Pressure-diuresis-natriuresis response in hyperthyroid and hypothyroid rats

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1. Renal responses to changes in renal perfusion pressure were studied in anaesthetized hyperthyroid (thyroxine, 300 μg day⁻¹ kg⁻¹) and hypothyroid (methimazole, 0.03% via drinking water) rats to determine whether an abnormality in the pressure-diuresis-natriuresis phenomenon is involved in the resetting of kidney function in these disorders.

2. There were no significant differences between control and hypothyroid rats with respect to the relationships between renal perfusion pressure and absolute or fractional water and sodium excretion. However, in hyperthyroid rats the pressure-diuresis-natriuresis mechanism was impaired.

3. Renal blood flow and glomerular filtration rate were well autoregulated and there were no differences between control and hypothyroid rats at every level of renal perfusion pressure. A significantly lower glomerular filtration rate was observed in hyperthyroid rats when data were expressed per gram kidney weight, but glomerular filtration rate was similar to that of control rats when normalized by body weight.

4. The shift in the pressure-diuresis-natriuresis response of hyperthyroid rats is mainly due to an increase in tubular reabsorption. Blunting of the renal pressure-diuresis-natriuresis mechanism in hyperthyroid rats may represent the functional resetting of the kidney necessary for sustained hypertension. However, a normal pressure-natriuresis response was observed in hypothyroid rats, in which blood pressure was markedly reduced.

INTRODUCTION

Increases in renal perfusion pressure (RPP) lead to increases in water and sodium excretion, a phenomenon commonly referred to as pressure-diuresis-natriuresis (PDN). Interest in the PDN mechanism is due in large part to the fact that this phenomenon is thought to be a central component of a feedback system for the long-term control of extracellular fluid volume and arterial pressure [1]. Thyroid disorders have important effects on blood pressure, renal function and salt and water metabolism [2-4]. Hypothyroid rats are unable to produce maximally concentrated urine, and show enhanced natriuresis when salt- or water-loaded [5, 6] and an impaired ability to conserve sodium, which results in a negative sodium balance and death when sodium intake is restricted [7]. Administration of thyroid hormones produces opposite changes in renal haemodynamics and sodium reabsorption [2]. Glomerular filtration rate (GFR) and renal blood flow (RBF) increase significantly, whereas the absolute and fractional sodium excretion decrease in thyroxine-treated rats [8], and we also have observed in hyperthyroid rats a reduced natriuresis after isotonic and hypertonic saline loads [9].

In spite of the importance of the PDN mechanism for the maintenance of sodium balance [10] and arterial pressure [1], the acute relationship between arterial pressure and kidney excretory function has not yet been characterized in thyroid disorders. The objective of the present study was thus to characterize the relationships between renal perfusion pressure (RPP) and water and sodium excretion in (thyroxine-treated) hyperthyroid and (methimazole-treated) hypothyroid rats.

METHODS

Male Wistar rats (Panlab, Barcelona, Spain), initially weighing 180–200 g, were maintained on standard chow and tap water, except where stated. The animals were divided into three groups: control, hyperthyroid and hypothyroid. Hyperthyroidism was induced by a subcutaneous injection of (thyroxine, T₄; Merck; 300 μg day⁻¹ kg⁻¹). Thyroxine was dissolved (1 μg/μl) in isotonic saline with 0.5 mol/l NaOH. Hypothyroidism was induced by administration of 0.03% (w/v) methimazole (Sigma) in the drinking water. This antithyroid drug was chosen because of its longer plasma half-life and more
potent inhibition of thyroid hormone synthesis than that of propylthiouracil, the other antithyroid drug more frequently used in therapy and experimental studies [11, 12]. Both thyroxine and methimazole treatments were administered for 6 weeks. The effectiveness of these treatments was assessed by comparing serum T4, serum triiodothyronine (T3), mean arterial pressure (MAP), heart rate (HR), and the final thyroid and body weights of control and treated rats.

Surgical preparation

All the experiments were performed as described previously [13] with rats fasted for 16 h before the experiment. The animals were anaesthetized with inactin (100 mg/kg, intraperitoneally; BYK Gulden, Konstanz, Germany) and placed on a heated surgical table to maintain rectal temperature at 36.5–37°C. The right femoral artery was cannulated, and basal MAP and HR were measured before any other intervention was performed (Hewlett-Packard 1280 transducer, Hewlett-Packard 8805D amplifier). Catheters were also inserted into the right jugular vein for infusions and into the right carotid artery for blood sampling and blood pressure monitoring. A tracheostomy tube was placed to facilitate respiration. The left kidney was exposed by a midline abdominal incision and denervated by stripping the adventitia from both the renal artery and vein, and by applying 95% ethanol containing 10% phenol to each vessel to destroy any remaining nerve fibres. The left kidney was denervated to avoid any neurogenic influence on water and sodium reabsorption produced by the changes in central blood pressure. The left ureter was catheterized to collect urine. Silk ligatures were placed around the superior mesenteric and celiac arteries, and two adjustable clamps were placed on the aorta above and below the renal arteries to allow for increasing or decreasing RPP. RBF was determined by a 2.5 mm flow probe placed around the left renal artery and connected to an electromagnetic flowmeter (model 501 D; Carolina Instruments, King, NC, U.S.A.). Finally, the abdominal opening was covered with a piece of Parafilm (American National Can, Greenwich, CT, U.S.A.) to minimize evaporation. All animals received an intravenous infusion of 0.9% NaCl solution containing 1% BSA at a rate of 2 ml h⁻¹ 100 g⁻¹. Plasma levels of sodium- and water-retaining hormones were maintained at fixed high levels by adding aldosterone (20 ng min⁻¹ kg⁻¹), corticosterone (10 ng min⁻¹ kg⁻¹), vasopressin (0.05 ng min⁻¹ kg⁻¹) and noradrenaline (100 ng min⁻¹ kg⁻¹) to the infusion solution. [³H]Inulin (1 μCi/ml; New England Nuclear, Itisa, Madrid, Spain) was included in the infusion solution to measure GFR. At least 60 min elapsed before the experiment was started.

Experimental procedure

RPP, measured either at the femoral or the carotid catheter, was continuously recorded throughout the experiment on a Hewlett-Packard model 7754A polygraph. After the stabilization period, RPP was lowered to about 100 mmHg by tightening the clamp above the renal arteries, and after 15 min of stabilization, urine and plasma samples were collected during two successive 10 min periods. The aortic clamp was then released so that the kidney was perfused to about 120 mmHg, and after a period of stabilization, two more clearance periods were consecutively recorded. Finally, the clamp below the renal arteries was occluded to further elevate RPP, and after a period of stabilization, two more clearance periods were obtained. In some animals it was necessary to momentarily occlude the celiac and mesenteric arteries to elevate RPP. Urine samples were collected in all periods into pre-weighed plastic vials. Blood samples (150 μl) for the determination of haematocrit and plasma inulin level were obtained from the femoral or carotid catheter into heparinized microhaematocrit tubes in the middle of each clearance period. At the end of the experiment, the animal was killed by an overdose of pentobarbital sodium, and both the thyroid and left kidney were removed and weighed.

Analytical techniques

[³H]Inulin in plasma and urine was measured by counting aliquots of the samples dissolved in scintillation fluid in a β-counter (Betamatic Basic, Kontron, Madrid, Spain). GFR was calculated as the clearance of radioactive inulin (urine to plasma concentration ratio multiplied by urine flow), and was normalized per gram kidney weight. Urine flow was determined gravimetrically. Sodium concentration was measured by flame photometry (Corning 435, Izasa, Barcelona, Spain). Serum T3 and T4 levels were determined by e.l.i.s.a. (Immunoassay System; Baxter, Miami, FL, U.S.A.)

Statistical analysis

Data are presented as means ± SEM. Values of MAP and RBF are the means of the values measured every minute in each experimental period. The significance of the differences in values measured within a group was evaluated using analysis of variance for repeated measures, followed by Duncan’s multiple range test. The differences in measured values between groups were analysed using a two-way analysis of variance followed by Duncan’s multiple range test. Regression analysis was performed by the least squares method. Slopes were compared with Student’s t-test. Values of each biological variable were compared by one-way analysis of variance. When the overall analysis of variance was significant, we then performed pairwise
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Table I. Morphological and haemodynamic variables in the experimental groups. Data are means ± SEM. Statistical significance: *P < 0.05, **P < 0.01 compared with the control group.

<table>
<thead>
<tr>
<th></th>
<th>Body wt. (g)</th>
<th>Left kidney wt. (g)</th>
<th>Thyroid wt. (mg)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>$T_3$ (µg/dl)</th>
<th>$T_4$ (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>389.5±22.6</td>
<td>1.27±0.1</td>
<td>34.7±1.2</td>
<td>119.2±1.9</td>
<td>361.2±5.7</td>
<td>3.7±0.2</td>
<td>53±8.3</td>
</tr>
<tr>
<td>Hyperthyroid (n=6)</td>
<td>298.0±7.7</td>
<td>1.28±0.1</td>
<td>22.4±1.7</td>
<td>146.8±2.8</td>
<td>408.8±4.0</td>
<td>40.7±2.8</td>
<td>345±7.2</td>
</tr>
<tr>
<td>Hypothyroid (n=6)</td>
<td>223.8±0.68</td>
<td>0.68±0.04</td>
<td>166.1±1.7</td>
<td>107.7±2.5</td>
<td>322.6±3.8</td>
<td>0.0±0.0</td>
<td>0±0.0</td>
</tr>
</tbody>
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comparisons by Bonferroni’s method. Differences were considered statistically significant at a $P$ level less than 0.05.

RESULTS

Biological variables

The effects of thyroxine or methimazole administration on biological variables are presented in Table 1. Animals given thyroxine or methimazole for 6 weeks gained significantly less weight than their age-matched controls during this period MAP, HR and serum $T_3$ and $T_4$ levels were decreased in hypothyroid rats and increased in hyperthyroid rats. Thyroid weight was increased in hypothyroid and decreased in hyperthyroid rats. Hence, rats given methimazole for 6 weeks developed characteristic manifestations of hypothyroidism, whereas those given thyroxine for a similar period developed hyperthyroidism.

Renal haemodynamics

The effects of changes in RPP on GFR and RBF are summarized in Fig. 1. In the control rats, GFR at the highest RPP level was significantly greater than at the two lower pressures. In contrast, GFR in hyperthyroid rats was significantly lower at the lowest RPP level than at the two higher pressures. GFR in hypothyroid rats did not change significantly at any level of RPP studied. When we compared GFR between groups, it was significantly decreased in hyperthyroid rats over the entire pressure range when data were expressed per gram kidney weight. However, this difference disappeared when data were expressed per 100 g of body weight (data not shown). RBF in control and hypothyroid groups did not change significantly and was well autoregulated at the levels of RPP studied. However, a significant increase in RBF was observed in hyperthyroid rats at the highest RPP level. RBF did not differ significantly, over the range of pressures studied, between hyper- or hypothyroid rats and the control group, when the data were expressed either per gram kidney weight or per body weight.

PDN response

The relationships between RPP and urine flow, and RPP and urinary sodium excretion, in control, hypothyroid and hyperthyroid rats normalized per gram kidney weight are shown in Fig. 2. In control rats, urine flow and sodium excretion increased from $38.2±7.4$ to $132.7±8.9$ µl min$^{-1}$ g$^{-1}$ and from $4.9±0.9$ to $20.1±1.4$ µmol min$^{-1}$ g$^{-1}$, respectively, in response to an elevation in RPP from 100 to 144 mmHg. In hypothyroid rats, urine flow and sodium excretion increased from $16.8±4.0$ to $109.1±12.2$ µl min$^{-1}$ g$^{-1}$ and from $3.5±0.9$ to $17.7±1.4$ µmol min$^{-1}$ g$^{-1}$, respectively, in response to an elevation in RPP from 101 to 133 mmHg.
There were no significant differences in the pressure-diuretic (slope; control, 2.17 ± 0.23; hypothyroid, 2.80 ± 0.23) and pressure-natriuretic (slope: control, 0.35 ± 0.04; hypothyroid, 0.38 ± 0.06) responses between control and hypothyroid rats. When data were normalized by body weight, the responses were very similar and only a significantly lower urine flow was observed in the hypothyroid group at the lowest level of RPP (data not shown).

In the hyperthyroid rats, the relationships between RPP and urine flow (slope 0.58 ± 0.17, \( P < 0.001 \)), and between RPP and urinary sodium excretion (slope 0.09 ± 0.03, \( P < 0.001 \)) were significantly blunted compared with the control rats. Thus, urine flow and sodium excretion increased from 4.8 ± 1.3 to 32.6 ± 8.6 \( \mu \)mol min\(^{-1} \)g\(^{-1} \) and from 0.4 ± 0.2 to 4.8 ± 1.3 \( \mu \)mol min\(^{-1} \)g\(^{-1} \) kidney weight, respectively, in response to an elevation in RPP from 101 to 150 mmHg. Similar results were observed when data were expressed per 100 g of body weight (data not shown).

The relationships between RPP and fractional excretion of water, and between RPP and fractional excretion of sodium, mirrored those described above for urine flow and sodium excretion (Fig. 2).

**DISCUSSION**

Thyroid disorders are accompanied by widespread alterations in renal haemodynamics and renal handling of sodium and water [2]. The present study characterized the chronic effects of thyroid hormones on the PDN response of rats in which neural and humoral influences on the kidney were controlled. The studies of renal function in thyroid disorders have the difficulty of normalizing the results in suitable physiological terms, since thyroid disorders affect body and kidney size disproportionately. Thus, renal mass related to body weight is usually increased in hyperthyroid and reduced in hypothyroid animals; thus, when data are adjusted to body weight, the amount of functional renal tissue is underestimated in hyperthyroid and overestimated in hypothyroid animals. These observations, together with the fact that we have studied the function of only one (left) kidney, are the reasons why we have preferred to express the data mainly related to kidney weight.

The results of our experiments indicate that hyperthyroid rats show a shift in the acute PDN response toward higher pressures, regardless of the manner of expression of data. This shift appears to be mainly due to an increase in tubular sodium and water reabsorption. The consequence of these changes in the PDN relationship is that for a given neural and endocrine background, RPP would have to be elevated in hyperthyroid rats in order to achieve the same rate of sodium and water excretion as in controls. These observations are consistent with previous findings in experimental hypertension, which suggested that the kidneys of hypertensive rats require elevated arterial pressure to excrete normal quantities of sodium and water [14, 15]. Moreover, our observations raise two possibilities. The first is that this renal dysfunction is necessary for the maintenance of this type of hypertension; the second, is that the change in the PDN relationship in hyperthyroid rats may be a consequence of increased blood pressure.

The structural or functional changes that shift the PDN relationship in the kidneys of hyperthyroid rats, regardless of whether this shift is primary or secondary to the development of hypertension, are unknown. The factors that might contribute to the...
abnormal PDN relationship in these animals include differences in the responsiveness of the renal tubules of hyperthyroid rats to the sodium- and water-retaining components of the hormone cocktail, intrinsic defects in the renal microvasculature or differences in the regulation of sodium and water reabsorption by endogenous circulating and intrarenal factors (e.g. angiotensin II, kinins and eicosanoids) not controlled in this preparation, but known to modulate the PDN response [16, 17]. However, it is important to note that the kidney was derenervated in the present study, and atrial natriuretic peptide levels are increased in hyperthyroidism [18]; thus, our findings cannot be attributed to alterations in renal nerve activity or to atrial natriuretic peptide levels.

Hypothyroidism induced by thyroidectomy or chemical means results in increased diuresis and natriuresis under basal conditions [2, 4], after saline expansion [5, 6, 19] or sodium restriction [6]. This tendency to lose sodium predisposes to shock and has been implicated as a mechanism by which hypothyroidism prevents experimental arterial hypertension [2]. However, the results of other authors [3, 8, 9] using different antithyroid drugs to induce hypothyroidism do not support these findings. The discrepancies may be due to the different protocols used, as many as there are authors, with changes in such fundamental factors as duration of hypothyroidism and the use of loads of the same volume as in control rats [19], without taking into account the reduced body weight of hypothyroid rats. The normal absolute and fractional excretion of sodium in this PDN experiment, when the data are normalized both by body or kidney weight, indicates that hypothyroidism induced by methimazole cannot be considered as a sodium-losing syndrome. However, in this study a direct effect of methimazole on renal function cannot be excluded, although we have observed in other experiments (F. Vargas et al., unpublished work) that chronic treatment with methimazole plus a replacement dose of thyroxine (12 µg/kg body weight) did not change renal function in comparison with control rats, indicating that methimazole per se does not modify renal function.

The findings in hypothyroid rats also indicate that arterial hypotension induced by the hypothyroid state is not accompanied by a shift to the left in the PDN curve, as it has been observed in chronically enalapril-treated hypertensive rats [20]. Our observations also contrast with the PDN curve obtained in hypotensive cirrhotic ascitic rats [21], which was shifted toward lower excretion rates. Hence, no clear pattern in the PDN curve is seen in hypotensive syndromes, in contrast with the clear shift to the right usually observed in hypertensive diseases.

Contradictory findings for RBF and GFR have been reported in hyperthyroid patients [22, 23] and rats [8, 24, 25], and a decrease in both variables has been found in hypothyroid humans and rats [2–5]. Our results contrast with these findings, as RBF and GFR were similar in our hypothyroid and control rats. These discrepancies may be due, at least in some studies [5, 24, 25], to the fact that RBF and GFR were not normalized by kidney weight. In this sense, Fregly et al. [7] noted that when RBF and GFR are related to kidney size, the ratios are the same as those for intact controls. Moreover, our rats were studied while neural and hormonal influences on the kidney were controlled, whereas these factors were not controlled in previous reports. In addition, we measured RBF with an electromagnetic flowmeter, whereas RBF was estimated from the renal clearance of p-aminohippuric acid in previous studies, and it has been reported [23] that thyroid disorders modify the tubular transport maximum for p-aminohippuric acid.

In summary, the diuretic and natriuretic responses to changes in RPP were markedly reduced in hyperthyroid rats compared with control rats, whereas no significant differences were found between controls and hypothyroid rats. This abnormal renal PDN response in hyperthyroid rats may represent the functional resetting of the kidney toward higher perfusion pressures, allowing the maintenance of this type of hypertension. The functional (uncontrolled hormones, renal autacoids, etc.) or structural changes within the kidney that blunt the PDN response in hypothyroidism remain to be identified.

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