Salt sensitivity in experimental thyroid disorders in rats

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1Facultad de Medicina, Departamento de Fisiología, Granada, Spain; 2Servicio de Nefrología, Unidad Experimental, Hospital Virgen de las Nieves, Granada; 3Departamento de Ciencias de la Salud, Universidad de Jaén, Jaén; and 4Facultad de Medicina, Departamento de Anatomía Patológica e Instituto de Biomedicina Regenerativa, Granada, Spain

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Perez-Abud R, Rodríguez-Gómez I, Villarejo AB, Moreno JM, Wangensteen R, Tassi M, O’Valle F, Osuna A, Vargas F. Salt sensitivity in experimental thyroid disorders in rats. Am J Physiol Endocrinol Metab 301: E281–E287, 2011. First published April 26, 2011; doi:10.1152/ajpendo.00690.2010.—This study assessed salt sensitivity, analyzing the effects of an increased saline intake on hemodynamic, morphological, and oxidative stress and renal variables in experimental thyroid disorders. Six groups of male Wistar rats were used: control, hypothyroid, hyperthyroid, and the same groups treated with salt (8% via food intake). Body weight, blood pressure (BP), and heart rate (HR) were recorded weekly for 6 wk. Finally, BP and HR were recorded directly, and morphological, metabolic, plasma, and renal variables were measured. High-salt intake increased BP in thyroxine-treated rats but not in control or hyperthyroid rats. High-salt intake increased cardiac mass in all groups, with a greater increase in hyperthyroid rats. Urinary isoprostanes and H2O2 were higher in hyperthyroid rats and were augmented by high-salt intake in all groups, especially in hyperthyroid rats. High-salt intake reduced plasma thyroid hormone levels in hyperthyroid rats. Proteinuria was increased in hyperthyroid rats and aggravated by high-salt intake. Urinary levels of aminopeptidases (glutamyl-, alanyl-, aspartyl-, and cystylaminopeptidase) were increased in hyperthyroid rats. All aminopeptidases were increased by salt intake in hyperthyroid rats but not in hypothyroid rats. In summary, hyperthyroid rats have enhanced salt sensitivity, and high-salt intake produces increased BP, cardiac hypertrophy, oxidative stress, and signs of renal injury. In contrast, hypothyroid rats are resistant to salt-induced BP elevation and renal injury signs. Urinary aminopeptidases are suitable biomarkers of renal injury.

These data indicate that thyroid disorders are accompanied by major changes in renal sodium handling.

Hypothyroidism reduces blood pressure (BP) and prevents experimental hypertension (46), whereas T4 administration to rats increases BP (6) and accelerates the course of hypertension (46). Although numerous systems can influence BP over the short term, long-term BP regulation depends on the renal excretion of sodium (22). An increased BP in response to dietary sodium (salt sensitivity) has been widely reported in humans and animals and is proposed as a major factor in the pathogenesis of hypertension (4).

With this background, the objective of this study was to examine the possible modulatory role of thyroid hormones on salt sensitivity in rats, analyzing the response of BP, morphological variables, renal function, and oxidative stress to increased saline intake in hyper- and hypothyroid rats.

METHODS

Animals. Male Wistar rats born and raised in the experimental animal service of the University of Granada were used. Experiments were performed according to European Union guidelines for the ethical care of animals. Experimental procedures were reviewed and approved by the Ethics Committee of the University of Granada. Rats initially weighing 250–280 g were maintained on standard chow and tap water ad libitum except where stated. The animals were divided into the following six groups: control, hyperthyroid, and hypothyroid rats allocated either to a diet of normal chow with 0.4% NaCl or to high-salt chow with 8% NaCl (n = 8 each group). For the control group, the high-salt diet was prepared by mixing 76 g of NaCl with 924 g of chow. In the hyper- and hypothyroid groups, the concentration of NaCl in the chow was adjusted according to weekly measurements of body weight and daily food intake records to deliver the same dosage as in control rats.

Hypothyroidism was induced by injecting T4 (75 µg·rat−1·day−1 sc), whereas hypothyroidism was induced by the continuous administration of 0.03% methimazole via drinking water, as previously reported (15, 43, 46). These treatments were administered for 6 wk. Tail systolic BP (SBP) and heart rate (HR) were recorded by using tail-cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter; Letica, Barcelona, Spain).

Experimental protocol. When the experimental period was completed, all rats were housed in metabolic cages (Panlab, Barcelona, Spain) with free access to food and their respective drinking fluid for a 4-day period (2 days for adaptation + 2 experimental days) during which food and fluid intake were measured and urine samples collected. We measured 24-h urine volume and total urinary sodium, potassium, proteinuria, creatinine, isoprostanes, H2O2 (as an index of oxidative stress), and aminopeptidases (APs) (as an index of renal injury). Mean values of all intake and urinary variables obtained during the two experimental days were compared among groups. Water and sodium balances were calculated with respect to renal losses, without taking extrarenal losses into account.

RENAI. FUNCTION AND SALT AND water metabolism can be considerably impaired by thyroid disorders (3, 46). Some authors reported that hypothyroid rats have a lesser ability to concentrate urine and show increased natriuresis after salt or water loading (10, 18, 25, 39) and have a reduced capacity to conserve sodium, which produces a negative sodium balance and death when subjected to dietary sodium restriction (13). However, our group did not observe increased sodium excretion in hypothyroid methimazole-treated rats under some of the above conditions (44) or in pressure-diuresis-natriuresis studies (43). On the other hand, thyroxine (T4)-treated rats showed changes in renal hemodynamics and sodium resorption (46), increased blood volume (34), polydipsia/polyuria (15), reduced ability to excrete sodium after hypertonic saline loading (44), and a blunted pressure-diuresis-natriuresis response (43).

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After completion of the metabolic study, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 units of heparin in isotonic sterile NaCl solution was inserted in the femoral artery to measure intra-arterial BP, HR, and pulse pressure in conscious rats and to draw blood samples. The catheter was tunneled subcutaneously and brought out through the skin at the dorsal side of the neck. Intra-arterial BP was measured at 24 h after femoral catheter implantation. Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (McLab; AD Instruments, Hastings, UK). BP and HR values obtained during the last 30 min were averaged for intergroup comparisons. Subsequently, blood samples taken with the femoral catheter were used to determine the following plasma variables: urea, creatinine, total proteins, electrolytes (sodium and potassium), and thyroid hormones (FT$_3$ and FT$_4$).

Finally, the rats were killed by exsanguination, and the kidneys and ventricles were removed and weighed. The heart was divided into right ventricle and left ventricle plus septum, and the kidney was dissected to separate the cortex and medulla.

Analytical procedures. Proteinuria was measured by the method of Bradford (2). Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer (Hitachi-912; Roche). Plasma thyroid hormone levels [free circulating triiodothyronine (T$_3$) and T$_4$] were determined with rat radioimmunoassay kits according to the manufacturer’s instructions (Diagnostic Products, Los Angeles, CA). An enzyme immunoassay kit (8-isoprostane EIA Kit; Cayman, Ann Arbor, MI) was used to measure urinary 8-isoprostane levels, and samples were previously purified by using the Affinity purification kit (Cayman). H$_2$O$_2$ was measured by the Hydrogen Peroxide Assay Kit (Cayman).

AP measurement. Glutamyl (GluAp)-, alanyl (AlaAp)-, aspartyl (AspAp)-, and cystinyl (CySAp) aminopeptidase activities were determined in duplicate in a fluorometric assay using glutamyl-, alanil-, aspartyl-, and cystinyl-β-naphthylamide as substrates (36). In brief, 20 μl of urine were incubated for 60 min at 37°C with 90 μl of their corresponding substrate solution [2.72 mg/dl glutamyl-β-naphthylamide, 10 mg/dl BSA, 10 mg/dl dithiothreitol (DTT), and 555 mg/dl CaCl$_2$ in 50 mM Tris·HCl, pH 7.4; 2.14 mg/dl alanyl-β-naphthylamide, 10 mg/dl BSA, and 10 mg/dl DTT in 50 mM phosphate buffer, pH 7.4; 2.58 mg/dl aspartyl-β-naphthylamide, 10 mg/dl BSA, and 39.4 mg/dl MnCl$_2$ in 50 mM Tris·HCl, pH 7.4; 5.63 mg/dl cystinyl-β-naphthylamide, 10 mg/dl BSA, and 10 mg/dl DTT in 50 mM Tris·HCl, pH 6]. The substrates were dissolved previously in 1 ml of DMSO and stored at −20°C. All reactions were stopped by adding 90 μl of 0.1 M acetate buffer, pH 4.2. The amount of β-naphthylamide released as a result of AP activities was measured fluorometrically at an emission wavelength of 412 nm and excitation wavelength of 345 nm and quantified using a standard curve for β-naphthylamide. Sample blanks were made in duplicate using an incubation solution that did not contain the substrate of the enzyme. Specific AP activities were expressed as nanomoles of substrate hydrolyzed per minute per milligram creatinine. Fluorogenic assays were linear with respect to time of hydrolysis and creatinine content.

Histopathological study. For conventional morphology, buffered 4% formaldehyde-fixed, paraffin-embedded longitudinal rat kidney sections in sagittal plane were stained with hematoxylin and eosin and periodic acid-Schiff stain. The extent of vascular injury (stenosis, hyaline arteriopathy, and myointimal hyperplasia) was assessed by examining profiles of arteries and arterioles in a single kidney section.
and counting affected vessels. The presence of glomerular lesions (glomerulosclerosis and capsular fibrosis) was assessed in at least 200 glomeruli. Tubular atrophy, necrosis, and tubular casts were also evaluated. The morphological study was done in a blinded fashion on 4-μm sections with light microscopy, using the most appropriate stain for each lesion.

**Morphometrical study.** Samples were fixed in buffered 4% formalin, embedded in paraffin, and serially sectioned at 5 μm thickness. Afterward, they were stained with 1% picro Sirius red F3BA (Gurr; BDH Chemicals, Poole, UK) for image analysis quantification. To improve staining, tissue sections were kept after deparaffination for 3–5 days in 70% alcohol as mordent. Picro Sirius red stains connective fibers deep red and cell nuclei and cytoplasmatic structures light red/bright yellow (38). Interstitial connective tissue and glomerular morphometry were automatically quantified on rat kidney histological sections by using various image-processing algorithms developed in a single image analysis application, Fibrosis HR (24).

**Statistical analyses.** The time course of SBP and HR was compared by using a nested design, with groups and days as fixed factors and rat as a random factor. When the overall difference was significant, Bonferroni’s method with an appropriate error was used. One-way ANOVA was used for comparisons of each variable. The parametric Kruskal-Wallis test and Mann Whitney U-test were used for morphometrical variables. P < 0.05 was considered significant.

**RESULTS**

**BP and HR.** Figure 1, left, shows the time course of tail SBP and HR, and Fig. 1, right, shows the final MAP and HR measured by direct recording in the experimental groups. SBP values were increased and decreased in hyperthyroid and hypothyroid rats, respectively, compared with controls. High-salt intake did not change the BP in control and hypothyroid rats but produced an increased SBP in the hyperthyroid group. HR was increased and decreased in hyperthyroid and hypothyroid rats, respectively, but was not modified by high-salt intake in any group. These data were confirmed by the direct recording measurements.

**Morphological variables.** At the end of the 6-wk study period, the body weight was significantly lower in hyper- and hypothyroid groups than in controls. High-salt intake produced an additional body weight decrease in hyper- and hypothyroid rats.

Kidney weight and kidney-to-body weight ratio were increased and decreased significantly in hyperthyroid and hypothyroid rats, respectively. High-salt intake produced an additional increase in kidney weight and kidney-to-body weight ratio in hyperthyroid rats.

Heart weight and heart-to-body weight ratio were increased and decreased significantly in hyperthyroid and hypothyroid groups, respectively. High-salt intake produced an additional increase in heart weight and heart-to-body weight ratio in all groups, and these effects were greater in hyperthyroidism. The left ventricular-to-heart weight ratio, an index of left ventricular cardiac hypertrophy, was not modified significantly by the treatments, indicating that they affected equally the left and right ventricular mass (Table 1).

**Plasma variables and thyroid hormone levels.** These results are summarized in Table 2. Plasma sodium was similar in all groups. Plasma potassium levels were increased and decreased in hyperthyroid and hypothyroid rats, respectively, and they were reduced by high-salt intake in control and hyperthyroid rats. Plasma creatinine levels were reduced and augmented in hyperthyroid and hypothyroid rats, respectively, but they were not modified significantly by high-salt intake in any group. Plasma calcium levels were reduced significantly in the hyperthyroid group but did not change significantly in the hypothyroid group; high-salt intake did not modify these levels in the control or hyperthyroid rats but reduced them in the hypothyroid group. Total plasma protein levels were reduced and

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Salt</th>
<th>Hypothyroid</th>
<th>Hypothyroid-Salt</th>
<th>Hyperthyroid</th>
<th>Hyperthyroid-Salt</th>
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<tbody>
<tr>
<td>FBW, g</td>
<td>388 ± 11</td>
<td>363 ± 7.4</td>
<td>265 ± 5.7***</td>
<td>253 ± 3.2***</td>
<td>302 ± 7.0***</td>
<td>283 ± 5.8***</td>
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<tr>
<td>KW, mg</td>
<td>1,028 ± 17</td>
<td>1,038 ± 35</td>
<td>606 ± 17***</td>
<td>621 ± 21***</td>
<td>1,149 ± 27**</td>
<td>1,265 ± 24**</td>
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<tr>
<td>HW, mg</td>
<td>943 ± 6</td>
<td>976 ± 9*</td>
<td>546 ± 9.2***</td>
<td>579 ± 11***</td>
<td>1,043 ± 9**</td>
<td>12.16 ± 11**</td>
</tr>
<tr>
<td>KW/BW, mg/g</td>
<td>2.66 ± 0.06</td>
<td>2.87 ± 0.11</td>
<td>2.29 ± 0.12**</td>
<td>2.55 ± 0.11</td>
<td>3.84 ± 0.09*</td>
<td>4.48 ± 0.06**</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>2.43 ± 0.04</td>
<td>2.69 ± 0.2*</td>
<td>2.07 ± 0.06**</td>
<td>2.38 ± 0.08*</td>
<td>3.41 ± 0.07**</td>
<td>3.96 ± 0.06**</td>
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<tr>
<td>LV/HW</td>
<td>0.780 ± 0.009</td>
<td>0.790 ± 0.006</td>
<td>0.785 ± 0.008</td>
<td>0.790 ± 0.005</td>
<td>0.802 ± 0.012</td>
<td>0.763 ± 0.021</td>
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<thead>
<tr>
<th>Groups</th>
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<th>Hyperthyroid</th>
<th>Hyperthyroid-Salt</th>
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<tbody>
<tr>
<td>Na, meq/l</td>
<td>140.0 ± 0.53</td>
<td>142.0 ± 0.76</td>
<td>143.0 ± 1.12</td>
<td>142.7 ± 1.86</td>
<td>139.8 ± 1.58</td>
<td>140.4 ± 0.98</td>
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<tr>
<td>K, meq/l</td>
<td>4.20 ± 0.08</td>
<td>3.89 ± 0.09*</td>
<td>2.94 ± 0.08***</td>
<td>3.05 ± 0.08***</td>
<td>6.03 ± 0.31*</td>
<td>3.95 ± 0.22**</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>10.2 ± 0.14</td>
<td>10.1 ± 0.11</td>
<td>10.1 ± 0.04</td>
<td>8.85 ± 0.39*</td>
<td>8.50 ± 0.04**</td>
<td>8.34 ± 0.18**</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>44.7 ± 2.2</td>
<td>42.6 ± 1.4</td>
<td>52.2 ± 2.1*</td>
<td>46.7 ± 2.5</td>
<td>41.3 ± 1.5</td>
<td>40.1 ± 3.1</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.41 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td>0.58 ± 0.01***</td>
<td>0.49 ± 0.02*</td>
<td>0.26 ± 0.04*</td>
<td>0.22 ± 0.01***</td>
</tr>
<tr>
<td>Total proteins, g/dl</td>
<td>5.56 ± 0.08</td>
<td>5.63 ± 0.10</td>
<td>6.01 ± 0.13*</td>
<td>5.54 ± 0.34</td>
<td>4.67 ± 0.04**</td>
<td>4.52 ± 0.10**</td>
</tr>
<tr>
<td>FT3, pg/ml</td>
<td>3.21 ± 0.05</td>
<td>3.49 ± 0.09</td>
<td>1.65 ± 0.11***</td>
<td>1.47 ± 0.07**</td>
<td>8.10 ± 0.30**</td>
<td>5.03 ± 0.37**</td>
</tr>
<tr>
<td>FT4, ng/ml</td>
<td>2.68 ± 0.13</td>
<td>2.98 ± 0.11</td>
<td>0.073 ± 0.005***</td>
<td>0.081 ± 0.005**</td>
<td>7.56 ± 0.23**</td>
<td>4.58 ± 0.58**</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. FT3, free triiodothyronine; FT4, free thyroxine. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the control group. +P < 0.05 and ++P < 0.01 vs. the respective saline untreated group.
augmented in hyperthyroid and hypothyroid rats, respectively, but they were not modified by high-salt intake in any group. FT3 and FT4 values were increased and decreased significantly in hyperthyroid and hypothyroid rats, respectively, whereas these levels were reduced by high-salt intake in hyperthyroid rats.

**Metabolic and urinary variables.** Food intake was increased and decreased in hyperthyroid and hypothyroid rats, respectively. High-salt intake reduced food intake in all groups, although the reduction did not reach statistical significance in the hypothyroid group. Water intake was increased markedly in hyperthyroid rats, and high-salt intake doubled water intake in control and hyperthyroid animals but did not modify fluid intake in hypothyroid rats. In consonance with these findings, daily urine volume was higher in hyperthyroid rats and was augmented by high-salt intake in control, hyperthyroid, and even hypothyroid rats (see Table 4). Water balance was higher in hyperthyroid rats and markedly reduced in the hypothyroid salt group. Sodium balance was higher in the hyperthyroid group, and high-salt intake increased the sodium balance in all groups with respect to their controls (Table 3). The increased positive water and sodium balances of untreated hyperthyroid rats are consistent with the increased extrarenal water and sodium losses (20) and higher blood volume (24, 34, 46) of these animals.

Total potassium excretion was increased and decreased in hyperthyroid and hypothyroid rats, respectively, and it was augmented by high-salt intake in control and hyperthyroid rats. Creatinine excretion was similar between control and hypothyroid rats. Proteinuria levels were twofold higher in hyperthyroid than in controls; high-salt intake did not change these levels in normal and hypothyroid rats but produced a marked increase in hyperthyroid rats. Creatinine clearance was similar in all groups, except for a higher value in the hyperthyroid salt group (Table 4).

**Oxidative stress variables.** These data are summarized in Fig. 2. Urinary isoprostanes and H2O2 were similar between control and hypothyroid rats and increased in hyperthyroid rats. They were increased by high-salt intake in all groups, more markedly in hyperthyroid rats.

**Urinary APs.** Urinary excretions of GluAP, AlaAP, CysAP, and AspAP were increased significantly in hyperthyroid rats, whereas the hypothyroid group showed similar levels as controls (Fig. 3). GluAP and AlaAP were increased by high-salt intake in the control group, and all APs were increased by high-salt intake in the hyperthyroid group, reaching higher values than the control group (Fig. 3), despite their higher creatinine excretion values, which were used to normalize AP values. High-salt intake did not significantly change urinary APs in hypothyroid rats, but GluAP and AlaAP were reduced compared with their respective salt-untreated group.

**Morphometrical results.** No significant differences were found between rat group analyses in area or percentage of renal interstitial connective tissue under normal saline intake (controls 4.1 ± 1.4%, hyperthyroid rats 4.3 ± 1.3%, and hyperthyroid rats 5.6 ± 2.4%) or after high-salt intake (controls 4.8 ± 1.1%, hyperthyroid rats 6.2 ± 2.4%, and hyperthyroid rats 3.9 ± 0.5%). Hyperthyroid salt-treated rats showed a significant increase in glomerular, mesangial, and tuft areas compared with hypothyroid salt rats (32,790 ± 406 vs. 28,678 ± 1,119 μm²; 6,795 ± 2,094 vs. 3,321 ± 623 μm²; and 25,071 ± 3,972 vs. 16,324 ± 4,043 μm², respectively (P = 0.029, Mann Whitney U-test), indicating a modulatory role of thyroid hormones in glomerular tuft when rats are under increased salt intake.

**DISCUSSION**

The main study findings were that increased dietary sodium exacerbated hypertension, cardiac hypertrophy, renal hypertrophy, and albuminuria and augmented sodium balance, oxidative stress, and urinary AP activity associated with T4-induced hyperthyroidism in rats, whereas hypothyroid rats were resistant to BP elevation, renal hypertrophy, and signs of renal injury after high-salt intake.

An increase in BP in response to dietary sodium (salt sensitivity) is well-documented in humans and is considered an important factor in the pathogenesis of hypertension (4). The present study shows that increased dietary sodium via food intake and high-salt intake increased BP and renal hypertrophy in rats. **Table 3. Metabolic variables in the experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Salt</th>
<th>Hypothyroid</th>
<th>Hypothyroid-Salt</th>
<th>Hyperthyroid</th>
<th>Hyperthyroid-Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/100 g</td>
<td>6.68 ± 0.29</td>
<td>4.71 ± 0.43***</td>
<td>4.59 ± 0.40***</td>
<td>3.87 ± 0.24***</td>
<td>14.27 ± 1.79***</td>
<td>11.50 ± 0.58***</td>
</tr>
<tr>
<td>Water intake, ml/100 g</td>
<td>6.27 ± 0.29</td>
<td>12.65 ± 1.88***</td>
<td>7.47 ± 0.79</td>
<td>7.73 ± 1.31</td>
<td>14.43 ± 2.73***</td>
<td>33.1 ± 4.65***</td>
</tr>
<tr>
<td>Water balance, ml/100 g</td>
<td>3.73 ± 0.17</td>
<td>5.25 ± 1.41</td>
<td>3.94 ± 0.61</td>
<td>0.76 ± 0.56***</td>
<td>9.33 ± 1.64*</td>
<td>7.3 ± 3.41</td>
</tr>
<tr>
<td>Sodium balance, mmol/100 g</td>
<td>0.14 ± 0.01</td>
<td>3.64 ± 0.47***</td>
<td>0.08 ± 0.02</td>
<td>4.05 ± 0.36***</td>
<td>0.42 ± 0.01*</td>
<td>5.7 ± 0.34***</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Water and sodium balances were calculated respect to renal losses, without accounting for extrarenal losses. All data are referred to 24 h. *P < 0.05 and ***P < 0.001 vs. the control group. +P < 0.05, + + P < 0.01, and + + + P < 0.001 vs. the respective saline untreated group.

**Table 4. Urinary and renal variables in the experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Salt</th>
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<th>Hypothyroid-Salt</th>
<th>Hyperthyroid</th>
<th>Hyperthyroid-Salt</th>
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<tbody>
<tr>
<td>Urine volume, ml/100 g</td>
<td>2.54 ± 0.20</td>
<td>7.40 ± 0.80***</td>
<td>3.08 ± 0.57</td>
<td>6.91 ± 0.70***</td>
<td>5.10 ± 1.31*</td>
<td>27.5 ± 1.23***</td>
</tr>
<tr>
<td>UaV, μeq/100 g</td>
<td>629 ± 35</td>
<td>794 ± 45*</td>
<td>505 ± 31*</td>
<td>407 ± 60</td>
<td>871 ± 62***</td>
<td>1,131 ± 71***</td>
</tr>
<tr>
<td>Proteinuria, mg/mg creatine</td>
<td>9.93 ± 0.74</td>
<td>9.71 ± 0.92</td>
<td>8.65 ± 0.60</td>
<td>9.21 ± 0.37</td>
<td>20.24 ± 1.17***</td>
<td>29.42 ± 1.92***</td>
</tr>
<tr>
<td>UcV, mg/kg kidney</td>
<td>4.86 ± 0.24</td>
<td>4.82 ± 0.19</td>
<td>5.35 ± 0.55</td>
<td>6.12 ± 0.22*</td>
<td>3.52 ± 0.16*</td>
<td>4.08 ± 0.16*</td>
</tr>
<tr>
<td>CrC, ml·min⁻¹·g kidney⁻¹</td>
<td>0.83 ± 0.06</td>
<td>0.84 ± 0.05</td>
<td>0.61 ± 0.08</td>
<td>0.88 ± 0.04</td>
<td>1.13 ± 0.24</td>
<td>1.48 ± 0.13***</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. UaV, total potassium excretion; UcV, total creatinine excretion; CrC, creatinine clearance. All data are referred to 24 h. *P < 0.05 and ***P < 0.001 vs. the control group. +P < 0.05, + + P < 0.01, and + + + P < 0.001 vs. the respective saline untreated group.
intake accelerates the increase in BP induced by T4 administration. However, the same sodium load did not increase BP in control or hypothyroid rats. These data contrast with a recent report from our laboratory that a chronic 2% NaCl load via drinking water produced a moderate BP increase in male Wistar rats (7). These discrepancies may reflect the distinct pathophysiological consequences of the different saline administration routes used. Thus a polyuria-polydipsia syndrome is produced when the saline load is given in drinking water and the sodium balance is disturbed by the high fluid intake. However, when it is administered in the food, the water intake dilutes the excess sodium and facilitates its excretion.

It has been reported that hyperthyroid rats have abnormal renal hemodynamics and an attenuated pressure-diuresis-natriuresis response (16, 26, 43), which can impair renal sodium handling and therefore participate in the salt sensitivity of BP in these animals. Angiotensin II (16) and oxidative stress (26) have been found to play an important role in these abnormalities. In the present study, urinary isoprostanes and H2O2, markers of increased endogenous oxidative stress (8, 11), were enhanced in hyperthyroid rats and augmented by the high-salt intake in all groups, more markedly in the hyperthyroid group. These results indicate that oxidative stress plays a role in the salt sensitivity of BP in the hyperthyroid state. However, the participation of other potential antidiuretic and antinatriuretic factors cannot be ruled out. Thus endothelin and vasopressin, which are stimulated in a compensatory manner when the systemic renin-angiotensin system is blunted (21), are elevated in hyperthyroid rats (46). In addition, activation of the intrarenal renin-angiotensin system by the metabolic receptor GPR91 for succinate, closely associated with oxidative stress, has a hormone-like signaling function in the distal nephron-collect-
ing duct system, which is the major source of (pro)renin in some diseases (32). Finally, thyroid hormones can produce sodium retention by acting directly on transport systems (e.g., Na\(^+\)-H\(^+\) and Na\(^+\)-K\(^+\)-ATPase) in renal tubules (19, 48).

The morphological data show that absolute and relative cardiac mass values were increased and decreased significantly in the hyperthyroid and hypothyroid groups, respectively. Left ventricular-to-heart weight ratio, an index of left ventricular cardiac hypertrophy, was not modified significantly by the treatments, indicating that thyroid disorders affected equally the left and right ventricular mass. These data are similar to previous observations by our group (46, 47).

Dietary sodium intake may modulate cardiac mass (35). Thus a high-sodium intake increases cardiac mass in normotensive rats (49) and exacerbates cardiac hypertrophy in hypertensive rats (14). Conversely, a low-sodium intake prevents cardiac hypertrophy associated with two-kidney, one-clip Goldblatt hypertension (30) and ANG II hypertension (28), regardless of BP reduction. In the present study, high-salt intake produced an increase in heart weight and the heart-to-body weight ratio in all groups, whereas hyperthyroid rats showed increased sensitivity to saline-induced cardiac hypertrophy.

High-salt intake reduced plasma thyroid hormone levels in the hyperthyroid rats, in agreement with the decreased thyroid hormone levels reported in saline models of hypertension (7, 23, 45). This phenomenon is believed to be mediated by the action of an unidentified substance, designated the "thyroid-depressing factor" by Threatte et al. (40). Renal injury is also associated with decreased thyroid hormone concentrations, due to central effects and changes in peripheral hormone metabolism and thyroid hormone-binding proteins (41). Moreover, it is known that comitant nonthyroidal illness can suppress serum T\(_4\) concentration in hyperthyroid cats (31), as observed in the present hyperthyroid salt group.

Plasma creatinine was reduced and augmented in hyperthyroid and hypothyroid rats, respectively. These findings are comparable with the plasma creatinine changes observed in humans (40) and cats (41). The reduction in serum creatinine concentration in hyperthyroidism may be due to decreased production by a reduced muscle mass (12), whereas the increase observed in hypothyroidism is caused by a creatinine generation from possible myopathy and rhabdomyolysis (37). Moreover, these changes in plasma creatinine may in part be explained by volume expansion and dilution and by volume depletion in hyper- and hypothyroid rats, respectively, as suggested by the plasma protein levels.

The unchanged creatinine clearance values in our untreated hyperthyroid rats contrast with the decreased glomerular filtration rates (GFR) observed in pressure-natriuresis experiments in anesthetized hyperthyroid rats (16, 26, 43). In the latter experiments, GFR was measured as the \([^{1}H]insulin\) clearance, and a hormone cocktail was administered to control neural and hormonal influences to which hyperthyroid rats could be more sensitive, as we suggested in our first publication on this topic (43).

Proteinuria levels were twofold higher in the hyperthyroid rats. Proteinuria is often present in hyperthyroid humans (12) and rats (27, 34) without being related to renin-angiotensin system activity, BP levels (34), or oxidative stress (27). Proteinuria was aggravated by the increased sodium intake in hyperthyroid rats. This observation is in line with reports that dietary sodium restriction prevents proteinuria in spontaneously hypertensive (1), 5/6 nephrectomized (9), and angiotensin II hypertensive rats (35).

There is an urgent need for improved biomarkers to permit a more timely diagnosis of kidney disease. They can be useful for early diagnosis and assessment of injury severity, for guiding targeted therapies, and for monitoring disease progression and resolution. When tubular cells are damaged, they release tubular enzymes into the ultrafiltrate, increasing urinary enzyme activities. Urinary markers of tubular damage (33), such as retinol-binding protein or N-acetyl-\(\beta\)-glucosaminidase, are elevated in hyperthyroid humans (12, 29) and cats (42). In this paper, we analyzed the urinary excretion of GluAP, AlaAP, CysAp, and AspAp as possible biomarkers of tubular dysfunction in thyroid disorders and after an increased saline intake. The increased urinary AP levels in saline- and T\(_4\)-treated rats suggest that the high-salt intake and hyperthyroid state have additive effects producing tubular dysfunction. In contrast, the hypothyroid state proved resistant to the effects of salt on urinary AP activity, supporting the proposition that the hypothyroid state is beneficial in chronic kidney disease. In this context, reduced proteinuria and a slower deterioration of renal function were reported in rats with induced renal insufficiency after thyroidection (5). Moreover, in another experimental setting, we observed that AP levels increase even before the elevation of total protein excretion (unpublished observations). Therefore, from a diagnostic standpoint, we can conclude that urinary AP values are suitable predictors of renal injury.

In summary, this study indicates that the hyperthyroid state is associated with increased salt sensitivity, manifested by an elevated BP, increased cardiac and renal hypertrophy, oxidative stress, and signs of renal injury in response to high-salt intake. In contrast, high-salt intake produced no BP increase, renal hypertrophy, or signs of renal injury in hypothyroid rats. It can also be concluded that urinary AP levels are a suitable biomarker of renal injury.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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