Kidney Function and Sodium Handling in the Pregnant Spontaneously Hypertensive Rat

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SUMMARY Effects of gestation on volume homeostasis and renal function were studied in awake spontaneously hypertensive rats (SHR). Systolic blood pressure was similar to that of virgin littermates during most of SHR pregnancy but decreased near term (p < 0.005). Plasma renin activity was lower in SHR than in age-matched Wistar-Kyoto (WKY) rats (p < 0.001), but values were similar in gravid and nonpregnant animals from each strain. Renal renin content and lipid volume fractions of papillary interstitial granules were significantly greater in pregnant animals of each strain and those of the gravid WKY were also greater than both pregnant and virgin SHR. Saralasin had no effect on mean arterial pressure in gravid and virgin rats from either group. Plasma volume increased significantly near term in animals of both strains. Kidney weight, glomerular filtration rate (GFR), and renal blood flow were lower in SHR compared to WKY, and the hypertensive rats failed to demonstrate an increase in GFR during gestation, unlike the WKY. All SHR and pregnant WKY excreted infused sodium better than the virgin WKY. Also, regular Wistar animals excreted a salt load better than the virgin WKY. Finally, uterine blood flow, pup number and conceptus weight were similar in SHR and WKY. We conclude that pregnancy induces a decrease in blood pressure in SHR, and that angiotensin II does not seem to play an important role in maintaining blood pressure during gestation in either SHR or WKY. Despite a lower GFR and its failure to increase during pregnancy, renal sodium handling is not impaired in the SHR. The virgin WKY has a decreased ability to excrete sodium which is ameliorated during gestation. (Hypertension 5: 498-506, 1983)

KEY WORDS • glomerular filtration rate • renal plasma flow • renal blood flow • renin-angiotensin system • plasma volume • sodium excretion • papillary interstitial cells

RESEARCH on hypertension during pregnancy has been hindered by the lack of suitable animal models. Okamoto and Aoki 1 have described a strain of rats (SHR) that develop hypertension spontaneously. The disease in these animals is said to resemble high blood pressure in humans in that they develop atherosclerosis and cardiovascular complications.2 Females of this strain of rats have a propensity to die of brain hemorrhage, which is of interest to us since intracranial bleeding is a major cause of death in pregnancy-associated hypertension in humans. The present study focuses on renal hemodynamics and volume homeostasis and compares the characteristics of hypertension in pregnant SHR to certain features of hypertensive disorders of human gestation.

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study, each animal was lightly anesthetized with ether; a PE 50 flanged polyethylene catheter was secured into the bladder, and the urethra was occluded with a clip at the vulva. Polyethylene tubing (PE 50) was also placed in a femoral artery to allow blood sampling and the measurement of mean blood pressure, and in a jugular vein for the infusion of saline and clearance markers. After cannulation of blood vessels and bladder, isotonic saline equal to 1% of body weight was infused and the animals were placed in restraining cages, secured in part with surgical silk sutured in the skin of the back, and allowed to recover from anesthesia. Priming doses of 20 mg inulin and 3 mg PAH were then given rapidly followed by a sustaining solution of 800 μg/min inulin and 120 μg/min PAH) which maintained plasma levels at approximately 50 and 3 mg per 100 ml, respectively. Fluid was delivered with constant infusion pumps at a rate of 0.02 ml per minute in both pregnant and control rats.

After a 1-hour equilibration period, urine was collected in preweighed test tubes and volumes determined by reweighing the tubes shortly after completing a collection. In each experiment, collections from four consecutive periods of 30 minutes each were obtained, and a heparinized blood sample was taken at the midpoint of each period. Experiments in which steady-state conditions did not prevail in both gravid and control animals were rejected. Plasma and urine inulin and PAH were measured by semimicro modifications of the anthrone and diazotization methods, respectively. 4, 5

In other studies, renal blood flow was measured in awake rats by a labeled microsphere technique. 6 Animals were prepared surgically under light ether anesthesia. A PE 10 catheter was passed in a retrograde fashion into the left ventricle via the right common carotid artery, and the opposite end was then tunneled subcutaneously to exit on the animal’s back just above the right scapula. PE 50 tubing was placed in the left femoral artery for blood withdrawal. Surgical fluid loss was replaced with isotonic saline equivalent to 1% of total body weight, the animal was placed in a restraining cage (as described above) and studied after an equilibration period of 60 minutes. To measure blood flow, microspheres labeled with either 85Sr or 141Ce which had a diameter of 15 ± 5 μm (3M Company, St. Paul, Minnesota) were diluted to a concentration of 6 × 105 spheres/ml in 10% dextran and suspended with a high speed vortex mixer for at least 5 minutes just prior to injection. (Microscopic examination of spheres handled in this manner revealed adequate suspension with no significant aggregation.) Sixty thousand microspheres of either label in a volume of 0.1 ml were rapidly injected into the left ventricle and the catheter was then flushed with 0.2–0.3 ml isotonic saline. During this procedure 0.68 ml of blood was withdrawn from the femoral catheter by a Harvard withdrawal/infusion pump (Model 600–950, Harvard Apparatus Company, Millis, Massachusetts) starting 10 seconds prior to the injection and continuing for 1 minute.

After injection of the microspheres, both kidneys were removed and the capsules stripped. One kidney was sectioned with a microtome (Sartorius Werke, Göttingen, Germany), and three 500 μm thick coronal sections were taken that represented three cortical zones: Zone I = outer cortex; Zone II = midcortex; and Zone III = inner or juxtamedullary cortex, including a thin rim of the outer medulla. Inclusion of this rim assured that all juxtamedullary glomeruli were included in Zone III. Each of these three sections and the opposite kidney were placed in preweighed vials and counted in a Packard Auto Gamma spectrometer (Packard Instruments Inc., Downers Grove, Illinois).

Saline Infusion Studies

Effects of acute sodium loading were studied in awake rats in restraining cages. Animals were prepared surgically as described above in the clearance protocols. The sodium load, calculated to equal 20% of the animal’s estimated extracellular space, was infused over a period of 30 minutes. These calculations were based on our previously derived data on the volume of distribution of inulin in 20-day gravid and virgin littermate Sprague-Dawley rats, 7 combined with similar results obtained during preliminary experiments in SHR and WKY animals. Extracellular fluid volume measured in the dams was considered to be 25% of anticipated nonconceptus body weight while that in the virgin rat was 21% of body weight. Urine was collected during the infusion, and for three successive 30-minute periods following its completion. Urinary sodium content was measured by flame photometry using lithium as an internal standard.

Plasma Volume

Plasma volume was measured in 20-day gravid animals and their virgin littermates by the Evans Blue dye technique as described for small animals by Belcher and Harris. 6 Briefly, 0.2 ml of a 0.5% (wt/vol) Evans Blue solution was injected intravenously and an arterial blood sample (0.6 ml) was taken after 5 minutes. In preliminary experiments, we verified that mixing had occurred by this time and that dye loss from the intravascular space was similar in gravid and virgin animals of both strains. Experiments were performed at the same hour of the morning to minimize diurnal variation in plasma volume.

Renin-Angiotensin System

Unanesthetized 20-day gravid SHR and WKY animals and their respective virgin littermates were guillotined and the blood issuing from their trunks was collected in iced tubes containing EDTA (1 mg/ml) for measurement of plasma renin activity. Immediately after sacrifice, the kidneys were removed from each animal and stored in liquid nitrogen for renin analysis. Plasma renin activity was measured by radioimmunoassay of generated angiotensin I as described by Haber et al. 8 Renal renin content was determined by a modification of the method of Haas et al. 9, 10 Schaffenberg et al., 11 and Boucher et al. 12
In separate studies, saralasin (whose lot potency was verified in rats treated with pressor doses of angiotensin II) was infused at a rate of 2.5 μg/min for 1 hour into unanesthetized animals placed in restraining cages and prepared as described in the clearance protocols. Blood pressure was measured with a Statham transducer and recorded on a Sanborn 780-6 videoscope (Hewlett-Packard Company, Palo Alto, California) for 60 minutes prior to, during, and after the infusion.

Papillary Interstitial Granules
Twenty-day pregnant and nongravid littermates were anesthetized and their kidneys perfused in retrograde fashion at a pressure of 180 mm Hg via the abdominal aorta with a glutaraldehyde fixative solution (osmolality = 1200 mOsm/kg) just prior to removal. The papillae from these kidneys were analyzed as follows: first, a preliminary survey was made by light microscopy to determine the density of interstitial cells in the papillary tip. Interstitial cell nuclei were counted in randomly oriented sections. Then, using a Siemens 101 electron microscope, every visible interstitial cell on a grid was photographed, the final micrograph being enlarged ×10,000. A single lattice grid system with points 0.5 cm apart was then superimposed on the micrograph and the number of hits on a cell, on the cytoplasm only, and on lipid droplets within the cell were computed. An arcsine transformation was then performed on the lipid:cell volume ratio, and a mixed model two-level nested analysis of variance for unequal sample size was used to test for significant differences between groups. Since this demonstrated that there were differences between groups, their means were compared using the Student's t test.

Statistics
Except as indicated above, all statistical analyses were performed with the Student t test for nonpaired samples.

Results
Blood Pressure
Figure 1 describes the course of systolic blood pressure during pregnancy in the SHR. Blood pressure levels were similar in gravid and virgin animals until the 17th gestational day, after which pressure declined significantly (p < 0.005) in the gravid animals. For this reason most protocols were performed on the day prior to anticipated term, when the differences in blood pressure would be expected to be near maximum. Also shown in Figure 1 are data from 20-day pregnant and control littermate WKY. Systolic pressure levels were slightly higher in the controls (126 ± 5 mm Hg) than the gravid (119 ± 4 mm Hg) animals, but the difference was not statistically significant.

Renal Hemodynamics
Results from clearance experiments are summarized in Table 1. Glomerular filtration rate (C\text{\textsubscript{inulin}}) was significantly lower in all SHR compared to either pregnant or virgin WKY rats (p < 0.05) of similar age, and no differences were noted between gravid and control SHR. In contrast, GFR was higher near term in WKY animals compared to their virgin littermate controls (p < 0.02). Kidney weights were also lower in SHR, and when C\text{\textsubscript{inulin}} was corrected for this, GFR was similar in all four groups (Table 1).

Effective renal plasma flow (ERPF), as measured by the clearance of PAH, was similar in gravid and pregnant animals of both strains, and was also significantly lower in SHR compared to age-matched WKY rats; again, these differences were abolished when the absolute values were factored by kidney weight. Since GFR rose in gravid WKY animals, but ERPF was unchanged, the filtration fraction increased during pregnancy in this strain (p < 0.001), while that of the pregnant SHR remained unchanged.

Total renal and uterine blood flow, measured with radioactive microspheres, are shown in Table 2. Total renal blood flow was similar in gravid and virgin SHR, and again values in this strain were significantly lower than in WKY (p < 0.005), but this time the difference persisted (p < 0.005) when corrected for kidney weight. Uterine blood flow was similar in the hypertensive and normotensive gravid animals.
TABLE 1. Renal Clearances of Inulin and p-Aminohippurate in Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Mean blood pressure (mm Hg)</th>
<th>NCBW (g)</th>
<th>Conceptus weight (g)</th>
<th>Pups (no.)</th>
<th>Kidney weight (g)</th>
<th>C inulin ml/min</th>
<th>C PAH ml/min</th>
<th>Filtration fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>151 ± 6</td>
<td>187 ± 3.3</td>
<td>31.9 ± 3.2</td>
<td>8.6 ± 0.5</td>
<td>1.49 ± 0.04</td>
<td>1.48 ± 0.06</td>
<td>0.99 ± 0.06</td>
<td>8.61 ± 0.24</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>167 ± 4</td>
<td>174 ± 4.1</td>
<td>1.46 ± 0.05</td>
<td>1.62 ± 0.09</td>
<td>1.13 ± 0.08</td>
<td>5.05 ± 0.35</td>
<td>1.20 ± 0.24</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.025</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>118 ± 5*</td>
<td>201 ± 3.5*</td>
<td>32.6 ± 2.1</td>
<td>9.7 ± 0.5</td>
<td>1.92 ± 0.07*</td>
<td>1.28 ± 0.16*</td>
<td>1.15 ± 0.05*</td>
<td>5.31 ± 0.34*</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>109 ± 6*</td>
<td>182 ± 1.5</td>
<td>1.77 ± 0.06*</td>
<td>1.87 ± 0.08*</td>
<td>1.09 ± 0.08</td>
<td>3.41 ± 0.27*</td>
<td>3.69 ± 0.21*</td>
<td>0.29 ± 0.01*</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.02</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NCBW = nonconceptus body weight (body weight minus uterus and its contents); C = clearance; kw = kidney weight; ± = SEM; PAH = p-aminohippurate.

*Significantly different from both pregnant and nonpregnant SHR.
†Significantly different from pregnant SHR.

Distribution of trapped microspheres differed in SHR and WKY, outer cortical trapping being greater in rats of the hypertensive strain (table 2).* However, gestation had no influence on the distribution of spheres in either SHR or WKY compared to their virgin littermates.

Saline Infusion Studies

Sodium excretion during the saline loading protocol is summarized in table 3 and figure 2. Gravid and control SHR and pregnant WKY all excreted a significantly greater percent of the load than the virgin WKY rats. This latter group appeared to handle infused sodium poorly, excreting but 31% ± 4% of the load. To investigate this further, we studied two other groups of virgin Wistar animals, the parent strain of WKY, one age- and the other weight-matched to the WKY. These latter animals handled infused sodium better than all other groups except the gravid SHR.

Plasma Volume

The distribution volume of Evans Blue dye was greater in the pregnant rats of both groups than in their nonpregnant controls (table 4). Values were 4.7 ± 0.2 ml/100 g in virgin WKY compared to 3.8 ± 0.2 ml/100 g in control SHR. Volume increased significantly during pregnancy, averaging 5.5 ± 0.3 ml/100 g body weight (p < 0.001) in the SHR and 5.5 ± 0.2 ml/100 g in the WKY (p < 0.01), both compared to their respective virgin controls.

TABLE 2. Renal Blood Flow and Intrarenal Distribution of Radioactive Microspheres in Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>NCBW (g)</th>
<th>RBF (ml/min)</th>
<th>RBF (ml/min/g kid wt)</th>
<th>Zone I (%)</th>
<th>Zone II (%)</th>
<th>Zone III (%)</th>
<th>UBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>7</td>
<td>201 ± 2.0</td>
<td>6.83 ± 0.95</td>
<td>5.05 ± 0.38</td>
<td>50.5 ± 1.40</td>
<td>29.0 ± 1.60</td>
<td>20.5 ± 0.80</td>
<td>3.45 ± 0.66</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>6</td>
<td>165 ± 1.0</td>
<td>6.61 ± 0.89</td>
<td>5.35 ± 0.83</td>
<td>49.6 ± 2.00</td>
<td>28.9 ± 1.50</td>
<td>21.5 ± 0.90</td>
<td>3.45 ± 0.66</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>11</td>
<td>221 ± 4.1*</td>
<td>11.7 ± 0.71*</td>
<td>7.37 ± 0.41*</td>
<td>38.7 ± 1.18*</td>
<td>34.3 ± 1.22*</td>
<td>26.9 ± 1.00*</td>
<td>3.04 ± 0.53</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>11</td>
<td>191 ± 4.0†</td>
<td>13.0 ± 0.86*</td>
<td>8.83 ± 0.46*</td>
<td>41.4 ± 1.50*</td>
<td>32.4 ± 1.00</td>
<td>26.1 ± 1.00*</td>
<td>3.04 ± 0.53</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

± = SEM; NCBW = nonconceptus body weight (body weight minus uterus and its contents); RBF = renal blood flow; kid wt = kidney weight; UBF = uterine blood flow.

*Significantly different from both pregnant and nonpregnant SHR.
†Significantly different from nonpregnant SHR.
TABLE 3. Sodium Excretion During and After Saline Infusion in Pregnant and Nongravid Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>NCBW (g)</th>
<th>Infusate (ml/30 min)</th>
<th>UV (% 2 hrs)</th>
<th>UNaV (% 2 hrs)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>7</td>
<td>181 ± 3.1</td>
<td>8.4 ± 0.25</td>
<td>1.30 ± 0.04</td>
<td>90 ± 12</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>6</td>
<td>169 ± 4.3</td>
<td>7.2 ± 0.19</td>
<td>1.12 ± 0.03</td>
<td>55 ± 9</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
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<tr>
<td>Wistar-Kyoto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>8</td>
<td>211 ± 3.1</td>
<td>9.6 ± 0.17</td>
<td>1.48 ± 0.03*</td>
<td>68 ± 8</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>8</td>
<td>192 ± 5.1</td>
<td>8.2 ± 0.21†</td>
<td>1.26 ± 0.03†</td>
<td>24 ± 3*</td>
<td>31 ± 4*</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>NS</td>
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<tr>
<td>Wistar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-matched</td>
<td>8</td>
<td>190 ± 3.4</td>
<td>8.12 ± 0.14</td>
<td>1.24 ± 0.02</td>
<td>85 ± 11</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Age-matched</td>
<td>4</td>
<td>247 ± 4.2</td>
<td>12.8 ± 0.90†</td>
<td>1.97 ± 0.32†</td>
<td>89 ± 13</td>
<td>78 ± 7</td>
</tr>
</tbody>
</table>

Abbreviations as in table 1. UV = water excretion; UNaV = sodium excretion.

*Significantly different from both pregnant and nongravid SHR.
†Significantly different from nonpregnant SHR.
‡Four animals inadvertently received saline equal to 30% of their extracellular volume. Water and sodium excretion were markedly ($p < 0.001$) lower in Wistar-Kyoto than in either age- or weight-matched Wistar rats.

Renin-Angiotensin System

Plasma renin activity was lower in SHR animals compared to age-matched WKY, but there were no significant differences between pregnant and virgin animals within each strain (table 4). Renal renin content was significantly greater in the gravid animals of each group and that of the pregnant WKY was also greater than both the pregnant and virgin SHR (table 4).

Saralasin infused for 60 minutes had no effect on the blood pressure of either gravid or virgin SHR or WKY animals. This was observed both on the 14th gestation-

TABLE 4. Plasma Volume, Renin Activity, and Kidney Renin Content in Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Plasma volume (ml/100 g NCBW)</th>
<th>PRA (ng/ml/hr)</th>
<th>Kidney renin content (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>5.51 ± 0.27†</td>
<td>6.8 ± 1.0</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>3.84 ± 0.18</td>
<td>5.2 ± 0.5</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>5.46 ± 0.19†</td>
<td>13.7 ± 2.3*</td>
<td>4.8 ± 0.3*</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 15)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>4.66 ± 0.15</td>
<td>18.0 ± 2.3*</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 17)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations as in table 1. PRA = plasma renin activity.
*Significantly different from both pregnant and virgin spontaneously hypertensive rats.
†Significantly different from spontaneously hypertensive nonpregnant rats.

Figure 2. Cumulative sodium excretion following a 30 min intravenous sodium chloride load. Pregnant animals of both the SH and WKY strains excreted sodium better than their respective virgin controls, although at lesser rates than age- or weight-matched Wistar rats. ○ WKY pregnant; □ SHR pregnant; ● WKY nonpregnant; ■ SHR nonpregnant; Δ Wistar age-matched; ▲ Wistar weight-matched.
TABLE 5. Volume Fraction of Interstitial Cells Occupied by Lipid Droplets in Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>No. of grids observed</th>
<th>Mean lipid volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>4</td>
<td>345</td>
<td>10.48 ± 0.31</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>4</td>
<td>277</td>
<td>8.36 ± 0.26</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>4</td>
<td>194</td>
<td>13.03 ± 0.48*</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>4</td>
<td>211</td>
<td>8.35 ± 0.50†</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

± = SEM.  
*Significantly different from both spontaneously hypertensive pregnant and nonpregnant rats.  
†Significantly different from spontaneously hypertensive pregnant rats.

al day, when systolic levels were similar in the gravid and virgin SHR, and near term, when blood pressure had fallen in the pregnant animal.

Papillary Interstitial Granules

An example of the grid lattice system used to count the cytoplasm or lipid droplets within interstitial cells is shown in figure 3. Results from four pregnant and virgin animals from each strain (194–345 observations per group) are summarized in table 5. There was a highly significant difference between gravid and control animals of each strain. Lipid volume fractions, lower in the gravid and control SHR than that measured in the pregnant WKY, were similar in the virgin animals from each group.

Discussion

These studies describe the course of blood pressure, renal hemodynamics, and the sodium excretory ability of the pregnant SHR. The major observations were that in these animals blood pressure decreases significantly near term, while GFR and the ability of the gravid animal to excrete a saline load remain unaltered. The results also suggest that virgin WKY have a decreased ability to excrete infused saline, which improves considerably during gestation.

Blood Pressure

Women with essential hypertension often experience decreases in blood pressure that start early in gestation and are maximal in midpregnancy, after

FIGURE 3. Lipid droplets in papillary interstitial cells and lattice grid used to determine lipid:cell volume ratios. × 10,000
which diastolic levels rise again toward or above non-pregnant means.\textsuperscript{13, 14} A gestation-induced decrease in blood pressure has also been described in rats with hypertension induced by a variety of experimental techniques\textsuperscript{15-18} and in these models the decrement occurs near term. In 1964 Takeda\textsuperscript{15, 16} observed decreases in blood pressure in gravid SHR that also occurred late in pregnancy and were ascribed to the developing fetal kidney. Aoi and colleagues\textsuperscript{17} confirmed these findings in a serial study of awake animals and noted that by the day of delivery systolic pressure had fallen to levels similar to these measured in term WKY. More recently, however, McCarty and Kopin\textsuperscript{18} found no difference in the mean blood pressure of 20-day gravid and control SHR. Their data suggested that blood pressure may even rise in the SHR during the last few days of pregnancy. Our results are similar to those of Takeda and Aoi et al. However, the decreases noted by us were not as marked as those noted by Aoi et al. In our study in which we measured both systolic (serial investigation) and mean pressures (renal hemodynamics and saline-loading protocols, tables 1 and 3), the blood pressure in 20-day gravid animals, although reduced below levels recorded earlier in gestation or in virgin controls, were (with rare exceptions) considerably higher than those of either pregnant or virgin WKY. Thus, declines in blood pressure are seen during pregnancy in SHR and in women with essential hypertension, but with different time courses. Furthermore, 10\% to 20\% of the women experience exaggerated increases in blood pressure during the third trimester (superimposed preeclampsia). This phenomenon was never noted in pregnant SHR.

We further studied factors that influence blood pressure control, focusing on the renin-angiotensin system. Renal renin content was increased during pregnancy in both SHR and WKY, but plasma renin activity (PRA) was similar and saralasin failed to decrease blood pressure in either gravid or virgin animals of either strain. (Our data are consistent with observations by some\textsuperscript{19-23} but not all\textsuperscript{24-27} that SHR manifest "low renin hypertension." The status of the renin-angiotensin system may also relate to the age of the animal.) These findings contrast with recent observations in Sprague-Dawley rats that PRA rises in late pregnancy when pressure in the gravid animal becomes angiotensin-dependent (i.e., decreases when saralasin is infused in doses comparable to those used by us) while levels in similarly treated virgins remain unchanged.\textsuperscript{28}

The lipid volume fraction of papillary interstitial cells increased during pregnancy in both strains but were lower in pregnant SHR than WKY. The meaning of this is unclear, as these lipids have been considered to represent vasodilatory prostaglandins (whose renal production may increase in pregnancy\textsuperscript{29}) or another hypertensive factor, yet a substantial decrement in pressure occurred only in the pregnant SHR.

Observation of similar lipid volume fractions of interstitial cells in virgin SHR and WKY contrasts with the results of Mandal et al.\textsuperscript{30} who suggested that there was diminished lipid granularity in the papilla of the hypertensive strain. The methodology utilized in their study differed from ours. For instance, we perfused the kidney with a fixative whose osmolality mimicked the hypertonicity normally present in the papilla and utilized a lattice system for counting. More important, Mandal et al. compared SHR to Wistar animals while the more appropriate control, the WKY rat, was used in the present study.

### Renal Hemodynamics

Glomerular filtration rate was significantly greater in term WKY compared to their virgin littermates, similar to changes seen during gestation in several other strains of rat.\textsuperscript{31-33} Renal hemodynamics also rise during normal human pregnancy, and similar increments occur in most pregnant women with essential hypertension.\textsuperscript{13, 14} In contrast, GFR was not higher in term pregnant SHR than in their littermate control (table 1), and both GFR and ERPF in both groups were lower than those of either gravid or virgin WKY. However, markedly decreased glomerular filtration rates, such as occasionally occur when preeclampsia complicates human pregnancy were not observed.

The absence of a physiological increase in glomerular filtration during late gestation in SHR is of interest, but the reason for this is not apparent from our data. One might speculate that the factors which normally cause a rise in filtration near term are counteracted by the simultaneous decrease in blood pressure. In this respect studies on the autoregulatory capacity of the SHR kidney might be revealing.

Little is known about the determinants of glomerular ultrafiltration in nongravid SHR, and there are only a few studies on morphological alterations in the kidney. Despite significant hypertension, there are no differences in the glomerular capillary pressures in 14- to 16-week-old SHR and WKY.\textsuperscript{34, 35} Feld et al.,\textsuperscript{36} using light microscopy, were unable to discern any structural changes in the kidneys of hypertensive rats below the age of 30 weeks. In contrast, Evan and colleagues,\textsuperscript{37} using scanning and transmission electron microscopy, noted that SHR had fewer glomerular endothelial fenestrae, apparent even before hypertension developed. The difference increased with age and by 12 weeks the density of endothelial fenestrae was 32\% below that in WKY; at this time the SHR also demonstrated morphological evidence of afferent arterial vasoconstriction. Whereas the relevance of the endothelial changes to hemodynamic parameters is not obvious, afferent vasoconstriction could explain, by itself, why GFR, ERPF, and total renal blood flow were lower in SHR compared to age-matched WKY, and why the former fail to increase GFR during pregnancy.

Although effective renal plasma flow and total blood flow were lower in SHR than WKY, values were similar in gravid and virgin littermates in each strain. Renal blood flow increases markedly in human gestation but there is controversy as to whether or not similar increments occur during rat gestation.\textsuperscript{31-33}
Plasma Volume

Plasma volume (ml/100 g nonconceptus body weight) was substantially greater in both gravid SHR and WKY compared to their respective virgin littersmates, the increment appearing to be greater in the former strain. In contrast, the physiological increase in plasma volume accompanying human pregnancy is often attenuated and occasionally abolished when hypertension occurs.

There is considerable disagreement concerning intravascular volume in the SHR, both plasma and blood volume having been described as increased, decreased or unchanged. In this respect we found 'low volume hypertension' when virgin SHR and WKY were compared.

Saline Infusion Studies

Patients with essential hypertension excrete infused saline more rapidly than normotensive controls. A similar type of "exaggerated natriuresis" has been described in some but not all gravid women with essential hypertension, while preeclamptic gravidas have a decreased ability to excrete infused saline. In this study both pregnant and virgin SHR demonstrated an enhanced ability to excrete infused saline.

It should be noted that, while some authors have described an exaggerated natriuretic response to volume expansion in nonpregnant SHR, others have found no differences, or even decrements in the ability of the hypertensive strain to excrete sodium. These discrepancies may reflect choice of appropriate controls, use and type of anesthesia, substrain variations and route of saline administration. An interesting alternative hypothesis, advanced by Vandewalle and colleagues, is that the SHR do not necessarily have an enhanced renal sodium excretory capacity but that the control WKY may actually have a decreased ability to excrete infused saline, and that this defect may be responsible for the outgrowth of the SHR strain. Our data demonstrating that both age- and weight-matched Wistar animals excreted sodium better than the virgin WKY are consistent with this view (table 3). Also, our results extend those of Vandewalle et al., by demonstrating that pregnancy in the WKY led to substantial improvement in their renal sodium secretory ability.

The enhanced natriuresis observed in hypertensive subjects has been ascribed to an exaggerated rise in blood pressure evoked by volume expansion. This was obviously not the case in the SHR, as the "exaggerated natriuresis" in the term pregnant animals occurred despite a significant decrease in blood pressure compared to their virgin littersmates.

The goal of this investigation was to describe blood pressure, volume homeostasis, and several renal functional parameters in gravid SHR and see how the findings compared with what is known to occur during gestation in pregnant women with essential hypertension. While there are some similarities, the SHR model obviously has many limitations. Pregnancy has a salutary effect on the blood pressure of both SHR and many gravidas with chronic hypertension, but the decrements occur during different phases of their respective gestations. Neither the hypertensive rat nor women with essential hypertension experience decrements in their renal sodium excretory ability. However, while the physiological increment in plasma volume is frequently limited in hypertensive gravidas, intravascular volume increased markedly in SHR. It should be noted, however, that complications seen during gestation in women with essential hypertension occur most often in the older gravidas who presumably have sustained a greater degree of end-organ damage than their younger counterparts. In this respect studies in older pregnant SHR might be more revealing.

Finally, our data are relevant to certain controversies regarding the nonpregnant SHR. Our animals manifested "low renin" hypertension and displayed an enhanced ability to excrete infused sodium in comparison with the subnormal renal excretory capacity in WKY rats. Further study of the pregnant WKY might explain this volume handling problem in the WKY strain.

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