Thyrotoxicosis-induced cardiac hypertrophy: influence of adrenergic nervous system versus renin-angiotensin system on myocyte remodeling

L. W. Hu, L. A. Benvenuti, E. A. Liberti, M. S. Carneiro-Ramos, and M. L. M. Barreto-Chaves. Thyrotoxicosis-induced cardiac hypertrophy: influence of adrenergic nervous system versus renin-angiotensin system on myocyte remodeling. Am J Physiol Regul Integr Comp Physiol 285: R1473–R1480, 2003. First published August 21, 2003; 10.1152/ajpregu.00269.2003.—The present study assessed the possible involvement of the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) in thyroxine (T4)-induced cardiac hypertrophy. Hemodynamic parameters, heart weight (HW), ratio of HW to body weight (HW/BW), and myocyte width were evaluated in absence of thyroid hormone (hypothyroidism) and after T4 administration. Male Wistar rats were used. Some were subjected to thyroidectomies, whereas hyperthyroidism was induced in others via daily intraperitoneal injection of T4 (25 or 100 μg·100 g BW⁻¹·day⁻¹) for 7 days. In some cases, T4 administration was combined with the angiotensin I-converting enzyme inhibitor enalapril (Ena), with the angiotensin type 1 (AT1) receptor blocker losartan (Los) or with the β-adrenergic blocker propranolol (Prop). Hemodynamics and morphology were then evaluated. Systolic blood pressure (SBP) was not altered by administration of either T4 alone or T4 in combination with the specific inhibitors. However, SBP decreased significantly in hypothyroid rats. An increased heart rate was seen after administration of either T4 alone or T4 in combination with either Los or Ena. Although the higher dose of T4 significantly increased HW, HW/BW increased in both T4-treated groups. Ena and Prop inhibited the increase in HW or HW/BW in hyperthyroid rats. Morphologically, both T4 dose levels significantly increased myocyte width, an occurrence prevented by RAS or SNS blockers. There was a good correlation between changes in HW/BW and myocyte width. These results indicate that T4-induced cardiac hypertrophy is associated with both the SNS and the RAS.

Cardiac myocytes; thyroid hormone; angiotensin-converting enzyme; angiotensin II receptors

Changes in hemodynamic loading appear to be an important stimulus for cardiac growth. However, disassociation between elevated arterial pressure and increased myocardial mass has been demonstrated in hypertensive cardiac hypertrophy in animals and humans (25). This dissociation suggests that, in addition to blood pressure, various stimuli are involved in the development and regression of cardiac hypertrophy (25). Over the past several decades, many neural and hormonal stimuli have been implicated in cardiac muscle growth, including, but not limited to, α- and β-adrenergic agonists, ANG II, glucocorticoids, insulin, growth hormone, glucagon, and thyroid hormone (T4) (25).

Mechanisms of cardiac hypertrophy produced by elevated T4 include a direct effect of the hormone on the heart and indirect effects related to stimulation of the adrenergic nervous system or altered left ventricular loading conditions. Decreased systemic vascular resistance and the subsequent increase in cardiac work contribute to this T4-induced hypertrophy (15, 25). However, in support of a direct effect of thyroid hormone on heart growth, some authors have observed that T4-induced cardiac hypertrophy in isolated heart preparations is accompanied by increased (31) quantity and rate of protein and ribosome synthesis (33).

It is well established that the enhanced hemodynamic parameters produced by increased sympathetic nervous system (SNS) activity constitute an important factor in the cardiac hypertrophy induced by hyperthyroidism (16). Moreover, chronic administration of the β-agonist isoproterenol also increases cardiac weight (20, 40). Thus many of the clinical findings of hyperthyroidism such as increased heart rate, cardiac output, and enhanced myocardial contractility are frequent and have led to the conclusion that hyperthyroidism is a hyperadrenergic state (22). However, there are conflicting reports regarding the preventative nature of sympathetic inhibition in hyperthyroidism-associated cardiac hypertrophy (11, 15).

In addition to the clear influences of the SNS, the renin-angiotensin system (RAS) may contribute to the...
cardiovascular modifications observed in hyperthyroidism. For example, RAS inhibitors are beneficial in the treatment of chronic heart failure and acute myocardial ischemia, as well as in regression of cardiac hypertrophy (9). Recently, Kobori et al. (17) reported that the local RAS plays a primary role in the development of hyperthyroidism-induced cardiac hypertrophy. These authors demonstrated that thyroid hormone activates the circulating and tissue RAS without involving the SNS. This may account for the cardiac hypertrophy observed in hyperthyroidism (18). Under these conditions, thyroid hormone directly stimulates renin mRNA in vivo (18) and in vitro (13) and increases both the synthesis and secretion of angiotensinogen and ANG II receptors (32). Conversely, thyroidectomy-induced (10) and methimazole-induced (25) thyroid dysfunction decrease plasma renin activity. These findings suggest that hyperthyroidism-induced cardiac hypertrophy could be involved in changes in circulating RAS or SNS activity.

The present study was designed to address the role of the β-adrenergic system versus that of the cardiac RAS in T4-induced cardiac hypertrophy. Two objectives were set. The first objective was to assess effects of two different dose levels [25 and 100 μg/100 g body weight (BW)] of T4 for 7 days on cardiac hypertrophy development. The second objective was to compare the effects of treatment with an angiotensin-converting enzyme (ACE) inhibitor or angiotensin type 1 (AT1)-receptor blocker versus those of a β-adrenergic blocker on T4-induced cardiac hypertrophy. β-Adrenergic blockade in hyperthyroidism has been analyzed previously in short-term (40) and long-term (12) experiments. However, the effects of drugs known to block RAS and prevent cardiomyocyte remodeling in other models of hyperthyroidism (6) have not yet been well established in thyroid hormone-induced cardiac hypertrophy.

MATERIALS AND METHODS

Animals

Care and use of laboratory animals were performed according to National Institutes of Health guidelines. Male Wistar rats weighing 170–250 g were obtained from the Instituto de Ciencias Biomédicas. They received a rat pellet diet and water ad libitum until the time of the experiment and were maintained in a temperature- and light-controlled (24°C; 12 h on/12 h off) environment. L-Thyroxine (T4) was purchased from Sigma (St. Louis, MO). Initially, rats were randomized into four groups: hypothyroid (Hypo), hyperthyroid (Hyper; T4-25 and T4-100), and control. In the Hypo group, the rats were surgically thyroidectomized and treated with methimazole (0.05%) added to the drinking water for 7 days. Hyperthyroidism was induced in T4-25 and T4-100 rats by daily intraperitoneal injections of T4 (25 and 100 μg·100 g BW⁻¹·day⁻¹, respectively) for 7 days. The Hyper group T4-100 was then subdivided into four groups: T4-100, T4 plus losartan (T4-Los), and T4 plus propanolol (T4-Prop). Enalapril, losartan, and propanolol were administrated in the drinking water once a day for 7 days at doses of 250 (19), 200 (29) and 750 (15) mg/l, respectively, during treatment. All rats were killed 24 h after the last dose of T4.

Hemodynamic Parameters

In all rats, systolic blood pressure (SBP) and heart rate (HR) were measured daily at approximately the same time of day by tail-cuff plethysmograph (Kent Scientific). Additionally, body weight (BW) was measured daily and SBP and weight were last determined immediately preceding death. Rats were familiarized with the apparatus for a total of 7 days before measurements were taken.

Heart Preparation

Morphological data were obtained with a previously reported method (35). After 1 wk, animals were anesthetized with pentobarbital sodium (45 mg/kg intraperitoneally) and killed by exsanguination from the abdominal aorta. The hearts were excised, immersed in ice-cold buffered sucrose solution for 20 min, and frozen in paraffin. The samples were sectioned at 5 μm, and 12 nonconsecutive sections were collected and stained with hematoxylin and eosin for morphometric analysis. To identify possible interstitial fibrosis, sections (10 μm in thickness) from Hypo and Hyper (T4-25 and T4-100) groups were stained by the Sirius red method and examined under polarized light to identify collagen fibers (14). A stereological method (number of points) was used to quantify collagen fibers in the myocardium of nine sections from three rats in each group according to Mandarim-De-Lacerda (24).

Following the method by Vliegen et al. (37), we measured the cardiomyocyte width of 50 cells per ventricular sample from eight rats in each group. The measurements were performed with a magnification of ×400 and a microscopic image analysis system (KS 300, Zeiss). The measurements were taken across the center of the nucleus, considering only myocytes showing longitudinal orientation, not branching, in the circular midwall bundles.

The left ventricular wall thickness and chamber size were evaluated in five rats each from the Hyper (T4-100) and control groups. The wall thickness was measured as the distance between the endocardium and epicardium on the lateral wall, disregarding the papillary muscles. The measurements were taken across a line perpendicular to the curvature of the ventricular wall, using the histological section presenting the fewest technical artifacts. The left ventricular chamber size was evaluated on the same histological section by measuring the cross-sectional area of the left ventricular lumen. The measurements were performed with a magnification of ×25 and a microscopic image analysis system (KS 300, Zeiss).
Table 1. Hemodynamic parameters before death

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>142.81 ± 7.08</td>
<td>378.72 ± 20.39</td>
</tr>
<tr>
<td>T4-25</td>
<td>10</td>
<td>142.23 ± 17.43</td>
<td>426.56 ± 30.50*</td>
</tr>
<tr>
<td>T4-100</td>
<td>8</td>
<td>142.80 ± 7.97</td>
<td>444.33 ± 37.27†</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>8</td>
<td>104.55 ± 14.07‡</td>
<td>361.70 ± 25.70</td>
</tr>
<tr>
<td>Enalapril</td>
<td>5</td>
<td>122.23 ± 12.35⁎</td>
<td>367.09 ± 32.98</td>
</tr>
<tr>
<td>Losartan</td>
<td>9</td>
<td>115.01 ± 24.13†</td>
<td>384.85 ± 22.42</td>
</tr>
<tr>
<td>Propanolol</td>
<td>5</td>
<td>138.98 ± 11.83</td>
<td>335.60 ± 11.59*</td>
</tr>
<tr>
<td>T4-100 + enalapril</td>
<td>5</td>
<td>164.48 ± 11.03</td>
<td>426.84 ± 32.98*</td>
</tr>
<tr>
<td>T4-100 + losartan</td>
<td>8</td>
<td>121.44 ± 23.31</td>
<td>452.25 ± 33.88$</td>
</tr>
<tr>
<td>T4-100 + propanolol</td>
<td>5</td>
<td>172.04 ± 22.86</td>
<td>396.41 ± 35.52</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. T4-25, rats treated with 0.025 mg·100 g⁻¹·day⁻¹ of thyroxine; T4-100, 0.1 mg·100 g⁻¹·day⁻¹ of thyroxine. *P < 0.05 vs. control; †P < 0.01 vs. control; ‡P < 0.001 vs. control; $P < 0.001 vs. losartan.

Statistical Analysis

The data are shown as means ± SE. Statistical analysis was performed using ANOVA, and values <0.05 were accepted as statistically significant. Tukey’s post hoc test was used to make individual comparisons between groups when a significant change was observed with ANOVA. Correlation coefficients were calculated with linear regression analysis.

RESULTS

Hemodynamic Parameters

The parameters of SBP and HR after T4 administration, after thyroidectomy (Hypo group), and after treatment with diverse inhibitors are summarized in Table 1. Neither dose of thyroid hormone affected SBP. However, SBP decreased significantly in hypothyroid rats (104.55 ± 14.07 vs. 142.81 ± 7.08 mmHg). In addition, both enalapril alone and losartan alone caused a marked reduction in SBP (122.23 ± 12.35 and 115.01 ± 24.13 mmHg, respectively).

Both doses of thyroid hormone (T4-25 and T4-100) significantly increased HR (426.56 ± 30.50 and 444.33 ± 37.27 beats/min, respectively, vs. 378.72 ± 20.39 beats/min in control animals). Neither losartan alone nor enalapril alone altered HR. However, HR was reduced 12% (P < 0.05) after propanolol treatment. T4 combined with either losartan or enalapril caused similar increases in HR, greater than those induced by T4 alone. However, propanolol treatment normalized HR of hyperthyroid rats (395.41 ± 35.52 vs. 378.72 ± 20.39 beats/min in controls).

Cardiac Hypertrophy

The data referring to BW before and after treatment and HW are summarized in Table 2. BW at the beginning of the experiment was the same in all groups of rats. However, BW gain during the 7-day study period was not similar in hypothyroid rats and was significantly different at death (191.1 ± 17.8 g in Hypo group vs. 224.6 ± 11.3 g in control). The T4-100 group presented a significant increase in HW (18%) compared with the control group (Table 2), and in both T4-treated groups (T4-25 and T4-100) a significant increase (P < 0.001) in HW/BW was observed (19.5% and 21.3%, respectively; Table 3). In contrast, HW/BW was significantly decreased in hypothyroid rats (P < 0.001). The hypertrophy induced by hormone in either ventricle was analyzed in some animals. Hypertrophy did not develop symmetrically; the percentage of weight gain in the left and the right ventricles was not equal. In fact, the weight gain of the right ventricle was more pronounced with both dose levels (+42.9% for T4-25 and +36.4% for T4-100) compared with the left ventricle (+9.6% for T4-25 and +17.3% for T4-100). This is also reflected by an increase in the right ventricle-to-left ventricle weight ratio (RV/LV) (Table 3). In addition, the hearts of hyperthyroid rats (T4-100) showed an increase in the ratio of left ventricle wall thickness to left ventricle chamber size (mm/mm²), which might suggest a concentric hypertrophy; however, this change was not significant (0.36 ± 0.12 vs. 0.43 ± 0.17 in control group; n = 5).

Compared with the higher dose of T4 alone (T4-100 group), the use of enalapril and propanolol combined with T4 significantly reduced BW, HW, and HW/BW, indicating that these drugs were able to prevent thyroid hormone-induced cardiac hypertrophy (Table 2; Fig. 1). However, the combination of T4 and losartan did not affect either BW, HW (Table 2), or HW/BW (Fig. 1). These results were confirmed when the dry heart mass-to-body mass ratio was determined (data not shown).

Table 2. Body weight before and after treatment and heart weight in all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body Weight (Day 0), g</th>
<th>Body Weight (End), g</th>
<th>Heart Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>202.90 ± 19.60</td>
<td>224.62 ± 11.35</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>T4-25</td>
<td>8</td>
<td>202.86 ± 22.65</td>
<td>221.75 ± 10.53</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>T4-100</td>
<td>8</td>
<td>201.53 ± 23.25</td>
<td>217.62 ± 18.11</td>
<td>0.73 ± 0.06b</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>7</td>
<td>196.00 ± 5.35</td>
<td>191.12 ± 17.75b</td>
<td>0.44 ± 0.06c</td>
</tr>
<tr>
<td>Enalapril</td>
<td>8</td>
<td>184.00 ± 9.62</td>
<td>212.50 ± 13.79</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>Losartan</td>
<td>4</td>
<td>217.00 ± 7.70</td>
<td>205.00 ± 7.61a</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>Propanolol</td>
<td>5</td>
<td>187.50 ± 6.19</td>
<td>203.87 ± 6.74b</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>T4-100 + enalapril</td>
<td>8</td>
<td>192.87 ± 5.96</td>
<td>190.25 ± 26.14b,c,d</td>
<td>0.52 ± 0.02b,e</td>
</tr>
<tr>
<td>T4-100 + losartan</td>
<td>8</td>
<td>247.00 ± 36.94</td>
<td>234.12 ± 40.87</td>
<td>0.71 ± 0.07a</td>
</tr>
<tr>
<td>T4-100 + propanolol</td>
<td>8</td>
<td>192.50 ± 7.23</td>
<td>180.87 ± 37.06b,d</td>
<td>0.60 ± 0.06a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. *P < 0.05 vs. control; †P < 0.01 vs. control; ‡P < 0.001 vs. control; §P < 0.05 vs. T4-100; †P < 0.001 vs. T4-100.
THYROXINE-INDUCED CARDIAC HYPERTROPHY

Table 3. Heart weight-to-body weight ratio, LV weight, RV weight, and RV-to-LV weight ratio in rats treated with thyroid hormone and in hypothyroid rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Heart wt/Body wt, mg/g</th>
<th>LV wt, g</th>
<th>Percent Change</th>
<th>RV wt, g</th>
<th>Percent Change</th>
<th>RV/LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>2.695 ± 0.097</td>
<td>0.520 ± 0.033</td>
<td></td>
<td>0.107 ± 0.015</td>
<td></td>
<td>0.220 ± 0.016</td>
</tr>
<tr>
<td>T4-25</td>
<td>5</td>
<td>3.221 ± 0.139‡</td>
<td>0.576 ± 0.055</td>
<td>± 9.6</td>
<td>0.153 ± 0.025†</td>
<td>+42.9</td>
<td>0.241 ± 0.038</td>
</tr>
<tr>
<td>T4-100</td>
<td>5</td>
<td>3.271 ± 0.081‡</td>
<td>0.617 ± 0.051‡</td>
<td>+17.3</td>
<td>0.145 ± 0.011†</td>
<td>+36.4</td>
<td>0.249 ± 0.019*</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>4</td>
<td>2.227 ± 0.071§</td>
<td>0.3495 ± 0.055‡</td>
<td>−32.6</td>
<td>0.075 ± 0.0028§</td>
<td>−29.9</td>
<td>0.219 ± 0.044</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. LV, left ventricle; RV, right ventricular free wall. *P < 0.05 vs. control; †P < 0.01 vs. control; ‡P < 0.001 vs. control; §§P < 0.001 vs. control.

Morphological Data

Cardiomyocyte analysis. Cardiac hypertrophy data from the different experimental groups were analyzed and also included measurements of transverse fiber diameter (myocyte width). Figure 1 shows the relation between cardiac hypertrophy expressed as HW/BW and myocyte width. At both dose levels (25 and 100 μg), the thyroid hormone promoted a significant increase in myocyte transverse diameter compared with the control group (18.0 ± 1.36 and 17.9 ± 1.31 μm, respectively, vs. 15.9 ± 1.6 μm; P < 0.05). Hypothyroidism tended to decrease the myocyte transverse diameter, although the difference was not statistically significant.

The administration of specific RAS inhibitors or an adrenergic response inhibitor induced a significant decrease (P < 0.01) in transverse myocyte diameter in animals treated with T4. Compared with the T4-100 group, transverse myocyte diameter was reduced by 18.4% in the enalapril group, 17.3% in the losartan group, and 14% in the propanolol group. However, there was no significant difference between the groups, and there was no significant difference in relation to the control group. Therefore, the increase in myocyte diameter promoted by T4 was significantly inhibited by administration of these drugs. Figure 2 shows the relation between HW/BW and average myocyte width and demonstrates a good correlation (r = 0.96) between these two parameters.

Interstitial connective tissue. To evaluate whether thyroid hormone-induced cardiac hypertrophy was accompanied by cardiac fibrosis, we examined the presence of collagen fibers in the transverse sections of control, Hypo, and T4-treated hearts (Fig. 3). Under polarized light, the Sirius red-stained sections exhibited scarce yellow or green collagen fibers in the myocardial region. The collagen quantification showed that there was no significant difference between the groups and there was no significant difference in relation to the control group (Fig. 3).

DISCUSSION

The major finding of this study is that specific RAS inhibitors or adrenergic response inhibitors are able to prevent the cardiac hypertrophy induced by acute hyperthyroidism. Some authors have already studied the action of RAS inhibitors in various models of hypertrophy induced by pressure overload. However, thyroid hormone-induced cardiac hypertrophy is a dissimilar model with peculiar and individual characteristics. Thus we developed a thyrotoxicosis model in Wistar rats to reproduce the cardiac pathology observed in acute hyperthyroidism and investigate the mechanisms of cardiac hypertrophy mediated by thyroid hormone.

Treatment with lower and higher doses (25 and 100 μg · 100 g BW⁻¹ · day⁻¹) of thyroxine promoted an increase in HW/BW of 19% and 21%, respectively. This
increase was not symmetrical with equal percentage weight gain of the left and the right ventricle. Van Lier et al. (36) and, later, Gerdes et al. (12) demonstrated that hyperthyroidism in rats causes greater enlargement of right ventricle than of left ventricle, in accordance with our data. In hyperthyroid animals, right ventricular pressure is increased by a much greater percentage than systemic blood pressure (1). Right ventricular pressure in hyperthyroid rats was not measured. However, in light of these previous studies, we can suppose that the greater degree of right ventricular involvement may result from pulmonary hypertension. Microscopic measurements confirmed an 

enlargement of cross-sectional myocyte width in hyperthyroid rats. Although cell length was not measured, we observed a clear correlation between cardiac hypertrophy expressed as HW/BW and that expressed as average myocyte width (r = 0.96). Therefore, we can assume that the cardiac hypertrophy induced in this model was provoked primarily by an increase in myocyte width. Gross anatomic changes in ventricular shape suggest that increased myocyte cross-sectional area is more pronounced in concentric hypertrophy, whereas increased cell length is a principal feature of volume overloading enlargement. In hearts of hyperthyroid rats, we observed a slight increase in the ratio of left ventricle wall thickness to left ventricle chamber size. This finding suggests a concentric hypertrophy, albeit to an insignificant degree. Furthermore, on the basis of hemodynamic and structural data, Gerdes et al. (12) suggested that excess thyroid hormones induce a combined eccentric and concentric cardiac hypertrophy that is more pronounced in the right ventricle.

Chronic hyperthyroidism has typically been associated with simultaneous increases in blood pressure (5). However, in this acute study, there was no difference in SBP between T4-treated animals and control group animals, indicating that the cardiac hypertrophy was not due to elevated hemodynamic parameters. Although dissociation between elevated arterial pressure and increased myocardial mass has been observed in this acute model of cardiac hypertrophy, hypothyroid rats presented decreased SBP, HW, BW, and HW/BW.

Fig. 2. Relationship of HW/BW to myocyte width in control, thyroidectomy and T4-treated [25 μg T4·100 g BW⁻¹·day⁻¹ (T4-25) and T4-100] rats. Note that there is a clear correlation between these 2 parameters. n, No. of animals.

Fig. 3. Transverse sections of the myocardium under polarized light. Top: representative section of collagen fibers in the myocardium of control group (A), T4-100 group (B), and Hypo group (C) (Sirius red; A–C: ×400). Bottom: quantitative analysis of collagen fibers in myocardium. Note that there is no significant difference between the groups.
This demonstrates that basal levels of thyroid hormone are very important for cardiac tissue metabolism and development and for maintenance of hemodynamic parameters. Interestingly, despite the fact that the hypothyroid group exhibited a significant reduction in HW/BW, transverse cardiomyocyte diameter decreased. However, it did not differ significantly in relation to that of the control group. Although the basis for this discrepancy is not clear, it is possible, considering that cardiac tissue is constituted by other cell types besides cardiomyocytes, that the situations of absence or deficiency of thyroid hormone that occur in hypothyroidism might compromise development and fibroblast proliferation, thereby reducing cardiac mass. According to Pernitsky and Anderson (28), triiodothyronine treatment induces proliferation of fibroblasts in vitro. In addition, it is possible that a significant reduction in cardiomyocyte diameter occurs after a longer period of hormone absence. Considering that decreased ventricular filling normally accompanies this condition, it is also possible that the drop in HW/BW observed in hypothyroid rats is due to decreased cell length. Another significant finding of the present investigation is that, despite the increased ratio of HW to BW, this hypertrophy was not accompanied by marked cardiac fibrosis, evidenced by collagen being poorly identified in the picrosirius-stained tissue sections. Yao and Eghbali (39) and Weber et al. (38) showed that, in contrast to what is usually seen in other models of hypertrophy, collagen deposition did not increase in the T4-induced myocardial hypertrophy model. These authors observed that, although thyroid hormone promotes the proliferation capacity of cardiac fibroblasts, it specifically inhibits the expression of cytoskeletal protein and collagen type I genes and is not likely to induce cardiac fibroblast hypertrophy.

**Hyperthyroidism and RAS**

Several mechanisms of hyperthyroid-induced cardiac hypertrophy have been proposed. Recently, some investigators have suggested that the RAS plays an important role in the development of cardiac hypertrophy in hyperthyroid rats, demonstrating an activated circulating RAS in hyperthyroidism (3, 18, 32). In the present study, enalapril or losartan was administered to hyperthyroid rats to confirm whether the RAS is involved in this model of cardiac hypertrophy and to compare the effects of these drugs in this model.

Our data show that acute treatment with the ACE inhibitor enalapril at a dose that did not decrease blood pressure attenuated cardiac hypertrophy (as estimated by the ratio of HW to BW) in rats induced to hyperthyroidism. Such a reduction was also observed in normal hearts treated with enalapril. In addition, this drug induced a proportional reduction of cardiomyocyte width. These data indicate that factors other than hemodynamic changes play an important role in the pathogenesis of T4-induced cardiac hypertrophy. Linz et al. (23) reported the same effect in another model of cardiac hypertrophy promoted by aortic banding in rats. Several studies have focused on the effect of angiotensin receptor blockers on the development of pressure-overload hypertrophy. These drugs prevent in vivo left ventricle pressure-overload hypertrophy and reduce the stretch-induced hypertrophic response in cardiac myocytes (6). In the present study, we also analyzed the effects of ANG II receptor blockade. Losartan was not able to prevent the increase in HW/BW of animals induced to hyperthyroidism. However, a significant decrease in the diameter of myocytes was observed in these animals. The reason for this discrepancy between HW/BW and morphometric analysis may be due to the varying distribution of AT1 receptors in the cardiac cells and consequently varying efficacy of losartan in prevention of cardiomyocyte hypertrophy in relation to growth of nonmyocyte cells, principally fibroblasts. Treatment with an AT1 receptor antagonist was not able, in this study, to prevent the increase in HW/BW. However, Crawford et al. (8) demonstrated that ANG II, acting through the AT1 receptor, stimulates fibronectin and expression of collagen types I and IV in association with the proliferation of interstitial fibroblasts by direct action and that this could be prevented by losartan, an AT1 receptor antagonist. However, it was recently reported that neither losartan, an AT1 receptor blocker, nor quinapril, an ACE inhibitor, was able to prevent isoproterenol-induced cardiac hypertrophy (21). Thus the observed diversity of response in these models indicates that specific stimuli sometimes influence the onset and development of cardiac hypertrophy. Furthermore, data obtained in our laboratory show that a longer period of treatment (14 days) with T4-100 and losartan prevented the increase in HW/BW (T4-100: 3.76 ± 0.22, T4-Los: 3.24 ± 0.22, P < 0.01; n = 7). Thus it is possible that losartan acts differently on myocytes than on nonmyocyte cells and that myocytes are more sensitive to the action of the drug than other cells.

Because ACE and AT1 are on successive pathways in the RAS, the primary effect of these agents and the underlying mechanism were previously believed to be identical (34). However, it has been reported that cardioprotection by ACE inhibitors includes different cascades from ANG II reduction or AT1 blockade, which implies that the pathways for preventing structural changes are different for ACE inhibitors than for AT1 antagonists (30). Considering that ACE inhibitors inhibit the breakdown of bradykinin, which causes an increase in NO production independent of the regulation of ANG II production (34), ACE inhibitors may exert beneficial effects through a bradykinin-NO pathway. Therefore, it is possible that the observed higher efficacy of enalapril treatment, compared with treatment with losartan, is due to the effects of bradykinin-independent pathways.

**Hyperthyroidism and Adrenergic Response**

The role of the β-adrenergic nervous system in the development of thyrotoxicosis is controversial. Hyperthyroidism has been found to be associated with in-
creased cardiac β-receptor density (4). Moalic et al. (26) showed an increased expression of β1-adrenergic receptor mRNA in response to thyroxine administration. However, this was associated with decreased expression in pressure overload-induced cardiac hypertrophy, suggesting that the adrenergic system could play a role in the development of cardiac hypertrophy in chronic hyperthyroidism. In our study of an acute hyperthyroidism model, propanolol was used to block the β-adrenergic system. Cardiac hypertrophy, as estimated by HW/BW and by cardiomyocyte width, decreased significantly in the propanolol-treated hyperthyroid hearts. These data are not in agreement with those of Cooper et al. (7), who reported that adrenergic stimulation in cats is not necessary for T4-induced hypertrophy. In addition, a morphological study showed that propanolol does not alter the degree of myocyte hypertrophy in hyperthyroid rats but modifies myocyte geometry, increasing cell length (12). This discrepancy may be related to differences in the dosages of propanolol and thyroid hormone, to differences in species or sex of animals, or to duration of treatment. As stated above, we did not measure the cell length. Nonetheless, the results of the present studies clearly show that the SNS plays an important role in the development of cardiac hypertrophy. Furthermore, Klein (15) demonstrated that propanolol administration prevents hyperthyroidism-induced cardiac hypertrophy.

Together, these results suggest that the effects of T4 are not mediated solely through the adrenergic nervous system and corroborate the findings of Asahi et al. (2) showing that the RAS is an important modulator of T4-induced cardiac hypertrophy.

Finally, the cardiovascular alterations of hyperthyroidism were reproduced with thyroid hormone injections in rats. Using the specific inhibitors enalapril, losartan, and propanolol, we demonstrated that the RAS and the adrenergic nervous system are involved in cardiac hypertrophy in hyperthyroid rats.

**Perspectives**

These observations may have important clinical implications for the treatment of patients with hyperthyroidism and cardiac hypertrophy. In light of previous literature reports and our results, it is clear that defining a trigger mechanism for the development of T4-induced cardiac hypertrophy is problematic. The mechanism by which the animal model of hyperthyroidism-induced cardiac hypertrophy occurs is multifactorial. Consequently, the complex interactions between the direct effect of thyroid hormone action at the cellular level and the RAS and/or the SNS have yet to be fully characterized.

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