Blood pressure, magnesium and other mineral balance in two rat models of salt-sensitive, induced hypertension: effects of a non-peptide angiotensin II receptor type 1 antagonist

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Abstract. The renin-angiotensin system is critically involved in regulating arterial blood pressure (BP). Inappropriate angiotensin type-1 receptor activation by angiotensin-II (Ang-II) is related to increased arterial BP. Mg has a role in BP; it can affect cardiac electrical activity, myocardial contractility, and vascular tone. To evaluate the relationship between high BP induced by a high sodium (Na) diet and Mg, and other mineral balances, two experimental rat models of salt-sensitive, induced-hypertension were used: Ang-II infused and Dahl salt-sensitive (SS) rats. We found that: 1) Ang-II infusion progressively increased BP, which was accompanied by hypomagnesuria and signs of secondary hyperaldosteronism; 2) an additive effect between Ang-II and a high Na load may have an effect on strontium (Sr), zinc (Zn) and copper (Cu) balances; 3) Dahl SS rats fed a high Na diet had a slow pressor response, accompanied by altered Mg, Na, potassium (K), and phosphate (P) balances; and 4) losartan prevented BP increases induced by Ang II-NaCl, but did not modify mineral balances. In Dahl SS rats, losartan attenuated high BP and ameliorated magnesemia, Na and K balances. Mg metabolism maybe considered a possible defect in this strain of rat that may contribute to hypertension.

Key words: angiotensin II, Dahl salt-sensitive rats, magnesium, minerals, losartan, angiotensin-II type-1 receptor

High BP is the major cause of cardiovascular disease and mortality in many countries. It affects ∼1 billion people in the world [1]. It has been reported that BP is determined by a combination of environmental (including nutrition) and genetic factors [2]. A link between salt-sensitive hypertension and a polymorphism of genes encoding the components of the renin-angiotensin system (RAS) has been established [3, 4]. Inappropriate angiotensin II (Ang II) activation via angiotensin type-1 receptor (AT1R) is responsible for abnormal water, electrolyte balance and vascular resistance, thereby increasing the extracellular fluid volume (ECFV) and arterial BP [3, 4]. Increased Ang II levels induce secretion of aldosterone. It has been shown that hyperaldosteronism decreases Mg and
Na reabsorption in proximal convoluted tubules (PCT) and the cortical thick ascending limb (cTAL) [5]. In Mg deficiency, basal aldosterone levels and the steroidogenic effects of Ang II are increased [6].

Mg is the second most abundant cation in the intracellular (IC) fluid. It is essential for many biochemical, and hence biological, activities [5, 7]. Mg has been linked to BP [8, 9]; it can affect cardiac electrical activity, myocardial contractility, and vascular tone, which have been associated with hypokalemia and other mineral disturbances [10, 11].

Many human and animal studies have shown an association between high Na intake and hypertension [4], and an inverse relation between K intake and arterial BP [12]. In rats fed a high Na and low K diet, hypercalciuria, hypermagnesuria and hypophosphaturia have been reported [13].

Many of these studies focused their research on cause and effect of an individual mineral in hypertension, but few of them [14-16], have evaluated combined minerals under the same experimental conditions. There are many experimental hypertension models [17]; however, we decided to evaluate two which are clearly salt-sensitive: the Ang II infusion model and Dahl salt-sensitive (Dahl SS) rats. Ang II infusion has been shown to induce the pressor response, associated with increased Na reabsorption. The effects of Ang II are potentiated when infusion is combined with a high salt diet [18]. In addition, Dahl SS rats, one of the most studied genetic models, developed high BP, with concomitant cardiovascular and renal injury when fed a high Na diet [19].

Hypertension may coincide with multiple mineral disorders, which could facilitate the onset of high BP or worsen it. In this sense, Mg has shown to be an important risk factor in the development of high BP.

The aim of the present study was to evaluate the relationship between high BP induced by a high Na diet, and Mg and other mineral balances in two experimental rat models of salt-sensitive, induced hypertension. Additionally, the effect of losartan, a non-peptide AT1R antagonist employed in the treatment of high BP, on Mg and mineral balances was also evaluated.

### Methods

#### Animals

**Experiment 1**

Six-week-old, male, Sprague Dawley rats, were used. During the first week, the rats were fed a powdered control diet (0.1% Na wt/wt) for adaptation. During the second week, to avoid endogenous Ang II release, rats were fed a high Na diet (3.0% Na wt/wt). The rats were randomly divided into five groups and each group was treated over the next 14 days as follows:

1. non-angiotensin II rats (non-Ang-II): sham-operated rats receiving a control diet (0.1% Na wt/wt);
2. non-angiotensin II-NaCl rats (non-Ang-II-NaCl): sham-operated rats receiving a high Na diet (3.0% Na wt/wt);
3. angiotensin II-infused rats (Ang-II): rats receiving an Ang II infusion at 450 ng/kg/min, and fed a control diet;
4. angiotensin II-NaCl rats (Ang-II-NaCl): rats receiving an Ang II infusion at 450 ng/kg/min, and fed a high Na diet (3.0% Na wt/wt);
5. angiotensin II-NaCl-losartan rats (Ang-II-NaCl-losartan): rats receiving an Ang II infusion at 450 ng/kg/min, and fed a high Na diet (3% Na wt/wt) plus losartan at 20.76 ± 1.68 mg/L per day.

Distilled water and food were available ad libitum. Rats were housed in metabolic cages for the last three days of the experiment. Urine and feces samples were collected, and water and food intake were recorded. At the end of the experiment, the animals were sacrificed and blood collected directly from the heart.

**Experiment 2**

Six-week-old, male, Dahl SS rats were used. During the first week of the experiment, Dahl SS rats were fed a powdered control diet (0.1% Na wt/wt). The rats were randomly divided into three groups and over the following eight weeks, each group was treated as follows:

1. Dahl SS rats: salt-sensitive Dahl rats receiving a control diet (0.1% Na wt/wt);
2) Dahl SS-NaCl rats: salt-sensitive Dahl rats receiving a high Na diet (3.0% Na wt/wt) and;
3) Dahl SS-NaCl-losartan rats: salt-sensitive Dahl rats receiving a high Na diet over eight weeks and losartan at 14.29 ± 0.59 mg/kg in drinking water from week 8- to 16. Based on water consumption, this was equivalent to 53.78 ± 2.61 mg/L per day.

After the final three days in metabolic cages, the rats were treated as above.,

All animals were housed in temperature-controlled rooms (22°C), with a 12:12-h light-dark cycle, and handled according to the recommendations of the IVIC Bioethical Committee and EC Directive 86/609/EEC for animal experiments.

Osmotic mini-pumps implants and Ang II infusion

ALZET 2002 mini-osmotic pumps (Durect Corporation, USA) were used for the Ang II infusions. For osmotic-pump implantation, rats were anesthetized with an anesthetic “cocktail” containing 5 mg/kg xylazine and 40 mg/kg ketamine in distilled water. Before osmotic pump implantation, rats were weighed, and data were used for the calculation of antibiotic, anesthetic and Ang II dosages. A small incision in the skin between the scapulae was made, forming a small pocket where the pump inserted. Oxytetracycline was administered at 20 mg/kg s.c., for three days after surgery at. Ang II (Sigma Aldrich, USA) was dissolved in 0.15 mM NaCl, containing 0.01N acetic acid. The infusion rate was adjusted to 450 mg/kg/min. Control rats received saline vehicle.

Blood pressure measurement

Indirect BP was measured in conscious animals by tail-cuff plethysmography using a non-invasive BP/heart rate monitoring system LE5002, and NIBPCHART V1.1 software for data collection, traceability and validation (Panlab, Harvard Apparatus, Barcelona, Spain). Rats were placed in restraint cages and habituated to experimental handling and conditions one week before the experiment began. BP was measured over 15 min at intervals of 3 min each, twice a week. The data analyzed corresponded to the mean of at least three measurements of the systolic (SBP), diastolic (DBP) and mean BP (MBP) per week. MBP was automatically calculated by the apparatus using the formula:

\[
MBP = DBP + 0.33 \times (SBP + DBP)
\]

After surgery in experiment 1, rats were allowed to recover for three days before their BP was measured.

Body weight

Body weight was measured twice weekly using a balance S/SI-1002 (Denver Instrument, USA).

Plasma collection

Blood from the heart was collected in heparin-containing tubes. Plasma was obtained by centrifugation (10 min, 3,500 rpm, 4°C), and frozen for later analysis.

Mineral analysis

Plasma and urine mineral concentrations were determined after adequate dilution with bi-distilled water. For food and feces analysis, samples were weighed, dried (24 h at 50°C). Samples were digested with 8 ml of an HNO₃ (14M) and H₂O₂ (30%) (4:1) mixture in a microwave digestion system (ETHOS One, Milestone Srl, Sorisole (BG), Italy) for 25 min at 220°C. When digested, the mineral solution was adjusted to 100 ml with bi-distilled water. To complete homogenization, the solution was sonicated for 20 min. In the samples, overall mineral content was determined using inductively-coupled plasma-atomic emission spectroscopy (ICP-OES) (Perkin-Elmer, optima 3000, Quebec, Canada) at different wavelengths: Mg, 279.077; Ca, 315.887; Na, 589.592 nm; K, 766.490 nm; P, 213.617; Mn, 257.610; Ni, 231.604; Sr, 407.771.

Statistical analysis

Values are expressed as means ± standard error of the mean (S.E.M.). Differences between experimental groups were determined by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer or the Dunnett test. Correlations
between groups were estimated by Pearson analysis. Differences were considered significant at \( P < 0.05 \). The software used was GraphPad Prism, version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

**Results**

**Experiment 1: Ang II-infused rats**

**BP**

Control rats fed a high Na diet showed no variation in systolic, diastolic and mean BP during the four-week study compared to the control rats fed a standard Na diet (**figures 1A-C**). The Ang II infusion with a standard Na diet induced, in 50% of animals, a significant increase in systolic (+27.2 \( \pm \) 9.3%), diastolic (+36 \( \pm \) 16.2%) and mean arterial pressure (+33 \( \pm \) 13.1%) from the first week of the Ang II treatment (week 3) compared to non-Ang-II rats. Ang II-infused rats fed a high Na diet showed high systolic, diastolic and mean BP from the first week of the Ang II treatment until the end of the study (+73.8 \( \pm \) 5.2%, 78.1 \( \pm \) 9.8% and 77.2 \( \pm \) 8.1%, respectively at week 4), compared to non-Ang-II-infused rats fed a high Na diet (**figures 1A-C**). Losartan treatment prevented this increase in systolic, diastolic and mean BP from the first week of the Ang II administration until the end of the experiment (**figures 1A-C**).

**Clinical parameters**

Non-Ang-II-infused, high salt-fed rats increased their water intake two-fold compared to those fed the control diet, which was related to higher urine excretion \((r = 0.94, p<0.001)\) (**table 1**).

Ang II infusion in standard Na-fed rats induced a small, not significant, increase in water intake. However, Ang II infusion in high salt-fed rats induced polydipsia, which losartan did not prevent. Urine excretion in Ang-II-infused, high salt-fed and Ang-II-infused high salt-fed, losartan-treated rats seemed to be unrelated to water intake \((r = 0.30, p>0.05)\) and \( r = 0.56, p>0.05 \), respectively).

Fractional water excretion increased significantly in both non-Ang-II-infused (1.9 fold) and Ang-II-infused, high salt-fed (1.4 fold) rats, but losartan, given to Ang-II-NaCl-treated rats did not modify the increase in fractional water excretion (**table 1**).

Even though no differences in food intake were found between groups, fecal egestion significantly decreased in non-Ang-II-infused, high salt-fed rats (-22.8 \( \pm \) 2.8%) and Ang-II treated rats (-22.9 \( \pm \) 4.6%) compared to non-Ang-II-infused rats. Losartan treatment of Ang-II-NaCl-treated rats had normal fecal egestion (**table 1**).

**Figure 2** shows that the five groups sustained their body weight gain during the four week study. Weight fell significantly in Ang II-infused rats compared to controls (-11.5 \( \pm \) 2.2% at week 4). Ang-II-infused, high salt-fed rats also had a smaller weigh gain compared to non-Ang-II-infused, high salt-fed rats at week four (-14.9 \( \pm \) 3.8%; **figure 2**). Losartan given to Ang-II-NaCl-treated rats normalized body weight gain.

**Biological parameters**

**Mineral balance.** The mineral composition of diets was similar in both groups of diets, except for Na content. Na intake was more than 32 times higher in high salt-fed rats compared to those fed a control diet (**table 2**). No variation was noted between the groups for the rest of the mineral intake.

**High Na diet effects on mineral balance.** When fed a high Na diet, non-Ang-II-infused rats presented a significant decrease (50.5 \( \pm \) 7.5%) in Mg urine excretion when compared to their counterparts. Therefore, net reabsorption showed an almost two-fold increase (**table 2**). Concerning the Ca balance, Ca reabsorption was significantly increased (0.91 \( \pm \) 0.12%).

Urine Na excretion in this group correlated positively with Na ingestion \((r = 0.81, p<0.01)\). These animals excreted 11 times more Na than those fed a standard diet. An increase in urine K excretion was also seen in this group (35.6 \( \pm \) 5.3%) compared to non-Ang-II rats, which was related to reduced, net, K reabsorption and a 1.36-fold increase in fractional K excretion (**table 2**). Hence, there was a higher urine Na/K ratio (1.44 \( \pm \) 0.03) compared to control rats (0.102 \( \pm \) 0.003).

A high Na diet in non-Ang-II-infused rats induced significant changes in the P balance (raising urine excretion to 173 \( \pm \) 45.3%), Sr fecal egestion (33.9 \( \pm \) 6.5%), and fractional Zn excretion (1.26 fold). No significant variation in Fe, Al, Ba, Cr, Mn and Ni was observed (data not shown).

**Ang II effects on mineral balance.** Regarding the Mg balance, Ang II rats fed a control diet
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Figure 1. Time course of blood pressure in control rats fed a standard diet (non-Ang-II), control rats fed a high sodium diet (non-Ang-II-NaCl), angiotensin II-treated rats fed a standard diet (Ang-II), angiotensin II-treated rats fed a high sodium diet (Ang-II-NaCl), and angiotensin II-treated rats fed a high sodium diet plus losartan in drinking water (Ang-II-NaCl-losartan). Systolic (A), diastolic (B) and mean blood pressure (C).

Values are expressed as means ± SEM in mmHg (n = 4-10 for each group). Statistical analysis was performed using one-way ANOVA followed by a Tukey test. ***P < 0.001 versus non-Ang-II, **P < 0.01 versus non-Ang-II; ###P < 0.001 versus non-Ang-II-NaCl, #P < 0.05 versus non-Ang-II-NaCl; †††P < 0.001 versus Ang-II, ††P < 0.01 versus Ang-II, †P < 0.05 versus Ang-II; and, &&&P < 0.001 versus Ang-II-NaCl rats.

showed lower Mg urine excretion when compared to non-Ang-II rats fed the same diet (table 2). This was related to an increased net Na reabsorption (2.9 fold). These rats also showed higher urine K excretion when compared to non-Ang-II rats. This effect was related to a reduced net K reabsorption of 47 ± 24.7%. Ang II infusion induced an marked decrease in the urine Na/K ratio (0.066 ± 0.014).

Lower net Sr reabsorption (-1.91 ± 0.09%) was observed in Ang II-infused rats compared to
Table 1. Water intake, urine excretion, fractional water excretion, food intake and fecal excretion in male control rats fed a standard diet (non-Ang-II), control rats fed a high sodium diet (non-Ang-II-NaCl), angiotensin II-treated rats fed a standard diet (Ang-II), angiotensin II-treated rats fed a high sodium diet (Ang-II-NaCl), and angiotensin II-treated rats fed a high sodium diet and losartan in drinking water (Ang-II-NaCl-losartan).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-Ang-II</th>
<th>Non-Ang-II-NaCl</th>
<th>Ang-II</th>
<th>Ang-II-NaCl</th>
<th>Ang-II-NaCl-losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (mL (24h)^{-1})</td>
<td>43.98 ± 2.36</td>
<td>101.56 ± 8.01***</td>
<td>50.83 ± 9.82</td>
<td>141.50 ± 5.28 #, †††</td>
<td>148.75 ± 12.29 ##, †††</td>
</tr>
<tr>
<td>Urine excretion (mL (24h)^{-1})</td>
<td>19.04 ± 0.96</td>
<td>82.77 ± 6.40 ***</td>
<td>32.33 ± 7.22</td>
<td>103.90 ± 2.45 † †</td>
<td>124.38 ± 8.07 ###, †††</td>
</tr>
<tr>
<td>Fractional water excretion (%)</td>
<td>44.06 ± 1.95</td>
<td>83.43 ± 3.91 ***</td>
<td>62.85 ± 5.34*</td>
<td>73.69 ± 2.30</td>
<td>85.59 ± 8.25 †</td>
</tr>
<tr>
<td>Food intake (g (24h)^{-1})</td>
<td>23.23 ± 0.39</td>
<td>22.02 ± 0.83</td>
<td>20.98 ± 3.21</td>
<td>21.33 ± 0.64</td>
<td>23.96 ± 1.16</td>
</tr>
<tr>
<td>Fecal excretion (mg/kg (24h)^{-1})</td>
<td>4.17 ± 0.16</td>
<td>3.22 ± 0.12 ***</td>
<td>3.22 ± 0.19*</td>
<td>3.17 ± 0.14</td>
<td>4.36 ± 0.21###</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM (n = 3-9/group). Statistical analysis was performed using one-way ANOVA followed by a Tukey–Kramer test. ***P < 0.001, *P < 0.05 versus non-Ang-II; ###P < 0.001, #P < 0.05 versus Non-Ang-II-NaCl; †††P < 0.001, †P < 0.05 versus Ang-II. Fractional water excretion (%) was computed as the percentage of dietary water excreted in the urine per day; these estimates do not include fecal and other water losses.

Figure 2. Time course of body weight in control rats fed a standard diet (non-Ang-II), control rats fed a high sodium diet (non-Ang-II-NaCl), angiotensin II-treated rats fed a standard diet (Ang-II), angiotensin II-treated rats fed a high sodium diet (Ang-II-NaCl), and angiotensin II-treated rats fed a high sodium diet and losartan in drinking water (Ang-II-NaCl-losartan).

Values are expressed as means ± SEM in g (n = 4-10 for each group). Statistical analysis was performed using one-way ANOVA followed by a Tukey test. ***P < 0.001, **P < 0.01 versus non-Ang-II; #P < 0.01 versus non-Ang-II-NaCl.
Table 2. Mg, Ca, Na, K, Ca, Mg, P, Sr and Zn balance in male control rats fed a standard diet (non-Ang-II), control rats fed a high sodium diet (non-Ang-II-NaCl), angiotensin II-treated rats fed a standard diet (Ang-II), angiotensin II-treated rats fed a high sodium diet (Ang-II-NaCl), and angiotensin II-treated rats fed a high sodium diet plus losartan in drinking water (Ang-II-NaCl-losartan).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Intake (mg/d)</th>
<th>Fecal (mg/d)</th>
<th>Urine (mg/d)</th>
<th>Percentage net reabsorption</th>
<th>Fractional excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mg</strong></td>
<td></td>
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</tr>
<tr>
<td>Non-Ang-II</td>
<td>21.20 ± 0.36</td>
<td>7.13 ± 0.33</td>
<td>12.29 ± 1.06</td>
<td>44.22 ± 4.37</td>
<td>88.01 ± 5.40</td>
</tr>
<tr>
<td>Non-Ang-II-NaCl</td>
<td>19.28 ± 0.72</td>
<td>6.39 ± 0.37</td>
<td>3.47 ± 0.55**</td>
<td>82.38 ± 2.45***</td>
<td>50.05 ± 2.37***</td>
</tr>
<tr>
<td>Ang-II</td>
<td>19.15 ± 2.93</td>
<td>6.76 ± 0.17</td>
<td>6.65 ± 0.50**</td>
<td>61.64 ± 7.51</td>
<td>75.70 ± 12.07</td>
</tr>
<tr>
<td>Ang-II-NaCl</td>
<td>18.68 ± 0.56</td>
<td>4.87 ± 0.57</td>
<td>6.55 ± 0.70#</td>
<td>64.86 ± 3.96#</td>
<td>61.41 ± 3.97</td>
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<tr>
<td>Ang-II-NaCl-losartan</td>
<td>20.99 ± 1.02</td>
<td>6.07 ± 0.52</td>
<td>5.86 ± 1.29</td>
<td>71.94 ± 6.82</td>
<td>56.97 ± 7.44</td>
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<td><strong>Ca</strong></td>
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</tr>
<tr>
<td>Non-Ang-II</td>
<td>173.74 ± 2.93</td>
<td>146.84 ± 6.19</td>
<td>3.64 ± 0.39</td>
<td>98.18 ± 0.17</td>
<td>83.32 ± 3.97</td>
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<tr>
<td>Non-Ang-II-NaCl</td>
<td>171.38 ± 6.44</td>
<td>101.71 ± 5.06***</td>
<td>1.56 ± 0.21**</td>
<td>99.07 ± 0.12*</td>
<td>60.61 ± 2.78**</td>
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<tr>
<td>Ang-II</td>
<td>156.93 ± 23.99</td>
<td>141.90 ± 9.12</td>
<td>3.34 ± 0.95</td>
<td>97.47 ± 0.98</td>
<td>97.69 ± 12.31</td>
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<tr>
<td>Ang-II-NaCl</td>
<td>166.03 ± 4.99</td>
<td>103.42 ± 10.83</td>
<td>5.57 ± 1.01#</td>
<td>96.65 ± 0.64#</td>
<td>66.17 ± 7.54</td>
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<tr>
<td>Ang-II-NaCl-losartan</td>
<td>186.55 ± 9.04</td>
<td>122.43 ± 10.70</td>
<td>5.73 ± 1.63##</td>
<td>96.98 ± 0.90##</td>
<td>68.27 ± 4.16</td>
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<tr>
<td><strong>Na</strong></td>
<td></td>
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<tr>
<td>Non-Ang-II</td>
<td>20.77 ± 0.35</td>
<td>2.36 ± 0.22</td>
<td>17.97 ± 0.79</td>
<td>14.83 ± 4.83</td>
<td>86.23 ± 2.49</td>
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<td>Non-Ang-II-NaCl</td>
<td>667.70 ± 25.10***</td>
<td>3.13 ± 0.31</td>
<td>205.00 ± 8.07***</td>
<td>69.76 ± 0.65***</td>
<td>28.73 ± 1.50***</td>
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<tr>
<td>Ang-II</td>
<td>18.76 ± 2.87</td>
<td>1.05 ± 0.04</td>
<td>9.45 ± 2.28*</td>
<td>43.67 ± 18.10*</td>
<td>62.34 ± 19.02*</td>
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<tr>
<td>Ang-II-NaCl</td>
<td>646.85 ± 19.43††</td>
<td>2.06 ± 0.35</td>
<td>176.27 ± 21.50††</td>
<td>73.00 ± 3.02†</td>
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<td>Ang-II-NaCl-losartan</td>
<td>726.80 ± 35.21††</td>
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<td>5.02 ± 1.79</td>
<td>197.41 ± 25.99††</td>
<td>72.42 ± 4.5†</td>
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<td><strong>K</strong></td>
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<td>Non-Ang-II</td>
<td>211.48 ± 3.56</td>
<td>13.16 ± 1.12</td>
<td>107.71 ± 7.36</td>
<td>49.42 ± 4.25</td>
<td>56.75 ± 4.78</td>
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<td>Non-Ang-II-NaCl</td>
<td>205.21 ± 7.72</td>
<td>14.65 ± 0.67</td>
<td>146.11 ± 5.66***</td>
<td>27.03 ± 3.40**</td>
<td>77.08 ± 4.34*</td>
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<td>Ang-II</td>
<td>191.01 ± 29.21</td>
<td>11.45 ± 0.21</td>
<td>140.97 ± 11.66*</td>
<td>26.39 ± 12.19**</td>
<td>86.30 ± 17.24*</td>
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<td>Ang-II-NaCl</td>
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<td>7.23 ± 1.16##</td>
<td>123.57 ± 11.85</td>
<td>38.08 ± 5.19</td>
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<td>Ang-II-NaCl-losartan</td>
<td>223.37 ± 10.82</td>
<td>11.10 ± 1.30#</td>
<td>129.91 ± 18.68</td>
<td>56.48 ± 4.04####,#</td>
<td>64.17 ± 10.15</td>
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<td><strong>P</strong></td>
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<tr>
<td>Non-Ang-II</td>
<td>148.88 ± 2.51</td>
<td>85.80 ± 3.62</td>
<td>0.45 ± 0.08</td>
<td>99.71 ± 0.06</td>
<td>52.72 ± 1.84</td>
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<tr>
<td>Non-Ang-II-NaCl</td>
<td>140.07 ± 5.27</td>
<td>70.59 ± 3.14*</td>
<td>1.23 ± 0.20**</td>
<td>99.10 ± 0.16**</td>
<td>51.90 ± 2.27</td>
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<td>Ang-II</td>
<td>134.47 ± 20.56</td>
<td>84.54 ± 4.72</td>
<td>0.54 ± 0.15</td>
<td>99.53 ± 0.17</td>
<td>66.67 ± 7.92</td>
</tr>
<tr>
<td>Ang-II-NaCl</td>
<td>135.70 ± 4.08</td>
<td>57.82 ± 6.45</td>
<td>1.90 ± 0.20†</td>
<td>98.58 ± 0.17</td>
<td>44.40 ± 5.47</td>
</tr>
<tr>
<td>Ang-II-NaCl-losartan</td>
<td>152.47 ± 7.39</td>
<td>78.20 ± 6.97</td>
<td>4.57 ± 2.90†</td>
<td>96.88 ± 2.08</td>
<td>54.15 ± 4.26</td>
</tr>
<tr>
<td><strong>Sr</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Ang-II</td>
<td>0.59 ± 0.01</td>
<td>0.412 ± 0.018</td>
<td>0.012 ± 0.001</td>
<td>98.08 ± 0.20</td>
<td>58.92 ± 6.79</td>
</tr>
<tr>
<td>Non-Ang-II-NaCl</td>
<td>0.56 ± 0.02</td>
<td>0.552 ± 0.027**</td>
<td>0.011 ± 0.001</td>
<td>97.91 ± 0.30</td>
<td>85.75 ± 2.24**</td>
</tr>
<tr>
<td>Ang-II</td>
<td>0.54 ± 0.02</td>
<td>0.448 ± 0.030</td>
<td>0.019 ± 0.001</td>
<td>96.20 ± 0.09*</td>
<td>81.63 ± 5.30</td>
</tr>
<tr>
<td>Ang-II-NaCl</td>
<td>0.54 ± 0.02</td>
<td>0.341 ± 0.034###</td>
<td>0.037 ± 0.004###</td>
<td>93.20 ± 0.87###</td>
<td>65.35 ± 7.33###</td>
</tr>
<tr>
<td>Ang-II-NaCl-losartan</td>
<td>0.61 ± 0.03</td>
<td>0.423 ± 0.037#</td>
<td>0.036 ± 0.003###</td>
<td>94.12 ± 0.59###</td>
<td>71.54 ± 3.67#</td>
</tr>
</tbody>
</table>
non-Ang II-infused rats fed the same diet. No changes in Ca, P, Zn (table 2), Fe, Al, Ba, Cr, Cu, Mn and Ni were observed (data not shown).

**Combined effects of Ang II-high Na diet on mineral balance.** The effects of Ang II infusion on some mineral balances were modified by the ingestion of high Na.

Magnesium urine excretion in this group remained low as it did for Ang II infusion alone and was not worsened by addition of a high Na diet. A high Na diet did not significantly affect the Ca balance in Ang II-treated rats, apart from fractional Ca excretion (table 2).

A high Na diet in Ang II-treated rats increased net Na reabsorption (1.7 fold versus Ang-II). Regarding the K balance, Ang II-NaCl rats showed significantly reduced fecal egestion. The Na/K ratio (1.41 ± 0.07) reached the values of those in non-Ang-II-NaCl rats.

As regards other mineral balances, higher P and Zn urine excretion were only seen when Ang II rats were fed a high Na diet (table 2). Cu fecal egestion was also reduced by supplying a high Na diet to Ang II rats (data not shown).

No significant variations in Sr (table 2), Fe, Al, Ba, Cr, Mn or Ni were observed (data not shown).

Treatment with losartan did not produce any important changes in mineral metabolism in Ang II rats fed a high Na diet.

**Plasma mineral analysis.** Plasma Mg remained normal in all of the groups studied (table 3). Only Na and K plasma concentrations seemed to vary among the groups during this experiment (table 3). A high Na diet in non-Ang-II rats increased the plasma Na concentration by +8.5 ± 3.8%, and decreased plasma K concentration (-31.3 ± 0.2% versus non-Ang-II rats; table 3).

The Na concentration in Ang II-infused rats fed a control diet increased by +9.7 ± 2.3%. No variation in plasma K concentration was observed in this group compared to non-Ang II-infused rats fed the same diet (table 3). When fed a high Na diet, Ang-II-infused rats showed a significant increase (8.2 ± 1.9%) in plasma Na concentrations compared to Ang II-infused rats fed a control diet. The plasma K concentration in this group showed a 1.4-fold increase compared to non-Ang-II-NaCl rats (table 3).

Losartan treatment of Ang-II-NaCl-treated rats did not prevent plasma Na increases (+12.3 ± 0.4% and +11.1 ± 0.4%), compared to non-Ang-II-NaCl and Ang-II rats, respectively (table 3).

Other mineral plasma concentrations analyzed: P, Fe, Cu, Zn showed no significant variation; Al, Ba, Cr, Mn, Ni and Sr were not detected by our equipment in any of the groups studied (data not shown).

**Experiment 2: Dahl SS rats**

**BP**

Dahl SS rats fed a control Na diet showed no significant variations in systolic, diastolic and
mean BP during the study (148 ± 4.6 mmHg, 101 ± 9.9 mmHg and 116.5 ± 7.7 mmHg for systolic, diastolic, and mean BP, respectively, at week 8). When fed a high Na diet, Dahl SS rats displayed a higher BP from week 3, which worsened during the study (211.3 ± 15.8 mmHg, 153.2 ± 6.2 mmHg, 172.36 ± 5.32 mmHg for systolic, diastolic, and mean blood pressure respectively at week 8), compared to rats fed a control Na diet (figures 3A-C).

To assess the effect of losartan on BP, Dahl SS rats were fed a high Na diet for eight weeks, when high BP became stabilized. At this point, losartan (14.3 ± 0.6 mg/kg) was added to the drinking water until the end of the study, while maintaining the high Na diet.

BP increased significantly from the first week of the high Na diet and did not differ from Dahl SS-NaCl rats (215 ± 26.0 mmHg, 171 ± 19.6 mmHg, 189 ± 25 mmHg for systolic, diastolic, and mean BP, respectively, for Dahl SS-NaCl-losartan rats at week 8) (figure 3D). Losartan induced a significant decrease in BP at week 9, but pressure remained high and unstable during the rest of the experiment. Notably, between week 15 and week 17, BP reduced (systolic: 150 ± 15.1 mmHg, diastolic: 131 ± 13.2 mmHg, mean: 137 ± 13.74 at week 17), but then increased slightly at the end of the study period (figure 3D).

Mortality in these rats was 22.2% (2/9) at week 8 and 14.3% (1/7) at week 17 during the losartan period.

Clinical parameters
Water intake, urine excretion and fractional water excretion significantly in Dahl SS rats fed a high Na diet compared to Dahl SS rats fed a standard Na diet. Food intake and fecal egestion did not differ between the groups studied (table 4). Losartan partly reduced water intake, urine excretion, and increased fractional water excretion induced by a high Na diet. A correlation between water intake and urine excretion was detected in Dahl SS, Dahl SS-NaCl and Dahl SS-NaCl-losartan rats (r = 0.85, p < 0.05; r = 0.97, p < 0.01 and r = 0.88, p < 0.05, respectively).

Dahl SS rats fed a standard diet showed a regular weight gain during the study. Weight gain was significantly lower in Dahl SS rats fed a high Na diet compared to control rats (figure 4A). Losartan did not modify the weight-gain rate (figure 4B).

Biological parameters
Mineral balance. The mineral composition of diets was similar in both groups of diets, apart from Na content. Na intake in Dahl SS rats fed a high Na diet, treated or not with losartan, increased >26 fold compared to Dahl SS rats fed a control diet. Intake of other minerals was unaffected (table 5).

As regards the Mg balance, urine Mg excretion decreased (74.0 ± 6.9%) in Dahl SS rats fed a high Na diet, and this was related to increased net Mg reabsorption (31.0 ± 3.6%). Treatment with losartan in this inherited rat model did not significantly alter the hypomagnesuria observed following a high Na diet.

As a result of a high Na intake, Dahl SS rats showed higher fecal and urine Na excretion (+97.9 ± 26.6%, 255.2 ± 39.1%, respectively) when compared to those fed a control diet, as demonstrated by a higher, net reabsorption
Figure 3. Time course of blood pressure in Dahl salt-sensitive (Dahl SS) rats fed a standard diet, Dahl SS rats fed a high sodium diet (Dahl SS-NaCl) and Dahl SS rats fed a high sodium diet plus losartan in drinking water (Dahl SS-NaCl-losartan). Systolic BP (A); diastolic blood pressure (B); mean blood pressure (C) in Dahl SS and Dahl SS-NaCl; and, systolic, diastolic and mean blood pressure in Dahl SS-NaCl-losartan rats (D).

Values are expressed as means ± SEM. in mmHg (n = 3-8 for each group). Statistical analysis was performed using the t-test followed by the Mann Whitney test. ***P < 0.001, **P < 0.01, *P < 0.05 versus Dahl SS rats (A, B, C) or versus week 0 (D).

(+623.5 ± 7.9%) and a lower fractional excretion (-35.2 ± 8.4%). Losartan increased by 2.5-fold urine Na excretion in Dahl SS rats fed a high Na diet, which was accompanied by a decrease in net Na reabsorption and an increase of 2.8 fold in fractional excretion (table 5).

A high Na diet decreased urine K excretion in Dahl SS rats, (53.2 ± 5.0%), which was linked to an increase in net K reabsorption (721.2 ± 97.3%) and a decrease in its fractional excretion (55.5 ± 5.7%; table 5). Moreover, the urine Na/K ratio (0.70 ± 0.06) was significantly higher compared to rats fed a control diet (0.09 ± 0.003). In this case, losartan treatment increased urine K excretion in Dahl SS-NaCl rats. This group had a higher urine Na/K ratio (1.17 ± 0.03) compared to Dahl SS-NaCl rats.

As regards P, Dahl SS-NaCl rats presented a 6.7-fold increase in urine P excretion (table 5). Losartan administration did not induce any changes in this mineral.

The other minerals - those not mentioned so far - did not show any significant differences between Dahl SS, Dahl SS-NaCl and losartan-treated Dahl SS-NaCl rats (table 5).

Plasma mineral analysis. Dahl SS rats fed a high Na diet did not show any variations
Table 4. Water intake, urine excretion, fractional water excretion, food intake and fecal excretion in male Dahl salt-sensitive (Dahl S/S) rats fed a standard diet, Dahl SS rats fed a high sodium diet (Dahl SS-NaCl), and Dahl SS rats fed a high sodium diet plus losartan in drinking water (Dahl SS-NaCl-losartan).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dahl SS</th>
<th>Dahl SS-NaCl</th>
<th>Dahl SS-NaCl-losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Intake (mL (24h)^{-1})</td>
<td>29.58 ± 2.27</td>
<td>103.33 ± 7.35 ***</td>
<td>74.74 ± 3.36 *** ##</td>
</tr>
<tr>
<td>Urine excretion (mL (24h)^{-1})</td>
<td>16.75 ± 1.62</td>
<td>81.46 ± 5.13 ***</td>
<td>61.97 ± 4.44 *** #</td>
</tr>
<tr>
<td>Fractional water excretion (%)</td>
<td>57.90 ± 2.47</td>
<td>79.18 ± 1.38 ***</td>
<td>85.29 ± 1.63 *** ##</td>
</tr>
<tr>
<td>Food intake (g (24h)^{-1})</td>
<td>16.91 ± 0.98</td>
<td>14.20 ± 1.58</td>
<td>15.36 ± 1.13</td>
</tr>
<tr>
<td>Fecal excretion (mg/kg (24h)^{-1})</td>
<td>3.31 ± 0.38</td>
<td>2.91 ± 0.23</td>
<td>2.80 ± 0.14</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM (n = 5-6/group). Statistical analysis was performed using one-way ANOVA followed by a Tukey test. ***P<0.001 versus Dahl SS rats; ##P<0.01, #P<0.05 versus Dahl SS-NaCl rats. Fractional water excretion (%) was computed as the percentage of dietary water excreted in the urine per day; these estimates do not include fecal and other water losses.

Figure 4. Time course of body weight in Dahl salt-sensitive (Dahl SS) rats fed a standard diet, Dahl SS rats fed a high sodium diet (Dahl SS-NaCl) and Dahl/SS rats fed a high sodium diet plus losartan in drinking water (Dahl SS-NaCl-losartan). Dahl SS and Dahl SS-NaCl (A); Dahl SS-NaCl-losartan (B). Values are expressed as means ± S.E.M. in g (n = 6-11 for each group). Statistical analysis was performed using the t-test followed by the Mann Whitney test. ***P<0.001, **P<0.01, *P<0.05 versus Dahl SS rats (A) or versus week 0 (B).
Table 5. Mg, Ca, Na, K, P, Sr and Zn in male Dahl salt-sensitive (Dahl S/S) rats fed a standard diet, Dahl SS rats fed a high sodium diet (Dahl SS-NaCl), and Dahl SS rats fed a high sodium diet plus losartan in drinking water (Dahl SS-NaCl-losartan).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Intake (mg/d)</th>
<th>Fecal (mg/d)</th>
<th>Urine (mg/d)</th>
<th>Percentage net reabsorption</th>
<th>Fractional excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>15.44 ± 0.90</td>
<td>9.93 ± 1.40</td>
<td>4.39 ± 1.10</td>
<td>69.48 ± 8.95</td>
<td>90.96 ± 9.12</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>12.44 ± 1.39</td>
<td>8.06 ± 0.88</td>
<td>1.14 ± 0.30*</td>
<td>91.00 ± 2.48*</td>
<td>76.21 ± 4.44</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>13.45 ± 0.99</td>
<td>6.14 ± 0.42*</td>
<td>1.36 ± 0.27*</td>
<td>87.24 ± 2.81</td>
<td>59.46 ± 4.04**</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>126.50 ± 7.36</td>
<td>123.83 ± 14.62</td>
<td>1.43 ± 0.35</td>
<td>98.74 ± 0.37</td>
<td>93.65 ± 7.90</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>110.56 ± 12.33</td>
<td>101.91 ± 7.73</td>
<td>1.08 ± 0.24</td>
<td>99.03 ± 0.23</td>
<td>99.71 ± 7.60</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>119.55 ± 8.77</td>
<td>111.02 ± 7.18</td>
<td>2.27 ± 0.38</td>
<td>97.91 ± 0.17†</td>
<td>96.53 ± 6.08</td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>15.12 ± 0.88</td>
<td>1.90 ± 0.27</td>
<td>13.12 ± 1.40</td>
<td>12.40 ± 5.23</td>
<td>99.56 ± 6.12</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>422.20 ± 53.08</td>
<td></td>
<td>46.61 ± 5.13</td>
<td></td>
<td>89.73 ± 0.98***</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>465.76 ± 34.18***</td>
<td>2.51 ± 0.40</td>
<td>118.74 ± 7.62***</td>
<td>73.65 ± 1.39***</td>
<td>26.92 ± 1.35***</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>153.97 ± 8.95</td>
<td>4.30 ± 0.59</td>
<td>143.23 ± 13.43</td>
<td>6.18 ± 4.63</td>
<td>96.47 ± 4.75</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>132.38 ± 14.77</td>
<td>4.31 ± 0.55</td>
<td>67.08 ± 7.19</td>
<td>56.60 ± 4.55***</td>
<td>42.90 ± 5.47**</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>143.14 ± 10.50</td>
<td>3.34 ± 0.40</td>
<td>100.67 ± 5.05*</td>
<td>24.94 ± 6.26**</td>
<td>77.43 ± 6.27##</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>120.44 ± 7.00</td>
<td>112.61 ± 12.25</td>
<td>0.31 ± 0.05</td>
<td>99.73 ± 0.05</td>
<td>88.80 ± 6.81</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>90.36 ± 10.08</td>
<td>101.74 ± 10.43</td>
<td>2.08 ± 0.54*</td>
<td>97.73 ± 0.58*</td>
<td>119.33 ± 4.91**</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>97.71 ± 7.17</td>
<td>92.18 ± 5.53</td>
<td>1.75 ± 0.37*</td>
<td>98.02 ± 0.41*</td>
<td>97.96 ± 6.09</td>
</tr>
<tr>
<td>Sr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>0.48 ± 0.03</td>
<td>0.53 ± 0.06</td>
<td>0.01 ± 0.001</td>
<td>97.78 ± 0.43</td>
<td>106.14 ± 9.18</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>0.36 ± 0.04</td>
<td>0.44 ± 0.05</td>
<td>0.01 ± 0.002</td>
<td>97.27 ± 0.12</td>
<td>133.89 ± 11.91</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>0.39 ± 0.03</td>
<td>0.42 ± 0.02</td>
<td>0.01 ± 0.001</td>
<td>97.55 ± 0.60</td>
<td>125.65 ± 2.00</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl SS</td>
<td>1.86 ± 0.11</td>
<td>1.69 ± 0.19</td>
<td>0.02 ± 0.004</td>
<td>99.02 ± 0.16</td>
<td>86.94 ± 6.83</td>
</tr>
<tr>
<td>Dahl SS-NaCl</td>
<td>1.49 ± 0.17</td>
<td>1.58 ± 0.17</td>
<td>0.02 ± 0.002</td>
<td>98.98 ± 0.09</td>
<td>111.52 ± 13.83</td>
</tr>
<tr>
<td>Dahl SS-NaCl-losartan</td>
<td>1.61 ± 0.12</td>
<td>1.45 ± 0.09</td>
<td>0.02 ± 0.001</td>
<td>98.98 ± 0.07</td>
<td>94.18 ± 6.36</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM (n = 5-6/group). Statistical analysis was performed using one-way ANOVA followed by a Tukey–Kramer test. ***P<0.001, **P<0.01, *P<0.05 versus Dahl SS rats; ###P<0.001, ##P<0.01, *P<0.05 versus Dahl SS-NaCl rats. Electrolyte intake and excretion were based on a two or three day average of individual animals. Percentage net reabsorption was calculated as the percentage of dietary electrolyte absorbed in the kidney (intake-urine excretion/intake) x 100. Fractional electrolyte excretion (%) was computed as the percentage of dietary electrolyte excreted in the urine and feces per day.

as regards plasma Mg, Ca, K, Fe, Cu and Zn concentrations (table 6). However, losartan produced an increase in plasma Mg concentration (1.3-fold), probably independently of the Na diet (table 6).

Dahl SS rats fed a high Na diet had high plasma Na concentrations compared to Dahl SS rats fed the control diet (a 1.2-fold increase). Losartan did not alter the increase in plasma Na concentrations induced by a high Na diet.

The other minerals analyzed here in plasma: P, Fe, Cu, Zn showed no significant variation; Al, Ba, Cr, Mn, Ni and Sr were undetectable by our equipment (data not shown).
**Table 6.** Plasma mineral concentration in male Dahl salt-sensitive (Dahl S/S) rats fed a standard diet, Dahl SS rats fed a high sodium diet (Dahl SS-NaCl), and Dahl SS rats fed a high sodium diet plus losartan in drinking water (Dahl SS-NaCl-losartan).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>0.72 ± 0.06</td>
<td>0.82 ± 0.07</td>
<td>1.07 ± 0.11*** ##</td>
</tr>
<tr>
<td>Ca</td>
<td>1.77 ± 0.17</td>
<td>1.95 ± 0.12</td>
<td>2.07 ± 0.08</td>
</tr>
<tr>
<td>Na</td>
<td>136.73 ± 7.14</td>
<td>161.98 ± 2.59**</td>
<td>162.87 ± 3.33**</td>
</tr>
<tr>
<td>K</td>
<td>5.81 ± 0.36</td>
<td>6.89 ± 0.43</td>
<td>6.22 ± 0.15</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. (n = 5-6/group). Statistical analysis was performed using one-way ANOVA followed by a Tukey–Kramer test. ***P < 0.001, **P < 0.01 versus Dahl SS rats; ##P < 0.01 versus Dahl SS-NaCl rats.

**Discussion**

We have shown that:

1) chronic Ang II infusion at 450 ng/kg/min in Sprague Dawley rats, progressively increased BP, which was accompanied by hypomagnesuria and signs of secondary hyperaldosteronism;
2) a high Na diet in this model exacerbated the BP response from the first week of treatment. It also produced a more complex mineral disorder, since Sr, Zn and Cu levels were altered;
3) Dahl SS rats fed a high Na diet showed a slow pressor-response, accompanied by signs of an activated RAS, since increases in Na reabsorption and hypokaluria occurred. Furthermore, hypomagnesuria and hyperphosphaturia were also observed;
4) losartan prevented BP increases in Ang II-NaCl, but not Mg and other mineral disorders, which suggests an extrarenal modulation of BP. However, losartan attenuated high BP and ameliorated magnesemia and the Na and K balance in Dahl SS rats, suggesting renal RAAS modulation.

The first part of our study was focused on a rat model of salt-sensitive, induced-hypertension by chronic infusion of a pressor dose of Ang II and a high Na diet. Ang II infusion alone induced hypertension with normomagnesemia, hypomagnesuria, increased Na reabsorption and K excretion, hypernatremia and increased fractional water excretion. It is worth noting that a gradual increase in BP occurred in half of the Ang II-infused rats, which has already been reported [20].

In our experiments, high doses of Ang II in rats fed a control diet, produced hypomagnesuria. This could be explained by the potentiating effects of Ang II over aldosterone secretion, which in distal convoluted tubule cells have been shown to stimulate Mg uptake [21], and/or by Ang-II-induced activation of the Na-dependent Mg exchanger in renal cells [22, 23]. Additionally, we observed normomagnesemia. This result contrasts with the observations of Touyz et al. [22], who reported hypomagnesemia after low dose Ang II infusions (150 ng/kg/min). The normomagnesemia observed could be a consequence of increased Mg reabsorption, as explained above, and/or increased Mg bone resorption. Bone resorption has been reported in bone marrow-derived mononuclear cells, after Ang II administration [24].

Ang II is also responsible, directly and indirectly, for the Na reabsorption and K secretion observed in our study. Directly, by its effect on active Na transport mechanisms in the epithelia [25, 26], and indirectly through its stimulatory action on aldosterone secretion [27], an effect demonstrated by the reduction in the urine Na/K ratio, a clinical sign of hyperaldosteronism.

Enhancement of Na reabsorption produces hypernatremia, which may raise plasma osmolality.

Baroreceptor induced-stimulation by high BP may be responsible for the increased fractional water excretion that we observed. This is in agreement with other studies that observed higher urinary volume excretion, decreased urinary osmolality and increased fluid intake after Ang II infusion [28]. Aldosterone may also be affecting intestinal water excretion, and in fact Ang-II-infused animals had reduced fecal excretion, probably due to increased water absorption in the colon [29].

Ang II-infused rats also presented retarded body weight gain, probably due to skeletal muscle wasting mediated by Ang II [30].
Ang II infusion has been shown to induce the pressor response, associated with increased Na reabsorption. The effects of Ang II are potentiated when the infusion is combined with a high salt diet [18].

Ang II-infused rats fed a high Na diet, presented a fast pressor response during the first week of treatment in all of the rats evaluated. As observed with Ang II infusion alone, increased BP was accompanied by hypomagnesuria, hypernatremia, hypernatriuria, and hyperkaluria. Additionally polydipsia, polyuria, and alterations in P, Zn and Cu balances were observed.

The hypomagnesuria induced by Ang II infusion was maintained when accompanied by a high Na diet. Under these conditions, aldosterone levels are not increased by the Na load.

The combined effects of Ang II, together with the increased Na supply may induce Na reabsorption and hence hypernatremia. Hypernatriuria in these Ang II Na-loaded rats seems to be secondary to a higher Na load on the kidneys, as observed in non-Ang II rats fed the same diet. Saline loading in dogs produces the same effects on Na excretion [31]. Polydipsia in Ang II-infused rats is a result of the additive effect of thirst-evoked sensation induced by both Ang II infusion and a high NaCl diet. It has been observed that rats increase their daily water intake in proportion to their dietary Na intake [32]. Polyuria and high fractional water excretion may be secondary to a higher water intake.

Hyperphosphaturia may suggest some effects on bone metabolism. In fact, it has been shown that a high NaCl diet may induce metabolic acidosis, which is responsible, among other things, for bone resorption [33, 34].

Ang II mediates the effects of the RAS system on kidney, vascular smooth muscle, adrenal cortex and brain, via the action of the Ang II receptor, type-1 (AT1R) or type-2 (AT2R) [35, 36]. Ang II-AT1R-mediated effects are responsible for vasoconstriction [37], aldosterone and vasopressin secretion [38], thirst and salt appetite [39]. Because losartan is used in the treatment of high BP, its effects on BP, and water and mineral balances were investigated. At 11 ± 0.8 mg/kg w/w given to Ang II-infused rats fed a high Na diet, it prevented high BP, as was also seen in Ang II-infused rats fed a control diet [40] and people with essential hypertension [41]. However, control of BP by losartan did not induce changes in Mg, or other mineral and water balances in our experiments. The absence of an effect of losartan on Mg and mineral balances may be masked by the high Na diet.

The low pressor response to losartan may be explained by inhibition of extrarrenal AT1R associated with RAS, an effect that could involve the central nervous system, or, more specifically, the sympathetic nervous system, which enhances sympathetic nervous transmission [42] to the kidney and heart, blunting arterial baroreflexes and hence inducing hypertension [43]. However, there are no conclusive studies showing that sympathetic nervous system activation contributes to the pressor response induced by exogenous Ang II [27, 42, 44].

Normal weight gain in losartan-Ang II-NaCl-treated rats was recovered, which is consistent with the blocking properties of losartan on the systemic effects of Ang II on the regulation of IGF-1 in skeletal muscle [30].

In the second part of our study, we measured BP and mineral metabolism in a genetic model of salt-sensitive hypertension: Dahl S/S rats fed a high Na diet [17]. A chronic rise in BP after salt loading may be related to abnormal central nervous system activation (increased sympathetic nervous system), renal Na and water handling, and hormonal changes [45]. As reported by others [46], a high Na diet in Dahl SS rats induces a time-dependent, slow pressor response. Moreover, in this study, a high Na diet in Dahl SS rats resulted in a dipsogenic and polyuric state, accompanied by an increase in fractional water excretion, hypomagnesuria, increased Na reabsorption, hypokaluria, and an increase in net K reabsorption.

High BP and SS in this model is caused by inappropriate activation of the renal RAAS, since the circulating RAAS has low activity [46, 47]. In fact, it has been shown that Dahl SS rats have increased levels of angiotensinogen [47] and Ang II [48] in kidney, and hypothalamus aldosterone [46], which may contribute to Mg and Na reabsorption.

In fact, the hypomagnesuria as seen in Dahl SS rats might be in response to aldosterone secretion [21], as a mechanism preventing hypomagnesemia. This is in contrast to the findings of others [13], where increased divalent cation excretion was observed in this model, which might be due to differences on dietary ions, especially K content.

It seems that high Na reabsorption in Dahl SS rats may also be attributed to their inability to
modulate Na reabsorption [49]. The higher Na reabsorption we found might have been responsible for the hypernatremia. Blunted regulation of NKCC2, as observed by others [50], could also explain higher K reabsorption in this strain of rat.

Phosphaturia found in this model was congruent with the findings of Goulding et al. [51] and our own observations on rats fed a high Na diet. The other minerals we examined did not show any significant differences between the experimental groups.

The reduction in body weight gain from the first week may be attributed to endogenous Ang II, as explained earlier.

It is not unexpected that suppression of the RAS could have effects on BP; indeed, when this strain was fed a high Na diet and treated with angiotensin-converting enzyme (ACE) inhibitors [52] or AT1R blockers [48, 53], BP was attenuated. Overexpression of AT1R occurs under a high Na diet in this strain [54]. Indeed, once hypertension had developed in Dahl SS rats fed a high Na diet, administration of losartan at 14.3 ± 0.6 mg/kg w/w attenuated, but did not normalize, BP by the end of the study. Morizane et al. [55] found that a similar dose of olmesartan (10 mg/kg) had no effect on BP. However, higher doses of AT1R blockers (30 and 100 mg/kg) attenuated and prevented salt-induced hypertension in this strain [48, 56]. Intracerebroventricular (ICV) infusion [57] or a high subcutaneous dose [53] of AT1R blockers could also prevent the sympathoexcitation and hypertension due to high-salt intake in SHR and Dahl SS rats.

We found that after losartan treatment, hypernatruria was exacerbated and percentage net reabsorption of Na was reduced, without effects on hypernatremia. Urine K excretion was decreased, but the percentage net reabsorption was normalized, supporting the idea of renal RAAS inhibition. However, there is a proposal that brain RAS is involved in sympathoexcitation and BP elevation caused by high salt intake in animal models of SS hypertension, including Dahl SS rats [53, 58].

After Ang II blockade, Mg was conserved by intestine and kidney, as reflected by a higher plasma Mg concentration. This may be a contributing factor in reducing BP, since hypomagnesemia may be related to hypertension [59].

Regarding P metabolism, phosphaturia was not corrected by losartan in this model, which may be related to the Na diet, and not to an Ang II effect.

Finally, losartan could ameliorate clinical parameters in Dahl SS rats fed a high Na diet. The diposogenic and hence polyuric state in these rats was significantly reduced, whereas fractional water excretion increased. The latter may be a consequence of the direct effect of Ang II on ADH secretion, as has been reported elsewhere [39]. With respect to body weight, weight gain was maintained in this group until it reached a plateau.

Overall, the results suggest that the Ang II-infused rat model of SS hypertension could be a consequence of an extrarenal RAS effect, and we speculate a major involvement of the central nervous system in the development of hypertension, specifically, sympathetic nervous system activation, since high BP and not Mg and mineral balance deregulation (specifically Na) were prevented after AT1R blockade. Hypomagnesuria was also evidenced after Ang II treatment, in the presence and absence of high salt intake, which may be responsible for normal magnesemia. An additive effect of Ang II and high Na load was seen on Sr, Zn and Cu balances.

In Dahl SS rats, SS hypertension could be a consequence of local renal RAAS deregulation, since BP is attenuated, and Na, K metabolism and magnesemia are ameliorated by losartan. Interestingly in this model, Mg metabolism may possibly be defective in this strain, and could be considered a contributing cause of the hypertension.

Disclosure

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