Effects of Thyroid Hormone on Angiotensinogen and Renin Messenger RNA Levels in Rats

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Abstract—We studied the expression of angiotensinogen and renin genes in rats treated with 3,3',5-triiodo-L-thyronine (T3) at doses of 0.1 and 1 mg/kg of body weight. Liver angiotensinogen mRNA increased by 2 to 3 times 8 to 12 hours after T3-treatment. This increase was dose-related. Plasma angiotensinogen concentration (PAC) increased 12 hours after T3-treatment. Brain and renal angiotensinogen mRNA levels and the renal renin mRNA level remained the same throughout the experimental periods. These results suggest that the thyroid hormone initially increases angiotensinogen mRNA and leads to an increase in the production of angiotensinogen in the liver followed by an elevation of PAC.

The renin-angiotensin system plays an important role in the control of blood pressure and the balance of electrolytes. Angiotensinogen, a precursor of angiotensin II, is a glycoprotein synthesized in the liver and other organs. Plasma angiotensinogen level is regulated by many factors such as glucocorticoids, estrogen, angiotensin II, thyroid hormone and prostaglandins (1). Thyroid hormone affects the production and secretion of angiotensinogen from liver slices (2, 3) and perfused liver (4). Plasma renin activity (PRA) increases during hyperthyroidism and decreases during hypothyroidism in both patients and rats (3, 5). Hence, thyroid hormone may affect both the production and release of renin and angiotensinogen. Our recent studies suggest that hepatic angiotensinogen mRNA level is a better indicator of its production ability than plasma angiotensinogen level is, especially when angiotensinogen consumption is accelerated by a high renin state (6).

In this study, we investigated the effect of thyroid hormone on hepatic angiotensinogen mRNA to determine its effect on the production of angiotensinogen in the liver. Renal renin mRNA levels in acute experiments were also determined.

Materials and Methods

Male 8-week-old Sprague-Dawley rats (SLC Inc. Japan), weighing 291–330 g, were used. They were fed standard rat diets (MF type, Oriental Yeast Co., Ltd. Japan) and divided into 2 groups (T3 of 0.1 and 1 mg/kg body weight treatment groups) of 30 animals each. We used the same protocol in each group’s experiments. Saline was administered to five control rats who were then immediately decapitated. Twenty-five rats who received subcutaneous injections of 3,3',5-triiodo-L-thyronine (T3; Nacalai Tesque, Inc.) were divided into 5 subgroups and decapitated 2, 4, 8, 12 and 24 hours after administration of T3.

Blood samples were collected into silanized glass beakers containing disodium ethylenediamine tetraacetic acid (1 mg/ml). The plasma was separated by centrifugation at 4°C and then stored at −80°C. Plasma renin activity (PRA), plasma renin concentration (PRC) and plasma angiotensinogen concentration (PAC) were measured using a radioimmunoassay for generated angiotensin I (6).

After decapitation, tissues (liver, kidney and brain) were immediately removed, frozen in liquid nitrogen and stored at −80°C until
total RNA extraction. Total RNA extractions were carried out according to the method of Chirgwin et al. (7). The amounts of total RNA obtained from the liver, kidney and brain were 6.7±0.2, 2.3±0.1 and 0.6±0.03 mg/g of tissue respectively. The amounts of RNA extracted from the same organs among the different groups were not significantly different.

Angiotensinogen and renin mRNAs were analyzed by Northern blot hybridization. Total RNAs extracted from the tissues were incubated for 1 hour at 50°C in a solution containing 1 M glyoxal and 50% dimethylsulfoxide at pH 7. Glyoxalated RNAs of 8-32 μg were electrophoresed on 1.5% agarose gels and transferred to Biodyne A membranes where they were heated at 80°C for 1 hour. The transferred membranes were prehybridized for 6 hours at 42°C in a solution of 50% for-mamide, 5×SSC, 10×Denhardt’s 50 mM phosphate buffer (pH 7.0), 2 mM EDTA, 0.1% SDS and 200 μg/ml salmon sperm DNA. Hybridization was performed for 16-24 hours at 42°C in the above prehybridization solution with approximately 4×10⁷ cpm/ml of 32P-labeled 712-bp Bam HI fragment derived from the cDNA insert of clone pRag 16 for rat angiotensinogen (8) or the 698-bp Kpn I fragment for rat renin cDNA (9). Membranes were washed with 0.2×SSC and 0.1% SDS at 42°C. Autoradiography was performed with an intensifying screen and Fuji-RX film at -80°C for 8 and 12 hours, respectively, to detect liver and brain angiotensinogen mRNA and about 7 days, to detect renal renin mRNA. Autoradiograms of membranes were scanned with a densitometer (Dual-wavelength TLC scanner, Shimadzu Ltd.). The absorbance units shown by the densitometer were plotted on a graph against the various amounts of total RNAs applied to the gels. Regression lines were calculated, and we estimated the relative levels of the angiotensinogen and renin mRNAs from the slopes of their regression lines. The mean values of the slopes of control samples were taken as 1, and the experimental slope values were divided by the mean values of the control groups in order to calculate the relative values.

Results are expressed as means±S.E.M. Analysis of variance followed by the Dunnett’s test was used for multiple comparisons, and P values of less than 0.05 were accepted as statistically significant differences.

Results

The effects of T₃-treatment on PRA, PRC and PAC are summarized in Table 1. PRA increased significantly 24 hours after T₃ administrations in both doses, but PRC did not change at all during the experiments. PAC increased significantly in the 12 to 24 hours after the treatments with both doses.

Table 1. PRA, PRC and PAC levels in rats from 2 to 24 hours after a single injection of triiodothyronine (T₃)

<table>
<thead>
<tr>
<th></th>
<th>T₃ (0.1 mg/kg)</th>
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<th>T₃ (1 mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>PRA</td>
<td>ng Ang I/ ml·hr</td>
<td>PRC</td>
</tr>
<tr>
<td>Control</td>
<td>5.8±0.4</td>
<td>27.4±4.4</td>
<td>1002±64</td>
</tr>
<tr>
<td>T₃ (2 hr)</td>
<td>5.2±0.4</td>
<td>29.0±3.2</td>
<td>1128±43</td>
</tr>
<tr>
<td>T₃ (4 hr)</td>
<td>4.3±0.6</td>
<td>23.5±3.0</td>
<td>1222±70</td>
</tr>
<tr>
<td>T₃ (8 hr)</td>
<td>7.0±1.3</td>
<td>33.1±5.3</td>
<td>1139±28</td>
</tr>
<tr>
<td>T₃ (12 hr)</td>
<td>7.1±0.9</td>
<td>25.4±2.8</td>
<td>1666±19*</td>
</tr>
<tr>
<td>T₃ (24 hr)</td>
<td>10.2±1.6*</td>
<td>27.3±4.8</td>
<td>1716±67*</td>
</tr>
</tbody>
</table>

Values are expressed as means±S.E.M. of duplicate determinations in five animals. PRA (plasma renin activity), PRC (plasma renin concentration) and PAC (plasma angiotensinogen concentration) are expressed as generation of angiotensin I (Ang I). *P<0.01 and †P<0.05, when compared with control values.
nucleotides. Compared with that in the liver, there was about one half as much angiotensinogen mRNA in the brain and one tenth as much in the kidney. There were significant increases in the rat liver angiotensinogen mRNA 8 hours after T3-treatments as shown in Fig. 1; however, no changes were induced in the brain or kidney.

We did a time course study of the effect of T3 on hepatic angiotensinogen gene expression. As shown in Fig. 2, when T3 was administered at 1 mg/kg, angiotensinogen mRNA in the liver increased gradually, and it reached a maximum level at 8 hours after treatment, which was maintained for 24 hours. At the maximum level of induction, the mRNA increased 3-fold over its control level. Dose-related responses were observed at 8 hours after T3 administration. Angiotensinogen mRNA levels in the brain and kidney, however, did not change throughout the experiments, renin mRNA levels in the kidney remained unchanged even at 1 mg/kg of T3 (Figs. 1 and 2).

**Discussion**

The results of this study show that the injection of T3 increases angiotensinogen mRNA levels and then increases PAC in rats.
Treatments with T₃ for 6 to 14 days enhance the production and secretion of angiotensinogen in liver slices (2, 3) perfused liver (4) and primary cultured hepatocytes (10), and plasma angiotensinogen which is also elevated in rats in vivo. Thyroidectomy, on the other hand, reduces the production of liver angiotensinogen and decreases PAC (2, 3, 11). T₃-treatment caused a dose- and time-dependent increase in angiotensinogen mRNA levels in the rat hepatoma cell line (12). Concentration of specific mRNA may be determined by its rate of synthesis and breakdown. The sequence analysis in the 5' flanking regions of rat and human angiotensinogen genes revealed hormone responsive elements including those for glucocorticoid, estrogen and thyroid hormones (13). Furthermore, it was demonstrated that thyroid hormone stabilizes a specific mRNA precursor to increase its concentration (14). Therefore the present study indicates that thyroid hormones may have the abilities both to increase the transcription rate of the gene and to stabilize mRNA for rat angiotensinogen, and that the production and secretion of angiotensinogen increase to elevate PAC.

We demonstrated that T₃ could not alter angiotensinogen mRNA levels in the brain and kidney. The angiotensinogen gene is also expressed in many other tissues (15–18). Different sodium states caused changes in the renal angiotensinogen mRNA level, but not in the brain angiotensinogen mRNA level (18). Dexamethasone, a potent stimulator of angiotensinogen production, could induce angiotensinogen mRNA accumulation in the liver and brain, but not in the kidney (19). Administration of angiotensin II also increased the liver angiotensinogen mRNA level, but did not change that in the brain or kidney (H. Iwao et al., unpublished data). These results suggest that angiotensinogen gene expression could be tissue specific.

Thyroid hormones also affected PRA and PRC. PRA levels in hyperthyroid patients were significantly greater than those in normal subjects, which in turn were greater than those in hypothyroid patients. The administration of propranolol or dibenzylene caused PRA levels to decrease in hyperthyroid patients (5). Thyroidectomy decreases PRA and PRC in rats (4, 10, 11). Isoproterenol increases PRA and PRC in control animals, but has no effect on thyroidectomized rats (11). These changes are related to either sympathetic or beta-adrenergic activity (5, 11). However, Dzau and Herrmann (2) reported that hyperthyroid rats show increased PAC without significant changes in PAR or PRC, and hypothyroid rats show a decreased PAC associated with unchanged PRA and PRC. In the present experiments, we used a single injection of T₃ to avoid beta-adrenergic modulation, and demonstrated that T₃-induced increase in PRA is associated with an increase in PAC. PRC, the indicator of renal renin secretion, did not change. Renal renin mRNA levels also remained the same after T₃-treatment. We believe that the rise in PRA was caused by stimulated renin production or release rather than elevated PAC, since the Kₘ value for the renin and angiotensinogen reaction is 2.4 μM, 2 to 3 times higher than the plasma angiotensinogen level (20).

In conclusion, we demonstrated the thyroid hormone's effects on angiotensinogen regulation of gene expression. The present experiments showed that the thyroid hormone rapidly stimulates the accumulation of angiotensinogen mRNA in the liver and then increase PAC. The thyroid hormone had no effect on the brain or renal angiotensinogen mRNA levels or the renal renin mRNA level.

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