Renal 20-hydroxyeicosatetraenoic acid synthesis during pregnancy

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We examined whether renal 20-hydroxyeicosatetraenoic acid (20-HETE) synthesis is altered during gestation. Renal microsomal arachidonic acid ω-hydroxylase activity increased by 50 and 48% in rats on days 12 and 19 of gestation, respectively. Renal microvessel 20-HETE synthesis increased by 50 and 82% in rats on days 6 and 12 of gestation, respectively. Renal microvessel 20-HETE synthesis in isolated medullary thick ascending limb was unchanged from control levels on days 6 and 12 of gestation, but it increased twofold on day 19 of gestation. This increase in day 19 of gestation was associated with a twofold increase in urinary 20-HETE excretion, and it coincided with a transient but significant reduction in systolic blood pressure. ABT treatment also decreased urinary sodium, urinary 20-HETE, and renal and nonpregnant rats in terms of the activity and expression of cytochrome P450 (CYP)4A proteins. Administration of the CYP4A inhibitor 1-aminobenzotriazole (ABT) for 2 days on day 12 of pregnancy or for 5 days starting on day 15 of pregnancy caused a transient but significant reduction in systolic blood pressure. ABT treatment also decreased urinary sodium, urinary 20-HETE, and renal and microvessel 20-HETE synthesis. This study, to our knowledge, is the first to demonstrate that 20-HETE synthesis in the kidney is altered in time- and site-specific manners during pregnancy. The localized pattern of changes suggests that there are distinct regulatory mechanisms for 20-HETE synthesis in the kidney during pregnancy.

Materials and Methods

20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) is a major metabolite of arachidonic acid in the rat kidney; its synthesis and actions have been localized primarily to the microcirculation and tubular segments such as the medullary thick ascending limb (mTAL) (18). 20-HETE of renal origin promotes renal vasoconstriction (17), contributes to myogenic tone in renal arterioles (7), and inhibits K⁺-channel conductivity in the mTAL (33). Inhibition of 20-HETE production has been shown to block autoregulation of renal blood flow (35) and tubuloglomerular feedback (36) in the rat. On the other hand, elevated chloride transport in the mTAL of Dahl salt-sensitive (SS/Jr) rats has been associated with diminished capacity to produce 20-HETE (34). The role of 20-HETE in the regulation of blood pressure has been further implied from studies showing that inhibition and stimulation of its formation affect arterial pressure (24, 25, 27, 28, 32).

Pregnancy-related changes in hormonal levels may have an impact on the tissue expression of enzymes that catalyze arachidonic acid ω-hydroxylation to 20-HETE. For example, a pulmonary prostaglandin ω-hydroxylase is greatly induced during pregnancy in rabbits (19). This induced enzyme, later identified as cytochrome P450 (CYP)4A4 isofrom, has the highest catalytic efficiency (Vₘₐₓ/Kₘ) value for arachidonic acid ω-hydroxylation compared with other substrates such as palmitate, 15-HETE, and prostaglandin E₁ (20). Normal pregnancy in humans and rats is associated with increases in the glomerular filtration rate and renal blood flow (16) along with a significant decrease in arterial pressure and total peripheral resistance (1, 12). The exact mechanisms mediating these physiological changes are not fully understood. We hypothesize that renal 20-HETE synthesis is affected during gestation and that 20-HETE is involved in the regulation of renal function and blood pressure during pregnancy. Hence, this study was designed to contrast pregnant and nonpregnant rats in terms of the activity and expression of CYP4A isofroms in whole kidneys and in isolated renal tissues including mTAL segments and microvessels. We also studied the effect of 1-aminobenzotriazole (ABT), a mechanism-based inhibitor of CYP-derived 20-HETE synthesis (28), on blood pressure and sodium excretion in rats on the third week of gestation.

Materials

[1-14C]-arachidonic acid (56 mCi/mmol) was obtained from DuPont-New England Nuclear (Boston, MA). Emulgen E911 was obtained from KAO Atlas (Tokyo, Japan). ABT was obtained from Aldrich Chemical (Milwaukee, WI). All solvents were HPLC grade.
Animals

Experiments were conducted on pregnant (timed pregnancy) and age-matched virgin Sprague-Dawley rats (8 wk old; Charles River Laboratories, Wilmington, MA) using protocols approved by the Institutional Animal Care and Use Committee. Animals were maintained under controlled housing conditions of light and temperature, and they received standard laboratory chow and water until used.

Protocols to Evaluate Renal CYP4A Expression and 20-HETE Production

Virgin and pregnant rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg) on the 6th, 12th, or 19th gestational day. The kidneys were removed, and microsomes were prepared as previously described (15). Renal microvessels were isolated, and their purity was estimated by phase-contrast microscopy as previously described (31). A preparation enriched with the mTAL was obtained by sequential digestion and sieving of tissue slices of the inner stripe of the outer medulla as described by Ito et al. (9).

20-HETE synthesis. Whole kidney microsomes (150 μg) were incubated with [1-14C]-arachidonic acid (0.4 μCi, 7 nmol) and reduced NADP (NADPH; 1 mM) in 0.3 ml potassium phosphate buffer (100 mM, pH 7.4) containing 10 mM MgCl2 for 30 min at 37°C. The reaction was terminated by acidification to pH 3.5–4.0 with 2 M formic acid, and metabolites were extracted with ethyl acetate. The final extract was evaporated under nitrogen, resuspended in 50 μl of methanol, and injected onto the HPLC column.

Reverse-phase HPLC was performed on a 5-μm ODS-Hypersil column, 4.6 × 200 mm (Hewlett Packard, Palo Alto, CA), using a linear gradient ranging from acetonitrile-water-acetic acid (50:50:0.1) to acetonitrile-acetic acid (100:0.1) at a flow rate of 1 ml/min for 30 min. The elution profile of the radioactive products was monitored by a flow detector (In/us System, Tampa, FL). The identity of 20-HETE was confirmed by its comigration with an authentic standard. 20-HETE formation was estimated on the basis of the specific activity of the added [1-14C]-arachidonic acid and was expressed as nanograms per milligram protein per hour.

For measurements of 20-HETE production in the mTAL and microvessels, homogenates (30 μg protein) were incubated with arachidonic acid (20 μM) in 1 ml of assay buffer containing 100 mM potassium phosphate buffer (pH 7.4), 10 mM MgCl2, 1 mM NADPH, and 2 μM indomethacin at 37°C for 60 min. After incubation, [20,20-3H]20-HETE (5 ng) was added as an internal standard, and the reaction mixture was acidified to pH 4 with 1 M formic acid. The mixture was extracted twice with 2 ml of ethyl acetate. The final extract was subjected to reverse-phase HPLC for isolation of 20-HETE as described above. Fractions coeluting with 20-HETE were collected, evaporated under nitrogen, and derivatized to pentfluorobenzyl bromine ester trimethylsilyl ether. 20-HETE was quantitated by negative chemical ionization-gas chromatography-mass spectrometry (NCI-GC-MS) by comparing the ratio of ion intensity (391:393) for derivatized 20-HETE with [20,20-3H]20-HETE (32).

Western blot analysis. Homogenates of microvessels (30 μg), mTAL (30 μg), or renal microsomes (10 μg) were separated by electrophoresis on a 10 × 20 cm, 8% SDS-polyacrylamide gel at 25 mA/gel at 4°C for 18–20 h. The proteins were transferred electrophoretically to an enhanced chemiluminescence (ECL) membrane in a transfer buffer consisting of 25 mM Tris-HCl, 192 mM glycine, and 20% methanol (vol/vol). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) containing 10 mM Tris-HCl, 0.1% Tween 20, and 150 mM NaCl for 90 min and then washed three times with TBS. The membranes were incubated for 1 h with a 1:1,000 dilution of goat anti-rat CYP4A11 antibody (Gentest, Woburn, MA) at room temperature, washed several times with TBS solution, and further incubated with a 1:5,000 dilution of horseradish peroxidase-coupled, rabbit anti-goat secondary antibody (Sigma Chemical, St. Louis, MO) for 1 h. The immunoblots were developed using an ECL detection kit (Amersham, Arlington Heights, IL). To ensure equal protein loading, membranes were stripped and incubated with a 1:5,000 dilution of mouse anti-chicken β-actin antibodies (Sigma) for 1 h. The second antibody was horseradish peroxidase-coupled, rabbit anti-mouse secondary antibody (1:5,000). Immunoreactive β-actin was detected as described above.

Measurement of urinary 20-HETE. Twenty-four-hour urine samples were collected, centrifuged at 3,000 g for 15 min, and stored at −20°C until the assay was performed. Urinary 20-HETE was measured by NCI-GC-MS as previously described (14). Briefly, [20,20-3H]20-HETE (2 ng/ml of urine) was added to rat urine samples. The mixture was acidified to pH 4 with formic acid, and metabolites were extracted with ethyl acetate. 20-HETE was isolated and purified by HPLC and further processed for NCI-GC-MS analysis as described above.

Protocol to Evaluate the Effect of Inhibition of 20-HETE Production by ABT

Rats were placed in metabolic cages on the 13th gestational day. On the 15th gestational day, rats (n = 4) were injected with ABT intraperitoneally at a dose of 25 mg·kg⁻¹·day⁻¹ for 5 days (days 15-19 of pregnancy). Pregnant rats in the control group were injected with 0.9% saline. Systolic arterial blood pressure was measured daily by tail-cuff sphygmography using a Natsume KN-210 apparatus (Peninsula Laboratories, Belmont, CA). Rats were warmed at 40°C for 10 min and allowed to rest quietly in a Lucite chamber before tail-cuff sphygmography; 10 pressure measurements were recorded for each rat, and the average systolic blood pressure was calculated. Twenty-four-hour urinary sodium was determined by standard flame photometry. Urinary 20-HETE was determined as described above. After treatment, the rats were killed on day 20 of gestation, and kidneys were removed for microsomal preparation to assess CYP4A expression and 20-HETE synthesis. In another set of experiments, rats on day 12 of pregnancy (n = 4 for each group) were administered ABT (25 mg·kg⁻¹·day⁻¹) or 0.9% saline for 2 days, and blood pressure was measured at 6, 12, and 24 h after injection. At the end of the treatment, renal microvessels were prepared and 20-HETE synthesis was measured as described above.

Statistical Analysis

Data are means ± SE. Data on the effect of ABT on systolic blood pressure were analyzed by a two-way analysis of variance followed by a Tukey test. All other data were analyzed by a one-way analysis of variance or the Student’s t-test for unpaired samples. Statistical significance was set at P < 0.05.

RESULTS

Changes in Systolic Blood Pressure, Urinary 20-HETE Excretion, Renal 20-HETE Synthesis, and CYP4A Isoform Expression During Pregnancy

As seen in Table 1, mean systolic blood pressure in pregnant rats on days 6 and 12 of gestation was not...
different from that of the control virgin rats. In contrast, mean systolic blood pressure was significantly (P < 0.05) decreased on the 19th day of gestation compared with control nonpregnant rats and pregnant rats on days 6 and 12 of gestation. Interestingly, urinary 20-HETE excretion on day 19 of pregnancy was, at least, twofold higher (P < 0.05) than the corresponding values in nonpregnant female rats or in rats on days 6 and 12 of pregnancy (Table 1). Furthermore, microsomal arachidonate ω-hydroxylase activity (formation of 20-HETE) in kidneys from rats on days 12 and 19 of gestation was significantly increased by 50 and 48%, respectively, relative to control data in nonpregnant female rats (Table 1).

Because microvessels and the mTAL are major sites of synthesis and action of 20-HETE in the kidney, we further examined the capacity of these structures to express CYP4A isoforms and to produce 20-HETE. A representative Western blot of renal CYP4A isoforms in the mTAL during pregnancy is shown in Fig. 1A. Because the expression of CYP4A2 is virtually undetectable in female rats (30), the major bands found in the Western blots are believed to be CYP4A1 and CYP4A3. The expression level of CYP4A1 and CYP4A3 in the mTAL was higher in rats on the 19th day of gestation than in either nonpregnant rats or rats on the 6th and 12th day of gestation. Densitometry analysis revealed a slight but significant increase in CYP4A protein levels at day 12 of pregnancy of 52% over the control levels and a marked 4.5-fold increase in CYP4A protein levels at day 19 of pregnancy (Fig. 1A). 20-HETE synthesis by the mTAL was comparable in nonpregnant rats and rats on the 6th and 12th day of gestation (Fig. 1B). However, 20-HETE synthesis by the mTAL on day 19 of pregnancy was increased (P < 0.05) by 84% relative to that in the mTAL from nonpregnant female rats (Fig. 1B).

In renal microvessels, the expression of CYP4A proteins on days 6 and 12 of pregnancy was higher than in control rats. Densitometry analysis indicated that the expression of CYP4A isoforms on days 6 and 12 of pregnancy was significantly increased by 57 and 160%, respectively, compared with control female rats; the expression of CYP4A isoforms returned to control level on day 19 of pregnancy (Fig. 2A). Also, as seen in Fig. 2B, the rate of 20-HETE synthesis by renal microvessels was 50 and 82% higher in rats on days 6 and 12 of gestation, respectively, relative to corresponding data in nonpregnant female rats. 20-HETE synthesis in renal microvessels returned to control level on day 19 of pregnancy (Fig. 2B).

Effect of ABT on Systolic Blood Pressure and Renal Excretory Functions in Pregnant Rats

Preliminary experiments were carried out in nonpregnant female rats to determine the efficacy and selectivity of ABT. Animals were injected intraperitoneally with saline vehicle or ABT at 25 or 50 mg/kg; renal cortical CYP content and 20-HETE synthesis were determined 16 h later. Arachidonic acid ω-hydroxylation in renal cortical microsomes was decreased (P < 0.05) by 35% (2,114 ± 75 ng·mg⁻¹·h⁻¹, n = 3) and 67% (1,083 ± 119 ng·mg⁻¹·h⁻¹, n = 3) after treatment

Table 1. SBP, urinary 20-HETE excretion, and whole kidney microsomal ω-hydroxylase activity in female control rats and pregnant rats at different gestational days

<table>
<thead>
<tr>
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<th>Nonpregnant Rats</th>
<th>Pregnant Rats</th>
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<tr>
<td></td>
<td>Day 6</td>
<td>Day 12</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>137 ± 3</td>
<td>134 ± 5</td>
</tr>
<tr>
<td>Urinary 20-HETE, ng/24 h</td>
<td>3.1 ± 2.4</td>
<td>4.2 ± 2.3</td>
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<tr>
<td>ω-Hydroxylase, ng·mg⁻¹·h⁻¹</td>
<td>3,346 ± 378</td>
<td>4,025 ± 735</td>
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Values are means ± SE (n = 5 rats. *P < 0.05 from control; †P < 0.05 from day 6 to day 12 of pregnancy). Systolic blood pressure (SBP), urinary 20-hydroxyeicosatetraenoic acid (20-HETE) excretion, and whole kidney arachidonic acid ω-hydroxylase activity were determined in female and pregnant Sprague-Dawley rats on the 6th, 12th, and 19th gestational days as described in MATERIALS AND METHODS.
with 25 and 50 mg/kg ABT, respectively, relative to control data in vehicle-treated rats (3,259 ± 138 ng·mg⁻¹·h⁻¹, n = 3). Renal cortical arachidonic acid epoxygenase activity was unaffected by ABT at 25 mg/kg (3,924 ± 43 ng·mg⁻¹·h⁻¹), but it was decreased (P < 0.05) at 50 mg/kg (1,937 ± 328 ng·mg⁻¹·h⁻¹, n = 3). Renal cortical CYP content measured by the method of Omura and Sato (21) was unaffected at the low dose but was significantly decreased following treatment with 50 mg/kg ABT (0.09 ± 0.03 vs. 0.19 ± 0.03 nmol/mg protein, n = 3, P < 0.05). We therefore used 25 mg/kg of ABT in subsequent studies.

Pregnant rats on day 15 of gestation were injected with vehicle or ABT (25 mg/kg) for 5 consecutive days (from day 15 to day 19 of gestation). As shown in Fig. 3A, in vehicle-treated rats, mean systolic blood pressure was 133 ± 12 mmHg on day 14 of gestation, decreased to 111 ± 8 mmHg (P < 0.05) on day 17 of gestation, and remained at about this level throughout the remainder of the observation period. In ABT-treated rats, mean systolic blood pressure decreased from 130 ± 8 mmHg on day 15 of gestation to 108 ± 6 mmHg on day 16 of pregnancy, 12 h after the first injection of ABT. Blood pressure remained lower on day 17 of pregnancy (91 ± 6 mmHg) and subsequently increased toward levels that are below those on day 14 of pregnancy. Of note, on days 16 and 17 of pregnancy, the systemic blood pressure of rats undergoing treatment with ABT was significantly lower than that of rats receiving saline vehicle only. Also, a significant decrease in urinary sodium excretion was noted 5 days after commencing ABT treatment (Fig. 3B).

As shown in Fig. 4, ABT treatment reduced (P < 0.05) urinary 20-HETE excretion by 54%. Moreover, ABT treatment resulted in a selective inhibition of 20-HETE synthesis and a loss in CYP4A immunoreactive protein in the kidney of pregnant rats. As seen in Fig. 5, A and B, following ABT treatment, conversion of
CYP4A expression in pregnant rats.

**DISCUSSION**

This study demonstrates that the activity of arachidonic acid metabolic pathways that yield 20-HETE is augmented at discrete time points during the course of pregnancy in rat renal vascular and tubular structures. This conclusion is based on observations that the ability of whole kidney microsomes to catalyze ω-hydroxylation of arachidonic acid to 20-HETE increases during gestation, along with the expression of CYP4A isoforms possessing arachidonic acid ω-hydroxylase activity and the synthesis of 20-HETE by both renal microvessels and mTAL segments. Interestingly, our study indicates that both CYP4A isoform expression and 20-HETE synthesis increase at different times in the mTAL and renal microvessels. For example, CYP4A expression and 20-HETE synthesis in the mTAL are elevated on day 19 of pregnancy compared with control female rats, in contrast to renal microvessels in which augmentation of CYP4A expression and 20-HETE synthesis are demonstrable on days 6 and 12 of pregnancy but not on day 19. It should be noted that, although CYP4A protein levels in the mTAL at day 12 of pregnancy were significantly higher (52% over the control levels), the rate of 20-HETE synthesis was not. This may be due to a loss of enzymatic activity of CYP4A proteins and/or enzymes/proteins that are essential for the CYP4A catalytic activity, such as NADPH cytochrome P450 reductase and cytochrome b5, during the relatively longer digestion procedure needed to obtain isolated mTAL.

In all, these observations suggest that the pathway of arachidonic acid metabolism to 20-HETE is dissimilarly regulated in the mTAL and renal microvessels during the course of gestation in rats. The factors responsible for augmentation of CYP4A expression and 20-HETE synthesis in microvessels during the first and second week of pregnancy or in the mTAL during late pregnancy are not known. It may be speculated that pregnancy-induced changes in hormonal background contribute to the regulation of CYP4A expression and 20-HETE synthesis. In this regard, ANG II and mineralocorticoids that are elevated during gestation (16) and that promote renal 20-HETE synthesis (3, 15) merit consideration for playing a role in the up-regulation of renal 20-HETE synthesis during pregnancy. One should also consider a role for nitric oxide (NO), which inhibits CYP4A activity and expression (22, 29) and is manufactured by distinct isoenzymes in the mTAL and renal microvessels (13). As NO production is increased in pregnancy (1), the chronology of both pregnancy-induced activation of vascular NO production and of pregnancy-associated elevation in fa-
tors that promote CYP4A expression may help to explain the observed differences in the chronology of changes in 20-HETE synthesis by the mTAL and microvessels.

Consideration should be given to the possibility that other CYP4 isoforms, distinct from CYP4A1 and CYP4A3 (the major CYP4A isoforms in the female rat), contribute to the synthesis of 20-HETE. CYP4F proteins (CYP4F4 and CYP4F5) are expressed in the rat kidney; however, the recombinant CYP4F4 and CYP4F5 proteins do not oxidize arachidonic acid (11). Even so, the possibility that these CYP4F isoforms contribute to 20-HETE synthesis still exists, because the catalytic activity of a recombinant CYP is measured under conditions that rarely exist in vivo. The lack of specific antibodies against the CYP4F proteins precludes a complete and accurate evaluation of their expression/activity at this time. All in all, the fact that changes in the expression of CYP4A1 and CYP4A3, the major CYP4A proteins in the female rat, correlate well with the production of 20-HETE in microvessels suggests that these isoforms contribute significantly to 20-HETE synthesis in this tissue.

20-HETE is endowed with vascular and renal actions that are relevant to the operation of vascular and renal mechanisms controlling blood pressure. For example, 20-HETE promotes vasoconstriction at renal and extrarenal sites (5, 8), and vascular 20-HETE contributes to the vasoconstriction induced by myogenic (17) and hormonal stimuli (2, 6). Synthesis of 20-HETE at vascular sites has been linked to the implementation of renal and extrarenal vasoconstrictor mechanisms and, consequently, to blood pressure elevation (32). On the other hand, 20-HETE produced by mTAL segments interferes with the function of the Na⁺-K⁺-2Cl⁻ co-transporter and, consequently, is expected to favor natriuresis and lowering of blood pressure (4, 24). It follows, then, that augmentation of renal 20-HETE synthesis at vascular and tubular sites during pregnancy may have different consequences on blood pressure and renal function during gestation.

In agreement with previous reports, we found that blood pressure falls during the 3rd wk of pregnancy, particularly on day 17 (1). Interestingly, this blood pressure reduction is more accentuated in pregnant rats undergoing treatment with ABT to inhibit CYP4A isoforms that manufacture 20-HETE. A plausible interpretation of this finding is that 20-HETE supports blood pressure during pregnancy. Previous studies documented renal vasodilation and augmentation of glomerular filtration rate in midterm pregnancy in rats (1). Because 20-HETE fosters renal vasoconstriction, it is intriguing that the increase in 20-HETE synthesis in renal microvessels during the second week of gestation, as reported in this study, coincides with lowering renal vascular resistance as reported by others (10). It is conceivable that, in such a setting, 20-HETE manufactured by renal microvessels functions to buffer renal vasoconstrictory mechanisms that are overactive during pregnancy (1). Inasmuch as 20-HETE may be metabolized by cyclooxygenase to 20-hydroxy prostanooids (26), one cannot exclude the possibility that such products also influence renal hemodynamics during pregnancy. The results showing that ABT, administered at mid-pregnancy, lowered blood pressure and inhibited renal microvessel synthesis of 20-HETE support a role for renal vascular 20-HETE in the regulation of blood pressure during pregnancy.

In this study, lowering of blood pressure during the 3rd wk of gestation was not accompanied by reduction of urinary sodium excretion, which implies a leftward shift in the pressure-natriuresis relationship (23). Augmentation of 20-HETE synthesis in the mTAL may be expected to produce such a leftward shift in the pressure-natriuresis relationship, because 20-HETE is known to interfere with ion transport in the mTAL (4, 33). This view is consistent with our findings that urinary sodium excretion falls significantly during treatment of pregnant rats with ABT. On the basis of our findings and the known actions of 20-HETE on vascular and renal function, the blood pressure response to inhibition of CYP4A with ABT in late pregnancy should be viewed as a composite that integrates the individual functional consequences of inhibiting 20-HETE synthesis in the mTAL and other segments of the nephron, the renal vasculature, and systematically.

In summary, this study is the first to demonstrate that 20-HETE and the expression of enzymes that catalyze its formation are altered in the kidney during pregnancy. The chronology of pregnancy-induced augmentation of 20-HETE synthesis differs in renal microvessels and mTAL segments, implying that renal 20-HETE synthesis is regulated in a site-specific manner during gestation. The study calls attention to the possibility that augmentation of 20-HETE synthesis at vascular and tubular sites during gestation impacts on the regulation of blood pressure and renal function.

**Perspectives**

Inasmuch as 20-HETE of vascular and tubular origins has effects that are opposite in their functional outcomes, malregulation of renal vascular and/or tubular CYP4A expression and 20-HETE synthesis during pregnancy may result in abnormalities of fluid volume regulation and blood pressure homeostasis. For example, upregulation of 20-HETE synthesis in renal microvessels may contribute to the pathogenesis of pregnancy-induced hypertension by promoting vasoconstriction and increased vascular resistance that, in turn, may bring about sodium retention by causing a rightward shift in the pressure-natriuresis relationship. Likewise, downregulation of CYP4A expression and 20-HETE synthesis in the mTAL is expected to promote sodium reabsorption, thereby contributing to the development of hypertension. The present study set the basis for further studies headed for understanding mechanisms that regulate 20-HETE synthesis in the renal microcirculation and tubular structures during pregnancy. Ultimately, this knowledge can uncover new therapeutic targets and provide novel loci for the...
control and treatment of pregnancy-induced hypertension.

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