High-sodium intake prevents pregnancy-induced decrease of blood pressure in the rat

Annie Beauséjour, Karine Auger, Jean St-Louis, and Michèle Brochu

Research Centre, Hôpital Sainte-Justine, and Department of Obstetrics and Gynecology, Université de Montréal, Montreal, Quebec, Canada H3T 1C5

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PREGNANCY, IN HUMANS AND RATS, is associated with important hemodynamic, hormonal, biochemical, and molecular changes. During normal pregnancy, plasma volume expands. In humans, it appears as early as the sixth week of pregnancy. Thereafter, blood volume increases by 40% until the thirtieth week to reach plateau, which is maintained until term (14). In pregnant rats, the increase in blood volume occurs during the last week of gestation (4, 31). Plasma renin activity (PRA), plasma ANG II, and aldosterone levels are increased during pregnancy (37). In humans, by the eighth week of pregnancy, PRA and aldosterone levels are higher than baseline and increase gradually until the end of pregnancy (42). In pregnant rats, aldosterone increases gradually from day 15 to 22 of gestation (9, 16). Despite blood volume expansion and activation of the renin-angiotensin-aldosterone system (RAAS), normal pregnancy is paradoxically accompanied by a significant fall in arterial blood pressure in women (28) and rats (36). However, the fall in blood pressure does not occur at the same time, relative to parturition, in the two species. In women, blood pressure already decreased by the end of the first trimester, and it reached its nadir in the second trimester and returned to pregestational values approaching term (28). In pregnant rats, blood pressure does not change until the seventeenth and eighteenth days and then gradually decreases until term (23rd day) (36). The cardiovascular changes observed in the last week of pregnancy in rats correspond to the ones occurring during the second trimester in pregnant women. We proposed that these characteristics make pregnant rats an excellent animal model to study the hemodynamic alterations of midpregnancy in humans.

Hypertensive disorders of pregnancy such as preeclampsia are the most common medical complications of pregnancy. They remain a major cause of maternal and perinatal morbidity and mortality worldwide and affect ∼5–10% of all pregnancies. Several pathophysiological mechanisms have been proposed for preeclampsia, such as defective placentation (2), alteration of immunological responses (38), or genetic defects (40). Compared with normal pregnancy, preeclampsia is characterized by maternal hypertension, decreased circulatory volume, proteinuria, and reduced activation of RAAS (11). Other manifestations such as an increase in maternal vascular tone, enhanced platelet aggregation, and reduced uteroplacental blood flow are observed (24). These coupled to disturbed renal functions can bring intrauterine growth restriction or worsen perinatal outcome. Moreover, as it has been observed that the vascular reactivity to...
ANG II is greatly enhanced in preeclampsia compared with that of normal pregnancies, it is believed that preeclampsia is a vasoconstricted condition (15). To try unravelling the physiological mechanisms of decreased blood pressure in pregnancy and, by the mean, the pathophysiological ones implicated in pre-eclampsia, animal models (for a review, see Ref. 30) have been investigated. These include inhibition of nitric oxide (NO) synthesis (45) and reduction of the uterine perfusion (1). However, although providing valuable information on cardiovascular regulatory mechanisms, these models are sometimes not specific to the pregnant condition and occasionally are associated with fetal mortality. Modifications of sodium intake have been shown to modulate RAAS activity (12, 31). It is well known that reduction of sodium intake increased RAAS activity. However, this maneuver did not modify the decrease in blood pressure observed during rat pregnancy (31). On the opposing side, high-sodium intake during pregnancy induces reduction in PRA and aldosterone (12). Such alteration of activity of RAAS can provide a valuable avenue to modify the reduction of blood pressure associated with the end of pregnancy in the rat. Of course, we are aware that there is no indication in the literature that the incidence of preeclampsia is increased by high-salt intake or, in the opposite, that low-salt intake prevents the development of gestational hypertension (13, 35). However, in the rat, salt restriction during gestation was associated with reduction in circulatory volume and reduced fetal growth (31). The purpose of the present study was not to find some links between high-salt diet and preeclampsia but only to alter the renin-angiotensin system.

We thus attempted to reduce the activity of RAAS by giving salt supplements to pregnant rats for the last week of gestation. The aim of this study was to define a possible link between RAAS and blood pressure during pregnancy and study the effects of salt supplementation on 1) plasma electrolytes, creatinine, and hematocrit; 2) urinary volume, electrolytes, and proteins; and 3) fetal and placentas weight and plasma sodium. Fetal weight was also measured to better characterize fetal growth. Because ANG II receptors were shown to be modulated by sodium intake (33), the expression of subtypes of these receptors (AT1 and AT2) was also measured in the kidneys, involved in water and electrolyte homeostasis, and in the placenta, responsible for fetomaternal exchanges.

MATERIALS AND METHODS

Animals

Experiments were performed on female Sprague-Dawley rats (Charles River Canada; St. Constant, Quebec) weighing between 225 and 250 g, and they were bed with a known fertile male in the animal room. Day 1 of pregnancy was established when spermatozoa were found in morning vaginal smears. Nonpregnant rats were picked up randomly through the estrous cycle and paired with pregnant ones. All animals were housed under controlled light (6 AM–6 PM) and temperature (21 ± 3°C). They were fed a normal diet containing 0.23% NaCl (Teklad global 18% protein rodent diet, Harlan Teklad; Montreal, Quebec, Canada). Control animals (pregnant and nonpregnant) had tap water during all the treatment period. The experimental groups received 0.9 or 1.8% NaCl solution as a beverage for 7 days, starting on day 15 of experimentation, corresponding to the last week of gestation. At the end of treatment (day 22 of gestation, term = day 23), the animals were decapitated (9–9:30 AM), and trunk blood was collected for hormones and electrolytes analyses. Kidneys and placentas were rapidly removed from the animal and snap frozen in liquid nitrogen. This study was approved by the local Animal Care Committee, which is accredited by the Canadian Council on Animal Care.

Physiological Measurements

Systolic blood pressure was measured as previously described (36) by the indirect tail-cuff method (50-001 rat tail blood pressure system; Harvard Apparatus, St-Laurent, Quebec, Canada) in unanesthetized rats. The animals were trained for blood pressure measurement on day 7 to 11 of experimentation. Systolic blood pressure was then recorded for day 12 to 22. Body weight was measured on day 1 and daily from day 12 to the end of treatment. Water and food intake were recorded every day from day 12. Placental weight, fetal weight, as well as fetal length (nose-rump length) were determined on day 22.

Sample Collection and Analysis

On day 20, rats were housed in individual metabolic cages for collection of 24-h urine samples. Urinary volume was determined, and an aliquot was used to measure the concentrations of sodium and potassium and the amount of proteins excreted per 24 h. On day 22, the animals were decapitated. The first blood sample (0.5 ml) was drawn into heparin sodium vacutainer tubes (Becton Dickinson; Franklin Lakes, NJ), and an aliquot was used for hematocrit measurement. After centrifugation at 3,000 rpm for 20 min at 4°C, plasma was stored at −80°C for hormone measurements. The second sample was drawn into heparin lithium vacutainer tubes (Becton Dickinson), centrifuged, and used for determination of plasma electrolytes and creatinine. Sodium and potassium were measured with specific electrodes, total calcium by colorimetric reaction, and creatinine by an enzymatic colorimetric test. Fetuses were also decapitated, and trunk blood was pooled to measure sodium concentration.

Hormonal Measurements

PRA was determined indirectly by radioimmunoassay of ANG I generated during a 2-h incubation period (17). The antibody used was purchased from Peninsula Laboratories (Belmont, CA). Plasma aldosterone and corticosterone measurements were obtained directly from plasma with commercial radioimmunoassay kits (aldosterone: Intermedico, Montreal, Quebec, Canada; corticosterone: Medicorp, Montreal, Quebec, Canada) Cross-reactivity of aldosterone antibody with progesterone antibody is <0.007%.

RNA Isolation

Total cellular RNA in the frozen kidney and placenta was extracted by a modification of the method of Chomczynski and Sacchi (10) using TRIzol Reagent (Life Technologies). Final RNA pellets were dissolved in an appropriate volume of 100% deionized formamide (Amresco; Solon, OH) and stored at −20°C. RNA concentration was determined from absorbance measurement at a wavelength of 260 nm, integrity of
samples was determined by the 260-to-280-nm ratio, and quality was verified by ethidium bromide fluorescence.

**RNAse Protection Assay**

*Probe preparation.* A 182-base DraI fragment excised from pcDNA1 plasmid (kindly provided by Dr. Kathy Griendling, Emory University, Atlanta, GA) was used for the preparation of a high-activity RNA antisense probe for the AT1 receptor. A 215-base HindIII-XbaI fragment excised from pcDNA1/Amp plasmid (gift from Dr. Gaëtan Guillemette, Université de Sherbrooke, Sherbrooke, Quebec, Canada) was cloned into the multiple cloning sites of the Bluescript vector, linearized with PvuII, and used for the preparation of a high-activity RNA antisense probe for the AT2 receptor. A 334-base fragment of β-actin gene (Ambion RNA; Austin, TX) was used as an internal control. The antisense probes were prepared by transcription in vitro with the Ambion Maxiscript technique (Ambion RNA) in the presence of T7 polymerase for AT2, SP6 polymerase for AT1 and β-actin, and [α-32P]UTP (Perkin-Elmer Life Sciences; Boston, MA) and washed with 75% ethanol-25% sodium acetate.

Cellular RNA (25 μg) was incubated at 65°C for 10 min with the antisense probes (100,000 counts/min for AT1 and AT2, 15,000 counts/min for β-actin) in a 5X PIPES buffer (200 mM 1,4-piperazine diethanesulfonic acid, pH 6.4, 2 M NaCl, and 5 mM EDTA) and 100% deionized formamide. After denaturation of RNA at 85°C for 5 min, the hybridization was performed overnight at 47°C. Nonhybridized RNA was digested by RNase T1/A (RNase cocktail from Ambion) in a buffer containing 1 M Tris-HCl (pH 1.5), 0.5 M EDTA, and 0.3 M NaCl for 1 h at 30°C. After treatment with RNase A/S, the hybridized RNA was purified by phenol-chloroform extraction, precipitated in 100% ethanol, and resuspended in 10 μl of electrophoresis buffer containing 80% deionized formamide, 1 mM EDTA, 0.1% bromphenol blue, 0.1% xylene cyanol, and 0.1% SDS. Preparations were denatured at 85°C for 5 min and then separated by electrophoresis in a 5% acrylamide-7 M urea gel at 40 W. After electrophoresis, the gel was fixed for 15 min in 10% methanol-5% acetic acid and dried for 1 h. The dried gel was exposed to X-ray film (Fuji medical X-Ray film, Fuji Photo Film; Tokyo, Japan) with intensifying screens at -80°C for 1–7 days. Relative intensities of AT1, AT2, and β-actin bands were determined by analysis of the gel with Scion Image computer software (National Institutes of Health). The steady-state mRNA levels of AT1 and AT2 were expressed in arbitrary units and standardized by comparison with hybridization results obtained from the housekeeper gene β-actin.

**Statistical Analysis**

Systolic blood pressures were compared in each group with stable pretreatment values by ANOVA for repeated measurements (GraphPad Prism; GraphPad Software). Body weight, plasma and urine parameters, RAAS activity, fetal parameters, and expression of ANG II receptors were performed using a one-way ANOVA to assess the impact of 0.9 and 1.8% sodium supplement compared with their control groups (BMDP, BMDP statistical software). All results are expressed as means ± SE. *P < 0.05 was considered to represent significant difference.

**RESULTS**

**Systolic Blood Pressure**

Figure 1 illustrates the effects of sodium supplementation on blood pressure in nonpregnant (Fig. 1A) and pregnant (Fig. 1B) rats from day 12 to day 22 of the experiment. Before the sodium supplement (day 12 to 14), systolic blood pressure was similar in all groups of rats. Sodium supplements (0.9 or 1.8%) did not affect systolic blood pressure in nonpregnant rats. However, pregnant rats receiving 0.9% NaCl supplement did not show the expected decrease of blood pressure observed in control pregnant rats. Pregnant rats on 1.8% sodium supplement had a significant increase in blood pressure compared with the pretreatment period and preg- nant control.

**Maternal Body Weight Gain, Food, and Water Intake**

Weight gain in the first 14 days of the experiment was not statistically different between animals randomly chosen to receive normal salt diet or salt supplementation and averaged 10 ± 2 and 53 ± 2 g in nonpregnant and pregnant rats, respectively. During

![Fig. 1. Effect of 0.9% and 1.8% sodium supplement on systolic blood pressure in nonpregnant (A) and pregnant (B) rats. Arrows indicate sodium supplement period. Number in parentheses represents number of rats used. Data points represent means; error bars represent ±SE. **P < 0.01 and ***P < 0.001 compared with the pretreatment period.](http://ajpheart.physiology.org/Downloaded from http://ajpheart.physiology.org/)
In pregnant rats, 1.8% sodium supplement induced a nary excretion in both nonpregnant and pregnant rats. nent (0.9 or 1.8%) induced an increased sodium uri-
ined an increased urinary volume. This resulted in a between the sodium-supplemented rats and their con-
duced an increased urinary volume from the quantity of water drunk during this period, all animals received normal diet and tap water. As shown in Table 1, during sodium (0.9 or 1.8%) supplements (day 15 to 22), water and sodium intake was increased in nonpregnant rats, but food intake was reduced by 44% in the group receiving 1.8% sodium supplement, accompanied by a decreased in body weight. Sodium intake was calculated from the water and food intake and was increased 10- and 20-fold with 0.9% and 1.8% salt water, respectively. In pregnant rats, water intake was increased only by the 0.9% sodium supplement. In the 1.8% sodium-supplement ed group, food intake was decreased by 44% and this is accompanied by an absence of body weight gain. Sodium intake was increased by 12- and 17-fold by 0.9% and 1.8% salt water, respectively.

**Urinary Parameters on Day 20**

Water intake and urinary volume were measured on day 20 to calculate water balance. For each animal, this was calculated by subtracting the quantity of urinary volume from the quantity of water drunk during 24 h. As shown in Table 2, sodium supplements (0.9 or 1.8%) increased water intake and urinary volume in nonpregnant rats. Water balance was not different between the sodium-supplemented rats and their controls. In pregnant rats, water intake was increased only by the 0.9% salted water. Both supplements induced an increased urinary volume. This resulted in a negative water balance in pregnant rats on 1.8% supplement compared with their controls. Sodium supplement (0.9 or 1.8%) induced an increased sodium urinary excretion in both nonpregnant and pregnant rats. In pregnant rats, 1.8% sodium supplement induced a decreased potassium excretion. Urinary protein loss was not different in nonpregnant groups. However, pregnant rats receiving 1.8% sodium supplement showed a 40% increased urinary protein excretion in their 24-h urine compared with the control pregnant rats.

**Plasma Electrolytes, Creatinine, and Hematocrit Levels**

As shown in Table 3, in nonpregnant animals, sodium supplementation (0.9 or 1.8%) had no effect on plasma electrolytes, osmolality, creatinine, and hematocrit. In pregnant rats, sodium supplementation of 0.9% induced a decrease of 9% in plasma creatinine level, whereas the 1.8% NaCl supplement induced increased plasma sodium and osmolality and decreased plasma potassium and creatinine, as well as an elevation of the hematocrit level.

**RAAS and Corticosterone Levels**

Sodium supplements (0.9 or 1.8%) decreased PRA (Fig. 2A) and plasma aldosterone (Fig. 2B) levels in nonpregnant and pregnant rats. Plasma corticosterone levels were increased only in the pregnant animals receiving 1.8% sodium supplement (Fig. 2C).

**Fetal Characteristics**

As shown in Table 4, 0.9% sodium supplementation did not affect fetal parameters. However, 1.8% sodium supplement was accompanied by decreased placental weight (19%), fetal weight (10%), and fetal length (5%) compared with control. Plasma sodium concentration

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**Table 1. Effects of 0.9 and 1.8% NaCl supplement on water intake, food intake, sodium intake, and weight gain in nonpregnant and pregnant rats from days 15 to 22**

<table>
<thead>
<tr>
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<th>Nonpregnant</th>
<th></th>
<th>Pregnant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not</td>
<td>0.9% NaCl</td>
<td>1.8% NaCl</td>
<td></td>
</tr>
<tr>
<td>Water intake, ml</td>
<td>238 ± 13(17)</td>
<td>326 ± 15(16)</td>
<td>358 ± 17(14)</td>
<td></td>
</tr>
<tr>
<td>Food intake, g</td>
<td>140 ± 9(17)</td>
<td>141 ± 3(16)</td>
<td>121 ± 5(14)</td>
<td></td>
</tr>
<tr>
<td>Sodium intake, g</td>
<td>0.32 ± 0.02(17)</td>
<td>3.2 ± 0.2(16)</td>
<td>6.6 ± 0.3(14)</td>
<td></td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>1.5 ± 2.4(17)</td>
<td>1.5 ± 1.4(16)</td>
<td>−3.7 ± 1.1(14)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (days 15 to 22) ± SE. Number shown in parentheses represent the number of rats used. *P < 0.05 and †P < 0.001 compared with nonsupplemented rats, same group. For weight gain, ‡P < 0.05 and §P < 0.001 compared with no weight gain.

**Table 2. Effects of 0.9 and 1.8% NaCl supplement on urinary parameters per 24 h on day 20**

<table>
<thead>
<tr>
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<th>Nonpregnant</th>
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<th>Pregnant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not</td>
<td>0.9% NaCl</td>
<td>1.8% NaCl</td>
<td></td>
</tr>
<tr>
<td>Water intake, ml/24 h</td>
<td>30 ± 2(10)</td>
<td>50 ± 3(9)</td>
<td>51 ± 7(8)</td>
<td></td>
</tr>
<tr>
<td>Volume, ml/24 h</td>
<td>13 ± 0(9)</td>
<td>25 ± 3(9)</td>
<td>31 ± 5(9)</td>
<td></td>
</tr>
<tr>
<td>Water balance, ml/24 h</td>
<td>15 ± 3(10)</td>
<td>22 ± 5(9)</td>
<td>17 ± 5(8)</td>
<td></td>
</tr>
<tr>
<td>Sodium, mmol/24 h</td>
<td>1.5 ± 0.1(8)</td>
<td>6.1 ± 1.1(8)</td>
<td>16.1 ± 1.8(13)</td>
<td></td>
</tr>
<tr>
<td>Potassium, mmol/24 h</td>
<td>2.9 ± 0.3(10)</td>
<td>2.5 ± 0.5(8)</td>
<td>2.6 ± 0.6(13)</td>
<td></td>
</tr>
<tr>
<td>Proteins, mg/24 h</td>
<td>7.9 ± 0.9(6)</td>
<td>8.7 ± 1.1(7)</td>
<td>9.6 ± 0.6(11)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Number shown in parentheses represent the number of rats used. *P < 0.05, †P < 0.01, and §P < 0.001 compared with nonsupplemented rats, same group.
was increased in fetuses of mothers receiving 1.8% sodium supplementation. These two supplements of sodium did not affect the number of fetuses.

**mRNA Expression of ANG II Receptors**

Sodium supplements (0.9 and 1.8% NaCl) similarly decreased AT₁ receptor mRNA steady state in the kidneys of both nonpregnant and pregnant rats (Fig. 3A). We also observed a decrease in AT₁ receptor mRNA in the placentas of the dams receiving sodium supplements (Fig. 3B). AT₂ mRNA was not detected in these tissues.

**DISCUSSION**

The purpose of the present investigation was to document the effects during gestation of increased sodium intake on blood pressure, salt homeostasis, and ANG II receptors in the kidney and placenta. We demonstrated that pregnant rats were more markedly affected by increased sodium intake than nonpregnant ones.

It is well established that a low-sodium diet enhances the activity of RAAS in nonpregnant and pregnant rats (31). On the opposite, high-sodium intake reduces the activity of this system (12). As expected, we observed that sodium supplements of 0.9 or 1.8% decreased the activity of RAAS, as measured by PRA and aldosterone levels. As shown previously (43), the decreased RAAS induced by NaCl supplements did not affect systolic blood pressure in nonpregnant rats. However, in pregnant rats, the 0.9% sodium supplementation prevented the decrease in blood pressure observed in normal pregnancy, whereas increased systolic blood pressure over pretreatment values was observed with the 1.8% NaCl supplement. These results suggest that the mechanisms controlling blood pressure are easily perturbed by high-sodium intake during pregnancy. In agreement with our results, Milton et al. (26) demonstrated that 0.9% saline in drinking water given to pregnant rabbits during the last week of gestation increased the mean arterial blood pressure compared with that of the pregnant rabbit on normal NaCl intake. This also corresponds to the finding of Barron et al. (3), who observed an increase of arterial pressure in pregnant rats on a high-sodium diet (8%) but not in nonpregnant rats. Whereas they used a high-sodium chow, we added sodium in the drinking water. Because pregnant rats on 1.8% of sodium in water drink around 50 ml of water a day, they ingested around 0.9 g of sodium. Because pregnant rats eat ~23 g of food each day, a diet containing 4% sodium corresponds to an ingestion of 0.92 g a day. Therefore, in the present study, the pregnant rats ingest much less sodium than the ones in Barron’s study (3). Moreover, they took a single measure of blood pressure at day 19 of gestation, whereas we were documenting it from midpregnancy to term.

In a parallel study, we have also measured contractile response to phenylephrine in the aorta rings of nonpregnant and pregnant rats on high-sodium intake (K. Auger, A. Beauséjour, M. Brochu, and J. St-Louis, unpublished observations). We observed an increased response in aortic rings of pregnant rats on 0.9 and 1.8% sodium supplement compared with pregnant rats on normal water. This suggested that the decreased sensitivity to vasoconstrictor normally observed in pregnancy is reversed, and this could explain the absence of decreased blood pressure. McEniery et al. (25) demonstrated that normotensive 18-wk pregnant women receiving a high-sodium diet (300 mmol sodium per day) for 7–10 days had no blood pressure change compared with pregnant women on a regular diet. The discrepancy with our results and other animal studies (3, 26) with those of the women of McEniery study could be explained by the level of sodium loading. In fact, the women in McEniery’s study consumed 0.11 g Na⁺·kg⁻¹·day⁻¹ during mid-pregnancy, whereas our rats (on 1.8% NaCl) received 3.29 g Na⁺·kg⁻¹·day⁻¹ at the end of gestation. Both regimens were administered for 7 days.

In nonpregnant rats, we observed that the supplement of 1.8% NaCl decreased food intake, accompanied by loss of body weight. This is in agreement with other reports showing that oral hypertonic solution (1.8% saline) reduces food intake in adult rats (22). In parallel to increased sodium and fluid intake, natriuresis and diuresis were also significantly augmented preserving normal water balance. Moreover, we observed decreased AT₁ mRNA levels in kidneys of nonpregnant rats receiving sodium supplements (0.9 or 1.8% NaCl). It has been suggested that renal AT₁ could be involved in sodium and water reabsorption from the proximal

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**Table 3. Effects of 0.9 and 1.8% NaCl supplement on plasma electrolytes, creatinine, and hematocrit in nonpregnant and pregnant rats on day 22**

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>P</th>
<th>Pregnant</th>
<th>P</th>
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<tbody>
<tr>
<td>Sodium, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td></td>
<td>1.8% NaCl</td>
<td></td>
</tr>
<tr>
<td>Osmolality, mmos/kgH₂O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>140 ± 1(18)</td>
<td>139 ± 1(19)</td>
<td>141 ± 1(20)</td>
<td>136 ± 1(16)</td>
</tr>
<tr>
<td>Osmolality, mmos/kgH₂O</td>
<td>299 ± 3(18)</td>
<td>297 ± 1(18)</td>
<td>299 ± 6(18)</td>
<td>293 ± 2(18)</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>6.4 ± 0.2(17)</td>
<td>6.5 ± 0.2(20)</td>
<td>6.5 ± 0.3(20)</td>
<td>5.7 ± 0.2(14)</td>
</tr>
<tr>
<td>Total calcium, mmol/l</td>
<td>2.69 ± 0.04(15)</td>
<td>2.69 ± 0.03(20)</td>
<td>2.63 ± 0.02(20)</td>
<td>2.29 ± 0.09(14)</td>
</tr>
<tr>
<td>Creatinine, µmol/ml</td>
<td>38.3 ± 1.7(17)</td>
<td>36.7 ± 1.7(21)</td>
<td>34.8 ± 1.3(20)</td>
<td>48.9 ± 1.6(16)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>46.2 ± 0.9(16)</td>
<td>45.1 ± 1.2(16)</td>
<td>47.6 ± 1.2(16)</td>
<td>31.7 ± 0.5(18)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number shown in parentheses represent the number of rats used. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with nonsupplemented rats, same group.
tubules (6). This provides an explanation for the increased sodium and water excretion. Consequently, plasma sodium, potassium, and total calcium, as well as creatinine and hematocrit levels, were not different in all groups of nonpregnant rats, demonstrating that physiological responses to sodium supplements appear to be adequate for maintaining homeostasis.

In pregnant rats, we showed that 0.9% sodium supplement increased water intake but did not affect food intake and body weight gain. However, pregnant rats drinking 1.8% saline did not increase their water consumption. These rats lost more weight because the total body weight at day 22 includes the fetoplacental unit. This is explained by a 44% reduction of food intake compared with control pregnant rats. This reduction in food intake is larger than the reduction observed in nonpregnant rats. It is well established that normal pregnancy in the rat is accompanied by increased sodium appetite and sodium retention (29), and both renal plasma flow and glomerular filtration rate are significantly increased (4). Several studies have shown that increased sodium intake (0.9% saline) leads to important diuresis and natriuresis in adult rats (41) and in pregnant rats (7, 12). In agreement with these, pregnant rats receiving 0.9% sodium supplement showed decreased plasma creatinine, increased diuresis, and natriuresis, which suggests an increased glomerular filtration compared with control pregnant rats. As argued above for nonpregnant rats, decreased AT1 mRNA levels were found in the kidney of pregnant rats receiving 0.9% NaCl. This is in accordance with the increased diuresis and natriuresis observed in this group.

Sodium supplement of 1.8% in water induced increased plasma sodium and corticosterone as well as decreased plasma potassium and creatinine levels and elevated hematocrit. Proteinuria was also observed suggesting that renal function was affected. Increases in diuresis and natriuresis without increase in water intake were observed in pregnant rats receiving 1.8% sodium supplement. This resulted in a negative water balance which, associated with high hematocrit value, is suggestive of decreased circulatory volume and dehydration. According to a mathematical derivation described by Van Beaumont (39), the percent difference between the original and final hematocrit ratios can be used in conjunction with a proportionality factor to estimate the percent change in plasma volume. Thus the increases in hematocrit reflect a significant decrease in plasma volume. It has been shown that intraperitoneal injection of 1.5 M saline solution (8.8% NaCl) to adult male rats induced dehydration (5). However, in our experiment, the suggested dehydration was only observed in pregnant rats on much sodium intake (1.8%), again suggesting that pregnant rats are more sensitive than nonpregnant rats to high-sodium intake.

Sodium supplement of 0.9% NaCl did not affect fetoplacental parameters. However, placental weight, fetal weight, and length were reduced when the dams received the 1.8% NaCl supplement. We suggest that this could be explained by the 44% reduction of maternal food intake and the suspected decrease in blood volume. Indeed, in humans and rats, decrease in maternal plasma volume (31, 32) and reduction of food intake (44) have been associated with decreased fetal growth. We showed a decreased AT1 mRNA in placentas of pregnant rats receiving 1.8% NaCl. This is in agreement with studies on human placenta (21, 23) from pregnancies complicated by preeclampsia and intrauterine growth retardation compared with normal term controls.

Several studies have attempted to prevent and reverse the pregnancy-induced decrease of blood pres-
ure in laboratory animals. For example, Simaan et al. (34) reported that activation of voltage-dependant calcium channels in pregnant rats causes a transient increase in blood pressure. Other studies have provided evidence for a potential role for NO. In fact, endothelial dysfunction is considered an important factor for the induction of gestational hypertension. In pregnant rats, subcutaneous injections of 50 mg/day of a NO synthase (NOS) inhibitor, L-nitro-L-arginine methyl ester (L-NAME) from day 17 to 22 of gestation resulted in sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation (27). Similarly, pregnant baboons were studied after the administration of an oral NOS inhibitor (N-nitro-L-arginine; 5 or 10 mg/kg) at different periods of the pregnancy (19). Increased mean arterial blood pressure in nonpregnant animals and in mid and late pregnancy was observed following NOS inhibition. Reduction in PRA and significant proteinuria were also observed. These results show that NOS inhibition induces changes in blood pressure that are not specific to the pregnant condition. However, in these rat studies, high doses of L-NAME were used. With a low dose of 1 mg/day, Kassab et al. (20) showed that the increase in blood pressure was observed in pregnant rats only, suggesting a role for NO in the regulation of blood pressure during pregnancy. No information about fetal growth and well being was reported for this model.

Candidate genes for hypertensive diseases in pregnancy, that have been proposed, include those of RAAS and NOS. Pregnant mice that had four copies of the angiotensinogen gene (Agt2/2) and that were heterozygous (eNOS+/−) or homozygous (eNOS−/−) for mutation of the endothelial NOS (eNOS) gene had higher blood pressures compared with controls (18). This indicates that eNOS and Agt are important genes in the regulation of blood pressure. These mice showed a decrease of blood pressure during pregnancy. Therefore, this model is thought to represent chronic hypertension during pregnancy more than preeclampsia. The blood pressure of mice eNOS−/− did not decrease during pregnancy, but there is no evidence of renal dysfunction (18). Fetal characteristics were not studied in all of these mutant mice. In our experiments, we demonstrated that 1.8% sodium supplement given to pregnant rats caused proteinuria, indicating some renal dysfunction. In the rat, transgenic females for the gene of human angiotensinogen were mated with males made transgenic for the gene of human renin (8). In this model, an increase in blood pressure was observed on the twelfth day of gestation; however, a decrease in blood pressure still occurred toward the end of gestation. In summary, we have observed that sodium supplements of 0.9 and 1.8% given to nonpregnant and pregnant rats induced inhibition of RAAS activity. Pregnant rats receiving 0.9% sodium supplement for the last week of gestation did not show the characteristic decrease in blood pressure, whereas the one on 1.8% NaCl did have an increase in blood pressure. This last effect of the 1.8% saline was accompanied by 1) an apparent reduction in blood volume expansion, 2) renal dysfunction as indicated by proteinuria, and 3) intrauterine growth restriction. All these findings resemble many clinical characteristics of preeclampsia in women (11, 24, 32, 37, 46). We believe that sodium supplementation during gestation, as described herein, represents an excellent tool that is specific to the pregnant

### Table 4. Effects of 0.9 and 1.8% NaCl supplement on fetal parameters

<table>
<thead>
<tr>
<th></th>
<th>Not Supplemented</th>
<th>0.9% NaCl</th>
<th>1.8% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight, g</td>
<td>0.46 ± 0.01(19)</td>
<td>0.49 ± 0.01(20)</td>
<td>0.40 ± 0.02*(13)</td>
</tr>
<tr>
<td>Fetal weight, g</td>
<td>5.05 ± 0.08(8)</td>
<td>5.08 ± 0.10(6)</td>
<td>4.41 ± 0.14*(8)</td>
</tr>
<tr>
<td>Fetal length, cm</td>
<td>4.57 ± 0.06(8)</td>
<td>4.67 ± 0.05(6)</td>
<td>4.36 ± 0.09*(8)</td>
</tr>
<tr>
<td>Fetal plasma sodium, mmol/l</td>
<td>135 ± 1(9)</td>
<td>135 ± 1(8)</td>
<td>142 ± 2(9)</td>
</tr>
<tr>
<td>Number of fetuses</td>
<td>15 ± 1(8)</td>
<td>16 ± 1(6)</td>
<td>14 ± 1(8)</td>
</tr>
</tbody>
</table>

Values are means of each litter ± SE. Number shown in parentheses represents the number of litter used. *P < 0.05, †P < 0.01 compared with not supplemented rats.

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**Fig. 3.** Effect of 0.9 and 1.8% sodium supplement on AT1 receptor mRNA steady state in target tissues. A: kidney in nonpregnant and pregnant rats; B: placenta in pregnant rats. Data are expressed as AT1-to-β-actin ratio (arbitrary units). Results shown are means ± SE of five separate experiments. *P < 0.5, **P < 0.01, and ***P < 0.001 compared with nonsupplemented animals, same group.
condition. Furthermore, pregnant rats treated this way should contribute to better understand the links between RAAS and the regulation of blood pressure during pregnancy.

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