EXPERIMENTAL STUDY

Atrial natriuretic peptide and aldosterone secretions, and atrial natriuretic peptide-binding sites in kidneys and adrenal glands of pregnant and fetal rats in late gestation in response to a high-salt diet

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Abstract

Objective: This study aimed at determining, in the term pregnant rat, whether maternal and fetal plasma atrial natriuretic peptide (ANP) concentrations were modified in response to an oral sodium load, and to investigate whether any changes in plasma concentrations were able to modify the density and affinity of the different ANP-binding site subtypes in maternal and fetal kidneys and adrenal glands.

Methods: Pregnant rats kept in metabolic cages were divided into two groups. The normal sodium diet group had free access to rat chow and tap water whereas the high sodium diet group received 1% NaCl as drinking water for 10 consecutive days from day 11 to day 21 of gestation with free access to standard rat chow. Pregnant rats from both groups were killed by decapitation on day 21 of gestation. The plasma ANP and aldosterone concentrations were determined by RIA. The density and affinity of ANP receptors were determined in the maternal and fetal adrenal glands and kidneys.

Results: In the pregnant rats on the high-salt diet, the sodium and water intakes, as well as the urine volume and sodium excretion, were significantly higher than in the control group. After 10 days of high-salt intake, water and sodium retentions were not significantly different in the two groups, indicating that the pregnant rats were able to excrete excess salt. The high sodium intake did not change the body weight of the pregnant rats but did increase the body weight of the fetal rats. Maternal and fetal hematocrits remained unchanged in both groups, the high sodium intake did not modify plasma sodium concentration in the maternal rats but increased that of the fetuses, indicating an accumulation of sodium in the fetal rats. The dietary sodium intake did not change the plasma ANP concentrations but significantly decreased the plasma aldosterone concentrations in both the maternal and fetal rats. In response to the high-salt diet, the density and affinity of total ANP, ANPβ and ANPγ receptors were not altered in the maternal isolated renal glomeruli or the adrenal zona glomerulosa membranes or the fetal adrenal gland and kidney membrane preparations.

Conclusion: These results suggest that ANP is not involved in the regulation of water and electrolyte balance in maternal and fetal rats during salt-loaded intake.

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Introduction

The regulation of water and sodium balance was studied throughout gestation in both rats placed on a normal diet (1–3) and those on a high-salt diet (4, 5). Gravid rats ingesting a high-salt diet present high diuresis and natriuresis (4, 5). Atrial natriuretic peptide (ANP) could play an important role in the regulation of water and sodium homeostasis after salt overloading, thanks to its natriuretic, diuretic and aldosterone-inhibiting properties (6, 7).

ANP is a cardiac hormone stored in atrial secretory granules (8) which vary in number during alterations of the electrolyte and fluid balance (9). ANP released into the blood circulation in response to atrial distension (10) exerts its biological activity through specific receptors mainly located in the renal glomeruli and adrenal zona glomerulosa (11). In the fetal rat, autoradiographic and binding studies have shown the presence of ANP receptors in the kidney and the adrenal gland (12, 13). In order to study the role of ANP in the regulation of sodium homeostasis, adult rats were subjected to chronic salt loading by being given NaCl solution (14–16) or rat chow containing NaCl (17, 18). The results of these studies were often controversial. Indeed, some investigators have described an increase of
plasma ANP concentration after salt-loading intake (14, 18, 19), while others have demonstrated a decrease of plasma concentrations after salt-overloading (20, 21) and others have shown that plasma ANP concentrations were not significantly altered by high-sodium intake (15, 16, 22, 23). These discrepancies can be explained by the differences in the protocols used in these experiments. Moreover, most studies showed that the ANP receptors were down-regulated in the adrenal glands and the renal glomeruli in response to salt-overloading (14, 15, 19, 24, 25).

The last week of gestation in the rat is marked by an increase in the volume of plasma, accompanied by a retention of sodium in both pregnant rats and their fetuses (1–3). In order to understand these modifications of water–sodium balance observed in late gestation, it is important to study the effects of a high-salt diet on the regulation of plasma ANP concentrations and the density of ANP receptors in pregnant rats and their fetuses in late gestation.

Our aim was to study the effects of a 10-day high-salt diet on water and sodium retention in pregnant rats, maternal and fetal plasma ANP and aldosterone concentrations, and the density and affinity of the different classes of ANP receptors in maternal and fetal kidneys and adrenal glands.

Materials and methods

Animals

Experiments were performed on Wistar rats bred in the laboratory. They were housed in a light-controlled room (light period 0700 to 1900 h). The females were mated with a male for one night. The following day was taken as day 0 of pregnancy if spermatozoa were found in vaginal smears. The pregnant females were kept in metabolic cages and divided into two groups: normal sodium diet (NSD) and high sodium diet (HSD). In the NSD group, the females had free access to standard rat chow (Usine d’Alimentation Rationnelle, Villemoisson-sur-Orge, France). In the HSD group, the females had free access to tap water and a sodium diet (NSD) and high sodium diet (HSD). In the NSD group, the females had free access to tap water and a sodium diet (NSD) and high sodium diet (HSD). In the HSD group, the females had free access to tap water and

Preparation of tissue samples

The adrenal glands and kidneys of fetuses and pregnant females were rapidly dissected and defatted. The adrenals of mothers were decapsuled in order to separate the capsule with zona glomerulosa from the inner tissue. All the tissues were frozen in liquid nitrogen and stored at −80 °C. For each experiment 6–8 maternal adrenals, 4 maternal kidneys, 180–200 fetal adrenals and 40–50 fetal kidneys were pooled to prepare tissue samples for one binding assay.

Fetal adrenals and kidneys with medulla and maternal capsules with zona glomerulosa were ground with a Teflon homogenizer in Tris–HCl buffer (50 mmol/L, pH 7.4) containing MnCl₂ (5 mmol/l), NaCl (120 mmol/l), aprotinin (1 µmol/l) and bacitracin (0.1%). Homogenates were centrifuged at 10000 g for 20 min at 4 °C and the supernatants were centrifuged at 20000 g for 30 min at 4 °C. The pellets containing the membranes

Hematocrit was determined by a microcapillary technique (Microcentrifuge, CRITSPIN, Poli Labo, Strasbourg, France).

In the NSD group, the amount of sodium ingested by the pregnant rats was determined by multiplying the amount of food eaten by the sodium content in standard chow (0.030 mEq/g). In the HSD group, the amount of sodium ingested by the pregnant rats was determined by adding the amount of sodium contained in the food which was eaten and the amount of sodium contained in the volume of the saline solution drunk (0.17 mEq/ml). The amount of sodium excreted was calculated by multiplying the urine volume by the urinary sodium concentration. The sodium excreted with the feces was not determined and the sodium concentration in tap water was very low (0.02 mEq/l).

Determination of aldosterone and ANP concentrations in maternal and fetal plasma

The plasma aldosterone concentrations were measured by RIA after extraction of aldosterone from the plasma with ethyl acetate after delipidation with iso-octane and assayed as previously described (26). Intra- and inter-assays were respectively ± 5.00% (n = 11) and 13.00% (n = 7) and recovery of aldosterone from plasma was over 95% (n = 10). Aldosterone concentration was expressed as nmol/l.

The plasma ANP concentrations were determined using an RIA kit (RIK-9103; Peninsula Laboratories, Belmont, CA, USA) after extraction of ANP from plasma with C₁₈ Sep-Pak cartridges eluted with a buffer containing 60% acetonitrile in 1% trifluoroacetic acid. Recovery of ANP from plasma was over 95% (n = 5). Immuno-reactive ANP concentration was expressed as pmol/l.
were rinsed with Tris–HCl buffer, centrifuged again at 20 000g for 30 min at 4°C and then diluted with Tris–HCl buffer. An aliquot was taken for protein determination and the remainder frozen in liquid nitrogen and stored at −80°C until required for the binding assay. The maternal glomeruli were prepared according to the technique previously described by Sraer et al. (27). The kidneys excised from the renal capsule were cut longitudinally in order to remove the inner tissue. The minced cortical tissue was passed successively through a 106 μm metal sieve which excluded the tubules and blood vessels and a 53 μm metal sieve which retained the glomeruli. The glomerular suspensions were rinsed three times with ice-cold Tris–HCl buffer and centrifuged at 10 000g for 5 min at 4°C and then diluted with Tris–HCl buffer. The purity of the preparation was verified by light microscopy and tubular fragments were always below 2% of the total number of glomeruli. An aliquot was taken for protein determination and the remainder frozen in liquid nitrogen and stored at −80°C until required for binding. The protein content was determined by the method of Lowry et al. (28) using bovine serum albumin (BSA) as standard.

**Binding assay**

The fetal and maternal membrane preparations at a concentration of 100 μg per tube were incubated in a final volume of 100 μl Tris–HCl buffer containing 0.5% BSA, 25 pmol/l 125I-rANP(1–28) (Amersham International plc, Amersham, Bucks, UK) and unlabeled rANP(1–28) (0.1 μmol/l) and cANP(4–23) (0.1 μmol/l). The density of ANP receptors was assessed with rANP(1–28) in the presence of an excess of a truncated ANP peptide which binds selectively to ANPc receptors. The density of ANPb receptors was obtained by subtracting the number of ANP receptors from the total receptor population. The binding studies were performed in duplicate at room temperature for 90 min. At the end of incubation, free 125I-rANP(1–28) was separated from that bound to membranes, by filtration through 0.1% polyethyleneimine-treated Whatman GF/C filters (Prolabo, Marcq-en-Barul, France), washed with 0.9% NaCl and counted in an LKB gamma counter. The non-specific binding was measured in the presence of an excess of rANP(1–28) (0.1 μmol/l) and cANP(4–23) (0.1 μmol/l). The specific binding was determined by subtracting the non-specific binding from the total binding.

**Statistical analysis**

The results are presented as means ± s.e.m. The significance of differences between mean values was estimated by Student’s t-test. The binding results were analyzed by the EBDA/LIGAND program to determine the affinity (Kd) and density (Bmax) of ANP receptors (29).

**Results**

**Maternal and fetal body weights (BW) in NSD and HSD groups**

In our experiments, we selected pregnant rats with between 10 and 14 fetuses each. The average number of fetuses per pregnant rat in the NSD group and the HSD group was 12.11 ± 0.70 and 12.33 ± 0.60 respectively (n = 10 for each group) (P > 0.05). The HSD pregnant rats were not significantly heavier (P > 0.05) than the NSD pregnant rats (378.34 ± 10.49 vs 359.00 ± 6.35 g respectively; n = 10 for each group). The quantity of food eaten by both groups was not significantly different (P > 0.05) (7.85 ± 0.50 vs 7.39 ± 0.22 g/100 g BW respectively; n = 10 for each group). The fetal BW of the HSD group was significantly higher (P < 0.001) than that of the NSD group (5.01 ± 0.05 (n = 53) vs 4.44 ± 0.44 g (n = 47) respectively).

**Water and sodium balance in NSD and HSD pregnant rats**

The water and sodium intakes were significantly higher in the HSD group than in the NSD group (P < 0.001) (Table 1). The urinary water and sodium excretions of the HSD group were significantly higher than those of the NSD group (P < 0.001) (Table 1). Water and sodium retention values, calculated as the difference between intake and excretion, were not significantly different between the two groups (P > 0.05) (Table 1).

**Table 1** Water and sodium balance in normal (NSD) and high-salt diet (HSD) pregnant rats in late gestation. Values are means ± s.e.m.; there were ten experiments for each result.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Water intake (ml/100 g BW/24 h)</th>
<th>Urinary water excretion (ml/100 g BW/24 h)</th>
<th>Water retention (ml/100 g BW/24 h)</th>
<th>Sodium intake (mEq/100 g BW/24 h) (Food)</th>
<th>Urinary sodium excretion (mEq/100 g BW/24 h) (Food + saline)</th>
<th>Sodium retention (mEq/100 g BW/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSD</td>
<td>11.00 ± 0.50</td>
<td>2.00 ± 0.50</td>
<td>9.00 ± 0.40</td>
<td>0.22 ± 0.01</td>
<td>0.20 ± 0.03</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>HSD</td>
<td>18.00 ± 1.50***</td>
<td>8.00 ± 1.30***</td>
<td>10.00 ± 0.85</td>
<td>3.69 ± 0.20***</td>
<td>3.65 ± 0.45***</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

***P < 0.01 compared with NSD.

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Maternal and fetal ANP response to a high-salt diet

Hematocrit, plasma sodium concentration, aldosterone and ANP concentrations in pregnant and fetal rats in late gestation

Salt-loading for 10 days did not change the maternal and fetal hematocrit values significantly (P > 0.05) (Table 2). The plasma sodium concentration was not significantly different between the HSD and NSD pregnant rats (P > 0.05) (Table 2). The plasma sodium concentration was significantly higher in the fetal rats than those in maternal NSD and HSD groups (P < 0.01) (Table 2). In pregnant and fetal rats, dietary salt-loading did not significantly change the plasma ANP concentrations (P > 0.05) (Table 2) but significantly decreased the plasma aldosterone concentrations (P < 0.01 and P < 0.001 respectively) (Table 2).

The effects of maternal salt-loading on density (Bmax) and affinity (Kd) of different ANP receptors in the kidneys and adrenal glands of pregnant and fetal rats in late gestation

The analysis of competition curves obtained from pregnant and fetal rats revealed that salt-loading for 10 consecutive days did not significantly alter the Bmax and Kd values of total ANP, ANPb and ANPc receptors, either in maternal renal glomeruli and adrenal zona glomerulosa membranes or in fetal kidney and adrenal membrane preparations (P > 0.05) (Figs 1 and 2). No significant difference in Kd of total ANP and ANPb receptors was observed in the maternal renal glomeruli and adrenal zona glomerulosa membranes or in the fetal kidney and adrenal membrane preparations (P > 0.05) (Table 3). The Kd values of total ANP receptors were three times higher than those in maternal NSD and HSD groups (P < 0.05) (Table 3). The Kd values of total ANP receptors did not significantly differ in the adrenal glands of fetal and maternal NSD and HSD groups (P > 0.05) (Table 3). The Kd values of ANPb receptors were not significantly different in fetal or maternal kidneys and adrenal glands in the two groups (P > 0.05) (Table 3).

Discussion

In the present study, our results showed that after 10 consecutive days of high-salt diet the pregnant rats maintained their weight balance, in spite of an increase in salt intake induced by sodium appetite, since their consumption of food and their body weight were not different from those of control pregnant rats. This observation is in agreement with previous reports (4, 5). Our results showed that the salt-loaded pregnant rats are able to excrete the entire sodium intake by increasing their urine flow. Their plasma sodium concentration, similar to excrete the entire sodium intake by increasing their urine flow. Their plasma sodium concentration, similar

Table 2 Effects of salt-loading on maternal and fetal hematocrit, plasma sodium, ANP and aldosterone concentrations in late gestation. Values are means ± S.E.M. with the number of experiments shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant rats</th>
<th>Fetal rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSD</td>
<td>HSD</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35.00 ± 0.80</td>
<td>36.00 ± 0.30</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/l)</td>
<td>155.00 ± 5.00</td>
<td>154.00 ± 10.00</td>
</tr>
<tr>
<td>Plasma ANP (pmol/l)</td>
<td>63.00 ± 9.00</td>
<td>68.00 ± 12.00</td>
</tr>
<tr>
<td>Plasma aldosterone (nmol/l)</td>
<td>4.50 ± 0.50</td>
<td>2.00 ± 0.35**</td>
</tr>
</tbody>
</table>

**P < 0.01, ***P < 0.001 compared with NSD.

Table 3 Effects of high-salt diet on affinity (Kd) (pmol/l) of total ANP and ANPb receptors in kidneys and adrenal glands of pregnant and fetal rats in late gestation. Values are means ± S.E.M.; there were seven experiments for each result.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant rats</th>
<th>Fetal rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSD</td>
<td>HSD</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ANP receptors</td>
<td>470 ± 72</td>
<td>558 ± 83</td>
</tr>
<tr>
<td>ANPb receptors</td>
<td>530 ± 47</td>
<td>590 ± 84</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ANP receptors</td>
<td>328 ± 47</td>
<td>339 ± 69</td>
</tr>
<tr>
<td>ANPb receptors</td>
<td>344 ± 55</td>
<td>352 ± 67</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with maternal NSD; †P < 0.05 compared with maternal HSD.
to that of the control pregnant rats, is additional proof of the complete elimination of excess salt. Several reports have shown that an increase in salt intake leads to an important diuresis and natriuresis in the adult rat (16, 19, 20, 23, 30), the pregnant rat (4, 5) or an increase in natriuresis in the monkey (31), man (32) and pregnant woman (33).

Our results showed that the high-salt diet of the pregnant rat increased fetal body weight and plasma sodium concentration. The increase in fetal body weight is probably the consequence of an increase in water retention associated with sodium accumulation. Indeed, previous data have shown that the fetal rat is able to retain a great deal of sodium in late gestation (1).

Figure 1 The effect of HSD (10 days) on the density (B_max) of the total ANP, ANPb and ANPc receptors in (a) isolated renal glomeruli and (b) adrenal zona glomerulosa membranes from NSD (open bars) and HSD (hatched bars) maternal rats. Inserts represent one example of Scatchard transformation of binding data; bound/free (B/F) versus bound ANP (fmol/mg protein) in (a) isolated glomeruli and (b) adrenal zona glomerulosa membranes. Curves of total ANP receptors in maternal control (○) and high-salted (●) tissues respectively; curves of ANPb receptors in maternal control (○) and high-salted (●) tissues respectively. Results are means ± S.E.M.; the number of experiments is given in brackets under the columns.
This discrepancy between pregnant and fetal rats is probably due to the immaturity of the fetal kidney which contains only a small number of filtering nephrons (34) and which cannot excrete the amount of sodium.

Several hormonal factors are involved in water–electrolyte balance. Arginine vasopressin (AVP), the renin–angiotensin–aldosterone system and ANP are among these main factors. Thanks to its natriuretic and diuretic properties, ANP can maintain the water–electrolyte balance by enhancing urinary excretion of sodium and water. Unfortunately, in our study, the plasma ANP levels in the fetal and pregnant rats were not significantly modified by a high-salt diet, suggesting that the amount of salt intake with saline solution does not interfere with ANP secretion. This result is in agreement with those of several investigations which showed that a high-salt diet did not modify the release of ANP in the adult rat (15, 16, 22, 23) or in

![Figure 2](image_url)

**Figure 2** The effect of maternal salt-loading on the density (Bmax) of total ANP, ANPb and ANPc receptors in (a) renal and (b) adrenal membranes from NSD (open bars) and HSD (hatched bars) fetal rats. Inserts represent one example of Scatchard transformation of binding data; bound/free (B/F) versus bound ANP (fmol/mg protein) in (a) renal and (b) adrenal membrane suspensions. Curves of total ANP receptors in control (○) and high-salted (●) tissues respectively; curves of ANPb receptors in fetal control (□) and high-salted (■) tissues respectively. Results are means ± S.E.M.; the number of experiments is given in brackets under the columns.
the pregnant rat (5, 35). Previous studies have shown that the blood volume expansion is the physiological stimulus of ANP secretion by increasing atrial distension in the adult rat (36, 37) and the fetal rat (38). In our study, the unchanged hematocrit values in both pregnant and fetal rats in response to a high-salt diet seem to indicate the absence of extracellular volume increase. The quantity of sodium used in our experiments may be inadequate to produce the increase in plasma volume expansion necessary to produce an atrial distension in both pregnant and fetal salt-loaded rats. From our results, ANP does not seem to be the main hormonal factor which maintains water and electrolyte balance during dietary salt intake. AVP does not seem to participate in this water and sodium homeostasis either, since the plasma AVP concentrations are not changed after a salt-loading treatment during pregnancy in the rat (5). Urodilatin is a peptide that belongs to the family of natriuretic peptides, located in the distal tubular cells and acting as a paracrine hormone within the kidney (39, 40). According to Bub et al. (23), urodilatin is stimulated by dietary salt loading and might be involved in the regulation of water and electrolyte balance in the rat. The renin–angiotensin–aldosterone system could play an important role in the regulation of sodium and water homeostasis. Our results show that plasma aldosterone concentrations are significantly reduced by about 50% in pregnant and fetal rats. Other studies have reported a significant suppression of the renin–angiotensin–aldosterone system after a high-salt diet in the adult rat (16, 30, 41–43) and pregnant woman (33).

The decrease in fetal plasma aldosterone concentrations in response to a high-salt intake suggests a decrease in the fetal renin–angiotensin system. We did not determine the fetal plasma renin concentrations in our fetal rats but in neonatal rats the renin–angiotensin system is more responsive to chronic sodium loading than that of the adult rat (44).

Previous studies have demonstrated the existence of at least two distinct types of ANP-binding sites in the renal glomeruli and adrenal glomerulosa cells (45, 46). One is the biological receptor coupled with guanylate cyclase (ANPb receptor), which is more abundant in the adrenal zona glomerulosa cells than in renal glomeruli, the other, not coupled with guanylate cyclase, is the clearance receptor (ANPc receptor), which is more abundant in renal glomeruli than in adrenal zona glomerulosa and can remove ANP from the blood circulation (47). Both types of ANP receptors are also present in the kidneys and the adrenal glands of the fetal rat at term, in proportions similar to those observed in the adult rat (13).

Our results show that a high-salt intake does not modify the density and affinity of total ANP, ANPb and ANPc receptors in both maternal and fetal kidneys and adrenal glands. These results are in agreement with those of Kollenda et al. (22) who demonstrated that in the renal glomeruli of the adult rat a high-salt diet did not change the density and affinity of total ANP, ANPb and ANPc receptors. However, they disagree with the studies showing a decrease in the number of ANP receptors in adrenal glomerulosa cells (19) and renal glomerulosa in the adult rat (14, 48).

Our results seem to demonstrate that the total number of ANP-binding sites is less affected by 10 days of salt-loading than by 3 days of dehydration. Indeed, in a recent study, we have shown that water deprivation significantly increased the total number of ANP receptors in renal glomeruli and adrenal zona glomerulosa membranes of the pregnant rat (49). The use of cANP(14–23) allowed us to demonstrate that the population of ANP receptors increases in the renal glomeruli and adrenal zona glomerulosa of the pregnant rat, whereas the ANPb receptor density remains unchanged (49). In contrast, the absence of up-regulation of ANP receptors in fetal kidneys and adrenal glands from dehydrated mothers could be associated with the immaturity of those organs (49).

In the fetal kidneys of both groups, the affinity of total ANP receptors is lower than that observed in control and high-salted glomeruloi of pregnant rats. This discrepancy could be due to an occupation of ANP receptors in fetal kidneys by endogenous circulating ANP. Indeed, in the fetal rat, the high concentrations of plasma ANP could occupy the small number of ANP receptors compared with the density of ANP receptors in maternal glomeruli; in those conditions, the endogenous peptide still present in fetal renal membrane preparations could compete with the radioligand during the binding studies, thus increasing the apparent $K_d$ in fetal renal preparations.

In our experiments, we did not determine the $K_d$ values of ANPc receptors in maternal and fetal preparations of both groups. The ANPc receptors in fetal kidneys might be mainly occupied with endogenous fetal plasma ANP since the $K_d$ values of ANPb receptors are not significantly different in fetal or maternal preparations of both groups. In isolated renal glomeruli washed with acid in order to reduce the occupation of ANP receptors with endogenous ANP, the affinity of ANPc receptors increased significantly (15).

In conclusion, our findings suggest that pregnant rats are able to maintain water and electrolyte homeostasis and ANP does not seem to be involved in the regulation of water–electrolyte balance during dietary salt intake. Other regulatory mechanisms are involved for maintaining water and sodium homeostasis.

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