Role of the renin-angiotensin system in the development of thyroxine-induced hypertension

Cipriano García del Río, María Rosario R Moreno, Antonio Osuna\(^2\), Juan de Dios Luna, Joaquín García-Estañ\(^1\) and Félix Vargas

Departamentos de Fisiología y Bioestadística and \(^1\)Facultades de Medicina de Granada y Murcia, and \(^2\)Servicio de Neftología. U. Experimental Virgen de las Nieves. Granada, Spain

(Correspondence should be addressed to F Vargas. Departamento de Fisiologia. Facultad de Medicina. E-18012 Granada. Spain)

Abstract

Objective: We evaluated the influence of chronic blockade of the renin-angiotensin system on hypertension induced by long-term thyroxine (T\(_4\)) administration. To this end, we determined the effects of chronic treatment with captopril on blood pressure, cardiac hypertrophy and other renal and metabolic variables of hypertensive hyperthyroid rats.

Methods: T\(_4\) was administered s.c. at 0·38 \(\mu\)mol/kg per day and captopril was given in the drinking water (1·38 mmol/l). Both treatments were maintained for 6 weeks. Control rats received tap water. After the treatment period, the rats were placed in metabolic cages. Later, blood pressure was measured in conscious rats by intra-arterial determination.

Results: T\(_4\)-treated rats showed an increased mean arterial pressure (MAP) whereas, in rats treated with T\(_4\) plus captopril, MAP was similar to that of the control group. Captopril did not affect the increased heart rate or ventricular weight/body weight ratio of hyperthyroid rats, but it improved the reduced creatinine clearance of these animals.

Conclusions: The elevation in blood pressure produced by long-term T\(_4\) administration was prevented by chronic blockade of the renin-angiotensin system. Captopril improved the renal function of hyperthyroid rats, but did not affect the relative cardiac hypertrophy of these animals.

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Introduction

Hyperthyroidism is an endocrine disease associated with important haemodynamic, renal and cardiac alterations such as increased cardiac output, reduced total peripheral resistance, increased blood pressure, a tendency to retain sodium and cardiac hypertrophy (1–3). These characteristic cardiovascular manifestations of hyperthyroidism are also reproduced by treatment with thyroid hormones in rats (1–3); these animal studies have shown a dose- and time-related increase in mean arterial pressure (MAP) (4, 5).

The importance of the renin-angiotensin-aldosterone system (RAAS) in the long-term control of arterial pressure, renal function and cardiac hypertrophy is well established (6–8). Hyperthyroidism is accompanied by hyperactivity of the RAAS (9–12). Thus, plasma renin activity and plasma levels of angiotensinogen, angiotensin II and aldosterone are directly related to plasma levels of thyroid hormones (10–12). Moreover, it has been shown that tri-iodothyronine (T\(_3\)) treatment increases angiotensin receptor density in kidney, liver and both cardiac ventricles (12). These studies indicate that the RAAS may play an important role in the increased blood pressure and cardiac and renal alterations of hyperthyroidism. Recent studies from our laboratory have shown that the acute administration of captopril markedly decreased MAP and improved renal haemodynamics and excretion in hypertensive hyperthyroid rats (13). However, long-term studies of the effects of RAAS blockade to prevent this type of hypertension have not been performed. Therefore the present study was undertaken to evaluate the influence of chronic blockade of the RAAS on the development of hypertension induced by the long-term administration of thyroxine (T\(_4\)). In addition, we also determined the effects of this treatment on cardiac hypertrophy and other renal and metabolic variables.

Materials and methods

Animals and experimental protocol

Male Wistar rats, born and raised in the experimental animal service of the University of Granada, were used. All experiments were performed according to the guidelines for the ethical care of animals of the European
Union. Rats initially weighing 150–175 g were randomly divided into four groups: control (C), control-captopril (CAP), thyroxine (T₄) and thyroxine-captopril (T₄-CAP) rats. All rats had free access to food and tap water. Captopril was given in the drinking water at an initial dose of 1·38 mmol/l (about 0·138 mmol/kg). The concentration of captopril in the drinking fluid was adjusted every 2 days according to the fluid intake of these animals, to ensure that a similar dose was administered to both captopril-treated groups. Hyperthyroidism was induced by injecting s.c. T₄ (Merck: 0·38 µmol/kg per day dissolved in 0·5 mol/l NaOH isotonic saline), a dose which has been previously used and known to produce arterial hypertension (5, 13). The treatments were administered for 6 weeks.

The effectiveness of these treatments was assessed by comparing plasma T₄, plasma T₃ and plasma levels of angiotensin II in control and treated rats. After the treatment period, all rats were housed in metabolic cages with free access to food and their respective drinking fluids for 5 days (2 days for adaptation and 3 experimental days) to measure food and fluid intake and to collect urine samples. Twenty-four hour urine volume, creatinine and total excretion of sodium and potassium were measured. The means of the values obtained in each intake or urinary variable during the 3 experimental days were used for statistical analyses between groups.

After the metabolic study was completed, the rats were anaesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 U heparin in isotonic sterile NaCl solution was inserted into the femoral artery for intra-arterial blood pressure and heart rate measurement and extraction of blood samples. The catheter was tunneled subcutaneously, and brought out through the skin at the dorsal side of the neck and protected with a silver spring. Twenty-four hours after the femoral catheter was implanted, intra-arterial blood pressure was measured by a TRA-021 transducer connected to a two channel Letigraph 2000 recorder (Letica SA, Barcelona, Spain). After 30 min of stabilization, values from the last 5 min recorded were averaged and used for comparisons between groups. Subsequently, blood samples from the femoral catheter were taken to determine thyroid hormones, angiotensin II, electrolytes, urea and creatinine. Immediately after exsanguination, the kidneys and ventricles were weighed. The heart was divided into the right ventricle and the left ventricle plus the septum.

**Analytical procedures and statistical analysis**

Plasma T₄ and T₃ levels were determined by a chemiluminescent enzyme immunoassay (IMMULITE: DPC, Los Angeles, CA, USA). Plasma angiotensin II levels were measured in methanol-extracted samples with an RIA kit purchased from Amersham Iberica SA (Madrid, Spain). Plasma and urinary electrolytes, urea and creatinine were measured in an autoanalyzer (Beckman CX4: USA).

Comparisons of each variable were done by performing a one-way ANOVA. When the overall ANOVA was significant, we then performed pairwise comparisons with Bonferroni’s and Newmann–Keul’s methods.
Table 1 Morphological variables. Body weight (BW), ventricular weight (VW), ventricular weight/body weight ratio (VW/BW), left/right ventricular weight ratio (LV/RV), kidney weight (KW) and kidney weight/body weight ratio (KW/BW), in C, CAP, T4 and T4-CAP rats. Values are means±S.E.M.; n = 7.

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>VW/BW (mg/g)</th>
<th>LV/RV</th>
<th>KW (mg)</th>
<th>KW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>421 ± 15.2</td>
<td>1118 ± 38</td>
<td>2.65 ± 0.06</td>
<td>3.59 ± 0.16</td>
<td>1300 ± 53</td>
<td>3.07 ± 0.07</td>
</tr>
<tr>
<td>CAP</td>
<td>380 ± 22.5</td>
<td>1001 ± 70</td>
<td>2.63 ± 0.16</td>
<td>3.46 ± 0.17</td>
<td>1262 ± 83</td>
<td>3.32 ± 0.07</td>
</tr>
<tr>
<td>T4</td>
<td>312 ± 28**</td>
<td>1293 ± 46</td>
<td>3.87 ± 0.2**</td>
<td>3.48 ± 0.06</td>
<td>1460 ± 59</td>
<td>4.35 ± 0.16**</td>
</tr>
<tr>
<td>T4-CAP</td>
<td>318 ± 23**</td>
<td>1123 ± 58</td>
<td>3.57 ± 0.16**</td>
<td>3.43 ± 0.10</td>
<td>1365 ± 76</td>
<td>4.33 ± 0.18**</td>
</tr>
</tbody>
</table>

**P < 0.01 compared with C and CAP groups.

Results

Blood pressure and heart rate

Figure 1 shows the systolic (SBP), diastolic (DBP) and MAP in the experimental groups at the end of the experimental period. These variables were significantly increased (P < 0.001 for each variable) in hyperthyroid T4-treated rats when compared with the control group. Co-administration of captopril during T4 treatment fully prevented the development of hypertension in these animals. Treatment with captopril also significantly reduced SBP, DBP and MAP (P < 0.05 each variable) in normal rats.

Heart rate (HR) was significantly increased (P < 0.01 for each group) in both T4-treated groups (T4 and T4-CAP) when compared with their respective controls (C and CAP). Chronic treatment with captopril did not affect the HR in control or hyperthyroid rats (Fig. 1).

Morphological variables

Body weight was significantly reduced in both T4-treated groups (P < 0.01 for each group) when compared with the control groups. Relative ventricular and renal weights were significantly increased in both T4-treated groups (P < 0.01 for each group and variable) in comparison with their corresponding controls (Table 1). The ratio of left ventricular to right ventricular weights, used as the index of absolute left ventricular hypertrophy, was similar in all four groups. Treatment with captopril did not significantly affect any morphological variable in control or hyperthyroid rats (Table 1).

Plasma, metabolic and urinary variables

Plasma T3 and T4 levels were markedly increased in both groups of hyperthyroid rats (P < 0.01 for each group), and plasma angiotensin II levels were significantly reduced (P < 0.01) in both captopril-treated groups. These data indicate the efficacy of both treatments. Plasma angiotensin II levels in the T4-treated group were greater (P < 0.01) than in the control group. Plasma sodium was significantly decreased (P < 0.01) in both groups treated with captopril. There were no significant differences in plasma potassium and creatinine levels between the experimental groups. Plasma urea was significantly decreased (P < 0.05) in the T4-CAP group (Table 2).

Creatinine clearance normalized per g kidney weight was significantly reduced in the T4 group (P < 0.01). This reduction in creatinine clearance was significantly improved (P < 0.05, T4-CAP vs T4) by captopril treatment, although it did not reach the control values (P < 0.05, T4-CAP vs C). Captopril treatment did not significantly change creatinine clearance in normal rats (Table 2).

Metabolic studies at the end of treatment showed increased food intake in both T4-treated groups (T4 = 10.81 ± 0.77, T4-CAP = 9.62 ± 0.47, P < 0.01) in

Table 2 Plasma variables and creatinine clearance (CrC). Plasma levels of electrolytes, urea, creatinine, angiotensin II (Ang II) and thyroid hormones measured in conscious rats after 6 weeks of treatment. Values are means±S.E.M.; n = 7.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>CAP</th>
<th>T4</th>
<th>T4-CAP</th>
</tr>
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<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>143.5 ± 0.8</td>
<td>138.0 ± 1.2**</td>
<td>142.7 ± 0.8</td>
<td>136.3 ± 0.9††</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.1 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>17.5 ± 0.67</td>
<td>17.2 ± 0.61</td>
<td>8.79 ± 0.56</td>
<td>7.96 ± 0.71†</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>273 ± 0.7</td>
<td>28 ± 1.0</td>
<td>28.7 ± 0.7</td>
<td>27.3 ± 2.1</td>
</tr>
<tr>
<td>CrC (ml/min per g kidney)</td>
<td>1.13 ± 0.04</td>
<td>1.20 ± 0.08</td>
<td>0.85 ± 0.04**</td>
<td>1.07 ± 0.21†</td>
</tr>
<tr>
<td>Ang II (pmol/l)</td>
<td>17.3 ± 2.02</td>
<td>3.08 ± 0.96**</td>
<td>37.6 ± 3.27**</td>
<td>4.7 ± 0.09††</td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>46.2 ± 3.7</td>
<td>47.5 ± 1.25</td>
<td>151.2 ± 11.25</td>
<td>140 ± 12.5</td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>0.92 ± 0.09</td>
<td>0.91 ± 0.05</td>
<td>2.54 ± 0.18**</td>
<td>2.39 ± 0.15</td>
</tr>
</tbody>
</table>

*P < 0.05 versus C; **P < 0.01 versus C; †P < 0.05, ††P < 0.01 versus T4.
comparison with their corresponding controls (C = 6.63 ± 0.24, CAP = 7.45 ± 0.28). Chronic treatment with captopril did not affect food intake in control or hyperthyroid rats (see above). Rats in the T4 group drank more fluid (14.3 ± 1.3, P < 0.01) than the control group (7.9 ± 0.39), and treatment with captopril increased fluid consumption in both normal (CAP = 14.8 ± 0.65, P < 0.01 vs C) and hyperthyroid (T4-CAP = 19.5 ± 1.19, P < 0.05 vs T4) rats.

Urine volume and total sodium and potassium excretion were significantly higher in both groups treated with T4 than in the control group (T4, P < 0.01; T4-CAP, P < 0.01, for each variable). Urine volume was also significantly increased (P < 0.01 vs control) in captopril-treated animals (Table 3).

Discussion

In a previous study we observed that the acute administration of captopril or losartan was followed by a significant decrease in MAP in hypertensive hyperthyroid rats, whereas no significant changes were observed in normal rats (13). The data reported in the present study extend these observations by showing that chronic treatment with captopril prevents T4-induced hypertension. Our data clearly indicate that the hyperactivity of the RAAS previously reported (9–12), and confirmed in this study, in hyperthyroid animals plays an important role in the increase in blood pressure produced by T4 treatment.

Increased blood pressure in the hyperthyroid state has been considered a model of cardiogenic hypertension (1–3). Thus, the increased blood pressure would be mainly maintained by an increased cardiac output, which is the result of the elevated stroke volume and increased heart rate. However, the fact that hyperthyroid rats treated with captopril showed normal blood pressure with increased heart rate, and probably also increased cardiac output, indicates that the increased cardiac output per se may not be the cause of hypertension in hyperthyroidism.

Hyperthyroidism resembles the clinical manifestations of a hyperadrenergic state. Despite this clinical impression, several studies have reported normal or low levels of catecholamines (14, 15) and an unaltered catecholamine responsiveness (16). However, it has been reported that, in experimental hyperthyroidism, the heart has an increased number of β-adrenergic receptors (17) and we also observed an increased concentration in the renal tissue (18), which may be related to the increased renin release observed in these animals. The results of the present paper seem to indicate that the increased renal concentration of β-adrenergic receptors plays a predominant role in the pathogenesis of T4-induced hypertension.

It is well known that the hyperthyroid state is associated with cardiac hypertrophy (2, 3, 19). Moreover, cardiac hypertrophy is a compensatory response secondary to the chronic increase in blood pressure, and hence usually observed in the different models of arterial hypertension (7, 8). The results of this study show that hyperthyroidism leads to an increase in the overall ventricular size and weight, which proportionally exceed the reduction in body weight so that heart-to-body weight ratio, a measure of relative ventricular hypertrophy, is increased. In addition, our results show that treatment with T4 did not influence the left-to-right ventricular weight ratio, a measure of absolute left ventricular hypertrophy. These results contrast with those reported in other models of arterial hypertension, where absolute left ventricular hypertrophy has been reported (20). The discrepancies between these data and those found in spontaneously hypertensive rats (SHR) may be explained by the fact that hypertrophy in spontaneous hypertension is produced by increased afterload, whereas in hyperthyroidism it is secondary to hyperdynamic circulation.

One of the factors that contributes directly, or in concert with elevated blood pressure, to the development of cardiac hypertrophy is the renin-angiotensin system (RAS) (7, 8). Angiotensin-converting enzyme inhibitors, by suppressing the cardiac RAS, have been shown to contribute to the regression of cardiac hypertrophy associated with systemic hypertensive states (7, 8). The RAS status was assessed in the present study by measuring its biologically active peptide angiotensin II. Plasma angiotensin II was higher in hyperthyroid rats than in control rats, suggesting its relation to left ventricular hypertrophy. However, chronic inhibition of the RAS with captopril did not significantly alter the relative ventricular hypertrophy in hyperthyroid rats. These findings agree with previous observations in normotensive hyperthyroid rats (21), but contrast with the efficacy of captopril in reversing or preventing cardiac hypertrophy in SHR (22). The reason for these discrepancies may be the absence of absolute left ventricular hypertrophy in hypertensive hyperthyroid rats.

Table 3 Urinary variables measured in the experimental groups after 6 weeks of treatment. Values are means ± S.E.M.; n = 7.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>CAP</th>
<th>T4</th>
<th>T4-CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume (ml/100 g)</td>
<td>3.19 ± 0.5</td>
<td>10.2 ± 1.1**</td>
<td>7.1 ± 0.62**</td>
<td>8.97 ± 0.73**</td>
</tr>
<tr>
<td>Sodium excretion (mmol/100 g)</td>
<td>0.40 ± 0.03</td>
<td>0.44 ± 0.02*</td>
<td>0.78 ± 0.05**</td>
<td>0.81 ± 0.05**</td>
</tr>
<tr>
<td>Potassium excretion (mmol/100 g)</td>
<td>1.11 ± 0.04</td>
<td>1.14 ± 0.07*</td>
<td>1.55 ± 0.08**</td>
<td>1.50 ± 0.07**</td>
</tr>
</tbody>
</table>

**P < 0.01 versus C.
The metabolic study at the end of treatment showed that control and hyperthyroid rats treated with captopril had an elevated fluid intake. This effect of chronic converting enzyme inhibition on fluid intake has been reported by others (23, 24), although the mechanism involved is not fully understood. Contradictory findings for glomerular filtration rate (GFR) have been reported in hypertensive patients (1, 25, 26) and rats (27, 28). These differences may be due to the fact that GFR was not normalized by kidney weight, especially because hyperthyroid rats have larger kidneys than controls. Creatinine clearance in control animals was not affected by captopril, but the reduced GFR of the hyperthyroid rats was significantly improved by the converting enzyme inhibition. These results agree with our previous study showing that the acute blockade of the RAAS reversed the reduced GFR and renal blood flow in hyperthyroid hypertensive animals (13).

In summary, this study shows that chronic treatment with captopril prevents the development of T₄-induced hypertension and improves the reduced creatinine clearance of hyperthyroid rats. Therefore, these results provide evidence for the influence of the RAAS on the increased blood pressure and renal alterations present in these animals. In addition, our data demonstrate the presence of a relative ventricular hypertrophy, which was unchanged by captopril, and the absence of absolute left ventricular hypertrophy in hypertensive hyperthyroid rats.

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