Renin-angiotensin system in hypothyroid rats: effects of potassium iodide and triiodo-L-thyronine

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Abstract. Kinetic studies of the renin-angiotensin system (RAS) were carried out by measuring plasma renin activity (PRA), plasma renin concentration (PRC) and plasma renin substrate (PRS). Changes in this system were studied during hypothyroidism, after administration of propylthiouracil (PTU), and in thyroidectomized rats. A significant decrease in PRA and PRC was observed in those animals previously treated with PTU. However, a significant increase in PRC, and a decrease in PRS, were found in T animals, but no changes in PRA were observed. In these animals, after daily administration of potassium iodide for 1 week (T+KI), no changes in RAS were observed in comparison with T rats. Nevertheless, administration of daily doses of triiodo-L-thyronine (T+T₃) induced a significant increase in PRA, leaving PRC unaltered. In this case the changes in PRA were related to the increase in PRS after T₃ treatment. These results suggest that two different mechanisms were involved in renin release, one activated in T rats and the other in pharmacological hypothyroidism.

The existence of a relationship between thyroid function and the renin-angiotensin system (RAS) has been recognized. Several investigators have noticed a decrease in plasma renin activity (PRA) in hypothyroid animals (Hauger-Klevene 1970; Hauger-Kleven & Levin 1976; Jiménez et al. 1982b) and patients (Hauger-Klevenc & Levin 1976) and an increase during hyperthyroidism (Hauger-Klevene 1970; Nara 1973; Jiménez et al. 1982a).

It has recently been noted that kidney extracts from thyroidectomized rats have a greater renin content than those of control animals (Bouhnik et al. 1981).

The aims of this work were to study 1) the changes within the RAS during hypothyroidism, after administration of propylthiouracil, and in thyroidectomized rats, and 2) the response to potassium iodide and triiodo-L-thyronine in thyroidectomized animals.

Material and Methods

Animals, thyroidectomy and treatment

Male Wistar rats were used in this study, fed with standard diets and tap water ad libitum.

A group of animals was selected after birth. For 3 weeks they were fed with a low iodine diet and subsequently thyroidectomized surgically. In order to ensure complete thyroid removal, 500 μCi ¹³¹I was administered to each animal 24 h after thyroidectomy. The rats were fed a low iodine diet supplemented with Ca-lactate. Signs of tetany were looked for. The groups of animals prepared in this way will be referred to as T animals.

Three different experimental groups of T animals were used in these experiments: 1) one, prepared as described before (T), 2) another group of thyroidectomized animals was given 3.0 mg/100 g body weight of potassium iodide (Merck), in daily doses, 1 week prior to the date of sacrifice (T+KI) and 3) a third group of thyroidectomized animals received, also 1 week prior to sacrifice, 3.3 μg/100 g body weight of 3,3',5-triido-L-thyronine (Sigma Co. St. Louis, USA) daily (T+T₃).

Pharmacological hypothyroidism was induced by daily administration of 0.5 mg/100 g body weight propylthiouracil (PTU, Carlo Erba) for 12 days prior to sacrifice.

Potassium iodide, T₃ and PTU were administered ip in phosphate buffered saline containing 0.1% bovine albumin.

A group of control animals received injection ip of phosphate buffered saline (euthyroid group, E).
Collection of samples
Before being killed the animals were put into individual metabolic cages and the urine was collected every 24 h for each experimental group.

Three hours after the last dose, an aliquot of blood was taken from the abdominal aorta, under sodium pento-barbitone anaesthesia (Nembutal, Abbot Lab. Madrid, Spain), and was then poured into a tube containing 80 µl of 6% EDTA. Another aliquot of blood was used to obtain serum. After centrifugation, at 2500 g at 4°C for 15 min, the plasma and serum obtained were stored at -20°C until used.

Plasma renin activity (PRA), plasma renin concentration (PRC) and plasma renin substrate (PRS) estimation
Angiotensin concentration (AI) was determined by radioimmunoassay (Cea-Ire-Sorin, Saluggia, Italy) as described elsewhere (Haber et al. 1969): 10 µl of 0.8 µL dimercaptopropanol and 20 µl of 0.34 µL 8-hydroxiquinoline were used as inhibitors of the converting enzyme and angiotensinases.

The basal AI and the amounts generated during plasma incubation at 37°C and pH 6.5, were measured at 2 hourly intervals up to 8 h. The PRA was determined for basal AI and for that generated during 2 h of incubation. The specific velocity constant of reaction (k) was used to deduce the PRC (Ryan et al. 1968). It was obtained from the expression: k = 1/t ln S0/S0-P, where S0 is the initial renin substrate concentration and P is the AI generated at time t. PRC was measured by a kinetic method (Campillo et al. 1976) as function of the AI generated during incubation (AI generated as t→∞). It was obtained by plotting the reciprocal value of AI generated against the reciprocal of correlated incubation time.

Thyroid hormone determination
Thyroxine (T₄) and T₃ serum levels were measured by radioimmunoassay methods (Radiochemical Centre, Amersham, England), adapted to rat serum, to follow the changes of both hormones.

Sodium and potassium measurements
Urinary excretion and serum levels of sodium and potassium were determined by flame photometry.

Statistics
All results are expressed as mean ± SEM. Statistical evaluation was performed by Student's t-test.

Results

Kinetic parameters of RAS
Fig. 1 shows the alterations in PRA, PRC and PRS after hypothyroidism induced by PTU treatment, and by surgical thyroidectomy. PTU administration provoked a significant decrease in PRA and PRC, while thyroidectomy produced an increase in PRC and a decrease in PRS. PRA remained unchanged.

After administering potassium iodide to a group of thyroidectomized rats, the changes observed on kinetic parameters of RAS were not significantly

Fig. 1.
Plasma renin activity (PRA), plasma renin concentration (PRC) and plasma renin substrate (PRS) in euthyroid animals (E), pharmacological hypothyroidism (PTU) and thyroidectomized rats (T), and in those treated with potassium iodide (T+KI) or triiodo-L-thyronine (T+T₃). Unpaired t-test was used (*P < 0.05, **P < 0.01, ***P < 0.001).
incubation time,
h
Fig. 2.
Time course of angiotensin I generation rate during incubation of plasma at 37°C and pH 6.5 from euthyroid (●), pharmacological hypothyroidism (●) and thyroidectomized rats (*), and in those treated with potassium iodide (▲) or triiodo-l-thyronine (■).

Angiotensin I generation during the incubation period is shown in Fig. 2. Only in pharmacological hypothyroidism is the generation of AI in linear relation with the incubation periods. After T₃ administration to the thyroidectomized rats, the AI formation was higher than in euthyroid specimens.

On the other hand, it was lower in the hypothyroid group than in the untreated thyroidectomized animals.

Table 1 shows, above the diagonal, the values obtained for the simple regression coefficients among the kinetic parameters in the RAS. The values of the multiple determination coefficients, R²₃(1) and R²₃(12), are shown under the diagonal.

**Sodium and potassium**
Sodium and potassium variations in serum and urine during induced changes of thyroid function are shown in Fig. 3. An increase in serum potassium concentration and a decrease in urinary potassium were observed in thyroidectomized animals. These also showed, together with those treated with potassium iodide, an increase of urinary sodium excretion. In PTU-treated animals and those thyroidectomized animals treated with T₃, no changes were observed in the serum or urinary concentrations of sodium and potassium.

**Table 1.**
Multiple regression analysis between the kinetic parameters of renin-angiotensin system (RAS).

<table>
<thead>
<tr>
<th></th>
<th>PRC</th>
<th>log PRS</th>
<th>PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRC</td>
<td>***</td>
<td>R₁,₂ = 0.79</td>
<td>R₁,₃ = 0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>log PRS</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>PRA</td>
<td>R₃(1) = 0.39</td>
<td>R₃(12) = 0.85</td>
<td>***</td>
</tr>
</tbody>
</table>

Other details are described in the text.

Fig. 3.
Serum and urine levels of sodium and potassium in different experimental conditions. Unpaired t-test was used (*P < 0.05, **P < 0.01).
In both pharmacological and surgical hypothyroidism, the T₄ serum levels were decreased. In T₃-treated rats, T₃ was increased.

In thyroidectomized rats, potassium iodide administration produced a significant increase in T₃ serum levels (Table 2).

**Discussion**

Previous studies performed in this laboratory have shown that in rats the PRA is not a useful index for evaluating the changes of renin release during experimental thyroid alterations induced by thyroid hormones or antithyroid drugs (Jiménez et al., in press).

Because of this, a more complete evaluation of RAS components was carried out during studies of induced pharmacological hypothyroidism, and in thyroidectomized animals by means of PRS and PRC estimation.

The results obtained in the present paper, are in agreement with Hauger-Klevene (1970), Hauger-Klevene et al. (1972) and Jiménez et al. (1982b), and show the existence of a functional relationship between thyroid hormones and RAS. However, the changes during induced hypothyroidism, in a group of adult rats, were different from those observed in another group of animals thyroidectomized at the 28th day of life, treated with ¹³¹I and kept on a low iodine diet to minimize the extra-thyroid synthesis of thyroid hormones.

In thyroidectomized rats no change in PRA was observed, in contrast to the marked decrease of this parameter seen in the animals treated with PTU, 0.5 mg/100 g BW, in rats treated with high doses of ¹³¹I, and also in hypothyroid patients (Hauger-Klevene & Levin 1976). On the other hand, while PRC decreases in states of pharmacological hypothyroidism, in thyroidectomized rats this parameter showed an increase. These results suggest that different mechanisms would be involved in renin release in each of the two groups of hypothyroid animals.

The relationship between PRA and PRC differed in the hypothyroid and thyroidectomized groups. In pharmacological hypothyroidism PRC and PRA were directly related in that a decrease in PRC was accompanied by a fall in PRA, but in the thyroidectomized animals PRC increase was not accompanied by any significant change in PRA.

In this context, simple regression analysis using determination coefficients shows that PRC can explain only 39% of the PRA changes. In multiple regression analysis however, taking into account the renin substrate and plasma renin levels, both components of the system are responsible for 85% of the RAS alteration.

These results confirm the biological importance of renin substrate in the increase or decrease of PRA during altered thyroid function, or, as also may be observed, in the course of dexamethasone treatment (Reid et al. 1973) and in other clinical disturbances (Skinner et al. 1972; Hassegawa et al. 1973).

The changes observed in angiotensin I production during sample incubation show the role played by renin substrate as the limiting factor in the reaction velocity (Quesada et al. 1975). In hypothyroid PTU-induced animals a linear relationship exists between angiotensin I generation and the incubation time, while in thyroidectomized animals

### Table 2.

Thyroxine (T₄) and triiodothyronine (T₃) serum levels in different experimental situations.

<table>
<thead>
<tr>
<th></th>
<th>E (7)</th>
<th>PTU (7)</th>
<th>T (6)</th>
<th>T+KI (6)</th>
<th>T+T₃ (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₄ (nmol/l)</td>
<td>81.7 ± 4.0</td>
<td>6.4 ± 0.4*</td>
<td>4.2 ± 0.30</td>
<td>7.9 ± 1.0*</td>
<td>4.0 ± 0.5*</td>
</tr>
<tr>
<td>T₃ (nmol/l)</td>
<td>1.5 ± 0.1</td>
<td>0.25 ± 0.03*</td>
<td>0.21 ± 0.03*</td>
<td>0.51 ± 0.07*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.8 ± 1.6*</td>
</tr>
</tbody>
</table>

Mean values ± SEM are given. N is the number of experiments. E: euthyroid. Unpaired t-test was used (* P < 0.001). a: significance levels in respect to thyroidectomized animals (P < 0.05).
a gradual decrease in production of angiotensin I is observed. This decrease occurs also in the control animals, where the renin substrate is not sufficient to maintain the reaction velocity rate (Carretero & Gross 1967).

The PRS levels during thyroid dysfunction are not only dependent on the plasma renin changes as shown in Table 1. According to Bouhnik et al. (1981) thyroid hormone administration increases PRS levels, thus confirming the important role these hormones play in hepatic angiotensinogen production (Murakami et al. 1981).

The mechanism by which thyroid hormones exert their action on renin release is not completely understood. Since during PTU-treatment there are no changes in sodium and potassium balance, the decreases in the adrenergic and adenyl-cyclase activities observed in hypothyroid patients and animals have been claimed to be the factor causing the PRA decrease in response to lower renin levels (Hauger-Klevene & Levin 1976). The cause of the increase in renal renin content, described by Bouhnik et al. (1981) in thyroidectomized rats, and of the increase in PRC in similar animals, described in this paper, has not been explained.

Histological studies performed on thyroidectomized rats showed evidence of glomerular immaturity, which suggests that the kidney is an organ very susceptible to changes in its development. On the other hand, it has been reported that surgical thyroid ablation produces significant alterations in renal cell metabolism (Holmes & DiScala 1971), together with a decrease in glomerular filtration rate and tubular transport (DiScala & Kinney 1971).

It is well known that during hypothyroidism induced in early stages of development there is a decrease in sodium re-absorption in the proximal and distal tubules (Michael et al. 1972), which increases the urinary sodium excretion (Michael et al. 1976), as confirmed in this paper and other studies.

Since thyroid hormones have an effect on either, or both, the membrane permeability to sodium and its active transport (Katz & Lindheimer 1977), the results suggest that the absence of thyroid hormones could produce an increase of renal and plasma renin levels in response to a feedback mechanism, possibly controlled by the increase in sodium concentration in the macula densa, according to Thurau (1974) in his model for the control of renin release by juxtaglomerular cells.

Following potassium iodide treatment, the urinary excretion and serum levels of potassium return to normal, possibly by an increase of the intracellular potassium concentration (Ismail-Beigi & Edelman 1973). However, the slight increase observed in thyroid hormone concentrations is not capable of restoring the sodium urinary excretion and renin concentration. These circumstances support the opinion that potassium is not directly involved in the renin release. The extrathyroidal hormone synthesis, induced by potassium iodide treatment, is not sufficient to restore the altered kidney function to normal.

The dose of triiodo-l-thyronine used in this work, lower than that used by Bouhnik et al. (1981) also using thyroidectomized animals, and biologically equivalent to the range of thyroxine administered by D'Angelo et al. (1976), is sufficient to suppress the levels of blood TSH during the period of treatment (Spira et al. 1979), and to restore urinary sodium excretion to normal. When triiodo-l-thyronine is present in excess, the PRC increases as a consequence of increased kidney renin release (Ganong 1982). The PRA is elevated by the PRS increase found concomitantly with that of PRC.

Acknowledgments

The authors express their appreciation to Dr. M. T. Miranda and Dr. L. Parras for their advice and assistance in performing the statistical study, and to Mr. D. W. Schofield for his correction of the manuscript.

References


Received on June 28th, 1983.