Salt-Dependent Renal Effects of an Angiotensin II Antagonist in Healthy Subjects

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This study was designed to evaluate in healthy volunteers the renal hemodynamic and tubular effects of the orally active angiotensin II receptor antagonist losartan (DuP 753 or MK 954). Losartan or a placebo was administered to 23 subjects maintained on a high-sodium (200 mmol/d) or a low-sodium (50 mmol/d) diet in a randomized, double-blind, crossover study. The two 6-day diet periods were separated by a 5-day washout period. On day 6, the subjects were water loaded, and blood pressure, renal hemodynamics, and urinary electrolyte excretion were measured for 6 hours after a single 100-mg oral dose of losartan (n=16) or placebo (n=7). Losartan induced no significant changes in blood pressure, glomerular filtration rate, or renal blood flow in these water-loaded subjects, whatever the sodium diet. In subjects on a low-salt diet, losartan markedly increased urinary sodium excretion from 115±9 to 207±21 μmol/min (P<.05). The fractional excretion of endogenous lithium was unchanged, suggesting no effect of losartan on the early proximal tubule in our experimental conditions. Losartan also increased urinary excretion of chloride, magnesium, calcium, and phosphate. In subjects on a high-salt diet, similar effects of losartan were observed, but the changes induced by the angiotensin II antagonist did not reach statistical significance. In addition, losartan demonstrated significant uricosuric properties with both sodium diets. Urinary uric acid excretion increased from 3.5±0.2 to 11.1±0.5 μmol/min (P<.01) in salt-depleted subjects and from 3.1±0.2 to 9.6±0.6 μmol/min (P<.01) in salt-loaded volunteers. This marked uricosuric effect of losartan was associated with a decrease in plasma uric acid. The time course of these changes in uric acid excretion suggests that it is a property of the parent compound rather than of a metabolite. These results demonstrate that in healthy subjects the angiotensin II receptor antagonist losartan is a natriuretic and kaliuretic compound, in particular during salt depletion. Moreover, losartan promotes uric acid excretion, an effect that appears to be independent of angiotensin II blockade.

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The kidneys contain large amounts of angiotensin II (Ang II) receptors distributed in the renal vasculature, glomeruli, proximal and distal tubules, and renomedullary interstitial cells.1,2 When activated, these receptors mediate the numerous effects of Ang II on renal hemodynamics, glomerular permeability, and urinary electrolyte excretion and thereby contribute to the control of blood pressure and renal sodium excretion. Thus, several investigators have demonstrated that intravenous or intra-arterial infusion of Ang II decreases renal plasma flow.3-6 At the glomerular site, Ang II constricts afferent as well as efferent arterioles and hence participates in the regulation of glomerular filtration rate (GFR).4,5,7 In addition, there is strong evidence that Ang II modulates renal filtration through direct effects on the glomerulus by reducing the ultrafiltration coefficient.4,5 Finally, Ang II per se induces sodium retention via a direct effect on renal tubules to stimulate proximal tubular sodium reabsorption.5-10

Conversely, blockade of the renin-angiotensin system with angiotensin converting enzyme (ACE) inhibitors causes an increase in renal blood flow, whereas GFR remains unchanged.11,12 These effects are generally more pronounced in animals or subjects whose renin-angiotensin system is stimulated, for example, by salt depletion.11,12 In animal models of chronic renal failure, converting enzyme inhibitors have also been shown to exert a protective effect on GFR.13,14 This effect is linked to a decrease in glomerular capillary pressure resulting from an attenuation of the action of Ang II on the afferent and efferent arterioles of the glomeruli.13 Finally, ACE inhibitors tend to enhance both absolute and fractional sodium excretion.7,15 These renal properties of ACE inhibitors are more likely due to the inhibition of Ang II generation. However, ACE inhibitors simultaneously inhibit bradykinin degradation and increase prostaglandin levels.16 Therefore, the exact contribution of Ang II reduction to the effects of ACE inhibitors on renal function is difficult to appreciate.

Blockade of renal Ang II receptors with specific antagonists should provide a better approach to under-
standing the renal properties of Ang II. Administration of saralasin, the first peptidic competitive antagonist of Ang II, has been shown to increase renal plasma flow, GFR, and sodium excretion.\textsuperscript{11} However, because this peptide is also a partial receptor agonist, opposite effects on renal hemodynamics and sodium excretion have sometimes been reported.\textsuperscript{18,19} Recently, several new nonpeptide imidazole Ang II receptor antagonists have been developed.\textsuperscript{20} These compounds have a high affinity for the type 1 Ang II receptors, have no agonistic activity, and have no effect on kinin or prostaglandin metabolism.\textsuperscript{21,22} Among these antagonists, losartan (DuP 753 or MK 954) has been shown to effectively inhibit Ang II binding in animal and human tissues.\textsuperscript{21,24,25} Recent studies have demonstrated that losartan displaces practically all Ang II bound to specific renal receptors.\textsuperscript{25} Administration of losartan to renal hypertensive rats causes a dose-dependent decrease in blood pressure.\textsuperscript{23} Moreover, losartan appears to increase renal blood flow and sodium excretion in some rat models\textsuperscript{26} and in normotensive and hypertensive dogs.\textsuperscript{27,28} In healthy volunteers, losartan abolishes the blood pressure response to exogenous Ang II, whereas in hypertensive patients, the first comparative studies suggest that the ability of losartan to lower blood pressure is comparable to that of an ACE inhibitor.\textsuperscript{29,30}

So far, the renal effects of losartan on renal function have never been evaluated in human subjects. The purpose of the present study was to compare the renal hemodynamic and tubular effects of losartan with those of a placebo in normal healthy volunteers on a low- and high-sodium diet. The trial was carried out according to a double-blind crossover design.

Methods

Twenty-four healthy male volunteers aged 21 to 37 years participated in this study. Each volunteer had a medical history taken and underwent a complete physical examination. Routine laboratory tests were done before and after administration of the drug. The nature and purpose of the study had been explained, and a written informed consent had been previously obtained. The protocol was approved by the Hospital Ethics Committee.

Study Design

Subjects were studied on a high-sodium (HS; 200 mmol sodium per day, 100 mmol potassium per day, 3500 calories per day) or low-sodium (LS; 50 mmol sodium per day, 100 mmol potassium per day, 3500 calories per day) diet using a randomized, crossover design. Two 6-day diet periods were separated by a 5-day washout period. The diets were provided under supervision of a dietician by the hospital restaurant where the subjects ate all their meals. Compliance to the diet was evaluated by 24-hour urine collections during the last 2 days of the diet.

On day 6 of each diet, the volunteers came to the hospital at 7 AM after an overnight fast to undergo clearance studies. On arrival, they were made comfortable on a bed. They remained supine, except for voiding, and fasted throughout the study procedure. Two intravenous catheters were inserted into antecubital veins, one for the infusion of inulin and p-aminohippuric acid (PAH) in a glucose/saline solution and a second into the contralateral forearm for drawing blood.

Between 7 and 8 AM, the volunteers drank an oral water load of 12 mL/kg. After a priming dose, the intravenous infusion of inulin and PAH was started; the infusions were calculated to provide plasma concentrations of approximately 400 and 20 \mu g/mL, respectively. The volunteers were asked to empty their bladder spontaneously every 30 minutes. After voiding, an equivalent amount of water was given orally to sustain a high urine output. After a 1.5-hour equilibration period, 30-minute baseline measurements were performed until the volunteers were in a steady state. The steady state was obtained when the volume of two consecutive urine collections was within 1 mL/min. At the end of the second baseline period (T0), the volunteers received in a randomized, double-blind fashion either a placebo (n=8) or a single oral dose of the Ang II antagonist losartan (100 mg, n=16). All subjects took either placebo or losartan twice on the two study days on the different diets.

Blood pressure, heart rate, urinary electrolyte excretion, and clearances of inulin (GFR) and PAH (effective renal plasma flow, ERPF) were measured at 30-minute intervals for 6 hours. Blood pressure was obtained with subjects in a supine position and was measured by the conventional auscultatory method. Blood samples for the measurement of electrolytes, osmolality, inulin, and PAH were drawn at half-hourly intervals. Blood samples for the determination of plasma renin activity (PRA) and plasma Ang II and aldosterone levels were drawn at T0 and again 4 and 6 hours after drug intake.

Drugs and Chemicals

Losartan was provided by Merck Sharp & Dohme Research Laboratories, Rahway, NJ. Inulin was purchased from Laevosan Gesellschaft, Linz, Donau, Austria, and PAH (Nephrotest, sodium salt of p-aminohippuric acid) from Biologische Arbeitsgemeinschaft GmbH, Lich, Essen, Germany.

Analytic Methods

Plasma and urinary inulin concentrations were measured by a microadaptation of a diphenylamine procedure on an autoanalyzer (Technicon, Tarrytown, NY).\textsuperscript{11} PRA concentrations were determined by spectrophotometry.\textsuperscript{31} Plasma and urinary sodium, potassium, and chloride were analyzed with selective electrodes (Helse, Beckman). Calcium, phosphate, uric acid, and magnesium were quantified photometrically (RAXT, Technicon). Endogenous trace lithium was measured by electrothermal absorption spectrophotometry as described by Durr et al.\textsuperscript{32} A hand-held digital pH meter in automatic temperature compensation mode (model 105, Corning, Halstead, Essex, UK) was used for the determination of urinary pH. Plasma and urinary osmolality were determined as freezing point depression on a microosmometer (Roebling, Berlin, Germany).

Aldosterone was measured by a direct radioimmunoassay.\textsuperscript{34} For the determination of PRA, generated angiotensin I was trapped and quantitated by high-affinity antibodies.\textsuperscript{35} For the quantitation of plasma Ang II levels, a new method using monoclonal antibodies against Ang II was used.\textsuperscript{36}
Renal Parameters and Statistical Evaluation

Clearances (C) were calculated by the traditional method using the formula C=kV/P, where U and P represent urine and plasma concentrations of x, and V is the urine flow rate in milliliters per minute. Fractional excretion (FE) was calculated as the clearance of x divided by the clearance of inulin or GFR (C/\text{GFR}). Plasma concentrations used for clearance determinations were calculated by averaging initial and final values of each clearance period. Filtration fraction was calculated as the ratio of inulin over PAH clearances (GFR/ERPF). Free water clearance (\text{C}_{w}) was calculated as urine flow rate (in milliliters per minute) minus osmolar clearance (\text{C}_{\text{o}}). Delivery of chloride to the distal tubule was calculated as (\text{C}_{w}+\text{C}_{\text{Na}})/\text{GFR} \times 100. Fractional sodium reabsorption in the post-proximal tubule was estimated as [(\text{FE}_{\text{Na}}-\text{FE}_{\text{Na}})/\text{FE}_{\text{Na}}] \times 100. Absolute distal reabsorption of sodium was estimated by the difference between the clearances of lithium and sodium multiplied by the plasma concentration of sodium. The absolute rate of proximal reabsorption of isotonic fluid corresponds to the difference between the clearance of inulin and that of lithium.

All results are expressed as mean ± SEM. The statistical significance of differences was evaluated by a two-way analysis of variance followed by the Fisher least significant difference test, with a value of P < .05 as the minimum level of significance. We looked for significant changes between the groups.

Results

The administration of losartan was well tolerated in all 16 subjects who were randomized to receive the Ang II antagonist. In the placebo group, one volunteer did not complete the study because he developed a benign, spontaneously reversible but symptomatic arrhythmia during the first phase on placebo. Thus, only seven subjects were included in the control group.

Systemic and Renal Hemodynamics

The changes in systolic and diastolic blood pressures, GFR, ERPF, and filtration fraction observed after administration of placebo or 100 mg losartan are shown in Table 1. At baseline, ie, before administration of the drug, systolic and diastolic blood pressures were both significantly lower in the losartan group compared with the placebo group (P < .05). A slight decrease in systolic and diastolic blood pressures was observed after administration of losartan in volunteers on the LS or HS diet, but these changes were not significant from baseline.

There was no significant change in GFR, ERPF, and filtration fraction with losartan. The slight increase in GFR and ERPF observed in salt-depleted volunteers on losartan was comparable to that obtained with placebo.

Hormonal Effects of Losartan

The changes in PRA and plasma Ang II and aldosterone levels are also shown in Table 1. As expected, the baseline activity of the renin-angiotensin-aldosterone system was stimulated in volunteers on an LS intake. Thus, PRA and plasma Ang II and aldosterone levels were significantly increased during the LS compared with the HS phase (P < .05). A slight decrease in systolic and diastolic blood pressures was observed after administration of losartan in volunteers on the LS or HS diet, but these changes were not significant from baseline.

The response of the renin-angiotensin system to Ang II blockade was more pronounced during salt restriction. A decrease in plasma aldosterone levels was also observed after losartan, but this change was not different from that obtained in the placebo group.

Fluid and Solute Excretion

The changes in urinary fluid and electrolyte excretion with the LS and HS diets are summarized in Tables 2 and 3.

Low-salt diet. The subjects received an oral water load to maintain a urine flow rate greater than 10 mL/min. In the placebo group, urine flow rate was at 12.6 ± 0.7 mL/min at baseline and did not increase throughout the study day. In this group, free water and osmolar clearances also remained unchanged. In the losartan group,
TABLE 2. Effect of Losartan on Fluid and Electrolyte Excretion in Normal Volunteers

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Placebo Baseline</th>
<th>Placebo Peak</th>
<th>Losartan Baseline</th>
<th>Losartan Peak</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>U_{NaV}</td>
<td>189±28</td>
<td>241±29</td>
<td>115±9.3</td>
<td>207±21</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{Kv}</td>
<td>5.11±0.3</td>
<td>5.56±0.5</td>
<td>4.54±0.2</td>
<td>5.35±0.18</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{Lv}</td>
<td>112±7.3</td>
<td>98±2.8</td>
<td>117±6.9</td>
<td>155±11</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{Ov}</td>
<td>147±15</td>
<td>138±15</td>
<td>106±11</td>
<td>155±22</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{V}</td>
<td>6.73±0.6</td>
<td>8.09±1.4</td>
<td>6.42±0.5</td>
<td>9.99±0.9</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{Mv}</td>
<td>14.9±1.8</td>
<td>21.2±1.2</td>
<td>12.4±1.0</td>
<td>21.8±1.7</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{Cv}</td>
<td>3.93±0.5</td>
<td>3.60±0.4</td>
<td>3.26±0.2</td>
<td>4.68±0.2</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>U_{UV}</td>
<td>4.11±0.3</td>
<td>3.98±0.2</td>
<td>3.56±0.2</td>
<td>11.1±0.5</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>Urinary pH</td>
<td>6.71±0.09</td>
<td>6.90±0.08</td>
<td>6.80±0.06</td>
<td>6.94±0.03</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>UV (mL/min)</td>
<td>12.6±0.7</td>
<td>12.8±0.6</td>
<td>10.5±0.4</td>
<td>13.1±0.6</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>Cl_{O}</td>
<td>9.22±0.6</td>
<td>9.23±0.5</td>
<td>7.61±0.4</td>
<td>9.36±0.5</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>Cl_{Ox}</td>
<td>3.41±0.1</td>
<td>3.46±0.1</td>
<td>2.91±0.2</td>
<td>3.74±0.2</td>
<td>*&lt;.05</td>
</tr>
<tr>
<td>Cl_{Ou}</td>
<td>35.5±2.9</td>
<td>34.2±4.2</td>
<td>29.0±2.6</td>
<td>31.3±2.2</td>
<td>*&lt;.05</td>
</tr>
</tbody>
</table>

V, volume; Osm, osmolar. Electrolyte excretions and clearances shown as U_{xV}, urinary excretion rate of x in micromoles per minute, and Cl_{x}, clearance of x in milliliters per minute.

For urine output was at 10.5±0.4 mL/min at baseline and increased significantly during the first 2 hours, with a peak value at 13.1±0.6 mL/min (P<.05 vs T0). Free water and osmolar clearances also increased significantly during the first hours after the administration of losartan (Table 2).

On day 5 of the diet, 24-hour urinary sodium excretion was 35±4.9 mmol/d in the placebo group and 36.1±3.4 mmol/d in the losartan group. As shown in Fig 1 (top), urinary sodium excretion of the subjects on placebo increased slightly during the first hour but remained stable thereafter. These changes were not significantly different from time 0. In contrast, sodium excretion increased significantly in the losartan group from the first to the sixth hour after drug intake. After baseline subtraction, the cumulative 6-hour sodium excretion was 16±6.6 mmol with placebo and 29.5±5.9 mmol with losartan (P<.02, Fig 1, bottom). Similarly, fractional excretion of sodium increased significantly with losartan, whereas no changes were found with placebo (Table 3).

The clearance of endogenous lithium was determined as an index of proximal sodium reabsorption. Urinary lithium excretion did not vary with placebo. After

TABLE 3. Effect of Losartan on Renal Function in Normal Volunteers

<table>
<thead>
<tr>
<th>Fractional excretion</th>
<th>Placebo Baseline 2 Hours</th>
<th>Placebo 2 Hours</th>
<th>Losartan Baseline 2 Hours</th>
<th>Losartan 2 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE_{Na} (%)</td>
<td>1.21±0.2</td>
<td>1.48±0.2</td>
<td>0.76±0.06</td>
<td>1.48±0.13</td>
</tr>
<tr>
<td>FE_{K} (%)</td>
<td>30.6±2.4</td>
<td>28.6±3.0</td>
<td>26.0±1.6</td>
<td>27.5±2.0</td>
</tr>
<tr>
<td>FE_{Li} (%)</td>
<td>25.9±3.2</td>
<td>21.3±1.2</td>
<td>27.6±1.8</td>
<td>34.4±1.9*</td>
</tr>
<tr>
<td>FE_{Ca} (%)</td>
<td>1.15±0.13</td>
<td>1.1±0.09</td>
<td>0.92±0.05</td>
<td>1.23±0.17</td>
</tr>
<tr>
<td>FE_{Cl} (%)</td>
<td>2.58±0.3</td>
<td>3.16±0.6</td>
<td>2.55±0.2</td>
<td>3.62±0.3</td>
</tr>
<tr>
<td>FE_{Cr} (%)</td>
<td>11.4±2.4</td>
<td>12.6±1.8</td>
<td>9.98±0.9</td>
<td>13.6±1.0</td>
</tr>
<tr>
<td>FE_{Mg} (%)</td>
<td>3.96±0.4</td>
<td>4.18±0.3</td>
<td>3.93±0.3</td>
<td>5.18±0.3</td>
</tr>
<tr>
<td>FE_{UM} (%)</td>
<td>11.3±0.8</td>
<td>10.9±0.9</td>
<td>10.5±0.9</td>
<td>30.3±2.21§</td>
</tr>
<tr>
<td>PRR (mL/min)</td>
<td>75.2±8.8</td>
<td>77.5±5.0</td>
<td>73.7±3.1</td>
<td>80.0±3.7</td>
</tr>
<tr>
<td>ADREN_{Na} (mmol/min)</td>
<td>4.66±0.3</td>
<td>4.41±0.5</td>
<td>3.87±0.3</td>
<td>4.11±0.27</td>
</tr>
<tr>
<td>FDR_{Na} (%)</td>
<td>95.8±0.7</td>
<td>94.4±1.0</td>
<td>97.0±0.2</td>
<td>95.0±0.15*</td>
</tr>
<tr>
<td>DD_{OCl} (%)</td>
<td>10.4±1.9</td>
<td>9.8±0.6</td>
<td>8.0±0.8</td>
<td>10.1±0.8</td>
</tr>
</tbody>
</table>

*P<.05 vs baseline.
†P<.05 vs placebo.
‡P<.01 vs baseline.
§P<.01 vs placebo.

FE_{x}, fractional excretion of x; PRR, proximal reabsorption rate; ADREN_{Na}, absolute distal reabsorption rate of sodium; FDR_{Na}, fractional distal reabsorption of sodium; DD_{OCl}, distal delivery of chloride.
administration of losartan, however, urinary lithium excretion increased significantly but only between the fourth and sixth hour after drug intake. Nonetheless, lithium clearance and the fractional excretion of endogenous lithium were not modified by the administration of losartan or placebo (Tables 2 and 3). Similarly, there was no change in proximal reabsorption rate with placebo and losartan. In contrast, a significant decrease in fractional distal reabsorption of sodium was found in the losartan group, suggesting a postproximal effect of the Ang II antagonist.

Urinary chloride excretion did not vary over 6 hours in the placebo group on the LS diet. With losartan, a transient increase in chloruria was observed, with a peak at 2 hours. However, analysis of chloride excretion in salt-depleted subjects was limited by the fact that many chloride concentrations were below the level of detection. Chloride delivery to the distal tubule tended to increase with losartan, but the changes were not statistically significant (Table 3). The changes in potassium excretion were shown in Fig 2 (top). The kaliuresis tended to decrease over the 6-hour observation in the placebo group. In contrast, a significant but transient rise in potassium excretion was seen after administration of losartan. Similar variations in fractional excretion of potassium were found (Table 3).

Among divalent ions, a transient increase in calcium excretion was observed with losartan but not with placebo ($P<.05$, Table 2). There was, however, no significant change in the fractional excretion of calcium.

Phosphate excretion rose after administration of the Ang II antagonist. The change in phosphate excretion became significant from the second to the sixth hour of investigation. However, a similar increase in phosphaturia was seen in the placebo group. As shown in Fig 2 (bottom), magnesium excretion was significantly enhanced during the first 3 hours after the administration of losartan. Fractional excretion of magnesium rose significantly from 3.93±0.3% at T0 to 5.18±0.3% at 2 hours ($P<.05$ within the group and $P<.05$ vs placebo).

Fig 3 (top) represents uric acid excretion with placebo or losartan. Urate excretion rate increased by a factor of 3 with losartan, from 3.56±0.2 to 11.1±0.5 μmol/min ($P<.01$), whereas it did not change with placebo (Table 2). The fractional excretion of uric acid also rose by a factor of 3. The uricosuric effect peaked at 2 hours, and no effect was observed beyond the fourth hour. Plasma uric acid (Fig 3, bottom) decreased slightly with losartan, from 322±12 μmol/L at T0 to 269±15 μmol/L at 6 hours, but the fall was not significant. As shown in Table 2, urinary pH was unchanged in the placebo and losartan groups.

High-salt diet. When the subjects were on the HS diet, several effects of losartan were similar to those obtained on the LS diet, but the response was generally attenuated and was often not statistically significant. After 5 days of the HS diet, the 24-hour urinary sodium excretion was 253.4±9.7 mmol/d in the placebo group (n=7) and 222.2±8.4 mmol/d in the losartan group (n=16). As shown in Table 2, urine output increased with losartan,
FIG 3. Line graphs show effects of losartan and placebo on urinary uric acid excretion (top) and plasma uric acid levels (bottom). ■, Placebo/low-salt diet; □, losartan/low-salt diet; ●, placebo/high-salt diet; ○, losartan/high-salt diet. *P<.05, **P<.01 vs time 0; +P<.05, ++P<.01, losartan vs placebo.

Discussion

The results of the present study demonstrate that a single dose of losartan has no significant systemic and renal hemodynamic effects in water-loaded healthy subjects maintained on a HS or LS diet. However, Ang II blockade with losartan induced significant changes in the renal handling of water, sodium, potassium, calcium, phosphate, magnesium, and uric acid.

Hemodynamic Effects

Although systolic and diastolic blood pressures tended to decrease by 6 mm Hg in subjects on an LS diet, the fall in systolic blood pressure was not significant, and the changes in diastolic pressure were only occasionally significant. The lack of a significant blood pressure reduction during sodium restriction may seem surprising, because blood pressure control relies very much on Ang II in this condition. Compliance to the diet was strictly monitored. At the end of salt depletion, 24-hour urinary sodium excretion was clearly less than 50 mmol in all subjects. The stability of blood pressure may reside rather in the fact that blood pressure was always measured with subjects in a supine position. Early findings with the administration of the Ang II antagonist saralasin to salt-depleted subjects have indeed shown that blood pressure does not necessarily drop with subjects in a supine position, whereas dramatic falls in blood pressure may occur after they assume the erect position. Volume expansion on the day of the renal studies is certainly another reason why blood pressure did not decrease in our salt-depleted subjects. In this regard, a dose-dependent fall in blood pressure in response to losartan has been reported recently in non-water-loaded healthy volunteers subjected to salt depletion with an LS diet and the concurrent administration of furosemide for 3 days.

Similarly, we could not demonstrate any effect of losartan on GFR or renal plasma flow, whatever the sodium diet. The absence of changes in GFR could be anticipated from the results of several studies performed with ACE inhibitors or Ang II antagonists. Indeed, there is now general agreement that ACE inhibition increases renal blood flow but has little if any effect on GFR. Moreover, Ang II blockade with saralasin does not substantially alter GFR in sodium-repleted dogs and may even induce a fall in GFR during salt depletion. In more recent studies, losartan had no effect on GFR in volume-expanded, anesthetized normotensive and spontaneously hypertensive rats, whereas it increased GFR dose dependently in conscious trained Penn hypertensive dogs and in normotensive dogs.

More surprising is the absence of a significant increase in renal blood flow after administration of losartan. Indeed, renal vasodilation characterized by a rise in renal blood flow and a fall in renal vascular resistance has consistently been found during ACE inhibition or Ang II receptor blockade with saralasin or losartan. There are several possible reasons why renal blood flow did not increase in our study even when the subjects were on an LS diet. The first factor that might have interfered with the vasodilator effect of losartan is volume expansion. Indeed, hypervolemia inhibits the activity of several vasoconstrictor systems,
including the renin-angiotensin system and vasopressin, thereby producing renal vasodilation. Hence, demonstration of a vasodilator effect of losartan may become very difficult as kidneys are already vasodilated. Under the same conditions of volume expansion, no increase in renal blood flow has been observed in normotensive rats after administration of losartan.32 Similarly, no change in renal blood flow was found in anesthetized rats.40 Among the other possibilities, losartan may have interfered with renal PAH secretion. An increase in renal blood flow measured with the PAH technique has been reported in hypertensive dogs treated with losartan.27 However, the accuracy of PAH clearance as a measure of renal plasma flow during losartan therapy has not been validated in these animals. A PAH-losartan interference may also be species dependent and specific to the human gender. Validation studies are needed to clarify this observation.

**Tubular Effects**

The most relevant findings of the present study are related to the influence of losartan on urinary electrolyte excretion. Indeed, losartan significantly increased urinary sodium, potassium, magnesium, calcium, and phosphate excretions, whereas placebo had no effect. Some arguments suggest that the mechanism whereby losartan increases urinary electrolyte excretion resides in the blockade of tubular Ang II receptors. On one hand, there was no change in GFR accounting for these effects, and the fractional excretion of these ions was also increased. On the other hand, these effects of losartan were always significant during salt restriction and attenuated during salt repletion, suggesting that the expression of these changes relies very much on the baseline activity of the renin-angiotensin system. Except for sodium and phosphate, the time course of these changes was characterized by an acute and transient elevation, with a peak at approximately 2 hours and a return to baseline at 4 hours. This excretion pattern is somewhat surprising, because Ang II receptor blockade with 100 mg losartan is supposed to last longer than 4 hours.41 The degree of Ang II blockade induced by receptor antagonists cannot easily be quantitated unless exogenous Ang II is infused.29 However, direct blockade of angiotensin receptors is generally associated with increases in PRA and Ang II levels that are linked to the suppression of the Ang II feedback on renin secretion. These two parameters can be used as indirect measures of Ang II blockade. Four hours after the administration of losartan, PRA and plasma Ang II levels were markedly elevated during the LS diet (fivefold to sixfold increase from baseline) as well as during the HS diet (threefold to fourfold increase). Even 6 hours after drug intake, PRA and plasma Ang II levels were significantly higher than baseline, although both values were already 50% lower than those measured 2 hours earlier. Accordingly, incomplete Ang II receptor blockade does not appear to be the main reason why the tubular effects of losartan are not sustained for 6 hours. Slight decreases in the plasma concentrations of these ions or activation of other counterregulatory mechanisms may have contributed to offset the drug-induced renal excretion.

The natriuretic effect of losartan is in agreement with many previous findings obtained with saralasin and converting enzyme inhibitors.7,15 More recently, losartan has been shown to induce sodium excretion in the rat and dog.26-28,40,43 The specific nephron site of action of the Ang II antagonist is not well established. Recent studies have suggested that losartan decreases fluid and electrolyte absorption in the first segment of the proximal convoluted tubule, a tubular segment that contains large amounts of Ang II receptors.40,43 However, a contribution of more distal sites has also been postulated to explain the natriuretic properties of ACE inhibitors or Ang II antagonists.3,44 In contrast to previous animal studies, our results suggest that in water-loaded subjects, the natriuresis induced by losartan is not linked to a direct effect of losartan on early proximal tubules but to changes in sodium reabsorption in postproximal segments.

The absence of a significant increase in fractional excretion of lithium in our experimental conditions does not completely rule out a possible effect of losartan on the early proximal tubule in other circumstances. During volume expansion, 50% to 60% of the glomerular filtrate may escape proximal reabsorption.42 Consequently, fractional excretion of lithium is very high and a further rise is difficult to demonstrate. Thus, the fractional excretion of lithium observed in our study (approximately 30%) is much greater than that reported with the same technique in normally hydrated subjects (15% to 20%).46 The discrepancy between our results and earlier findings may also be due to technical differences. Indeed, in contrast to other studies, fractional excretion of lithium was calculated on the basis of plasma and urinary endogenous trace lithium.39 This approach has the advantage of avoiding the administration of exogenous lithium, which per se may increase sodium excretion. Some preliminary studies have shown that this technique is very effective in demonstrating changes in proximal sodium reabsorption induced by various maneuvers or diseases.33,47 Therefore, it does not appear that this new technique is responsible for this apparent discrepancy.

It is not inconceivable that Ang II receptor blockade affects postproximal sodium reabsorption. Autoradiographic studies have demonstrated the presence of Ang II receptors in large amounts in the outer medulla, which contains medullary and cortical thick ascending limbs as well as thin limbs of Henle’s loops.1,2 Moreover, the medullary vasa recta flow is under the control of Ang II.44 A decrease in sodium reabsorption in the loop of Henle could be mediated by an increased perfusion of these vasa recta. The excretion pattern of the other electrolytes is also compatible with a postproximal effect of losartan. Thus, magnesium excretion increased markedly in salt-depleted volunteers treated with losartan. Although 20% to 30% of the filtered magnesium is reabsorbed in the proximal tubule, close to 70% of magnesium reabsorption occurs in the proximal straight tubule and in the thick ascending limb of Henle, with little if any tubular handling beyond the distal tubule. A decrease in proximal reabsorption of magnesium is generally not accompanied by an increased magnesiumuria, because the thick ascending limb of Henle compensates the proximal loss. The presence of magnesiumuria with losartan therefore points to an effect of this agent on magnesium reabsorption in the loop.
Losartan also enhanced urinary calcium and phosphate excretions in salt-restricted subjects. The great bulk of calcium and phosphate reabsorption occurs in the proximal convoluted tubule, but 20% to 30% of the filtered load is handled by proximal segments. The presence of a losartan-induced calciuria also points to a postproximal effect of the Ang II antagonist. Indeed, administration of proximally acting diuretics, such as acetazolamide, inhibits sodium and calcium reabsorption in the proximal tubule and enhances the distal delivery of calcium. Yet calcium excretion is not increased, because there appears to be a preferential reabsorption of the delivered calcium in the distal tubule.49 This observation again indicates that the calciuria observed with losartan results at least in part from a decrease in postproximal reabsorption.

Surprisingly, losartan significantly increased urinary potassium during salt depletion and to a lesser degree in the salt-loaded volunteers. In general, prolonged blockade of the renin-angiotensin system with ACE inhibitors is rather associated with a potassium retention due to a decrease in plasma aldosterone levels. Our observation is not unique. Indeed, Ang II receptor blockade with losartan in the dog has also been shown to reduce dose dependently the fractional reabsorption of potassium.27-28 A kaliuresis has been noted in the Munich-Wistar rat submitted to whole-kidney clearances.42 There are some possible explanations for this kaliuretic effect of losartan. First, the mother compound losartan is a potassium salt, and its administration will transiently increase the filtered load of potassium. The dose dependency obtained in the dog and the very transient pattern observed in our subjects, corresponding to the half-life of losartan, would go along with this explanation.

Besides, the increased potassium loss may be consecutive to an increased sodium delivery to the distal tubule and to an increased sodium-potassium exchange at this site of the nephron. In this latter case, however, a sustained but not transient effect on potassium excretion would be expected, because sodium excretion increased for 6 hours after administration of losartan. It is possible that other yet unknown mechanisms may have played a role in promoting potassium excretion.

Among the effects of losartan on renal tubular function, its ability to promote uric acid excretion is certainly the most impressive, observed in the present study as urinary uric acid excretion increased by 300% during treatment. Interestingly, this effect developed during salt restriction as well as during salt repletion and was associated with a decrease in plasma uric acid levels. A similar effect of losartan on uric acid has been reported recently in healthy male Japanese volunteers.50 In that study, uricosuria was dose dependent and persisted during repeated administration. In accordance with these results, uricosuria increased mainly during the 4 hours after drug intake. The mechanism or mechanisms whereby losartan enhances urate excretion are not known. Uric acid excretion depends on several factors, including extracellular fluid volume, urine flow rate, and urinary pH. As discussed above, our volunteers were volume expanded, and urine output increased significantly with losartan, whereas urinary pH was not affected. However, the changes in urine flow rate (+30% at peak during LS) are out of proportion and cannot explain the threefold increase in uric acid excretion. An effect of losartan on the renal tubular handling of uric acid must therefore be postulated. The renal handling of uric acid is characterized by filtration, proximal tubular reabsorption, secretion, and possibly postsecretory reabsorption. As discussed above, GFR was unchanged during treatment, and we have no evidence for a decrease in proximal reabsorption with losartan. Under these conditions, the increase in uric acid excretion is probably due to a stimulation of tubular secretion or to an inhibition of the postsecretory reabsorption process.

Another important question is whether this uricosuric effect of losartan is linked to Ang II receptor blockade or to a proper effect of the drug. The time course of the uricosuric effect is compatible with a direct effect of the parent compound, and our finding that uricosuria develops whatever the degree of activity of the renin-angiotensin system would actually suggest that this effect is not Ang II dependent. Yet the data are not sufficient to entirely rule out this latter possibility. Infusion of low doses of Ang II reduces uric acid excretion, probably by increasing its proximal reabsorption (personal unpublished observation). Moreover, a slight increase in uric acid excretion (approximately 30%) has been observed during ACE inhibition.51,52 Yet there is no clear evidence that uric acid excretion is directly under the control of the renin-angiotensin system.

Taken together, these results demonstrate that the Ang II antagonist losartan promotes sodium excretion and has no effect on renal hemodynamics in water-loaded healthy subjects. This natriuretic property of losartan may play an important role in its ability to lower blood pressure in hypertensive patients.30 In addition, losartan increases uric acid excretion and lowers plasma uric acid levels. Clinically, this effect might become useful, because hyperuricemia is found in close to 30% of patients with untreated mild hypertension and may contribute to the deterioration of renal function.53 The results of larger studies will tell us the real effect of this drug-induced increase in uric acid excretion.

References


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