Ang II receptor expression and effect of Ang II receptor blockade in thyrotoxic rat myocardium

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Abstract. – AIM: To investigate the relationship between expression of the angiotensin II (Ang II) receptors and thyroid hormones in the myocardium of rats with thyrotoxicosis.

MATERIALS AND METHODS: Forty-four adult male Sprague-Dawley rats were divided into four groups: control group (saline), losartan group (10 mg/kg), thyrotoxicosis group (0.5 mg/kg L-thyroid hormone sodium) and thyrotoxicosis-plus-losartan group (0.5 mg/kg L-thyroid hormone plus 10 mg/kg losartan) and treated intragastrically daily for four weeks. The heart weight (HW), body weight (BW) and HW/BW ratios were determined. The Ang II protein contents in cardiac homogenates and serum were determined by ELISA. The serum concentrations of levothyroxine (T3), triiodothyronine (T4) and thyroid stimulating hormone (TSH) were measured by radioimmunoassay. The expression of angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) were quantified by real-time PCR and Western blotting.

RESULTS: The thyrotoxicosis group had an increased BW/HW and higher cardiac AT1R and AT2R expression compared to controls. AT1R and AT2R expressions significantly reduced in the thyrotoxicosis-plus-losartan group, compared to the thyrotoxicosis group.

CONCLUSIONS: Thyroid hormone upregulated cardiac AT1R and AT2R, leading to cardiac remodeling, which was reversed by losartan. Cardiac damage in thyrotoxic rats may be related to upregulation of the Ang II receptors.

Key Words: Thyrotoxicosis, Ang II receptor, Remodeling, AT1 receptor, AT2 receptors.

Introduction

The renin angiotensin system (RAS) plays a significant role in regulating blood pressure and sustaining the balance of the internal environment. The RAS consists of interactive hormones and prohormones, such as renin-angiotensin, angiotensin I (Ang I), Ang II, angiotensin-converting-enzyme (ACE), and the Ang II receptors: angiotensin II type 1 receptor (AT1R), AT2R, AT3R and AT4R. At the formative stage of hypertensive cardiac hypertrophy, the local RAS is activated and AT1R is expressed at abnormally high levels in the cardiac tissue. The abnormally high expression of AT1R further intensifies the role of RAS in remodeling of the left ventricle. AT2R is involved in the apoptosis of endothelial cells, fibroblasts and smooth muscle cells, and has been shown to play a pro-apoptotic role in rat cardiocytes, rat fibroblasts and cultured human umbilical vein endothelial cells.

Thyroid hormones exert a broad range of effects on the cardiovascular system under physiological conditions. Excessive thyroxin directly or indirectly results in increased cardiac output, decreased peripheral resistance, increased blood circulation in the organs and a highly dynamic state in the blood vessels, via neuroendocrine changes such as activation of the sympathetic adrenal system and RAS.

The biologically active thyroid hormone affects heart rate, cardiac contractility, diastolic function, and systemic vascular resistance. However, the role of RAS in hyperthyroid heart disease is still not completely understood. The heart is one of the major target for thyroid hormone. Previous studies have indicated that different doses of thyroid hormone to the thyroidectomized rats alter the electrophysiological properties of the cardiac myocytes measured in vitro. RAS may play a part in muscular hypertrophy and interstitial changes in hyperthyroid cardiac disease. In rat models of hyperthyroid, higher concentrations of Ang II in the blood plasma and myocardium increase the expression of ACE activity in different tissues. ACE inhibitors (such as imidapril) and angiotensin re-
ceptor blockers (such as valsartan) can markedly inhibit the induction of cardiac hypertrophy and fibrosis by levothyroxine. The complicated molecular mechanisms which regulate the interactions between thyroxin hormones and RAS are still unknown and need to be further characterized. This study aimed to investigate the relationship between expression of AT1R and AT2R and thyroid hormones in thyrotoxic rats. The results are expected to provide insights for the development of novel therapeutic strategies for cardiovascular diseases.

Materials and Methods

Medicines and Reagents
L-thyroid hormone sodium (#100599, Merck KGaA, Darmstadt, Germany) and losartan (#09266, Hangzhou Moshadong Medicine Ltd. Co., China) were dissolved in saline. T3 and T4 (Catalog #IMK439, IMK440) were purchased from Beijing Atomic Energy Ltd. Co., China. The Ang II Sandwich ELISA kit including two antibodies was purchased from Shanghai Hufeng Biochemistry Ltd. Co., China.

Animal Selection and Molding
Forty-four adult male Sprague-Dawley rats (specific pathogen free; 260-330 g) were purchased from the Animal Experimental Center of Xi’an Jiaotong University, China. The rats were provided pellet fodder and water and housed at constant a temperature of 21±3°C and relative humidity of 55±5%. The rats were randomly divided into four equal groups: the control group (saline), the losartan group (10 mg/kg losartan), the thyrotoxicosis group (0.5 mg/kg L-thyroid hormone sodium) and the thyrotoxicosis-plus-losartan group (0.5 mg/kg L-thyroid hormone plus 10 mg/kg losartan), and were treated intragastrically once a day for four weeks.

Measurements of Arterial Blood Pressure and Pulse
Tail arterial systolic pressure and pulse were measured on day 0 and at the end of the fourth week of treatment using the BP-6A noninvasive blood pressure measurement system (Chengdu Taimeng, Chengdu, China), according to the manufacturer’s instructions. The incubator linked to a tail-cuff gasbag, a sensing device and an energy transducer was preheated at 35°C for 10 min before the rat was placed into the incubator in a cage. The tail of the rat was passed through the tail-cuff gasbag, fixed and the gasbag was aerated. The measurement system automatically loosened and slowly released the gas when the pressure reached 180 mmHg. During the pressure drop process, the pressure corresponding to the first pulse was recorded as the rat systolic blood pressure. The pulse number was counted during the five sec when the pulse amplitude range was clearly observed, and the pulse rate was calculated. The tail arterial systolic pressure and pulse was measured 3-5 times for each rat, and the average values were calculated.

Blood Collection and HW/BW Measurement
The body weight of each rat was measured after four weeks. Blood (5 ml) was collected from the abdominal aorta under chloral hydrate anesthesia, centrifuged at 3000 rpm for 10 min and the serum was stored at –20°C. After blood collection, the rat was sacrificed by left ventricular injection of potassium chloride (1.34 mol/L), and the heart was quickly removed, washed with saline and weighed after the excess water was removed using filter paper. The ratio of heart weight to body weight (HW/BW) was calculated. Immediately after weighing, 200 mg cardiac tissue was frozen in liquid nitrogen for 3-5 sec and stored at –80°C.

Measurement of Serum T3 and T4 Concentrations
The serum concentrations of T3 and T4 were determined using the microsphere solid-phase separation radioimmunoassay method. Standard solutions were prepared by placing 125I-T3 or 125I-T4 (200 µl) in separate tubes with different concentrations of standard T3 or T4 solution (50 µl) and T3 or T4 microsphere antibodies (200 µl). The serum samples (50 µl) were placed in samples tubes with 125I-T3 or 125I-T4 (200 µl) and T3 or T4 microsphere antibodies (200 µl). The tubes were well mixed, incubated at 37°C for 45 min, centrifuged at 3500 rpm for 20 min, the serum was discarded and the radioactivity of the deposits was counted using a GC400 Gamma radioimmunoassay counter (Keda Chuangxin Company Ltd, China) for 60 sec. Curve-fitting software with the 4 parameter logistic (4PL) model equation was used to automatically calculate the binding ratio, create the standard curve and determine the total T3 (TT3) or total T4 (TT4) concentration in each sample.
Measurement of Serum and Cardiac Ang II Concentrations

The serum samples were defrosted on ice. Frozen cardiac tissue (−80°C) was thawed on ice, then 100 mg of the tissue was ground with saline and centrifuged at 1500 rpm for 5 min at −4°C. The supernatant was collected and used to prepare 10% tissue homogenates. The standard curves and serum and cardiac Ang II concentrations were determined using the ELISA reagent kit, according to the manufacturer’s instructions. After the addition of enzyme, incubation and color development, absorbance (optical density) was measured at 450 nm using an ELISA reader (DG5033A; Nanjing Medical Equipment Co., Ltd., Nanjing, China). The Ang II concentrations were calculated using linear regression from the standard curve and multiplied by the sample dilution factor (serum: x1; cardiac tissue: x10).

Measurement of AT1R and AT2R mRNA Expression

AT1R and AT2R mRNA expression were quantified by real-time reverse transcription-polymerase chain reaction (qRT-PCR). Total RNA was extracted using the RNA Fast 200 Total RNA Extraction Reagent kit (#220010; Shanghai Feijie Biology Company, China) and stored at −4°C until analysis. First strand cDNA synthesis was performed using the RevertAid™ Reverse First Strand cDNA Synthesis Kit (#1622; MBI Fermentas, Vilnius, Lithuania). After reverse transcription, the cDNA products were stored at −20°C and analyzed within one week. The PCR primers were designed using Beacon designer software version 4.0 (Premier Biosoft, Palo Alto, CA, USA) and synthesized by Beijing Sanbozhiyuan Biotechnology Ltd. Co., China. The sequences of the primers were as follows: R-AT1R-p1 5’-CTGGCTGATGGCTGGCTTG-3’ and R-AT1R-p2 5’-GCTGGTGAATAGTTAAGGG-3’ (187 bp product); R-AT2R-p1 5’-TTGGATGCTGCTGACCTGGATG-3’ and R-AT2R-p2 5’-ATGGGACACTTAAACACACTAGG-3’ (180 bp product); R-actin-p1 5’-CTATCGGCAATGACGCTTCC-3’ (827 bp product) and R-actin-p2 5’-TGGTGGCATAGGTCTTTACG-3’ (972 bp product).

The qRT-PCR was performed in volumes of 25 µl containing 0.5 µl forward and reverse primers (10 µM; final concentration 0.2 µM), 9.5 µl DEPC-treated, double distilled water, 12.5 µl SYBR Green Realtime PCR Master Mix (QPK-201; Toyobo, Osaka, Japan) and 2 µl cDNA template in a PCR machine (IQ 5.0; BioRad, Hercules, CA, USA) using the following conditions: 95°C for 60 s; 40 cycles of 95°C for 15 s and 60°C for 15 s, followed by one step of 72°C for 45 s. The C values for AT1R, AT2R and β-actin were determined in each sample and relative expression was calculated using the 2-∆∆Ct method.

AT1R and AT2R Protein Expression Analysis

AT1R and AT2R expression were determined using Western blotting. Protein was extracted from 50 mg frozen cardiac tissue (−80°C) in a 50:1 mixture of lysis buffer (#PE-01, Shannxi Xinanfeng Biology Co., Ltd., China) containing protein enzyme inhibitor cocktail (#539131, Calbiochem, Darmstadt, Germany). Protein concentration was measured using the BCA Protein Assay Kit (Beijing Kangwei Biotech, Inc., China). The protein concentrations of all samples were between 1.03 mg/ml and 2.1 mg/ml; within the optimal concentration range of 1-3 mg/ml required for electrophoresis. Lysate (50 µl) from each sample was separated on polyacrylamide gels, transferred to nylon membranes at a constant current of 100 mA for 1.5 h, blocked in 10% non-fat milk in Tris Buffered Saline with Tween (TBS-T) for 1-2 h, washed three times in TBS-T, incubated with primary rabbit anti-A T1R or anti-AT2R antibodies overnight at 4°C and then incubated in goat anti-rabbit IgG (#CW0097; Beijing Kangwei Century Biology Co., Ltd., China) for 1 h at room temperature. The bands were visualized using horseradish peroxidase and enhanced chemiluminescence and quantified using the public domain ImageJ software (http://www.softpedia.com/Download/ImageJ-Download-29167.html).

β-actin was used as an internal control.

Statistical Analysis

Results were expressed as mean ± SD and analyzed using single factor variance analysis with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA); p < 0.05 was considered statistically significant.

Results

BW, HW and HW/BW Ratio

Table I shows the HW/BW ratios of the different groups after the four weeks treatment. The HW/BW ratio of the thyrotoxicosis group (332 ± 21) was significantly higher than the control group (260 ± 32).
Table I. Effect of losartan on the cardiac and serum levels of body weight (BW), heart weight (HW) and heart weight: body weight (HW/BW) ratio in a rat model of thyrotoxicosis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>BW (g)</th>
<th>HW (g)</th>
<th>HW/BW ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>413 ± 34</td>
<td>1.02 ± 0.42</td>
<td>260 ± 32</td>
</tr>
<tr>
<td>Losartan</td>
<td>11</td>
<td>376 ± 21</td>
<td>0.94 ± 0.08</td>
<td>249 ± 12</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>11</td>
<td>386 ± 22</td>
<td>1.28 ± 0.15</td>
<td>332 ± 21*</td>
</tr>
<tr>
<td>Thyrotoxicosis+ Losartan</td>
<td>11</td>
<td>397 ± 28</td>
<td>1.20 ± 0.11</td>
<td>299 ± 16*+</td>
</tr>
</tbody>
</table>

Values are mean ± SD; *p < 0.05 vs. control group; †p < 0.05 vs. losartan group.

Table II. Effect of losartan on the cardiac and serum levels of total T3 (TT3), total T4 (TT4) and thyroid stimulating hormone (TSH) in a rat model of thyrotoxicosis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>BW (g)</th>
<th>HW (g)</th>
<th>HW/BW ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>0.72 ± 0.21</td>
<td>4.5 ± 0.11</td>
<td>94 ± 23.6</td>
</tr>
<tr>
<td>Losartan</td>
<td>11</td>
<td>0.58 ± 0.15</td>
<td>3.5 ± 1.4</td>
<td>91.8 ± 32.1</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>11</td>
<td>0.81 ± 0.31</td>
<td>18.2 ± 4.3</td>
<td>86.1 ± 13.5*</td>
</tr>
<tr>
<td>Thyrotoxicosis+ Losartan</td>
<td>11</td>
<td>0.64 ± 0.24</td>
<td>24.8 ± 10.9</td>
<td>96.7 ± 42.3*†</td>
</tr>
</tbody>
</table>

Values are mean ± SD; *p < 0.05 vs. control group; †p < 0.05 vs. thyrotoxicosis + losartan group.

Table III. Effect of losartan on the cardiac and serum levels of angiotensin-converting-enzyme (ACE), ACE 2 and angiopeitn-2 (AngII) in a rat model of thyrotoxicosis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Cardiac (U/100 g)</th>
<th>Serum (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACE</td>
<td>ACE2</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>38.1 ± 3.6</td>
<td>19.3 ± 1.9</td>
</tr>
<tr>
<td>Losartan</td>
<td>11</td>
<td>38.8 ± 4.2</td>
<td>20.7 ± 2.3</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>11</td>
<td>39.8 ± 3.2</td>
<td>21.0 ± 2.0</td>
</tr>
<tr>
<td>Thyrotoxicosis+ Losartan</td>
<td>11</td>
<td>36.8 ± 2.6</td>
<td>21.6 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD; *p < 0.05 vs. control group.

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and losartan group (249 ± 12; both p < 0.05); however, the HW/BW ratio of the thyrotoxicosis + losartan group (299 ± 16) was significantly lower than the thyrotoxicosis group (p < 0.05).

Thyroid Hormone Levels

Table II shows the serum concentration of thyroid hormones and TSH after the four weeks of treatment. The TT3 concentration was not significantly different in any group (p > 0.05). The TT4 concentration of the thyrotoxicosis group was significantly higher than the control group and losartan group (p < 0.05), and the TT4 concentration of the thyrotoxicosis + losartan group was significantly higher than the thyrotoxicosis group (p < 0.05). The TSH concentration of the thyrotoxicosis group was lower than the control group and losartan group, and the TSH concentration of the thyrotoxicosis + losartan group was higher than the thyrotoxicosis group. However, these differences were not statistically significant (p > 0.05).

Serum and Cardiac Tissue ACE, ACE 2 and Ang II Contents

Table III indicates that the ACE, ACE2 and Ang II contents in cardiac tissue did not vary significantly in the control group, losartan group, thyrotoxicosis group or thyrotoxicosis + losartan group. Additionally, the serum Ang II content did not vary significantly amongst the rats from any treatment group. The serum ACE content of the thyrotoxicosis group was significantly higher than the control group, losartan group and thyrotoxicosis + losartan group (p < 0.05). The expression of ACE2 was significantly higher in the losartan group, thyrotoxicosis group and thyrotoxicosis + losartan group than the control group (p < 0.05).
Expression of AT1R and AT2R mRNA

Table IV and Figure 1: shows that the expression of AT1R and AT2R mRNA were significantly higher in the thyrotoxicosis group than the control group or losartan group (p < 0.05); however, there was no significant difference in the expression of AT1R and AT2R mRNA in the thyrotoxicosis group and thyrotoxicosis + losartan group.

Expression of AT1R and AT2R Protein

Table V and Figure 2 shows the expression of AT1R and AT2R protein significantly increased in the thyrotoxicosis group, compared to the control group and losartan group (p < 0.05). However, there was no significant difference in the expression of AT1R and AT2R in the thyrotoxicosis group and thyrotoxicosis + losartan group.

Discussion

Thyroid Hormone Induces Cardiac Hypertrophy via the RAS

In this study, we induced a rat model of hyperthyroidism by four weeks daily intragastric administration of thyroid hormone to investigate the
A high level of thyroid hormones can also activate ATPase, increase expression of the α-myosin heavy chain, enhance cardiac contractility, induce cardiac hypertrophy and ultimately enlarge the heart.\(^9\)

**Effects of Hyperthyroidism and Angiotensin Receptor Blockade on Cardiac Tissue and Plasma ACE and Ang II Content**

The serum levels of ACE significantly increased in the thyrotoxicosis group compared to the control group and losartan group, suggesting that thyroid hormone increases the synthesis or secretion of ACE in rats with hyperthyroidism. In rats treated daily with 0.025 mg or 0.100 mg L-thyroid hormone for 14 days, Forhead et al.\(^11\) reported thyroid hormone play an important part in the developmental control of fetal concentrations of ACE in the organs such as the lung and kidneys. L-thyroid hormone may promote the activation and expression of ACE via two mechanisms: thyroid hormones may directly affect ACE gene expression or protein synthesis, or thyroid hormones may indirectly affect the expression of ACE by influencing other components of the RAS.

The levels of Ang II in cardiac tissue did not increase significantly in the thyrotoxicosis, thyrotoxicosis + losartan or losartan groups, compared to the control group. The levels of Ang II slightly increased in the plasma of the thyrotoxicosis + losartan and losartan groups, and reduced slightly in the thyrotoxicosis + losartan group compared to the control group; however, these differences were not statistically significant. This data indicates that, although thyroid hormone activated the RAS in the heart, it did not significantly increase the local expression of Ang II in cardiac tissue. The administration of losartan had no significant effect on the cardiac tissue and plasma ACE or Ang II contents, indicating that the ability of losartan to inhibit RAS and reduce cardiac hypertrophy was not linked to altered expression of Ang II or ACE.

**Effects of Hyperthyroidism and Angiotensin Receptor Blockade on Expression of AT1 in Cardiac Tissue**

Real-time fluorescence quantitative PCR and Western blotting analysis showed that the levels of AT1R significantly increased in the thyrotoxicosis group, compared to the control group and losartan group. Incubation of cardiac muscle

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Figures:

**Figure 2.** Western blot of AT1R and AT2R protein expression.

- **A.** AT1R protein.
- **B.** AT2R protein:
  1: thyrotoxicosis group; 2: losartan group; 3: control group; 4: low thyroid hormone; 5: thyrotoxicosis-plus-losartan group.

Relationship between expression of the Ang II receptors and cardiac damage in rats with thyrotoxicosis.

The body weight of the rats in each group did not change significantly; however, the HW/BW ratios of the thyrotoxicosis group and the thyrotoxicosis + losartan group increased significantly compared to the control group. This suggests that the high levels of thyroid hormones induced significant cardiac hypertrophy. However, thyroid hormone-induced cardiac hypertrophy was partially suppressed by administration of the angiotensin receptor blocker losartan, which indicated that the RAS may be involved in the development of thyroid hormone-induced cardiac hypertrophy.

The RAS has previously been linked to the development of hyperthyroidism-induced cardiac hypertrophy in rats. Hu et al.\(^8\) reported that cardiac hypertrophy induced by T4 in rats was related to the RAS and the sympathetic nervous system, and that administration of angiotensin receptor or ACEI inhibitors significantly reduced hyperthyroidism-induced cardiac hypertrophy. It has also been shown that cardiac hypertrophy can be suppressed by reducing the pressure loads induced by hyperthyroidism in rats. There are several reports describing that high levels of thyroid hormones induced significant cardiac hypertrophy via the receptor- and voltage-dependent calcium channels and increased cardiac contractility.\(^9\)
The effects of AT1 antagonists reported by Pantos et al. confirmed that AM increased in thyrotoxic rats. However, the peripheral vascular resistance reduced. RAS plays a significant role in the modulation of blood pressure. Therefore, when blood pressure decreases, RAS is activated, leading to increased levels of AngI/Ang II, ACE, renin and upregulation of AT1R. Sohluter reported that AT1R is significantly increased by thyroid hormone-induced activation of RAS in the heart, which may be attributed to the effects of AT1R on the 70 kDa nucleoglucoprotein S6 kinase and the Akt signal transduction pathway. It has already been shown that thyroid hormone can enhance the expression of AT1R, and binding of Ang II to AT1R stimulates the growth of cardiac muscle cells and leads to cardiac hypertrophy. AT1R activates membrane G-proteins, leading to phospholipase C (PLC) activation and accelerated phosphatidylinositol hydrolysis to generate phosphoinositide and digalactosyl diacylglycerol. In turn, phosphoinositide activates the sarcoplasmic reticulum, leading to increased release of free Ca2+ and enhanced excitation-contraction coupling in cardiac myocytes and cardiac contractility. AT1R can also activate nuclear genes via a specific ion stream and increase cytoplasmic protein synthesis, as well as inducing the expression of proto-oncogenes by regulating the growth factor signaling network to consequently lead to abnormal cellular proliferation, increased cell size and cardiac hypertrophy. Taken together, this data indicates that thyroid hormone activates the RAS in the heart, which stimulates the expression of the AT1R which binds with Ang II to accelerate cardiac hypertrophy.

Losartan is an angiotensin receptor blocker which is used commonly in the clinic. In this study, the AT1R antagonist losartan significantly inhibited hyperthyroidism-induced cardiac hypertrophy and suppressed RAS, in agreement with the effects of AT1 antagonists reported by Pantos et al. However, losartan had significant effect on the expression of AT1R in the thyrotoxic cardiac tissue, compared to the thyrotoxicosis group. Research has indicated that intracellular Ca2+ overload may provide a link between RAS and thyroid hormones; thus, losartan may inhibit cardiac hypertrophy by altering the intracellular calcium content. The ability of losartan to modulate the effects of AT1R in cardiac tissue may be the major mechanism by which this angiotensin receptor blocker inhibits cardiac hypertrophy or ventricular re-building and prevents the development of cardiac dysfunction.

**Effects of Hyperthyroidism and Angiotensin Receptor Blockade on AT2R Expression in Cardiac Tissue**

Real-time quantitative PCR and Western blotting showed that expression of AT2R mRNA and protein significantly increased in the myocardium of the thyrotoxicosis group, compared to the control and losartan groups; however, the expression of AT2R was not significantly different in the thyrotoxicosis group and thyrotoxicosis-plus-losartan group. AT2R is expressed at high levels in embryonic cardiac tissue and at low levels in adult cerebral and cardiac tissue. AT2R evoked increased collagen production in VSMC and mesangial cells, but expression of AT2R is dysregulated in patients with cardiac hypertrophy, ischemic inflammation or cardiac function failure. The pathological and physiological significance of up-regulation of AT2R is an area of intensive research and the effects of AT2R in cardiac hypertrophy are controversial.

Heinic et al. reported that AT2R knockout (KO) mice had a significantly higher blood pressure than wild-type (WT) mice. Kijimad et al. demonstrated that pressure overload lead to increased expression of AT2R in cardiac hypertrophy. Activation of AT2R leads to blood vessel dilatation and reduced blood pressure, and the effect of AT2R on blood pressure reduction has been associated with the release of NO and bradykinin. A number of studies have revealed that AT1R inhibitors can reduce thyroid hormone-induced cardiac hypertrophy, but do not affect the expression of AT2R in cardiac muscle tissue. However, Horiuchi et al. demonstrated that the AT2R repressor PD123319 could inhibit the Ang II-induced vascular contractility of spontaneously hypertensive rat (SHR) mesenteric arterioles, suggesting that AT2R potentially plays a role in the contractility of SHR arterioles. Additionally, AT2R has been related to water and sodium retention, as body temperature and urine sodium excretion are decreased in AT2R KO mice, which leads to water and sodium retention and increased blood pressure.

AT2R can also accelerate cellular growth, in parallel to the effects of AT1R. A repressor of AT2R can inhibit DNA synthesis, suggesting...
that AT2R may promote cardiac hypertrophy. In a study of thyrotoxic rats, AT2R promoted cardiac hypertrophy\(^2\), whereas, thyroid hormone-induced cardiac hypertrophy was decreased by the administration of an AT2P repressor.

It has been observed that increased expression of AT1R and AT2R mRNA and protein in the rat models of cardiac hypertrophy are due to spontaneous high blood pressure or pressure overload\(^1\). Circulating and local RAS systems may affect cardiac remodeling in heart-failure patients, mainly via AT1R-mediated growth and hypertrophy of cardiac cells and AT2R mediated apoptosis\(^1\), which is regulated in part via activation of cytokinin protein kinase\(^2\). Based on the above analysis, it remains unclear whether the AT2R is upregulated or downregulated during cardiac remodeling and the pathological significance of variations in the expression of AT2R requires further investigation.

### Conclusions

This study suggests that the induction of cardiac hypertrophy by thyroid hormone is associated with the upregulation of AT1R and AT2R. The angiotensin receptor blocker losartan reduced the expression of AT1R, but had no significant effect on the expression of AT2R. In addition, losartan effectively blocked the Ang II-AT1R system in the rat myocardium and inhibited cardiac hypertrophy in thyrotoxic rats. Our results provide further confirmation that the RAS is involved in the occurrence and development of thyroid hormone-induced cardiac hypertrophy and cardiac remodeling via AT1R and AT2R.

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**Conflict of Interest**

The Authors declare that they have no conflict of interests.

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