Urinary excretion of digoxin-like immunoreactive factor and arginine-vasopressin in hyper- and hypo-thyroid rats

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SUMMARY

1. Urinary excretion of digoxin-like immunoreactive factor and arginine-vasopressin and other parameters related to salt and water metabolism were studied in hyper- and hypo-thyroid rats after different tests.

2. Urinary excretion of arginine-vasopressin was increased in hyperthyroid and reduced in hypothyroid rats with respect to controls, in response to water deprivation or a hypertonic saline load.

3. Control and hypothyroid rats showed the highest urinary excretion of digoxin-like immunoreactive factor after a hypertonic saline load. However, hyperthyroid rats had the highest urinary levels of digoxin-like immunoreactive factor under normal conditions.

4. From these results it is suggested that: (a) hyper- and hypo-thyroid rats exhibit hyper- and hypo-responsiveness of arginine-vasopressin secretion to osmotic stimuli, respectively; (b) an unidentified digoxin-like immunoreactive factor measured in unextracted rat urine may be related to diuresis and natriuresis in control and hypothyroid rats; however, dissociation between this factor and natriuresis is observed in hyperthyroid rats.

Key words: arginine-vasopressin, digoxin-like immunoreactive factor, electrolytes, hyperthyroidism, hypothyroidism, urinary excretion.

INTRODUCTION

Thyroid disorders have important effects on renal function and salt and water metabolism [1]. In the rat, hypothyroidism causes a decrease in glomerular filtration rate [2-4] and in effective plasma flow [3, 5], together with a defect in tubular sodium reabsorption [3, 4] and alterations in urine-concentrating ability [4, 5]. Thus hypothyroidism in rats is considered to be a disease that produces water and sodium loss. Although the effects of hypothyroidism on renal function have been studied extensively, there is less information concerning the effect of hyperthyroidism, in which a tendency toward sodium retention is seen.

The impairment of sodium reabsorption in the hypothyroid kidney is attributed to a decrease in Na⁺-K⁺-ATPase activity, which has been shown to occur when the plasma thyroxine (T₄) concentration is decreased [3, 6]. Several studies suggest that renal sodium excretion might be regulated by an endogenous digitalis-like factor with putative natriuretic properties [7]. Its activity as an inhibitor of the Na⁺-K⁺ pump makes it similar to digitalis compounds; furthermore, a number of observations also suggest that endogenous digitalis-like factor may cross-react with digoxin-binding antibodies [8]. Although this issue remains controversial, alterations in levels of endogenous digoxin-like immunoreactive factor (DLIF) have been observed in several diseases with abnormal salt and water balance [9, 10]. However, the possible role of DLIF in the changes produced by thyroid abnormalities in salt and water metabolism has not been evaluated.

Arginine-vasopressin (AVP) has only been measured in plasma from hypothyroid humans [11] and rats [12, 13], with scarce and conflicting results. We therefore determined the urinary excretion of AVP, a better index of AVP production, after osmotic stimulus in hyper- and hypothyroid rats.
The present study was designed to evaluate the renal handling of sodium and water in hyper- and hypo-thyroid rats treated for identical periods. As the kidney is able to adjust to the disturbances that may result from thyroid diseases [1], urine collected from control and rats with thyroid dysfunction was studied under normal conditions and after marked changes in urine water and sodium concentration. These changes were produced by water deprivation and by isotonic and hypertonic saline loads. Moreover, we also examined the possible role of a putative natriuretic hormone, measured as DLIF, in the changes induced in urinary sodium excretion.

**METHODS**

**Animal preparation**

Male Wistar rats initially weighing 100–150 g were maintained on standard chow and tap water except where stated. The animals were divided into three groups: control, hyperthyroid and hypothyroid rats (n = 10 in each group). Hyperthyroidism was induced by subcutaneous injection of T4 (Merck; 200 μg day⁻¹ kg⁻¹, dissolved in 0.5 mol/l NaOH isotonic saline). Hypothyroidism was induced by the continuous administration of 0.02% (w/v) methimazole (Sigma) via the drinking water.

The effectiveness of these treatments was assessed by comparing rectal body temperature, serum T4 concentration, serum tri-iodothyronine (T3) concentration, heart rate and the final body weight of control and treated rats. Rectal body temperature was measured with a small lubricated probe connected to a thermometer (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Blood pressure and heart rate were recorded as described below.

**Experimental protocol**

Five weeks after treatment with T4 or methimazole, control, hyperthyroid and hypothyroid rats were placed in individual metabolic cages with food and tap water provided ad libitum. After a 48 h adaptation period, four tests were performed. Urine samples were taken after: (a) 24 h under normal conditions, (b) 24 h of water deprivation with free access to food, (c) 5 h of an intraperitoneal isotonic saline [0.9% (w/v) NaCl] load and 5 h of an intraperitoneal hypertonic saline [3% (w/v) NaCl] load.

The water deprivation test was performed on the day after 24 h under normal conditions. A 48 h recovery period in the metabolic cages between the water deprivation test and the isotonic saline load was allowed. Isotonic and hypertonic saline solutions were administered at a rate of 3 ml/100 g body weight, with an interval of 24 h between each saline load. During the 5 h of urine collection, rats were deprived of food and water. Before and after each urine collection, light suprapubic pressure was applied to ensure emptying of the bladder. The following parameters were measured in urine samples: urine volume (UV), urinary sodium (U_NaV), potassium (U_KV), AVP (U_AVPV) and digoxin-like immunoreactive factor (U_DLIFV) excretion, and urinary osmolality (U_Osm).

After the urinary studies had been completed, the carotid artery was cannulated. After a 24 h recovery period, mean arterial pressure and heart rate were recorded directly (Bell and Howell type 4 transducer connected to a two-channel Devices MX2 recorder). When blood pressure had stabilized (30 min), blood samples from the carotid catheter were taken for determination of serum electrolyte, T4 and T3 concentrations and serum osmolality.

**Cannulation procedures**

Rats were anaesthetized with sodium pentobarbital (Nembutal, Serva, Heidelberg, Germany; 40 mg/kg intraperitoneally). A polyethylene catheter (PE-10 connected to PE-50), containing 100 units of heparin (Leo, Madrid, Spain) in isotonic sterile NaCl solution, was inserted into the right carotid artery for either direct blood pressure measurements or for extracting blood samples. The catheter was placed subcutaneously and exteriorized at the dorsum of the neck.

**Analytical procedures**

DLIF was quantified in unextracted urine samples by r.i.a. using a commercially available kit (Digoxin MAIA kit; Biodata S.p.A., Milan, Italy) in which the sensitivity was enhanced as previously described [10]. The lower limit of detection for urine was 83 pg/ml, with intra- and inter-assay coefficients of variation of 4.9% and 7.7%, respectively. AVP was measured by r.i.a. in unextracted urine; the intra- and inter-assay coefficients of variation were 6.8% and 10.5%, respectively. Serum T4 and T3 levels were determined with r.i.a. kits purchased from Diagnostic Products Corporation (Los Angeles, CA, U.S.A.). Serum and urinary sodium and potassium concentrations and osmolality were measured on the day by flame photometry (Corning Instruments 435, Halstead, Essex, U.K.) with lithium as the internal standard and by freezing point depression (Automatic Osmometter, Osmette A; Precision Systems Inc., Sudbury, MA, U.S.A.), respectively.

**Statistical analysis**

An analysis of the nested design was carried out [14, 15] to compare groups and tests; the design had two fixed effect factors (group and test) and one random effect factor (the rat), this factor being nested in the group. When the different tests for factors and the group-test interactions were significant, the groups were compared at different tests using the Bonferroni rule to keep the overall error as small as possible. The Welch approximation to Student's t-test was performed when the variance was unhomogeneous under the Bonferroni rule. Comparisons of each biological parameter were carried out with one-way analysis of variance. When the overall analysis of variance was significant, we then performed pairwise comparisons by Bonferroni's method.
RESULTS

Effects of T₄ or methimazole administration on biological parameters in the rat (Table 1)

Animals given T₄ or methimazole for 5 weeks gained significantly less weight than their matched controls during this period. Heart rate, rectal temperature and serum T₄ and T₃ levels were decreased in hypothyroid rats and were increased in hyperthyroid rats. Hence rats given methimazole for 5 weeks developed characteristic manifestations of hypothyroidism, whereas those receiving thyroxine for a similar period developed hyperthyroidism. There were no significant differences in mean arterial pressure, serum sodium and potassium concentrations and serum osmolality when hyperthyroid and hypothyroid rats were compared with control rats.

Table 1. Biological parameters of hypothyroid (treated with 0.02% methimazole in drinking water), control and hyperthyroid (treated with T₄, 200 μg day⁻¹ kg⁻¹, subcutaneously) rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypothyroid rats</th>
<th>Control rats</th>
<th>P</th>
<th>Hyperthyroid rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Body wt. (g)</td>
<td>277.5±24.3</td>
<td>340.7±10.2</td>
<td>&lt;0.01</td>
<td>249.0±31.0</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94.3±1.9</td>
<td>102.0±4.5</td>
<td>NS</td>
<td>116.8±6.7</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>321.0±7.7</td>
<td>361.5±8.8</td>
<td>&lt;0.01</td>
<td>471.1±16.00</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.1±0.80</td>
<td>38.4±0.80</td>
<td>&lt;0.05</td>
<td>39.2±0.07</td>
</tr>
<tr>
<td>Serum T₃ concn. (μg/dl)</td>
<td>0.5±0.04</td>
<td>3.5±0.3</td>
<td>&lt;0.01</td>
<td>34.9±8.9</td>
</tr>
<tr>
<td>Serum Na⁺ concn. (mmol/l)</td>
<td>19.2±2.2</td>
<td>48.4±6.0</td>
<td>&lt;0.01</td>
<td>344.0±18.00</td>
</tr>
<tr>
<td>Serum Na⁺ concn. (mmol/l)</td>
<td>142.0±8.1</td>
<td>141.8±0.5</td>
<td>NS</td>
<td>140.8±0.85</td>
</tr>
<tr>
<td>Serum K⁺ concn. (mmol/l)</td>
<td>4.1±0.06</td>
<td>4.2±0.13</td>
<td>NS</td>
<td>4.3±0.09</td>
</tr>
<tr>
<td>Serum osmolality (mosm/kg)</td>
<td>288.2±3.0</td>
<td>288.2±1.6</td>
<td>NS</td>
<td>284.9±2.4</td>
</tr>
</tbody>
</table>

Table 2. Uᵥ, Uᵥᵥᵥ, Uᵥᵥᵥ and Na⁺/K⁺ ratio, in control (C), hyperthyroid (T₄) and hypothyroid (Me) rats

These parameters were measured after normal conditions (NC), water deprivation (WD), an isotonic saline (0.9% NaCl) load (ISL) and a hypertonic saline (3% NaCl) load (HSL). Values are expressed as means±SEM (n=10, each group). Statistical significance: *P<0.05, **P<0.01, ***P<0.001 compared with control rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>WD</th>
<th>ISL</th>
<th>HSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uᵥ (μl h⁻¹ 100 g⁻¹ body wt.)</td>
<td>221±38</td>
<td>81±11</td>
<td>115±26**</td>
<td>621±52</td>
</tr>
<tr>
<td>Me</td>
<td>161±6</td>
<td>121±4</td>
<td>387±53</td>
<td>676±32</td>
</tr>
<tr>
<td>C</td>
<td>499±50**</td>
<td>106±4</td>
<td>222±33</td>
<td>428±33**</td>
</tr>
<tr>
<td>T₄</td>
<td>1342±165</td>
<td>2087±171</td>
<td>1755±223**</td>
<td>975±54</td>
</tr>
<tr>
<td>Me</td>
<td>1698±78</td>
<td>2121±81</td>
<td>736±43</td>
<td>1011±30</td>
</tr>
<tr>
<td>C</td>
<td>984±123*</td>
<td>2722±79*</td>
<td>1485±180*</td>
<td>1534±54*</td>
</tr>
<tr>
<td>T₄</td>
<td>10±1.0</td>
<td>13±1.2</td>
<td>19±3.0</td>
<td>191±8.8</td>
</tr>
<tr>
<td>Me</td>
<td>12±0.8</td>
<td>18±0.5</td>
<td>35±5.9</td>
<td>198±8.3</td>
</tr>
<tr>
<td>C</td>
<td>20±0.8**</td>
<td>12±0.6**</td>
<td>14±3.2*</td>
<td>130±8.5***</td>
</tr>
<tr>
<td>T₄</td>
<td>30±1.5</td>
<td>20±1.7*</td>
<td>19±1.0*</td>
<td>50±1.7**</td>
</tr>
<tr>
<td>Me</td>
<td>30±1.9</td>
<td>36±1.3</td>
<td>41±3.3</td>
<td>76±2.6</td>
</tr>
<tr>
<td>C</td>
<td>66±2.3**</td>
<td>44±1.4</td>
<td>40±4.1</td>
<td>80±3.9</td>
</tr>
<tr>
<td>Na⁺/K⁺ ratio</td>
<td>0.33±0.03</td>
<td>0.64±0.03*</td>
<td>0.97±0.10*</td>
<td>3.82±0.21***</td>
</tr>
<tr>
<td>Me</td>
<td>0.32±0.02</td>
<td>0.51±0.01</td>
<td>0.87±0.07</td>
<td>2.62±0.09</td>
</tr>
<tr>
<td>C</td>
<td>0.31±0.01</td>
<td>0.28±0.02**</td>
<td>0.32±0.04**</td>
<td>1.62±0.07**</td>
</tr>
</tbody>
</table>

UV, Uᵥᵥᵥ, Uᵥᵥᵥ and Uᵥᵥᵥ (Table 2)

In hyperthyroid rats UV was increased under normal conditions and was reduced after the hypertonic saline load when compared with control rats. In hypothyroid rats no significant differences were found in UV, except for a reduction after the isotonic saline load. Obviously,
changes in the opposite direction were found in $U_{\text{Osm}}$ in all three groups. No clear pattern in $U_{\text{Na}}V$ was seen in rats with thyroid dysfunction when the results of the different tests were compared with those in control rats. Thus hyperthyroid rats showed a higher $U_{\text{Na}}V$ after 24 h under normal conditions, and a significant reduction in $U_{\text{Na}}V$ in the remaining tests. No differences were found between hypothyroid and control rats. Hypothyroid rats showed a reduction in $U_{\text{Na}}V$ in all tests when compared with control rats, although the differences were not significant under normal conditions. Hyperthyroid rats showed an elevation after 24 h under normal conditions. The Na$^+$/K$^+$ ratio was higher and lower in hypo- and hyper-thyroid rats, respectively, when compared with control rats, except after 24 h under normal conditions. $U_{\text{Osm}}$ was significantly increased in hyperthyroid rats in all tests except under normal conditions. However, in hypothyroid rats, only an increased $U_{\text{Osm}}$ was seen after the isotonic saline load.

$U_{\text{AVP}}V$ (Fig. 1)

In hyper- and hypo-thyroid rats $U_{\text{AVP}}V$ did not significantly differ from that in control rats under normal conditions and after the isotonic saline load. Both water deprivation and the hypertonic saline load produced a significant increase ($P<0.001$) in $U_{\text{AVP}}V$ with respect to normal conditions or the isotonic saline load in all three experimental groups, these responses being higher in hyperthyroid rats and lower in hypothyroid rats with respect to that observed in control rats.

$U_{\text{DLIF}}V$ (Fig. 1)

In control rats $U_{\text{DLIF}}V$ was increased after the isotonic saline load with respect to normal conditions and water deprivation, although the greatest increase was recorded after the hypertonic saline load ($P<0.001$ compared with normal conditions and water deprivation). This pattern was not observed in the hyperthyroid group, which showed the highest $U_{\text{DLIF}}V$ under normal conditions. Hypothyroid rats also showed the highest $U_{\text{DLIF}}V$ after the hypertonic saline load, as did control rats.

DISCUSSION

Diuresis and $U_{\text{Na}}V$

Several studies provide evidence that hypothyroidism in the rat is accompanied by increased diuresis and natriuresis under basal conditions [5, 13, 16], after water deprivation [1] or after an isotonic saline load [17, 18]. This tendency toward sodium loss has been implicated as a mechanism by which hypothyroidism prevents experimental arterial hypertension [1]. Our results, however (Table 2), do not support these findings. The discrepancy may be due to the different protocols used, as many as there are authors, with changes in such fundamental factors as duration of hypothyroidism, the use of anaesthetic [5] and the use of loads of the same volume as in control rats [17], without taking into account the reduced body weight of hypothyroid rats. On the other hand, other authors, using equally varied protocols, found no elevation in diuresis or natriuresis [2, 3] in hypothyroid rats.

Na$^+$/K$^+$ ratio and $U_{\text{Osm}}$

Our results show that the Na$^+$/K$^+$ ratio was higher and lower in hypo- and hyper-thyroid rats, respectively, when compared with control rats, except after 24 h under normal conditions. In this sense, a high Na$^+$/K$^+$ ratio and a reduction in $U_{\text{K}}V$ have also been recognized in hypothyroid rats fed a diet deficient in sodium and potassium [19] or after a saline load [2]. Nevertheless, either no significant differences [6, 18] or contradictory results have been reported, depending on the method of induction of hypothyroidism [17]. On the other hand, increases in kaliuresis related to the T4 dose have been found in hyperthyroid rats after 24 h of water deprivation [1].

The data on $U_{\text{Osm}}$ indicated that concentrating ability was not impaired in methimazole-treated rats, as has been previously reported in other models of experimental hypothyroidism [4, 5]. However, hyperthyroid rats showed an increased concentrating capacity after water deprivation.
**U_{AVP}V**

$U_{AVP}V$ was used as an index of AVP production under normal conditions and after the different stresses [10, 20]. Contradictory results have been observed with respect to plasma AVP levels in hypothyroid rats [12, 13, 21]. We found no significant differences in $U_{AVP}V$ in the three experimental groups under normal conditions and after the isotonic saline load. However, hyper-responsiveness in hyperthyroid and hyporesponsiveness in hypothyroid rats occurred to stimuli such as 24 h of water deprivation or a hypertonic saline load. The reduced $U_{AVP}V$ after the hypertonic saline load in hypothyroid rats agrees with the data obtained in plasma by Ali et al. [12]. These authors showed that in propylthiouracil-treated rats under a salt load (5%, 2 ml/100 g body weight), plasma AVP levels are lower than those in normal rats throughout the time course of AVP release. However, no data are available (to our knowledge) on AVP values in hyperthyroid rats under normal conditions or after stimulation.

**U_{DLIF}V**

Several authors have described an endogenous factor with digoxin-like immunoreactivity, measurable by digoxin i.r.s.i.s in the plasma and urine of humans and animals [10, 22]. The relationship between these factors and sodium metabolism has been observed in several situations [10, 23, 24] in humans and animals. The higher $U_{DLIF}V$ after the hypertonic saline load in control and hypothyroid rats agrees with these previous observations. However, a clear dissociation between $U_{DLIF}V$ and $U_{NA}V$ was observed in hyperthyroid rats. Dissociations have also been observed between digoxin immunoreactivity and natriuresis [25] and with other biochemical [26] and pharmacological [27] properties of digoxin-like factor.

The changes in $U_{DLIF}V$ in the three experimental groups were similar to those observed in diuresis: the highest $U_{DLIF}V$ in control and hypothyroid rats was obtained after the hypertonic saline load, and in hyperthyroid rats, $U_{DLIF}V$ was highest under normal conditions. In these situations, the highest values of UV were also observed. These data agree with a previous report that showed $U_{DLIF}V$ to be related to UV in humans [25]. Moreover, it should be recalled that this putative natriuretic factor, in spite of intensive investigation, has not been clearly identified, and different workers have used different preparations of samples and antiserum to measure this factor.

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