Renal interstitial hydrostatic pressure and natriuretic responses to volume expansion in pregnant rats

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During normal pregnancy, there is a significant increase in extracellular fluid volume, plasma volume, and extracellular fluid volume are primarily dependent on sodium content; therefore, the regulation of sodium excretion is essential in volume regulation. The conservation of sodium and water is critical for fetal growth and development during normal pregnancy. A positive correlation exists between the extent of plasma volume expansion (VE), amniotic fluid volume, and fetal growth (2, 5, 6, 13). It is likely that the mechanisms that are responsible for the increase in plasma volume during normal pregnancy involve adaptations in renal function, including changes in the volume-sensing mechanisms, to allow for continued renal sodium retention and the subsequent VE. However, it is also likely that the alterations in renal volume-sensing mechanisms adjust during the course of pregnancy, because the demand for and the rate of fluid retention are probably different in early pregnancy compared with that observed as the pregnancy approaches term.

In normal nonpregnant rats, VE increases renal interstitial hydrostatic pressure (RIHP) and sodium and water excretion (11); however, these renal responses to VE during pregnancy are controversial (4). Therefore, the objective of this study was to determine RIHP, natriuretic, and diuretic responses to VE in nonpregnant, midterm pregnant, and late-term pregnant Sprague-Dawley (SD) rats. Furthermore, fractional excretion of lithium (FELi), which has been shown to correlate (inverse relationship) well with fractional sodium reabsorption in the proximal tubule (8, 20, 21), was utilized as an index of proximal tubule reabsorption and was determined during control and VE periods in pregnant and nonpregnant rats.

METHODS

All rats in these studies were female SD rats purchased from Harlan Sprague Dawley (Indianapolis, IN). All rats were fed a normal Purina Rat Chow containing 0.1 meq sodium/g and had free access to water. All protocols in this study were conducted in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee.

Polyethylene Matrix Implantation

The implantation procedure of the polyethylene (PE) matrix has been previously described (12). RIHP was directly and continuously measured by means of a PE matrix that...
was implanted in the left kidneys of rats when they were 11–16 wks old (12).

Monitoring of Estrous Cycle and Induction of Pregnancy in Rats

Monitoring of the estrous cycle and induction of pregnancy in rats has been previously described (10). Approximately 1 wk after PE matrix implantation, vaginal swabs were taken daily in all rats to monitor their estrous cycle. To determine the stage in the estrous cycle, the female rats were restrained manually and a wet swab was inserted in the vagina and smeared on a slide. As previously described, the slide was immediately fixed with 1% toluidine blue solution (with a few drops of 1 N potassium hydroxide), and it was observed under the microscope for cells that characterize each stage of the estrous cycle. A male breeder and a female SD rat were housed together for 1 day when the female was found to be in the estrous stage. The female was tested for the presence of sperm in the vagina on the day after −24 h of being in the same cage with the male breeder SD rat. The presence of sperm on the fixed slide of the vaginal smear indicated day 1 of pregnancy.

Three groups of female SD rats were studied in these experiments.

Nonpregnant SD rats (n = 7). These were rats that were mated, as evidenced by the presence of sperm on the vaginal smear on the following day, but found to be nonpregnant during the acute experiments.

Midterm pregnant SD rats (n = 8). These rats were pregnant for 12–14 days when the VE studies were performed.

Late-term pregnant SD rats (n = 8). These rats were pregnant for 19–20 days when the VE studies were performed.

Surgical Procedure for VE Experiments

On the day of the acute experiment, rats were anesthetized with Inactin (100 mg/kg), and catheters were placed in the trachea (PE-240) and left jugular vein (PE-50) for intravenous infusion of 1.5 ml·100 g body wt−1·h−1 of saline with 6 mM lithium chloride (LiCl) and 1.5 ml·100 g body wt−1·h−1 of a solution of 3% inulin and 6.25% bovine albumin in saline (with 6 mM LiCl). A PE-50 catheter was implanted in the left carotid artery for mean arterial pressure (MAP) measurement and blood withdrawal. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. The rats were allowed 1 h to recover after completion of the surgical procedures. Then, a control period of 30 min was started. During the 30-min clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period, −1 ml of blood was withdrawn from the left carotid artery for plasma electrolyte, lithium, and inulin measurements. At this time, VE (5% body wt/30 min) was started. Urine was collected for 20 min, starting 10 min after the initiation of VE. Again, during the second clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period, −1 ml of blood was withdrawn from the left carotid artery for plasma electrolyte, lithium, and inulin measurements. All rats were killed by air embolism at the end of the experiment while still under deep anesthesia, and both kidneys were excised and weighed. This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Glomerular filtration rate was calculated from the clearance of inulin, and inulin concentrations were measured by the anthrone method (7). Sodium and lithium concentrations in plasma and urine were measured by using flame photometry (model 943, Instrumentation Laboratory, Lexington, MA).

Standard paired Student’s t-tests were used for comparisons between the first and second clearance periods in the same group of rats. Standard unpaired Student’s t-tests were used for group comparisons at equivalent periods. All data are means ± SE, and P < 0.05 was accepted as a statistically significant difference.

RESULTS

The results of these experiments are shown below. Basal RIHP, functional excretion of sodium (FE\(_{\text{Na}}\)), and urine flow rate (V) were all significantly lower (P < 0.05) in midterm pregnant and late-term pregnant compared with nonpregnant rats (Table 1). VE significantly increased RIHP (Fig. 1), FE\(_{\text{Na}}\) (Fig. 2), and V (Table 1) in all groups. As shown in Fig. 1 and Table 2, with VE, ΔRIHP was highest for nonpregnant (3.0 ± 0.3 mmHg) compared with midterm pregnant (1.6 ± 0.1 mmHg; P < 0.05 vs. nonpregnant rats) and late-term pregnant rats (1.2 ± 0.1 mmHg; P < 0.05 vs. both midterm pregnant and nonpregnant rats). ΔFE\(_{\text{Na}}\) and ΔV were similar in all groups (Table 2): 5.8 ± 1.0% and 231 ± 27 μl/min for nonpregnant, 6.8 ± 1.3% and 173 ± 16 μl/min for midterm pregnant, and 7.6 ± 1.2% and 203 ± 10 μl/min for late-term pregnant rats, respectively. The calculated value of natriuretic sensitivity (ΔFE\(_{\text{Na}}\)/ΔRIHP; Fig. 3) and diuretic sensitivity (ΔV/ΔRIHP; Table 2) to increases in RIHP during VE increased gradually with pregnancy and were both significantly higher in late-term pregnant rats compared with nonpregnant rats. These increases in ΔFE\(_{\text{Na}}\)/ΔRIHP and ΔV/ΔRIHP were associated with a significant increase in ΔFE\(_{\text{Na}}\)/ΔRIHP (Fig. 4) in midterm pregnant (18.7 ± 5.4%/mmHg) and late-term pregnant rats (27.8 ± 7.9%/mmHg) compared with nonpregnant rats (4.6 ± 1.7%/mmHg; P < 0.05 vs. both midterm pregnant and nonpregnant rats).

![Fig. 1. Renal interstitial hydrostatic pressure (RIHP) during control period and acute saline volume expansion (VE) in pregnant rats.](http://ajprenal.physiology.org/)

Fig. 1. Renal interstitial hydrostatic pressure (RIHP) during control period and acute saline volume expansion (VE; 5% body wt/30 min) in nonpregnant (NP), midterm pregnant (MP), and late-term pregnant (LP) groups of Sprague-Dawley (SD) rats. Values are means ± SE. n, No. of rats. *Significant difference (P < 0.05) between control and VE periods in the same group of rats compared with Student’s paired t-test. †Significant difference (P < 0.05) between NP rats and MP or LP rats at equivalent periods compared with Student’s unpaired t-test.
0.05 vs. midterm pregnant and late-term pregnant rats), suggesting a significant inhibition of proximal tubule reabsorption with increases in RIHP resulting from VE in pregnant rats. The weight of both kidneys was similar in all groups: 1.95 ± 0.08 g for nonpregnant, 2.06 ± 0.03 g for midterm pregnant, and 2.04 ± 0.06 g for late-term pregnant.

DISCUSSION

The results of the present study show that, with acute VE, the increase in RIHP was attenuated in pregnant compared with nonpregnant SD rats. The lower basal RIHP and the smaller increase in RIHP with VE in pregnant rats (Fig. 1) further support the previously introduced proposal (10) that renal interstitial compliance is increased during pregnancy. The results of the present study are consistent with previous findings (10) showing that basal RIHP and sodium and water excretions are significantly lower in pregnant compared with nonpregnant rats (Figs. 1 and 2, Table 1). In addition to the attenuated pressure natriuretic and diuretic responses (10, 16), pregnant rats have been shown to be refractory to the natriuretic actions of atrial natriuretic peptide (15). In contrast, in the present study VE caused a similar increase in sodium excretion in all groups, indicating an intact response to VE during pregnancy, although the increase in RIHP was significantly attenuated in pregnant compared with nonpregnant rats (Figs. 1 and 2). Therefore, it appears that there is no generalized attenuation in natriuretic and diuretic responses to various stimuli during pregnancy, suggesting that these attenuations are not caused by a single factor such as reductions in RIHP. In addition, both sodium-retentive hormones, such as deoxycorticosterone and aldosterone, and natriuretic hormones, such as progesterone, are increased in pregnancy (1). This suggests that the plasma volume regulation during pregnancy is likely to be multifactorial and is not the result of a simple increase in the levels of one or more of the hormones that can cause sodium retention.

In the present study, VE, which increases RIHP in normal rats (11), was utilized to determine RIHP, natriuretic, and diuretic responses and sensitivities to acute saline VE in nonpregnant, midterm pregnant, and late-term pregnant SD rats. The results of the present study show that, with VE, natriuretic and diuretic sensitivities to increases in RIHP (dFE Na/dRIHP and dV/ΔRIHP) increase gradually with pregnancy and reach a maximum during late term (Fig. 3, Table 2). The gradual increase in natriuretic and diuretic sensitivities might provide an adaptive mechanism to protect against excessive VE, especially close to term. The findings in the present study that natriuretic and diuretic sensitivities are altered during pregnancy are not unprecedented or unique to pregnancy. Natriuretic and diuretic sensitivities to increases in RIHP have been shown to be significantly increased in spontaneous hypertension (9), whereas they are attenuated in streptozotocin-induced diabetes mellitus (17). Pressure natriuretic and diuretic responses are decreased in pregnant (10) and spontaneously hypertensive rats (12), and these attenuated responses are associated

Table 1. Renal responses to acute saline volume expansion (5% body wt/30 min) in nonpregnant, midterm pregnant (12–14 days after conception), and late-term pregnant (19–20 days after conception) groups of Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant (n = 7)</th>
<th>Midterm Pregnant (n = 8)</th>
<th>Late-Term Pregnant (n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>Control VE</td>
<td>Control VE</td>
<td>Control VE</td>
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<tr>
<td>MAP, mmHg</td>
<td>121 ± 4</td>
<td>111 ± 4*</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>2.26 ± 0.25</td>
<td>3.39 ± 0.47*</td>
<td>1.92 ± 0.19</td>
</tr>
<tr>
<td>RIHP, mmHg</td>
<td>5.8 ± 0.3</td>
<td>8.8 ± 0.4*</td>
<td>2.9 ± 0.2†</td>
</tr>
<tr>
<td>FE Na, %</td>
<td>4.17 ± 0.36</td>
<td>9.94 ± 1.07†</td>
<td>2.23 ± 0.37†</td>
</tr>
<tr>
<td>FE Li, %</td>
<td>26.6 ± 4.2</td>
<td>40.8 ± 4.7*</td>
<td>23.9 ± 5.0</td>
</tr>
<tr>
<td>U, μl/min</td>
<td>89.1 ± 7.1</td>
<td>320.0 ± 28.7*</td>
<td>50.2 ± 10.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats; VE, volume expansion; MAP, mean arterial pressure; GFR, glomerular filtration rate; RIHP, renal interstitial hydrostatic pressure; FE Na, fractional excretion of sodium; FE Li, fractional excretion of lithium; V, urine flow rate. *Significant difference (P < 0.05) between control and VE periods in the same group of rats compared with Student’s paired t-test. †Significant difference between nonpregnant Sprague-Dawley rat group and midterm or late-term pregnant Sprague-Dawley rat group at equivalent periods compared with Student’s unpaired t-test.
with attenuated increases in RIHP with increased renal perfusion pressure during pregnancy (10) and spontaneous hypertension (12). It can be speculated that these renal adaptations may be important in regulating plasma volume under different physiological and pathophysiological conditions.

Lithium excretion, an index of proximal tubule reabsorption (8, 20, 21), was determined during VE in pregnant rats, and the responses of changes in RIHP to changes in RIHP during VE were calculated. It should be noted that, although the lithium excretion method of evaluating proximal tubule reabsorption of sodium is acceptable as a valid indirect method, some studies indicate that this indirect method might not be appropriate to utilize as an index of proximal tubule reabsorption in sodium-depleted animals (22).

In the present study, the significant increase in FE\(_{Li}\) per increase in RIHP (Fig. 4) in midterm pregnant and late-term pregnant compared with nonpregnant rats suggests a greater inhibition of proximal tubular reabsorption in pregnant rats to VE. The exact mechanism of how increases in RIHP are translated to increases in sodium excretion is not known; however, several mechanisms to explain this relationship have been previously suggested. These mechanisms include an increase in the back-leak of fluid and solutes into the proximal tubule (3, 18) and/or increases in medullary blood flow as a result of increases in RIHP, which would ultimately result in a decrease in sodium reabsorption and an increase in sodium excretion (19).

In a study by Mahaney et al. (14), a reduction in Na\(^+\)-K\(^+\)-ATPase activity was observed in mid term pregnant and late-term pregnant SD rats compared with virgin rats. The authors noted that the observed reduction in Na\(^+\)-K\(^+\)-ATPase activity and abundance in the renal cortex of pregnant rats, especially in late pregnancy, was unexpected, because this might cause natriuresis that would not promote the massive volume retention that is observed in late-term pregnant rats. It was concluded that whatever promotes net sodium and water retention during pregnancy must also be capable of overwhelming the natriuresis that can result from a reduction in Na\(^+\)-K\(^+\)-ATPase activity (14). Whether a relationship exists between RIHP and Na\(^+\)-K\(^+\)-ATPase activity under basal conditions or during pregnancy is not yet determined.

In conclusion, basal RIHP and the increase in RIHP during VE were attenuated during pregnancy. VE resulted in a similar increase in sodium and water excretions in nonpregnant and pregnant groups of rats,
indicating an intact excretory response to VE during pregnancy.

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REFERENCES


