Renal interstitial hydrostatic pressure and pressure natriuresis in pregnant rats

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Khraibi, Ali A. Renal interstitial hydrostatic pressure and pressure natriuresis in pregnant rats. Am J Physiol Renal Physiol 279: F353–F357, 2000.—The objective of this study was to test the hypothesis that a decrease in renal interstitial hydrostatic pressure (RIHP) accounts for the blunted pressure natriuresis during pregnancy. RIHP was measured in nonpregnant (NP; n = 9), midterm pregnant (MP; 12–14 days after conception; n = 10), and late-term pregnant (LP; 18–21 days after conception; n = 12) female Sprague-Dawley rats at two renal perfusion pressure (RPP) levels (99 and 120 mmHg). At the lower RPP level, RIHP was 5.8 ± 0.3 mmHg for NP, 3.4 ± 0.4 mmHg for MP (P < 0.05 vs. NP), and 2.9 ± 0.1 mmHg for LP (P < 0.05 vs. NP) rats. The increase in RPP from 99 to 120 mmHg resulted in pressure natriuretic and diuretic responses in all groups; however, the increases in fractional excretion of sodium (ΔFENa), urine flow rate (ΔV), and ΔRIHP were significantly greater (P < 0.05) in NP compared with both MP and LP rats. ΔFENa, ΔV, and ΔRIHP were 2.06 ± 0.28%, 81.44 ± 14.10 μl/min, and 3.0 ± 0.5 mmHg for NP; 0.67 ± 0.13%, 28.03 ± 5.28 μl/min, and 0.5 ± 0.2 mmHg for MP; and 0.48 ± 0.12%, 18.14 ± 4.70 μl/min, and 0.4 ± 0.1 mmHg for LP rats. In conclusion, RIHP is significantly lower in pregnant compared with nonpregnant rats at similar RPP levels. Also, the ability of pregnant rats to increase RIHP in response to an increase in RPP is blunted. These changes in RIHP may play an important role in the blunted pressure natriuresis and contribute to the conservation of sodium and water that is critical for fetal growth and development during normal pregnancy.

IN NORMOTENSIVE NONPREGNANT animals, acute increases in renal perfusion pressure (RPP) provide a potent natriuretic stimulus that is referred to as the “pressure natriuresis phenomenon.” Increases in RPP are transmitted to the renal interstitium and increase renal interstitial hydrostatic pressure (RIHP), urinary sodium excretion, and urine flow rate in normotensive Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats (10–12). When the increases in RIHP that are produced by increases in RPP are attenuated by acute renal decapsulation in normotensive rats, the increases in sodium and water excretion that are observed with increases in RPP are almost completely abolished (12, 13). Therefore, in normotensive rats, increases in RIHP are required for the full expression of pressure natriuretic and diuretic responses, suggesting a causal relationship between increases in RIHP and sodium and water excretions. In a recent study by Masilamani et al. (16) it was demonstrated that acute pressure natriuresis is blunted in pregnant rats. Such a change in the relationship between RPP and sodium excretion tends to reduce sodium and water excretion and consequently promote sodium and water retention and the volume expansion, which occurs during normal pregnancy. However, the role of RIHP in the reduced sodium and water excretion that characterizes normal pregnancy has not been studied.

Normal pregnancy is characterized by a significant extracellular and plasma volume expansion. There is a positive correlation among the extent of plasma volume expansion, amniotic fluid volume, and fetal growth (1, 3, 4, 14). Extracellular fluid volume is primarily dependent on sodium content, and therefore, the regulation of sodium excretion is critical to its maintenance. The kidney plays a critical role in the long-term regulation of extracellular fluid volume and blood pressure; therefore, it is likely that the mechanisms responsible for the changes in plasma volume and blood pressure during normal pregnancy involve alterations in renal function. Changes in the volume-sensing mechanisms must occur for continued volume expansion in the presence of sodium retention. Small changes in RIHP have been demonstrated to play an important role in sodium excretion under various physiological conditions (8). These conditions include changes in RPP, systemic volume expansion (6, 11, 15), and direct renal interstitial volume expansion (DRIVE) (9). Therefore, the objective of this study was to measure RIHP in pregnant normotensive rats and determine its role in the attenuated pressure natriuresis response that has been demonstrated in these rats.

METHODS

All rats in these studies were female SD rats purchased from Harlan Sprague Dawley (Indianapolis, IN). All rats

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were fed a normal Purina Rat Chow containing 0.1 meq sodium/g and had free access to water.

Polyethylene Matrix Implantation

The implantation procedure of the polyethylene (PE) matrix has been previously described (10). RIHP was measured directly and continuously via a PE matrix that was implanted in the left kidney of rats when they were 11–16 wk old (10).

Monitoring of Estrous Cycle and Induction of Pregnancy in Rats

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, female rats were restrained manually and a wet swab was inserted in the vagina and then smeared on a slide. The slide was immediately fixed with 1% toluidine blue solution (with few drops of 1 N potassium hydroxide) and observed under the microscope for cells that characterize each stage of the estrous cycle.

The estrous cycle of female rats is ~5 days and can be divided into three consecutive periods: the diestrous stage (2–3 days; characterized by smaller mutinucleated vaginal cells), the proestrous stage (1 day; characterized by larger vaginal cells with a well-defined nucleus), and the estrous stage (1 day; characterized by the appearance of cornified epithelial cells). 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 the next 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 rats (NP; n = 9). These were rats that were mated, as evidenced by the presence of sperm on the vaginal smear on the next day, but found to be nonpregnant during the acute experiments. On the day when the acute pressure natriuresis studies were performed, one of these rats was mated 13 days earlier, and the rest were mated at least 15 days earlier.

Midterm pregnant rats (MP; n = 10). These rats were pregnant for 12–14 days when the pressure natriuresis studies were performed.

Late-term pregnant rats (LP; n = 12). These rats were pregnant for 18–21 days when the pressure natriuresis studies were performed.

Surgical Procedure for Acute Pressure Natriuresis 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 ml/100 g body wt/h of saline and 1 ml/100 g body wt/h of a solution of 3% inulin and 6.25% bovine albumin in saline. A PE-50 catheter was implanted in the left carotid artery for mean arterial pressure (MAP) measurement and blood withdrawal. A PE-50 catheter was implanted in the left femoral artery for the measurement of RPP. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. A small Blalock clamp was placed around the abdominal aorta above both renal arteries and was utilized to control RPP. The rats were allowed 1 h to recover after completion of the surgical procedures. Then RPP was controlled at the lower level (~100 mmHg) by tightening the Blalock clamp around the abdominal aorta and thus reducing RPP. Ten minutes after RPP was set at the lower level, the first clearance 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 electrolytes, phosphate, and inulin measurements. At this time the Blalock clamp was loosened to allow RPP to increase and then controlled at the higher level (~120 mmHg). The second clearance period of 30 min was started 10 min after RPP was set at the higher level. Again during the second clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period, about 1 ml of blood was withdrawn from the left carotid artery for plasma electrolytes, phosphate, 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 death is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

The glomerular filtration rate (GFR) was calculated from the clearance of inulin, and inulin concentrations were measured by the anthrone method (7). Sodium concentrations in plasma and urine were measured by using flame photometry (model NOVA 1+, NOVA Biomedical, Waltham, MA). Phosphate concentrations in plasma and urine were measured according to the method of Chen et al. (2).

Standard paired Student’s t-tests were used for comparisons in the same group of rats between the first and second clearance periods. 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 in Fig. 1 and Table 1. At the lower RPP level, RIHP was 5.9 ± 0.3 mmHg for NP, which was significantly higher (P < 0.05) than it was for MP (3.4 ± 0.4 mmHg) and LP (2.9 ± 0.1 mmHg) rats at similar RPP level (Fig. 1). The increase in RPP from ~100–120 mmHg resulted in pressure natriuretic and diuretic responses in all groups; however, increases in fractional excretion of sodium, ΔV, and ΔRIHP were significantly greater (P < 0.05) in NP compared with both MP and LP rats. ΔFENa, ΔV, and ΔRIHP were 2.06 ± 0.28%, 81.44 ± 14.10 μl/min, and 3.0 ± 0.5 mmHg for NP, 0.67 ± 0.13%, 28.03 ± 5.28 μl/min, and 0.5 ± 0.2 mmHg for MP, and 0.48 ± 0.12%, 18.14 ± 4.70 μl/min, and 0.4 ± 0.1 mmHg for LP rats. GFR was well autoregulated in all groups with the changes in RPP. However, GFR was significantly higher (P < 0.05) in MP at both low and high RPP (4.43 ± 0.23 and 4.15 ± 0.20 ml/min, respectively) compared with NP (3.19 ± 0.21 and 3.25 ± 0.26 ml/min) rats (Table 1). Thus during midterm pregnancy, enhanced sodium and water reabsorption occurred despite the significant increase in their filtered loads. Fractional excretion of phosphate (FEp) increased significantly (from 15.39 ± 3.75 to 26.54 ± 7.06%) in NP rats as RPP was allowed to increase from low to high level; whereas, FEp did not increase significantly in MP (10.55 ± 1.17 to 13.82 ± 2.38%) or LP (from 13.05 ± 1.67 to 17.31 ± 5.44%) rats (Table 1). The increase in FEp (ΔFEp) was 11.15 ± 4.23% for LP rats, 3.27 ± 1.95% for LP rats, and 4.26 ± 5.14% for LP rats as RPP was...
allowed to increase from a low to a high level. The weight of both kidneys was 2.24 ± 0.07 g for MP rats, which was significantly higher \((P < 0.05)\) than for NP (1.93 ± 0.05 g) or LP (1.92 ± 0.05 g) rats.

**DISCUSSION**

The results of the present study are consistent with those in a previous study (16) that demonstrated that acute pressure natriuresis is attenuated in pregnant rats. Furthermore, the present study shows that RIHP is significantly lower in MP and LP rats compared with NP rats at similar RPP levels. As shown in Fig. 1, the blunted pressure natriuretic and diuretic responses that are observed in MP and LP rats are associated with a lower RIHP and a significant attenuation of the increase in RIHP with increases in RPP. The cause of

| Table 1. Renal responses to changes in RPP in nonpregnant female, midterm pregnant (12–14 days after conception), and late-term pregnant (18–21 days after conception) groups of Sprague-Dawley rats |
|-----------------|-----------------|-----------------|-----------------|
|                 | Non Pregnant SD \((n = 9)\) | Midterm Pregnant SD \((n = 10)\) | Late-Term Pregnant SD \((n = 12)\) |
| RPP, mmHg       | 99 ± 1           | 118 ± 2*         | 123 ± 2*         | 100 ± 1           | 119 ± 2*         |
| RIHP, mmHg      | 5.9 ± 0.3        | 8.9 ± 0.7*       | 3.4 ± 0.4*       | 4.0 ± 0.4†        | 2.9 ± 0.1†       | 3.3 ± 0.1*†      |
| MAP, mmHg       | 121 ± 2          | 118 ± 2          | 122 ± 3          | 124 ± 3          | 117 ± 3          | 119 ± 2          |
| GFR, ml/min     | 3.19 ± 0.21      | 3.35 ± 0.26      | 4.43 ± 0.23†     | 4.15 ± 0.20†     | 3.58 ± 0.34      | 3.60 ± 0.27      |
| U\(_{\text{Na}}\)V, μEq/min| 5.76 ± 1.04     | 15.98 ± 1.89     | 1.13 ± 0.29†     | 5.01 ± 0.76†     | 2.99 ± 0.76      | 5.49 ± 1.16†     |
| FE\(_{\text{Na}}\), % | 1.22 ± 0.19      | 3.28 ± 0.28      | 0.18 ± 0.04†     | 0.85 ± 0.12†     | 0.62 ± 0.15†     | 1.10 ± 0.21†     |
| FE\(_{\text{Pi}}\), % | 15.39 ± 3.75     | 26.54 ± 7.06*    | 10.55 ± 1.17     | 13.82 ± 2.38     | 13.05 ± 1.67     | 17.31 ± 5.44     |
| V, ml/min       | 43.37 ± 7.06     | 124.81 ± 17.73*  | 15.20 ± 2.04†    | 43.23 ± 4.85†    | 20.86 ± 5.41†    | 39.00 ± 7.61†    |

Values are mean ± SE. \(n = \) No. of rats; SD, Sprague-Dawley; RPP, renal perfusion pressure; RIHP, renal interstitial hydrostatic pressure; MAP, mean arterial pressure; GFR, glomerular filtration rate; \(U_{\text{Na}}V\), urinary sodium excretion; FE\(_{\text{Na}}\), fractional excretion of sodium; FE\(_{\text{Pi}}\), fractional excretion of phosphate; \(V\), urine flow rate. *Significant difference \((P < 0.05)\) between low and high RPP periods in the same group of rats compared with Student’s paired \(t\)-test. †Significant difference between nonpregnant female SD rat group and midterm or late-term pregnant SD rat group at equivalent periods (similar RPP level) compared with Student’s unpaired \(t\)-test.
the lower basal RIHP or the blunted increase in RIHP with increases in RPP in pregnant rats is not known. It can be speculated that increased renal interstitial compliance (change in renal interstitial volume/change in RIHP) during pregnancy can lead to a reduced RIHP and less of an increase in RIHP. Renal interstitial compliance can be affected by changes in the interstitial matrix or changes in the stiffness characteristics of the renal capsule. An increase in the elasticity of the renal capsule would increase the compliance of the renal interstitium. In this context, renal decapsulation can be thought of as a case of infinite elasticity. Renal decapsulation has been shown to significantly attenuate the increases in RIHP that are observed with increases in RPP or with systemic volume expansion in normotensive rats (11, 13). An attenuated pressure natriuresis response or the possibility of increased renal interstitial compliance is not unique to pregnancy. In the Okamoto spontaneously hypertensive rat (SHR), the pressure natriuresis and diuresis responses are attenuated and this attenuation is associated with a blunted increase in RIHP compared with the normotensive WKY rat (12). However, when RIHP is increased by either systemic saline volume expansion or by DRIVE in the SHR, the increase in RIHP is attenuated (9, 11), yet the natriuretic and diuretic responses are exaggerated compared with those in WKY rats (5, 9, 11). These data suggest that in the SHR, the compliance of the renal interstitium is greater than it is in normotensive WKY rats. The large renal compliance results in an attenuated transmission of RPP to the renal interstitium and smaller increases in RIHP and the attenuated pressure natriuresis response.

The results of the present study and previous studies (9) suggest that renal interstitial compliance is increased similarly during pregnancy and spontaneous hypertension. It is important to note that in the SHR, the attenuation in pressure natriuresis does not lead to cumulative increases in plasma volume. When volume is retained in the SHR due to the attenuated pressure natriuresis, the additional plasma volume expansion results in increases in RIHP (9, 11) and an exaggerated natriuresis and diuresis response (5, 9, 11). This might be an important compensatory mechanism in the SHR to regulate plasma volume in the absence of a normal pressure natriuresis and diuresis. The data in the present study and others (16) show that during pregnancy in rats, acute pressure natriuresis is blunted. This would suggest that there is an increased renal interstitial compliance in pregnant normotensive rats leading to a blunted increase in RIHP with increases in RPP. Although renal interstitial compliance during pregnancy has not been studied, it is likely that changes in compliance could occur because the renal interstitium is composed of a gel of glycosaminoglycans, and estrogens are known to modify the viscosity of this gel (17). An increase in the viscosity of the renal interstitial gel, and an increase in the elasticity of the renal capsule, would result in an increase in renal interstitial compliance. These changes would significantly reduce or prevent the transmission of RPP to the renal interstitium and further reduce transmission of increased pressure in the renal interstitium to the renal tubules. Renal interstitial compliance may gradually increase during the course of pregnancy. These changes can result in a gradual attenuation in the relationship among RPP, RIHP, and sodium and water excretions. These alterations can lead to sodium and water retention and the gradual volume expansion during the course of normal pregnancy.

In the present study, GFR was well autoregulated in all groups with the changes in RPP. However, GFR was significantly higher in MP compared with NP rats at both low and high RPP (Table 1). Thus, during mid-term pregnancy, the blunted pressure natriuresis response and the enhanced sodium and water reabsorption occurred despite the significant increase in their filtered loads. Also, FE\textsubscript{Na}, increased significantly in NP rats as RPP was allowed to increase from a low to a high level, whereas FE\textsubscript{Na}, did not increase significantly in MP or LP rats (Table 1).

In conclusion, RIHP is significantly lower in pregnant compared with nonpregnant rats at similar RPP levels. Furthermore, the ability of pregnant rats to increase RIHP in response to an increase in RPP is blunted. Thus the combination of lower basal RIHP and the attenuated increase in RIHP with increases in RPP may play an important role in the blunted pressure natriuresis and contribute to the conservation of sodium and water that is critical for fetal growth and development during normal pregnancy.

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