Renal and Hormonal Effects of Water Deprivation in Late-Term Pregnant Rats

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Abstract—Water-retaining hormones are stimulated during pregnancy allowing normal volume expansion. Because pregnant rats actively retain water, we postulate that water deprivation (WD) would cause a greater reduction in plasma volume in pregnant than in nonpregnant rats. To test this hypothesis, Sprague-Dawley pregnant and nonpregnant rats were water-deprived for 48 hours. At day 19 of pregnancy, or in the corresponding day in nonpregnant rats, they were randomly assigned to either a WD or a control (C) pair-fed group (n=10 to 12 per group). WD significantly reduced body weight, food intake, and creatinine clearance, and increased increased urinary osmolality in nonpregnant and pregnant rats. WD reduced plasma volume in a similar proportion in nonpregnant and pregnant rats (nonpregnant rats C=13.1±0.4, WD=11.0±0.2; pregnant rats C=19.4±0.7, WD=16.8±0.5 mL, P<0.001). Both groups of pregnant rats had a similar reduction in blood pressure. Plasma renin activity (nonpregnant rats C=6.1±1.1, WD=20.5±2.0; pregnant rats C=49±9.7, WD=94±12 ng angiotensin I/mL per hour, P<0.001) and plasma aldosterone levels were increased by pregnancy and further increased by WD. WD significantly reduced urinary kallikrein. WD caused a significant reduction in fetal but not placental weights. Present data indicate that 48-hour WD reduced renal kallikrein and further stimulated water-retaining hormones. We speculate that these are compensatory changes contributing to the maintenance of pregnancy in response to WD. (Hypertension. 2004;44:334-339.)

Key Words: preeclampsia ■ pregnancy ■ renin ■ bradykinin ■ aldosterone ■ kallikrein ■ mineralcorticoids

Normal pregnancy is characterized by several hemodynamic changes that allow a progressive increment in uteroplacental blood flow and adequate fetal growth. The initial event that triggers these hemodynamic changes is thought to be a drop in systemic resistance caused by an increase in vasodilator substances, such as NO and renal kallikrein. This systemic vasodilatation would stimulate the renin-angiotensin system (RAS) that, in turn, produces renal water and sodium retention.1-3 The increase in total body sodium is exceeded by the increase in total body water, causing a reduction in both plasma sodium and serum osmolality during normal gestation. Although in nonpregnant animals these changes would cause suppression of arginine vasopressin release and a water diuresis, volume-sensing arginine vasopressin release mechanisms appear to adjust as pregnancy progresses, so that the new volume is sensed as normal.4 Thus, the central regulation of arginine vasopressin release maintains body tonicity at a lower steady-state value during pregnancy and, furthermore, the threshold for thirst is reset to a lower plasma osmolality.5 Responses to sodium loading or to chronic mineralocorticoid administration indicate that pregnant and nonpregnant rats respond similarly. However, during salt restriction, gravid rats fail to expand their plasma volume normally, and this relative hypovolemia activates mechanisms leading to free water retention and pathological hyponatremia, responses not observed in nonpregnant animals.6

Water deprivation (WD) is a potent stimulus, which modifies the water and electrolyte balance, decreasing both extracellular and intracellular fluid volume. Consequently, we hypothesized the existence of quantitative differences in the hormonal and hemodynamic responses to WD between nonpregnant and pregnant rats. Specifically, we expected that because pregnant rats actively retain water, WD would cause a greater impact on plasma volume expansion in this group. The experiments reported here were performed to determine the influence of WD on plasma volume expansion, plasma renin activity (PRA), plasma aldosterone, and urinary kallikrein activity, as well as in renal function and reproductive outcome, in nonpregnant and in near-term pregnant rats.

Methods

Experimental Design
Female Sprague-Dawley rats, 230 to 250 g initial weight, were maintained at the Center for Medical Research animal care facilities in a controlled environment (22 to 24°C and a 12-hour light/dark cycle). All procedures met international guidelines for animal wel-
Hormonal and Biochemical Measurements

PRA was determined by radioimmunooassay of generated angiotensin I under control conditions.⁶ Plasma aldosterone was measured by radioimmunooassay with the use of a commercial kit (Diagnostic Products Corporation). Urinary kallikrein activity was determined by the amidase method with the use of the synthetic substrate DL-Val-Leu-Arginine-p-nitroanilide (Sigma).⁷ Plasma and urinary electrolytes were measured with a Berkman autoanalyzer. Plasma and urinary creatinine levels were measured with a Beckman autoanalyzer. Urinary protein concentration was determined by the Bradford method (bio-rad protein assay). Serum and urinary osmolality were determined by freezing point (Advanced Instruments, model 3DI).

Statistical Analysis

Statistical analysis was performed using the computer program Stat View II (Abacus Concepts Inc). All data were expressed as mean ± SEM. Statistical significance was accepted at a level of P<0.05, and data were analyzed by a single-factor factorial design ANOVA or by a simple linear regression.

Results

Maternal general characteristics are shown in Table 1. Main findings include a significant reduction in body weight in WD rats and a reduction in food intake after the second day of WD; this was more pronounced in pregnant than in nonpregnant rats. Systolic blood pressure was reduced during pregnancy and was unaffected by WD. Hematocrit values were lower in pregnant than in nonpregnant rats and were significantly increased by WD. Plasma volume levels were increased by pregnancy and reduced in both groups of WD rats. The highest plasma volume levels were observed in control pregnant rats (Table 1). Interestingly, percentage reduction in plasma volume after WD was similar in nonpregnant than in pregnant rats (16% versus 14%, nonsignificant). When all groups were combined, a significant negative correlation was observed between plasma volume and hematocrit levels (r = −0.9, P<0.001).

The main renal changes caused by WD are shown in Table 2. As expected, WD reduced urine output. Serum creatinine was similar in all groups (nonpregnant rats Control C = 0.4 ± 0.03 versus WD = 0.4 ± 0.03, pregnant rats C = 0.5 ± 0.03 versus WD = 0.5 ± 0.08 mg%). Urinary creatinine excretion was significantly reduced by WD (nonpregnant rats C = 8.3 ± 0.9 versus WD = 5.0 ± 0.5, P<0.001; pregnant rats C = 7.6 ± 0.4 versus WD = 4.6 ± 0.4 mg per day, P<0.001). In consequence, creatinine clearance was lower in both groups of WD rats. Serum osmolality was not affected

| TABLE 1. General Effects of 48-Hour Water Deprivation in Nonpregnant and Pregnant Rats |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Parameter                                | Nonpregnant |          | Pregnant |          |
| Weight day 19, g                           | 265 ± 2.5     | 266 ± 1.9     | 357 ± 5.5†   | 356 ± 6.7†     |
| Weight day 21, g                           | 246 ± 1.8     | 234 ± 1.8*    | 335 ± 6.7†   | 320 ± 5.4†     |
| Net weight, g                             | 202 ± 1.0     | 191 ± 2.1*    | 205 ± 3.0    | 196 ± 3.1*     |
| Water intake day 19–20, mL                 | 25.2 ± 1.4    | 0*            | 21.9 ± 1.3   | 0*             |
| Water intake day 20–21, mL                 | 19.2 ± 1.3    | 0*            | 14.4 ± 2.0   | 0*             |
| Food intake day 19–20, g                   | 10.6 ± 0.3    | 9.7 ± 1.2     | 9.2 ± 0.1    | 9.5 ± 1.2     |
| Food intake day 20–21, g                   | 3.4 ± 0.06    | 3.4 ± 0.5     | 0.8 ± 0.1†   | 0.5 ± 0.2†     |
| Systolic blood pressure, mm Hg             | 110 ± 3.1     | 117 ± 2.8     | 67 ± 3.0†    | 60 ± 3.5†     |
| Hematocrit                                | 0.42 ± 0.005  | 0.45 ± 0.006* | 0.34 ± 0.005†| 0.39 ± 0.01†  |
| Plasma volume, mL                         | 13.1 ± 0.4    | 11.0 ± 0.2*   | 19.4 ± 0.7†  | 16.8 ± 0.5†   |

Values are mean ± SEM.
* Differences between control vs water-deprived groups.
† Differences between nonpregnant vs pregnant rats, P<0.05, ANOVA.
by WD in nonpregnant rats and was significantly reduced in WD pregnant rats. Urinary osmolality was significantly increased by WD. WD decreased daily solute excretion in nonpregnant rats but did not change it in pregnant rats (nonpregnant rats $C=12.2\pm1.0$ versus WD $=6.5\pm0.7$, $P<0.001$; pregnant rats $C=8.3\pm0.9$ versus WD $=6.5\pm0.6$, Osm per day). Urinary osmolality to serum osmolality ratio was increased in both groups of WD rats (nonpregnant rats $C=8.7\pm1.7$ versus WD $=18.7\pm2.5$, $P<0.001$; pregnant rats $C=8.4\pm1.1$ versus WD $=20.9\pm2.0$, $P<0.001$). WD significantly reduced urinary sodium excretion in nonpregnant rats but did not modify it in pregnant animals; both groups of pregnant rats had significantly lower sodium excretion than their corresponding nonpregnant groups. WD significantly reduced sodium fractional excretion in pregnant rat but not in nonpregnant rats. WD reduced osmolar clearance and free water reabsorption only in nonpregnant rats. (Table 2). When water reabsorption was expressed as a proportion of creatinine clearance, values were similar in all groups except for pregnant WD rats, which exhibited a greater value (data not shown).

As shown in the Figure, both groups of pregnant rats had significantly higher PRA levels than their corresponding nonpregnant groups. WD further increased PRA levels in pregnant rats, but the rise observed in nonpregnant rats was not statistically significant ($6.1\pm1.1$ to $20.5\pm2.0$ ng angiotensin I (Ang I)/mL per hour). WD significantly increased aldosterone levels in both nonpregnant and pregnant rats; the highest values were observed in WD pregnant rats. Plasma aldosterone to PRA ratio was significantly reduced in pregnant rats when compared with the corresponding nonpregnant groups and was unaffected by WD (nonpregnant rats $C=88.4\pm12$ versus WD $=89.7\pm18.3$; pregnant rats $C=41\pm7.9$, WD $=38.2\pm5.4$, $P<0.01$). When all groups were combined, a positive and significant correlation was observed between PRA and aldosterone levels ($r=0.68$, $P<0.001$). WD significantly reduced urinary kallikrein activity in nonpregnant and in pregnant rats (Figure). Urinary kallikrein activity had a positive correlation with creatinine excretion ($r=0.86$, $P<0.001$) and when urine kallikrein activity was expressed per creatinine excretion, differences disappeared (data not shown).

Results of selected maternal and fetal variables obtained in rats that underwent 48-hour or 72-hour WD during late pregnancy are shown in Table 3. Maternal weight at term was significantly lower in 72-hour WD rats when compared with controls. Systolic blood pressure was unaffected by WD (not shown). WD did not modify litter size, and fetuses from all groups were alive when extracted from the uterus. WD decreased fetal weight, particularly in the 72-hour group, and increased the number of pups whose birth weights were below the 10th percentile (42/139 and 41/49 for 48-hour and 72-hour WD, respectively, $x^2=86$, $P<0.001$). Placental weight was unaffected by WD. Hematocrit (0.42±0.006), plasma volume (16.6±0.8 mL), serum creatinine (0.57±0.2 mg%), creatinine clearance (0.8±0.2 mL/min), PRA, and aldosterone levels were similar between 48-hour and 72-hour WD pregnant rats (Table 3). When compared with the 48-hour WD rats, serum osmolality was even lower (303±5.5 mOsm/L, $P<0.05$) and urinary osmolality was higher in the 72-hour WD group (8197±1047 mOsm/L, $P<0.001$); thus, urinary to serum osmolality ratio increased progressively with WD (Table 3).

### Discussion

In the present study, we provide data showing quantitative differences between late pregnant and nonpregnant rats in
response to WD. Late-term pregnant rats were able to further stimulate renin and aldosterone above the already elevated values observed in normal pregnant rats. In addition, although the ability to concentrate urine in response to WD was similar in pregnant and nonpregnant rats, they exhibited differences in some parameters of renal function, such as a decrease in serum osmolality and changes in renal sodium excretion and in water reabsorption after WD.

Normal pregnancy, in humans and rats, is associated with important hormonal, biochemical, hemodynamic, and renal changes. In the pregnant rat, the increase in plasma volume occurs mainly during the last week of gestation, at the time this study was performed. Also, there is a marked increase in volume retaining hormones, such as renin and aldosterone, particularly near term. The renin-aldosterone system is a major determinant of sodium balance in pregnancy, opposing the natriuretic effects of several factors, such as filtered sodium, progesterone, and atrial natriuretic factor, among others. In the present study, 48-hour WD in late-term pregnant rats caused a marked increase in renin and aldosterone, above the already high values observed in normal pregnancy, indicating that this system is able to respond physiologically during pregnancy. Similar results have been reported in pregnant rats fed a low sodium diet, which was also able to stimulate renin and aldosterone. In the present study, when WD increased to 72 hours, PRA and aldosterone levels were not further elevated, indicating that the ability to secrete both hormones had reached a plateau. Aldosterone rise in response to WD is consistent with previous studies and is most likely secondary to stimulation of the renin-angiotensin system, as suggested by the positive and significant correlation observed between PRA and aldosterone levels.

WD caused a significant reduction in urinary kallikrein activity, both in nonpregnant and pregnant rats. Similar results were reported in male rats. The mechanisms by which WD decreased urinary kallikrein activity are beyond the scope of the present study; however, we can speculate that it was secondary to the reduction in renal function, because differences disappeared when kallikrein values were expressed per creatinine excretion. This reduction may have

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>48-Hour WD (n=12)</th>
<th>72-Hour WD (n=4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at term, g</td>
<td>335±6.7</td>
<td>320±5.4</td>
<td>302±9.8*</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Litter size, n</td>
<td>11.2±0.4</td>
<td>11.6±0.4</td>
<td>12.0±0.9</td>
<td>NS</td>
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<tr>
<td>Fetal weight, g</td>
<td>5.0±0.05</td>
<td>4.7±0.05*</td>
<td>4.0±0.3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>0.53±0.02</td>
<td>0.51±0.02</td>
<td>0.53±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Fetal to placental ratio</td>
<td>9.5±0.4</td>
<td>9.3±0.2</td>
<td>7.5±0.1*†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PRA, ng Ang I/mL per hour</td>
<td>49.0±9.7</td>
<td>94.2±12.1*</td>
<td>94.0±20*</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Aldosterone, nmol/L</td>
<td>3.96±0.6</td>
<td>8.91±0.8*</td>
<td>9.2±2.3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary to serum osmolality ratio</td>
<td>8.4±1.1</td>
<td>20.9±2.0*</td>
<td>27±3.8*†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

* Differences between control and water-deprived groups.
† Differences between 48-hour WD and 72-hour WD, P<0.05, ANOVA.
In fact, sodium fractional excretion was reduced in normal rats compatible with an active renal sodium and water retention. In pregnant rats, the highest kallikrein levels are observed at midpregnancy. We have previously demonstrated that urinary kallikrein is increased in normal rat pregnancy. In the rat, because fractional excretion of both electrolytes was unchanged, the lower daily sodium excretion in pregnant rats could contribute to this finding, remains to be elucidated.

The lower birth weight observed in WD rats is compatible with the hemodynamic changes associated with WD. However, we cannot rule out the possibility of some direct nutritional effect caused by WD. Although control rats were pair-fed with WD, absolute maternal weight at term and net maternal weight were lower in WD rats. Moreover, pregnant rats seemed to be more affected by WD than nonpregnant animals, because food intake, particularly during the second day of WD, was lower than that of nonpregnant rats. A greater effect on birth weight was observed when WD was extended to 72 hours. Placental weight remained essentially unchanged, probably reflecting that at the moment when WD started, placenta had reached its maximum growth. Interestingly, plasma volume and hematocrit did not change further. However, kidneys were still able to produce more concentrated urine. Previous studies have shown that 3-day WD in late-term pregnant rats decreased plasma atrial natriuretic peptide concentration, which might contribute to the water-retaining mechanisms set in motion during WD.

**Perspectives**

In the present study, we demonstrated that 48-hour WD in late pregnant rats caused a significant reduction in volume expansion and in fetal weight. In addition, it further stimulated volume-retaining hormones, such as renin and aldosterone, above the already elevated values observed in normal pregnancy. However, after a 72-hour WD period, the RAS did not increase further, suggesting a plateau of the response. Nevertheless, kidneys were still able to produce more concentrated urine. It is of interest that the blood pressure-lowering effect of pregnancy, which is believed to be caused by systemic vasodilatation, was still present in water-deprived rats, despite their high renin and low kallikrein levels. Taken altogether, present data suggest that volume and hemodynamic regulation during pregnancy is a far more complex process in which many counterregulatory mechanisms can be involved to maintain adequate fetal survival.

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**References**
