-pregnant values. Theoretically, serum concentration of uric acid is determined by several factors during pregnancy including dietary intake of purines, metabolic production of uric acid by the mother, fetus and placenta, as well as renal and gastrointestinal excretion (39,44,45). Alterations in any one or in several of these factors could underlie the changes of serum uric acid that occur in normal pregnancy. However, altered renal handling likely makes a major contribution. Depending on the study (41,43), the renal clearance of uric acid is increased during gestation by virtue of raised GFR and filtered load, reduced tubular reabsorption, or both. In one study (41), the restoration of serum uric acid toward non-pregnant levels near term was explained by a progressively increasing renal tubular reabsorption.

6. RENAL HANDLING OF PROTEIN DURING NORMAL PREGNANCY

An extensive review of this topic was recently published (1). Briefly, the urinary excretion of total protein, albumin, low molecular weight (LMW) proteins and of several renal tubular enzymes increases during normal human pregnancy. The increase in renal excretion of LMW proteins and of renal tubular enzymes is consistent with a physiological impairment of proximal tubular function. (This concept is also supported by the finding of elevated urinary excretion or fractional excretion of glucose, amino acids, uric acid and calcium during normal human gestation (46, and see Renal Handling of Uric Acid, above). In addition to insufficient proximal tubular reabsorption, the elevated GFR of pregnancy, and possibly an alteration in the electrostatic charge of the glomerular filter also contribute to gestational albuminuria. Available evidence does not indicate an increase in the glomerular permselectivity on the basis of size or molecular weight. As in the non-pregnant condition, much of the protein in normal pregnancy urine is probably Tamm-Horsfall protein secreted by renal tubules, and whether its secretion is increased during gestation has not been studied in detail (47).

7. RENAL HEMODYNAMICS AND GLOMERULAR FILTRATION IN PREECLAMPSIA

Numerous studies have reported that renal function is reduced in preeclamptic pregnancies. In Conrad and Lindheimer's detailed review of the subject (1), 23 studies reporting glomerular filtration rate (GFR) and renal plasma flow (RPF) in preeclamptic women are summarized. These are particularly noteworthy because (1) an appropriate control group of late pregnant women was used, and in nine of the studies, nonpregnant controls were included; (2) reasonably clear criteria for the diagnosis of preeclampsia was presented; and (3) many of these studies also used renal clearances of inulin and para-aminohippurate to reliably measure GFR and RPF, respectively. On average, GFR and effective RPF (ERPF) were reduced by 32% and 24%, respectively, in preeclamptic women compared to normal, late-pregnant values. GFR was reduced in all of these studies and ERPF was also found to be reduced except for the report by of one group of investigators (48,49). Both GFR and ERPF were decreased by 22% compared to nonpregnant control subjects. On further scrutiny of the methodologies employed, it is important to note that a clear definition of preeclampsia was explicitly stated in the work of Assali and colleagues: "…the presence of hypertension, edema, and proteinuria after the 24th week of gestation, together with the absence of a history of hypertension prior to pregnancy, and the return of the blood pressure to normal levels following delivery" (50). In the studies of McCartney et al and Sarles et al, the histopathologic finding of glomerular endotheliosis was used to confirm the clinical diagnosis of preeclampsia (51,52). In these studies, the lower GFR and ERPF in preeclamptic subjects was noted when such rigorous criteria for the diagnosis of preeclampsia were employed (50-52). More recent work confirms the decline in GFR and ERPF in preeclampsia (53,54). Investigations by Lafayette and colleagues performed 3-5 hours post-cesarean section also revealed that GFR, as measured by inulin clearance, was decreased in women with preeclampsia compared to normal postpartum controls; in contrast, ERPF, measured by para-amminohippurate clearance, was no different between the two groups (55). Unfortunately, these immediate postpartum findings are difficult to interpret, because they are confounded by significant hemodynamic changes that occur following cesarean delivery.

The precise mechanism(s) responsible for the compromise of the renal circulation in preeclampsia is unknown. However, the reduced ERPF is due to high renal vascular resistance. Conrad and Lindheimer estimated renal vascular resistances from 5 studies (97 normal pregnancies and 65 preeclamptic pregnancies) (1,56). The mean total renal vascular resistance was increased by approximately 3-fold in preeclampsia compared to normal pregnancy. A comparably elevated renal afferent (pre-glomerular arteriolar) resistance is the major contributor to this increased total renal vascular resistance. The increased afferent arteriolar tone in preeclampsia may protect the glomerulus from damage due to high systemic arterial pressures. Both reduced ERPF, ultrafiltration coefficient (K_f, which reflects the surface area for filtration and the intrinsic permeability of glomerular capillaries), or both are possible mechanisms for the reduced GFR in preeclampsia (1, 54-56).

8. RENAL HANDLING OF URIC ACID IN PREECLAMPSIA

The association between preeclampsia and hyperuricemia was first reported in 1917 (57). Since that time numerous investigators have substantiated this finding (1). Furthermore, there have been a number of studies that have reported a strong positive correlation between the degree of hyperuricemia and the severity of preeclampsia (summarized in (1,56)). Specifically, the hyperuricemia correlated with the severity of the renal histologic finding of glomerular endothelioisis (58), and with perinatal morbidity and mortality (59,60,61). The increase in serum

uric acid is an early change in pregnancies complicated by preeclampsia occurring as early as 24-25 weeks gestation (59) and even earlier in women who develop preeclampsia or gestational hypertension with hyperuricemia at diagnosis (62). The predictive value of uric acid in preeclampsia remains controversial. Of two recent systematic reviews on the subject, one concludes insufficient evidence for the accuracy of uric acid in predicting preeclampsia (63) and the other that uric acid is a poor predictor of maternal and fetal complications in preeclampsia (64).

As discussed under RENAL HANDLING OF URIC ACID DURING NORMAL PREGNANCY, above, the possible sources of increased circulating uric acid include elevated production via purine breakdown or dietary intake and/or decreased clearance by the liver or kidney. Of these, reduced renal excretion appears to be the major source of elevated uric acid level in pregnancies complicated by preeclampsia (1). Diet is unlikely to be a major contributing factor (1). Even when preeclamptic and normal pregnant women were given a rigidly controlled diet, including purine intake, hyperuricemia in preeclampsia was observed (65). Since humans lack the liver enzyme uricase that degrades uric acid to allantoin, this cannot be an explanation for hyperuricemia in preeclampsia. Controversy remains regarding the feto-placental unit as an influence on circulating levels of uric acid in pregnancy and its potential contribution in preeclampsia (1,66). Early work using injected urate isotope suggested that the rate of uric acid production was increased in preeclampsia (67). However, these results were later retracted by the authors (65). It was also postulated that multiple infarcts in the placenta associated with preeclampsia may contribute to hyperuricemia; however, concentrations of uric acid and oxypurine, a metabolite of uric acid, were not greater in the uterine venous circulation (68) or "intervillous space" (69) compared to peripheral circulation arguing against a major placental source. Adding to the controversy was the now outdated and disproven belief that the placenta did not contain xanthine dehydrogenase/oxidase activity (70). A recent review (66) has suggested that increased fetal and/or placental production may contribute to circulating uric acid based on the finding of elevated uric acid in multifetal gestations (71) and that purine metabolites are elevated in the umbilical cord blood specimens from newborns with clinical signs of intrauterine hypoxia during labor (72). Trophoblast shedding into the maternal circulation has also been proposed as a source of purine availability for breakdown (66).

Most agree, however, that the major source of elevated uric acid in preeclampsia is reduced renal excretion as a consequence of reduced GFR and increased net reabsorption of uric acid by the renal tubules (1,56). In reviewing a series of studies evaluating the renal handling of uric acid during preeclampsia, enhanced tubular reabsorption is implicated as the primary mechanism (1). On average, plasma uric acid concentration was 6.7 mg% in preeclampsia compared to 3.8 mg% in third trimester uncomplicated pregnancies with GFRs of 96 ml/min and 124 ml/min, respectively. The fractional reabsorption of uric acid was 93.5% in subjects with preeclampsia compared to 89.4% in normal, late-pregnant controls. Studies by Czaczkes et al and Hayashi et al using probenicid to inhibit tubular reabsorption of uric acid further support the concept that increased tubular reabsorption is the major reason for elevated circulating uric acid (73,74). In preeclamptic women, probenecid increased renal clearance of uric acid from 3.7 to 18.8 ml/min, reduced the fractional reabsorption from 96.3% to 81%, and restored circulating plasma uric acid levels from 6.9 to 3.2mg%. In fact, circulating uric acid levels in preeclamptic women were comparable to values observed in normal pregnant women receiving probenecid, 3.2 vs 3.3 mg% (73). This implicates enhanced tubular reabsorption of uric acid in preeclampsia rather than reduced glomerular filtration or tubular secretion. This increased proximal tubular reabsorption of uric acid is coupled to sodium, and the latter is most likely increased in preclampsia as a consequence of reduced plasma volume (1). Interestingly, Hayashi and colleagues noted that although probenecid reduced uric acid levels, it did not alter the clinical course of preeclampsia suggesting that hyperuricemia is not an important factor in the pathogenesis of preeclampsia (74). In contrast, a more recent review of the subject hypothesizes that uric acid may indeed have a pathophysiologic role in preeclampsia (66) citing animal studies in which elevated uric acid is associated with hypertension and afferent arteriolar thickening (75,76). These deleterious effects are prevented with allopurinol, a xanthine oxidase inhibitor (75,76). In humans, hyperuricemia predicts hypertension and is an independent predictor of renal disease progression in certain conditions (75). A recent, small, randomized, clinical trial comparing probenecid treatment to placebo in 40 women with early-onset preeclampsia demonstrated lower circulating uric acid concentrations, higher platelet counts, with no apparent change in blood pressure or clinical course of preeclampsia (77). Although an interesting concept, further investigations are warranted in this regard.

9. RENAL HANDLING OF PROTEIN IN PREECLAMPSIA

The association between eclampsia and proteinuria was first reported in 1843 (78). Currently, proteinuria is a hallmark of preeclampsia and along with new onset hypertension, total urine protein excretion in excess of 300 mg in 24 hours is widely recognized as a criterion in the research and clinical definitions of the syndrome. The alteration in circulating levels of proteins in preeclampsia varies beyond those changes seen in normal pregnancy (reviewed extensively in (1)). In general, the circulating levels reflect the pattern of heavy urinary loss of intermediate molecular weight proteins and relative retention of proteins with higher molecular weights. For example, serum concentration of albumin, total protein, thyroxine-binding prealbumin, IgG and transferrin are reduced, whereas levels of α_2-macroglobulin, β-lipoprotein and β_1- A-C complement fractions are increased. As indicated above, the urinary proteins are primarily from the plasma including albumin, α_1 and α_2-globulin, β-globulin, γ-globulin (IgG, IgA, and occasionally IgM),

ceruloplasmin, pseudo-cholinesterase, and α_2-macroglobulin (1). While some reports suggest that the magnitude of the proteinuria is correlated with the histologic severity of the renal lesion (79), others have shown that the glomerular lesion can also be seen without proteinuria and in normal pregnancies (80,81). Interestingly, the degree of proteinuria does not appear to predict maternal and fetal outcomes (82).

As previously discussed, GFR and ERPF are reduced in preeclampsia; therefore the increased proteinuria most likely results from the altered glomerular permeability (size and/or charge selectivity) and/or changes in tubular handling of filtered proteins. Since glomerular capillary pressure (P_{GC}) has not been studied directly in humans and preeclampsia, elevated P_{GC} cannot be definitively ruled out as a contributor. Urinary protein excretion of greater than 1 gram/day saturates the tubular resorptive capacity; therefore, renal clearances of plasma proteins and their molecular weights can be used to evaluate the size selectivity of the glomerular barrier when urinary protein excretion is greater than the tubular resorptive capacity. Studies have used renal clearances of intravenously administered dextran or inert polymer polyvinylpyrrolidone (PVP) in preeclamptic women to evaluate glomerular size selectivity. The advantage is that these compounds are available in various molecular weights and are neither reabsorbed nor secreted by the tubule; thus, urinary excretion reflects glomerular filtration. Using these approaches, the protein selectivity was noted to be in an "intermediate range" in preeclampsia (1,56,83,84). More recent work using the infusion of the neutral dextrans and incorporating mathematical modeling of fractional clearance data in normal pregnancy suggests that a significant nondiscriminating shunt appears concomitantly with increasing gestational age and proteinuria (6,56,85). These investigators have suggested that the reduced filtration in preeclampsia is caused by a reduced ERPF and reduced K_f, the latter by 50% prepartum compared to their own postpartum values (54). It seems paradoxical that the filtration of water is reduced in preeclampsia, but larger molecules such as proteins are not. However, by using theoretical models to analyze the neutral dextran sieving data in preeclampsia, there was an increase in pore size variation around the mean and the shunt component that accounts for the free passage of large molecules was also increased (54). This would possibly correspond to a broadening of the foot processes and disruption of the integrity of the slit diaphragm allowing for high loss of protein in small areas of the glomerular wall, without an increase in the flux of water (56).

Whether changes in charge selectivity of the glomerular barrier occur in preeclampsia remains an unresolved issue. The glomerular filtration of albumin, a negatively charged protein, is reduced to a greater extent than would be expected with size alone. Furthermore, studies of glomerular charge selectivity in nonpregnant subjects has revealed that neutral dextrans are more freely filtered than its anionic counterparts of a similar molecular weight (reviewed in (56)). One reason that this phenomenon has not been studied extensively in pregnancy and preeclamsia is that administration of charged dextrans may not be safe in pregnancy. Investigations by Moran and colleagues provide indirect evidence for the loss of glomerular charge selectivity in preeclampsia. Despite a 100-fold increase in urinary albumin excretion, fractional neutral dextran clearances of comparable molecular weight were reduced (54). Histopathologic studies of renal biopsies performed in the postpartum period of women with pregnancies complicated by preeclampsia revealed enlarged glomeruli, fusion of the foot processes and thickened glomerular basement membrane in combination with a decrease in the amount of anionic heparin sulfate in the glomerular basement membrane. This loss of negative charge may, in turn, permit collapse of the slit in the epithelial layer allowing passage of negatively charged proteins (86,87)

The increase in urinary protein excretion during preeclampsia may also be a consequence of further impairment of the proximal tubular function beyond that seen with normal pregnancy (see RENAL HANDLING OF URIC ACID IN NORMAL PREGNANCY, above). This is particularly evident for low molecular weight proteins (e.g. β2-microglobulin) that are freely filtered at the glomerulus and are noted to be higher in the urine of women with preeclampsia compared to normal pregnancy (1).

10. GLOMERULAR LESION OF PREECLAMPSIA

Preeclampsia is associated with a characteristic renal lesion termed "glomerular capillary endotheliosis" first noted as early as 1924 (88). The reader is referred to two excellent reviews for histologic presentations and detailed discussion (89,90). The glomeruli are enlarged, but not remarkably hypercellular. The glomerular enlargement is caused primarily by swelling of the endothelial cells with some swelling of mesangial cells; this hypertrophy leads to encroachment on the glomerular space, and consequently, the glomerulus, itself, appears "bloodless". The glomerular epithelial cells (podocytes) often appear swollen with PAS-positive hyaline droplets. Deposition of fibrin and fibrin derivatives may also be seen. Electron microscopy is essential to confirm the endotheliosis and identify the loss of endothelial fenestrae. Extensive vacuolization of the endothelial cells, and less often, the mesangial cells, are also noted on ultrastructural examination. Interestingly, the foot processes are relatively preserved, despite the extensive proteinuria seen with preeclampsia. Since there are currently very few indications for third trimester renal biopsies, it is useful to review past studies when renal biopsies were performed more frequently. In a large series of 176 renal biopsies in hypertensive pregnant women diagnosed clinically as having preeclampsia, Fisher et al demonstrated that 18% of primiparous women had another parenchymal renal disorder and that in 25% of primiparous women the diagnosis of preeclampsia was "incorrect" based on histologic diagnosis. These numbers were even higher for multiparous women, 51% had an underlying renal disorder and in approximately 76% the clinical diagnosis of preeclampsia was "incorrect" (79). Thus, based on renal histopathology, the clinical diagnosis of preeclampsia is

more likely to be correct in primigravid women. Recent publications by Strevens and colleagues demonstrated that glomerular endotheliosis can be observed in normotensive pregnancies (80,81). The ethical issues surrounding these investigations have been questioned and the reader is referred to the letters exchanged in this regard (91). However, the glomerular volume was greater, and the lesion more severe in women with hypertension and proteinuria. The glomerular endotheliosis usually resolves within a few weeks after delivery. On balance, it may be more accurate to consider glomerular endotheliosis as "characteristic" of preeclampsia rather than "pathognomonic" as initially claimed.

11. ANGIOGENIC FACTORS AND THE KIDNEY IN PREECLAMPSIA

Recent work by Karumanchi and colleagues (reviewed in detail elsewhere in this volume on preeclampsia) has implicated the role of angiogenic factors in the pathophysiology of preeclampsia (92). Here we will review their findings as they specifically pertain to the kidney in preeclampsia. The hypothesis that antagonism of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) may play a role in preeclampsia is partly derived from findings in the renal literature. VEGF has been shown to have vasoactive effects by inducing nitric oxide and vasodilatory prostacyclins in endothelial cells, thereby decreasing vascular tone and blood pressure (93). Furthermore, VEGF is important in glomerular endothelial cell health and repair in animal models (94,95) and its absence results in proteinuria and glomerular endotheliosis (96,97). VEGF, which is constitutively expressed in the glomerular podocyte of the kidney and in other organs with fenestrated endothelium, is important for induction, maintenance, and health of the fenestrae (96,98). Finally, antiangiogenic factors such as VEGF neutralizing antibodies or VEGF receptor inhibitors used in clinical oncology trials can produce hypertension and proteinuria in humans (99,100).

Soluble Flt-1 (soluble fms-like tyrosine kinase receptor –1) is a potent VEGF and PlGF antagonist and binds these proangiogenic factors in the circulation (101). Using a rat model, Maynard and colleagues demonstrated that administration of soluble Flt-1 resulted in functional, renal morphological and clinical features similar to preeclampsia (102). With regards to the vasodilatory effects, sFlt-1 inhibited the dose-dependent vasorelaxation induced by VEGF and PlGF using an *in vitro* assay of renal microvascular reactivity. The authors suggest that elevated levels of circulating sFlt-1 in preeclamptic women may oppose the physiologic vasodilation of pregnancy, thereby resulting in hypertension. Adenovirus transfection of rats with sFlt-1 also resulted in a renal lesion consistent with glomerular endotheliosis producing hypertension and proteinuria, all features of preeclampsia.

Circulating soluble Flt-1 levels are increased and PlGF levels are decreased in preeclampsia compared to uncomplicated pregnancies as well as prior to the clinical syndrome of preeclampsia (103, and reviewed in 92). Urinary excretion of angiogenic factors has been studied as a potential marker to predict preeclampsia. Levine et al and other groups demonstrated a strong association between decreased urinary PlGF at midgestation and subsequent early development of preeclampsia (104,105). Levine and colleagues did not study urinary excretion of sFlt-1 or VEGF reasoning that sFlt-1 (~100 kDa) is too large of a molecule to be filtered into urine in the absence of renal damage and that although VEGF (~45 kDa) is readily filtered, the major source in the urine is from endogenous production by cells in the kidney (106,107). Buhimschi and colleagues assessed serum and urinary levels of angiogenic factors sFlt-1 and VEGF, in addition to PlGF, in women with severe preeclampsia (108). Urinary levels of these angiogenic factors was significantly increased in severe preeclampsia. Fractional excretion of sFlt-1 and VEGF were significantly higher than expected based on their estimation of glomerular "leakage" suggesting a more complicated mechanism than a compromised glomerular barrier. These conclusions are not unexpected since glomerular podocytes produce angiogenic factors such as VEGF. Certainly, further investigation is warranted to fully understand the source(s) and effects of these various angiogenic factors in pregnancy and preeclampsia.

Soluble endoglin, a soluble TGF-β co-receptor expressed by the placenta may contribute to the pathophysiology of preeclampsia (109). Circulating endoglin is elevated in the serum of preeclamptic women and correlates with disease severity. In the rat, its effects are amplified by sFlt-1 resulting in features similar to HELLP syndrome. Renal histopathologic effects include focal glomerular endotheliosis, which is exacerbated when co-administered with sFlt-1. In addition, renal microvascular reactivity studies indicate that soluble endoglin inhibits a TGF- β mediated endothelial nitric oxide synthase dependent vasodilation. The investigators propose that endoglin may oppose the physiologic nitric oxide dependent vasodilation in pregnancy, thereby contributing to the hypertension of preeclampsia.

12. PERSPECTIVES

12.1. Maternal renal, cardiovascular and osmoregulatory adaptations in normal pregnancy: important phenomena or epiphenomena?

Theoretically, the renal, cardiovascular and osmoregulatory changes of pregnancy may be beneficial to the fetus. When the maternal kidneys and other non-reproductive organs vasodilate during early pregnancy, they effectively serve as "arterial-venous shunts", thereby creating a marked decline in systemic vascular resistance that, in turn, initiates a rise in cardiac output by reducing cardiac afterload. This early gestational rise in cardiac output clearly anticipates the large increase in uterine blood flow, as well as oxygen and nutrient demands of the fetus and placenta that occur later in pregnancy. Indeed, the arterial-mixed venous oxygen content difference narrows during early gestation in both rats and humans signifying that oxygen delivery exceeds demands (110, and citations therein). Moreover, the reduction in systemic vascular

resistance, in concert with a modest decline in arterial pressure and concomitant increase in global arterial compliance, instigate arterial underfilling, thereby stimulating renal retention of sodium and water through activation of the sympathetic and renin-angiotensin-aldosterone systems resulting in the expansion of extracellular fluid and plasma volumes. Increase in red cell mass subsequently occurs secondary to elevated circulating levels of erythropoietin (111, and citations therein), thereby contributing to expansion of blood volume. Osmoregulatory changes occur simultaneously (and likely by the same hormone responsible for maternal vasodilation, i.e., relaxin—vide supra), that lead to retention of solute-free water which distributes to the total body water space. Thus, it is possible that the hyperdynamic maternal circulation and expansion of extracellular fluid and blood volumes, as well as total body water serve as buffer systems for the fetus and placenta in the event of hemorrhage, sodium deprivation, dehydration or other stressful conditions.

To our knowledge, this general concept has not been tested. However, our newly acquired and growing knowledge of the hormonal and molecular mechanisms of these maternal adaptations to pregnancy will facilitate investigation of their overarching purpose.

12.2. Therapeutic potential of relaxin in preeclampsia

During active disease, the circulating concentrations of *immunoreactive* relaxin are not significantly different from those measured in normotensive, gestational-aged matched controls (112). However, whether *bioactive* serum concentrations may be reduced in preeclampsia, or whether there may be a deficiency of vascular relaxin receptors or a defect in post-receptor signaling is unknown. Moreover, in women destined to develop preeclampsia, the status of circulating relaxin during the first trimester when peak levels are reached is also unknown. It is possible that abnormally high or low serum concentrations at that time may result in exaggerated or deficient maternal renal and cardiovascular adaptations that, in turn, predispose women to develop preeclampsia.

By virtue of its potent renal vasodilatory properties, relaxin administration should improve RPF and GFR in women with preeclampsia. By increasing the filtered load and/or fractional excretion of uric acid, a potential perpetuator of vascular damage in the disease, serum levels would be decreased. In addition, relaxin treatment would be expected to reduce systemic vascular resistance and increase global arterial compliance, thereby improving cardiac output and maternal organ perfusion. There is limited information indicating that relaxin may be a uterine vasodilator, too (113). Possibly, relaxin could improve uteroplacental blood flow by vasodilating unremodeled spiral arteries containing vascular smooth muscle, thereby improving oxygenation of the intervillous space and reducing placental expression of hypoxia-inducible transcription factors and their target genes such as sflt-1 (114). Because relaxin appears to be mainly an arterial vasodilator, preload is not compromised, and therefore, cardiac output is reciprocally increased, so that hypotension should not be a limitation of relaxin therapy. Rather, blood pressure reduction can be achieved using the standard anti-hypertensive agents.

Is more relaxin better in preeclampsia? As mentioned above, immunoreactive serum concentrations are comparable to normotensive controls, but they are only 50% or so of the peak levels observed in the first trimester of normal pregnancies. Ultimately, the answer to this question can only be resolved by clinical investigation. Will the vasodilatory properties of relaxin which ultimately depend on endothelial nitric oxide production be preserved in the face of the "endothelial dysfunction" that accompanies preeclampsia? As a partial answer to this question, we have observed that relaxin is effective in SHR and angiotensin II-infused rats, two animal models of hypertension associated with endothelial dysfunction (30).

Finally, based on both circumstantial evidence and preliminary experiments from our laboratory, we postulate that vascular endothelial growth factor is an intermediary molecule in the relaxin vasodilatory pathway situated between the relaxin receptor and gelatinase. Thus, our own work in normal pregnancy, and that of Karumanchi and colleagues in preeclampsia may ultimately be joined together at this molecule. On the one hand, circulating anti-angiogenic molecules such as sFlt-1 may disrupt the relaxin vasodilatory pathway, thereby contributing to the pathogenesis of preeclampsia. On the other hand, relaxin supplementation may restore endothelial cell health in the disease by augmenting *local* production of VEGF within the arterial wall, thereby partly or wholly offsetting the deleterious effects of circulating anti-angiogenic factors.

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