Effects of hyper- and hypothyroid on expression of thyroid hormone receptor mRNA in rat myocardium

C R Liu¹, L Y Li, F Shi, X Y Zang, Y M Liu¹, Y Sun and B H Kan

Key Laboratory of Hormones and Development, Ministry of Health China, Endocrinology Institute of Tianjin Medical University, Tianjin Medical Institute, Tianjin 300070, China

¹Department of Pathology, Medical College of Chinese People’s Armed Police Force, Tianjin 300162, China

(Correspondence should be addressed to L Y Li; Email: lily@tijmu.edu.cn)

Abstract

Thyroid dysfunction is classified into hyperthyroidism and congenital hypothyroidism (CH). Both hyperthyroidism and CH can cause heart lesions; however, the mechanisms involved remain unclear. The left ventricle was collected from eu-, hyper-, and hypothyroid rat. RNA was extracted and reverse-transcribed to cDNA. Real-time fluorescence quantitation-PCR was used to quantify the differential expression of thyroid hormone receptor (TR) subtype mRNA among eu-, hyper-, and hypothyroid rat myocardium. Here, we show that compared with the normal myocardium, TRα1 mRNA expression was upregulated by 51% (P<0.01), TRα2 mRNA expression was downregulated by 58% (P<0.01), and TRβ1 mRNA expression remained unchanged in hypothyroid rat myocardium (P>0.05). TRα1, TRα2, and TRβ1 were expressed in normal and hypothyroid rat myocardium throughout the developmental process. In hypothyroid rats, myocardial TRα1 mRNA expression was generally downregulated and the expression peak appeared late. Myocardial TRα2 mRNA expression was generally upregulated and the expression peak appeared late. Myocardial TRβ1 mRNA expression was generally downregulated and changed similarly with the control group. In addition, the hypogenetic myocardium can be seen in the hypothyroid rat by pathology study. Taken together, the abnormal expression of TR subtype mRNA may have a close relationship with the pathogenesis of CH and hyperthyroidism heart disease.

Introduction

The heart is a major target organ for thyroid hormone (triiodothyronine, T₃) action. Thyroid hormone exerts its biological effects largely by influencing thyroid hormone-regulated gene expression via interactions with the high-affinity thyroid hormone receptor (TR) located in the nucleus. TR plays an important role in mediating the cardiac physiologic actions of T₃ (Sussman 2001, Dillmann 2002).

Normal thyroid hormone level is essential for maintaining normal heart structure and function. In clinical settings, patients with hyperthyroidism or congenital hypothyroidism (CH) may present abnormalities in myocardial structure and heart function. Changes in thyroid status exert marked influences on the contractile and electrophysiological function of the heart. The molecular basis of alterations in electrophysiological function caused by changes in different thyroid hormone status has not been completely explored.

Besides aggravating preexisting ischemic heart disease or other types of heart disease, hyperthyroidism itself may cause atrial fibrillation, cardiac dilatation, heart failure, angina pectoris, or myocardial infarction. The incidence of hyperthyroid heart disease was 10 to 20% among hyperthyroidism patients. Hyperthyroidism is frequently complicated by arrhythmia and, most commonly by atrial fibrillation (Klein & Ojamaa 2001). Current studies have demonstrated the possible association of atrial fibrillation with Na⁺–K⁺-ATPase. The maintenance of atrial fibrillation depends on the formation of multiple reentrant cycles. In hyperthyroidism, the activity of Na⁺–K⁺-ATPase is enhanced in myocardial cells, which promotes Na⁺ outflow and K⁺ inflow and thus shortens the duration of action potential of individual atrial muscle cell (Rensma et al. 1988, Wijffels et al. 1995, Maixent et al. 2000, Workman et al. 2003). In addition, shortening of the action potential duration in the ventricular muscle and concomitant tachycardia may render the ventricle susceptible to developing heart failure known as thyrotoxic heart (Klein 1990, Polikar et al. 1993). Hyperthyroidism that has been existing for a long time and has not been treated can result in marked morphological changes of the heart, including atrial or ventricular dilatation, heart weight gain, hypertrophy of myocardial cells, widened spaces between the muscle fibers of the heart (Henle’s fissures), and ultimately necrosis of myocardial cells and heart failure. The factors below may be associated with these myocardial pathologies: 1) hyperthyroid symptom is similar to sympathetic nerve system. Furthermore, tachycardia, increased pulse pressure, higher cardiac output, and oxygen wastage caused by hyperthyroidism can be successfully alleviated using sympathetic nerve or β-receptor retarder. Therefore, it was...
estimated that hyperthyroid myocardial injury may be related with sympathetic-adrenalin system (Morgan & Baker 1991). 2) In hyperthyroidism, the renin–angiotensin–aldosterone system (RAAS) is activated, the plasma rennin activity is enhanced, the concentrations of angiotensinogen and angiotensin (AT) are increased, and the density of AT receptor in the heart is upregulated. Activation of the RAAS adversely influences the cardiovascular system: on the one hand, it increases blood volume and the heart's burden and, on the other, AT binds to high-affinity AT receptors on myocardial cells or vascular smooth muscle cells and stimulates c-fos gene transcription in the nucleus through the intracellular signal transduction system. The c-fos protein can promote growth of myocardial or vascular smooth muscle cells and lead to myocardial hypertrophy. As a result, compensated myocardial function turns decompensated followed by heart failure (Katz 1990, Morgan & Baker 1991, Marchant et al. 1993, Sernia et al. 1993, Basset et al. 2001). These findings were derived from biochemical research on the pathogenesis of hyperthyroid heart disease. As for molecular research on the pathogenesis of hyperthyroid heart disease, thyroid hormone-regulated genes have been the major concern. It was demonstrated that myocardial genes upregulated by thyroid hormone include HCN2, HCN4, myosin heavy chain α (MHCα), and sarcoplasmic reticulum calcium-activated ATPase2 (SERCA2), and myocardial genes downregulated by thyroid hormone include MHCβ and Na+/Ca2+ exchanger (Dillmann 1990, 1996, Kahaly & Dillmann 2005). Despite the aforementioned findings, TR, a factor closely associated with the physiologic functions of thyroid hormone, has not been fully investigated and few studies of the association between hyperthyroid heart disease and TR subtypes have been reported.

CH is a relatively common newborn endocrine disorder that occurs with a frequency of ~1:3500. CH may cause cretinism and myxedema in different age groups. During the fetal and infantile periods, inadequate thyroxin derived from the maternal body or synthesized may cause CH (cretinism). The patient usually presents with myocardial structural developmental abnormalities and serious heart dysfunction, a condition called hypothyroid heart disease. Adult hypothyroid heart disease was first reported by Zondek in 1918. In CH patients, heart damage is very common. It is now thought that 70–80% of hypothyroidism patients have cardiovascular lesions, typically hypothyroid coronary heart disease, hydropericardium, cardiomyopathy, or pericarditis with cardiomyopathy (Balducci et al. 1991, Di Meo et al. 1994, Chao et al. 1997). Many patients visit the cardiovascular departments because of heart damages as initial manifestations. Due to diversified clinical manifestations and a chronic disease course, it is very easy to misdiagnose CH as another disease. Hence, to deepen the understanding of the pathogenesis of hypothyroid heart disease is important to increase the life quality of hypothyroid patients. To date, there have been only some pathomorphological reports on hypothyroid heart disease. Nevertheless, few studies of the pathogenesis of hypothyroid heart disease have been reported. In particular, the effect of CH on myocardial TR subtype mRNA expression at different developmental stages has not yet been reported.

Taken together, thyroid hormone exerts crucial regulatory actions on maintaining normal relaxation and contraction and electrophysiology of the heart. Abnormal thyroid hormone concentrations may lead to myocardial structural and functional abnormalities. Thus far, the pathogenesis of hyper- and hypothyroid heart disease has not been completely elucidated. TR plays an important role in mediating the physiologic actions of thyroid hormone. Whether abnormal thyroid hormone concentrations influence myocardial expression of TR, and whether the pathogenesis of hyper- and hypothyroid heart disease is associated with TR are issues worthy of investigation.

It is believed that unraveling these issues will open a new avenue to clinical treatment of these disorders. In order to explore the association between TR and heart lesions during thyroid dysfunction and to provide a new therapeutic strategy for hyper- and hypothyroid heart disease at the molecular level, differential expressions of TR subtype mRNAs among normal, hyperthyroid, and hypothyroid rat myocardium were analyzed using fluorescence quantitation-PCR (FQ-PCR).

Materials and Methods

Experimental animals

All animals were obtained from the Experimental Animal Centre of the Academy of Military Medical Sciences of China. They were raised in a temperature-controlled room (21–23 °C) under a 12 h light:12 h darkness cycle. Food and water were freely available.

Hyperthyroid animals Sixteen Wistar rats (specific pathogen-free animals, weighing 150–180 g) were equally randomized to the control group and the experimental group (n = 8). Thyroxin tablets (Shanghai Great Wall Pharmaceutical, Shanghai, China) were ground and diluted with normal saline into a 40 g/l suspension. Each rat from the experimental group and the control group (Morreale de Escobar et al. 1988) was intragastrically administered 1 ml suspension and normal saline respectively, once a day, for 3 successive weeks. After 3 weeks of treatment, the animals were weighed and anesthetized with 1% pentobarbital, followed by thoracotomy and blood sampling from the heart. Blood samples were centrifuged and the supernatants were obtained for detecting serum free T3 (FT3) and free thyroxine (FT4) levels by RIA (completed by radiology Institute of Tianjin Medical University). The heart was obtained and blood in the cardiac chamber was washed out quickly with diethylpyrocarbonate (DEPC) water. The heart was wiped dry with a sheet of filter paper, weighed, and stored in liquid nitrogen.

Hypothyroid animals Twelve pregnant Wistar rats (specific pathogen-free animals) were equally randomized into the experimental group and the control group. From the 15th day of gestation, each rat from the experimental group was
intragastrically administered with 2.5 ml of 1% propylthiouracil (Dr Herbrand KG, Chem–Pharm. Works, D–77723 Gengenbach, Germany) daily until postnatal 15-day. Litters were CH rats (Gilbert & Paczkowski 2003). In contrast, from the 15th day of gestation, each rat from the control group was intragastrically administered with 2.5 ml normal saline daily until postnatal 15 days. At postnatal days 0 (P0d), 14 (P14d), 21 (P21d), and 45 (P45d), the rats were weighed and killed, and their blood samples were collected from the heart. Serum FT₃ and FT₄ were dynamically determined by RIA (completed by radiology Institute of Tianjin Medical University). The heart was obtained and blood in the cardiac chambers was washed out quickly with DEPC water. The heart was wiped dry with a sheet of filter paper, weighed, and stored in liquid nitrogen. There were 40 neonate rats in total and each subgroup contained 5 of them (n = 5).

Total RNA extraction

Total RNA was isolated from the left ventricle (because this part of the heart makes the major contribution to total cardiac RNA; Gloss et al. 2001) with Trizol reagent, as described by the manufacturer (Gibco, Invitrogen Corporation). Total RNA was quantified by absorbance at 260 nm. The integrity of RNA was assessed by electrophoresis in agarose–formaldehyde gels. Equal amounts of RNA were subjected to reverse transcription to synthesize cDNAs (Reverse Transcription System, Promega Co).

PCR

PCR was carried out in a total reaction volume of 20 μl containing 4 μl cDNA. The 30 cycles of PCR amplification were preceded by a heat denaturing step at 95 °C for 5 min. Each cycle of PCR consisted of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The PCR was terminated with a prolonged extension step at 72 °C for 5 min. Primers were designed with Gene Runner Analysis software (Hastings Software Inc., Hastings on Hudson, NY, USA). The nucleotide sequences for the primers were listed in Table 1. Products were electrophoresed in 1.5%-agarose gels, stained with ethidium bromide, and visualized under u.v. light.

Preparation of FQ-PCR standards

TRα1, TRα2, TRβ1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) target fragments were recovered and purified from 1%-agarose gel using a PCR product purification kit (Beijing BioDev–Tech Co., Ltd, Beijing, China). The target fragments were cloned into PMD18-T vector (Tiangen Biotech Co., Ltd, Beijing, China) respectively and the recombinants were transformed into competent DH5α bacteria, followed by screening of positive clones. Recombinant plasmid–carrying bacteria suspension was sent to Shanghai Bioasia Co., Ltd for sequencing analysis. Meanwhile, recombinant plasmids were extracted and A260 values were measured to clear the concentration of each recombinant plasmid. The copy number of recombinant plasmids was converted as follows: copy number = mass (g) × 6.023 × 10²³/660 × base number. The solution of plasmids was diluted to 10⁶, 10⁵, 10⁴, and 10³ copy/μl respectively. Thus, FQ-PCR standards for TRα1, TRα2, TRβ1, and GAPDH were obtained. The standards were stored at −20 °C before use.

Plotting FQ-PCR standard curve

TaqMan probes (with the fluorescent substance 6-carboxy–fluorescein (FAM) at the 5’-terminal and the fluorescence quencher 6-carboxytetramethylrhodamine (TAMRA) at the 3’-terminal) were synthesized according to the sequences as listed in Table 1 (Shanghai Bioasia Co., Ltd, Shanghai, China). FQ-PCR amplification was carried out using the following cycle conditions: Stage 1 (denaturation), 95 °C for 5 min; Stage 2 (amplification), 40 cycles of 95 °C for 15 s, 63 °C for 40 s; Stage 3 (melting curve), 95 °C for 0 s, 45 °C for 15 s, 95 °C for 0 s; and Stage 4 (cooling), 40 °C for 30 s. FQ-PCR standards were amplified using Roche Lightcycler System for real-time PCR to determine the correlation of the standard curve.

Table 1 Nucleotide sequences for thyroid hormone receptor α1 (TRα1), TRα2, TRβ1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers and TaqMan probe

<table>
<thead>
<tr>
<th>Target</th>
<th>Nucleotide sequences</th>
<th>Amplification site</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRα1</td>
<td>Sense 5'-TGCCCTTACTCACCCTACCA-3'</td>
<td>1897–1916</td>
</tr>
<tr>
<td></td>
<td>Antisense 5'-AAGCCAAGCGCTGCTCTGCT-3'</td>
<td>2009–2028</td>
</tr>
<tr>
<td></td>
<td>Probe 5'-TTCAGGGGACACGCTACTCC-3'</td>
<td>1960–1984</td>
</tr>
<tr>
<td>TRα2</td>
<td>Sense 5'-TGAGCAGGAGTTGTTGAGAG-3'</td>
<td>1211–1230</td>
</tr>
<tr>
<td></td>
<td>Antisense 5'-GAATGGAGAATTCCGCTCG-3'</td>
<td>1308–1327</td>
</tr>
<tr>
<td></td>
<td>Probe 5'-TTCAGGACGACACGAGAGAG-3'</td>
<td>1255–1276</td>
</tr>
<tr>
<td>TRβ1</td>
<td>Sense 5'-AGCCAGCAGCAAGAGATGG-3'</td>
<td>4017–4036</td>
</tr>
<tr>
<td></td>
<td>Antisense 5'-CCGCCAGAGACTGAGCTG-3'</td>
<td>4116–4136</td>
</tr>
<tr>
<td></td>
<td>Probe 5'-AGCAAGGCGGCTATGAACCGTG-3'</td>
<td>4068–4092</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense 5'-CTGGGAAACCTGCGAAGGT-3'</td>
<td>1587–1605</td>
</tr>
<tr>
<td></td>
<td>Antisense 5'-TGGGAGTGGTGTTGAAGTC-3'</td>
<td>1696–1715</td>
</tr>
<tr>
<td></td>
<td>Probe 5'-CATCAAGAGGGTGAGCAGGC-3'</td>
<td>1614–1637</td>
</tr>
</tbody>
</table>
FQ-PCR detection of samples

Total RNA was extracted from myocardial samples. cDNAs were obtained by reverse transcription and then amplified along with the standards using Roche Lightcycler System. The amplification conditions were the same for both the sample cDNAs and the standard cDNAs. Thus, the copy number of TRα1, TRα2, TRβ1, and GAPDH mRNA per μl was calculated for each of the samples. Following each FQ-PCR, a melting curve analysis was performed to ensure the specificity of FQ-PCR.

Pathologic study

Rat hearts from the CH group and the control group were washed with DEPC water to remove blood in the cardiac chambers and then stored in liquid nitrogen. Rat hearts were sectioned into a thickness of ~10 μm using a thermostatic freezing microtome CM1850 (Leica, Leica Microsystems GmbH, Wetzlar, Germany) at −20 °C. The tissue was loaded onto glass slides, fixated in a mixture of 95 ml of 95% ethanol and 5 ml glacial acetic acid for 1 min, and washed with running water. The sections were subjected to routine hematoxylin and eosin (HE) staining, mounting with neutral resin, and photography using an Olympus image analyzer (Olympus, Tokyo, Japan).

Statistical analysis

Each experiment was repeated thrice. All data were expressed as means ± s.d. TRα1, TRα2, and TRβ1 mRNA levels were analyzed by independent samples t-test to determine whether the expression of mRNAs encoding TR receptors varied between normal and hyperthyroid groups or between normal and congenital hypothyroid groups. Levels of expression of each mRNA detected were normalized to GAPDH mRNA. P<0.05 was considered to be significant.

Results

Hyperthyroid animals

Compared with the control group, the weight of rats from the hyperthyroid group increased more slowly, the heart rate (HR) was faster, and serum FT3 and FT4 levels were higher (P<0.01). Hyperthyroid rats were irritable, they ate and drank significantly more, and their hair lacked luster. Macroscopic observations revealed obvious myocardial hypertrophy and cardiac chamber narrowing in hyperthyroid rats (5/8), and heart weight was higher in the hyperthyroidism group than in the control group (P<0.01; Tables 2 and 3).

Hypothyroid animals

The neonate mortality rate was higher in the hypothyroid group (30%) than in the control group. Hypothyroid rats grew slowly and were small with a short tail. They opened their eyes late. Their hair grew slowly and was sparse. They were weaned late. Their balancing capability was poor and they reacted to stimuli slowly. The body weights (BW) of P0d, P14d, P21d, and P45d hypothyroid rats were lower than those in the corresponding control group (t=3.97, 8.66, 8.91, 5.72, P<0.01). The FT3 levels of P0d, P14d, and P21d hypothyroid rats were lower than those in the corresponding control group (t=2.84, 5.81, 5.20, P<0.01). The FT4 levels of P0d, P14d, and P21d hypothyroid rats were lower than those in the corresponding control group (t=3.64, 4.97, P<0.01; Table 4).

Table 2 Body weight (BW) changes in control and hyperthyroid rats (n=8, X±S, g)

<table>
<thead>
<tr>
<th>Group</th>
<th>0 w</th>
<th>3 w</th>
<th>BW changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>175.64±11.80</td>
<td>275.53±35.47</td>
<td>99.89±24.36</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>173.17±10.90</td>
<td>231.19±10.01</td>
<td>58.01±14.78*</td>
</tr>
</tbody>
</table>

The weight of rats from the hyperthyroid group increased more slowly than the control group. t=4.158.

*P<0.01 versus control group.
Differential expression of mRNA for TRα1, TRα2, and TRβ1 between normal and hypothyroid rats myocardium

TRα1, TRα2, and TRβ1 were expressed in normal and hypothyroid rat myocardium throughout the developmental process.

In normal rat myocardium, TRα1 mRNA expression was the highest at birth and then decreased gradually to the adult levels. Compared with normal rat myocardium, TRα1 mRNA expression was downregulated by 90% at P0d (t=18.91, P<0.01), 25% at P21d (t=3.49, P<0.01), and 41% at P45d (t=5.88, P<0.01) in hypothyroid rat myocardium, with the expression peak appearing until 2 weeks postnatally. Hence, in hypothyroid rats, myocardial TRα1 mRNA expression was generally downregulated and the expression peak appeared late (Fig. 4).

In normal rat myocardium, TRα2 mRNA expression was the highest at birth and then decreased gradually to the adult levels. Compared with normal rat myocardium, TRα2 mRNA expression was upregulated by 27% at P0d (t=7.60, P<0.01), 71% at P14d (t=20.94, P<0.01), 78% at P21d (t=22.05, P<0.01), and 36% at P45d (t=9.46, P<0.01) in hypothyroid rat myocardium, with the expression peak appearing until 2 weeks postnatally. Hence, in hypothyroid rats, myocardial TRα2 mRNA expression was generally upregulated and the expression peak appeared late (Fig. 5).

In normal rat myocardium, TRβ1 mRNA expression reached a peak at P14d and then decreased gradually to the adult levels. Compared with normal rat myocardium, TRβ1 mRNA expression was downregulated by 75% at P0d (t=61.97, P<0.01), 64% at P14d (t=9.44, P<0.01), 66% at P21d (t=30.86, P<0.01), and 67% at P45d (t=11.25, P<0.01) in hypothyroid rat myocardium. Hence, in hypothyroid rats, myocardial TRβ1 mRNA expression was generally downregulated and changed similarly with the control group (Fig. 6).

Pathologic study

Observations of HE-stained sections showed no morphological differences of myocardium between control P0d and hypothyroid P0d rats. In all P0d rats, myocardial pedomorphism was observed, i.e., myocardial cells contained a little sarcoplasm, but no myofibrils or transverse striations, and cell boundary was indistinct. In the control group, myocardial sarcoplasm became abundant and obvious myofibrils or transverse striations were observed at P14d, P21d, and P45d. In contrast, in hypothyroid group, myocardial cell maldevelopment was observed at 14 and 21 days postnatally, with a little sarcoplasm, a thin and long cell shape, and

Table 3 Serum free tri-iodothyronine (FT₃) and free thyroxine (FT₄) levels, heart rate (HR), and heart weight (HW) in control and hyperthyroid rats (n=8, X±S)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hyperthyroid</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT₃ (pmol/l)</td>
<td>1.01±0.23</td>
<td>9.99±2.22*</td>
<td>11.37</td>
</tr>
<tr>
<td>FT₄ (pmol/l)</td>
<td>18.46±4.54</td>
<td>62.99±12.27*</td>
<td>9.63</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>439.13±25.71</td>
<td>527.75±26.47*</td>
<td>6.79</td>
</tr>
<tr>
<td>HW (g)</td>
<td>0.95±0.16</td>
<td>1.32±0.16*</td>
<td>4.57</td>
</tr>
</tbody>
</table>

Serum FT₃ and FT₄ levels, heart rate, and heart weight were higher in the hyperthyroid group than in the control group. *P<0.01 versus control group.

Table 4 Body weight (BW), serum free tri-iodothyronine (FT₃) and free thyroxine (FT₄) levels in each group (n=12, X±S)

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>FT₃ (pmol/l)</th>
<th>FT₄ (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0d</td>
<td>6.80±0.68</td>
<td>0.20±0.08</td>
<td>3.98±0.70</td>
</tr>
<tr>
<td>14d</td>
<td>35.11±2.73</td>
<td>1.31±0.29</td>
<td>92.18±30.43</td>
</tr>
<tr>
<td>21d</td>
<td>61.19±3.79</td>
<td>1.4±0.32</td>
<td>53.20±6.85</td>
</tr>
<tr>
<td>45d</td>
<td>169.55±13.12</td>
<td>1.51±0.28</td>
<td>73.41±19.78</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0d</td>
<td>5.48±0.53*</td>
<td>0.10±0.05*</td>
<td>1.77±0.25*</td>
</tr>
<tr>
<td>14d</td>
<td>24.04±2.66*</td>
<td>0.55±0.11*</td>
<td>4.69±1.82*</td>
</tr>
<tr>
<td>21d</td>
<td>39.19±4.55*</td>
<td>0.86±0.26*</td>
<td>28.24±4.46*</td>
</tr>
<tr>
<td>45d</td>
<td>124.26±14.13*</td>
<td>1.42±0.48</td>
<td>65.57±6.31</td>
</tr>
</tbody>
</table>

The body weight, FT₃, and FT₄ of P0d, P14d, and P21d hypothyroid rats were lower than those in the P0d, P14d, and P21d control group respectively. *P<0.01 versus control group.

www.endocrinology-journals.org
indistinct transverse striations. Moreover, Henle’s fissures (spaces between myocardial cells) were widened and myocardial cells were deranged focally. At 45 days postnatally, in the hypothyroid group, myocardial transverse striations can be observed, but sarcoplasm was still not abundant and cells were thin and long, with widened Henle’s fissures (Fig. 7).

Discussion

The heart is a major target organ for thyroid hormone (T3) action, and marked changes occur in cardiac function in patients with hypo- or hyperthyroidism. T3 significantly influences cardiac contractile and electrophysiological function with increasing amounts of T3 enhancing cardiac contractile function and accelerating HR. These effects are mediated by the binding of T3 to specific nuclear TRs. TRs are members of a family of nuclear receptors that includes steroid, retinoic acid, and vitamin D receptors, all closely related to the v-erbA oncogene. TRs are encoded by two different genes: TRα on chromosome 17 and TRβ on chromosome 3. TRα and TRβ isoforms have a similar structure, characterized by an amino-terminal domain, a DNA-binding domain comprises two zinc finger motifs, and a carboxy-terminal ligand-binding domain. The TRα gene generates the TRα1 and TRα2 isoforms that are identical for the first 370 amino acids but differ as a consequence of differential splicing at their C terminus: the last 40 specific residues of TRα1 are replaced by 122 amino acids encoded by the TRα2-specific exon 10, the last exon in the TRα locus. As a consequence of the C-terminal change, the TRα2 protein is unable to bind TH and no other ligand has been identified. TRα2 binds DNA weakly and only binds a subset of T3-responsive sites. Furthermore, it dimerizes poorly with RXR and lacks the activating function domain 2 that interacts with coactivators. Both TRα1 and TRα2 mRNAs are widely distributed. Alternative promoters or splicing of the amino-terminal region of the TRβ mRNA forms TRβ1 and TRβ2 in rat, both of which bind T3. The TRβ2 mRNA is concentrated primarily in the anterior pituitary and the central nervous system of the rat, whereas the TRβ1 mRNA is widely distributed. Therefore, in our experiments,
we detected TRα1, TRα2, and TRβ1 mRNA alterations except β2. In addition, TRα1 and TRβ1 are thyroid hormone functional receptors except TRα2 (Sap et al. 1986, Weinberger et al. 1986, Thompson et al. 1987, Lazar & Chin 1990).

In our experiment, the phenotype of the hyperthyroid rat has features of hyperthyroidism, including slowly increased BW, elevated HR, cardiac hypertrophy, and higher serum FT3 and FT4 levels. The phenotype of the hypothyroid rat also has features of CH, such as slow growth, late weaning, poor balancing capability, slower reaction to stimuli, and lower serum FT3 and FT4 levels. Thus, the animal models were made successfully.

Our results have shown that TRα1 mRNA expression was upregulated, TRα2 mRNA expression was downregulated, but TRβ1 mRNA expression remained unchanged in the hyperthyroid rat myocardium compared with the normal myocardium. This may be because: 1) TRα1 is the major functional receptor in the myocardium; deficiency in TR isoforms caused discrete alterations in HR and ventricular function. The TRα1−/− mice exhibited lower HR, lower body temperature, and slower ventricular repolarization (prolonged P–Q, QRS, and Q–T durations in the ECG) as compared with normal mice. The TRβ−/− mice exhibited severely impaired hearing, normal HR as well as normal contractile function of papillary muscle (Forrest et al. 1996a,b, Rüsch et al. 1998, Wikström et al. 1998, Göthe et al. 1999, Johansson et al. 1999). Comparison of the cardiac phenotype between TRα and TRβKO mice clearly indicates that TRα had predominant contractile and electrophysiological function in the heart. Furthermore, TRα1 mRNA was present at a 3:1 ratio to TRβ mRNA in the ventricle of the heart (Swanson et al. 2003). These findings indicated that the cardiac phenotype regulated by T3 was primarily mediated by the more predominant TRα in the heart. The lack of TRα cannot be compensated by TRβ in the heart, but the lack of TRβ can be compensated by TRα in the heart (Gloss et al. 2001). The TRα1 exerts a predominant effect on cardiac electrophysiological functions. Thus, at high thyroid hormone level, the expression of TRα1, the major functional

Figure 5 FQ-PCR analysis of TRα2 mRNA expression differentiations between control and hypothyroid rat myocardium (X±S, n=5). TRα2 mRNA expression was upregulated by 27% at P0d, 71% at P14d, 78% at P21d, and 36% at P45d in hypothyroid rat myocardium, with the expression peak appearing until 2 weeks postnatally, *P<0.01 compared with the control group.

Figure 6 FQ-PCR analysis of TRβ1 mRNA expression differentiations between control and hypothyroid rat myocardium (X±S, n=5). TRβ1 mRNA expression was downregulated by 75% at P0d, 64% at P14d, 66% at P21d, and 67% at P45d in hypothyroid rat myocardium. *P<0.01 compared with the control group.

Figure 7 Observe the morphological differences of myocardium between control group and hypothyroid group rat. (A) Rat myocardium in P0d control group, HE staining, 100×. (B) Rat myocardium in P45d hypothyroid group (myocardial transverse striations can be observed, but sarcoplasm was still not abundant, with widened Henle’s fissures), HE staining, 400×. (C) Rat myocardium in P21d control group, HE staining, 200×. (D) Rat myocardium in P21d hypothyroid group (with widened Henle’s fissures, myocardial cells were deranged locally), HE staining, 200×. (E) Rat myocardium in P21d control group, HE staining, 400×. (F) Rat myocardium in P21d hypothyroid group (with a little sarcoplasm and indistinct transverse striations), HE staining, 400×.
receptor of thyroid hormones in the myocardium was upregulated in a compensatory manner. 2) The TRα2 has been suggested to exert a suppressive function on other TRs. Suggested mechanisms for suppression include competition for binding to thyroid hormone response elements (TREs) on target genes, formation of inactive heterodimers, or squelching (Rentoumis et al. 1990, Katz et al. 1992, Katz & Lazar 1993, Liu et al. 1995, Tagami et al. 1998). Transient transfection of the cDNA coding for TRα2 has been shown to block the effect of a co-transfected α1 or β1 cDNA in facilitating T3 regulation of a third co-transfected reporter gene (Koenig et al. 1989, Strait et al. 1990). This blockade has been ascribed to an ability of the TRα2 protein to compete with α1 and β1 for the TRE situated in the 5′-flanking region of the reporter gene. In addition, selective ablation of TRα2 resulted in an inevitable, concomitant overexpression of TRα1 (Saltó et al. 2001). So downregulation of TRα2 mRNA expression attenuates the inhibition of TRα1 by TRα2 and leads to upregulation of TRα1 expression. Upregulated TRα1 can lead to heart lesions through modulating the expression of T3-regulated genes. MHC has a high ATPase activity and is closely related to fast myocardial contraction. The TRα1 expression upregulation may upregulate the expression of MHCα mRNA and enhance myocardial inotropy (Kimugawa et al. 2001). A long-term hyperkinetic state of myocardium causes myocardial strain to progress from the compensated to the decompensated stage. Na+/H+ exchanger-1 (NHE1) is a plasma membrane protein that removes an intracellular proton, exchanging it with extracellular sodium. By doing so, the NHE1 raises intracellular pH. The TRα1 can activate the NHE1 exchanger promoter (Li et al. 2002). The TRα1 expression upregulation may lead to NHE1 overactivation, thus causes acidosis in myocardial cells and progressive degeneration and even necrosis of myocardial cells. These changes are consistent with myocardial pathological changes in patients with chronic hyperthyroid heart disease.

Thyroid hormone signaling is critical for proper heart development and function. T3 can increase expressions of myocardium-specific TH-responsive genes. Myocardium-specific genes comprise a list of proteins responsible for cardiomyocyte function: MHC, SERCA2, α- and β-adrenergic receptors, several plasma membrane ion transporters, such as Na+/Ca2+ exchanger, NHE, and a variety of ion channels, including HCN2, HCN4, Kv1.5, Kv4.2, and Kv4.3. They can be regulated at both the transcriptional and post-transcriptional levels by T3 binding with specific nuclear TRs, thus coordinating the electrochemical and mechanical responses of the myocardium (Ma et al. 2003, Kahaly & Dillmann 2005). TH-responsive gene promoters are selectively regulated by the various TR isoforms. For example, TRα1 can activate the α-MHC, HCN2, and HCN4 promoter and TRβ1 can activate the HCN4 promoter and repress the β-MHC promoter (Gloss et al. 2001). Our experiment showed that in hypothyroid rats, TRα1 mRNA expression was generally downregulated and the expression peak appeared late, and TRβ1 mRNA expression was generally downregulated. Downregulated TRα1 and TRβ1 mRNA expression will lead to the decreased expression of HCN2, HCN4, MHCα, and SERCA2. HCN2 and HCN4 have been identified as components of an ion channel that constitutes the I_s current, which contributes to HR generation. Markedly decreased expression of HCN2 and HCN4 occurs in TRαKO mice and euthyroid TRβPV with a decreased HR (Gloss et al. 2001). MHCα mRNA code protein that is linked to force generation. SERCA2 mRNA code protein that is linked to calcium lowering during diastole, so decreased levels of MHCα mRNA and SERCA2 mRNA may provide the cause for slowed force development and prolonged relaxation time. Thus, the abnormal expressions of myocardium-specific genes caused myocardial structural developmental abnormalities and serious heart dysfunction. These changes are consistent with myocardium pathological changes in patients with chronic hypothyroid heart disease.

Abnormal expressions of TR subtypes in hypothyroid adult rat myocardium have been reported previously, but the results were inconsistent, possibly due to different preparation methods of animal models and different detection methods adopted by those laboratories. In the present study, we established congenital hypothyroid rats and monitored the influence of CH on myocardial TR subtypes expressions from birth through adulthood. We not only reported the changes of myocardial TR expressions in normal rats throughout the developmental process, but also demonstrated delayed peaks and downregulation of expressions of functional receptors of thyroid hormone in hypothyroid rats. In addition, the study confirmed that CH caused myocardial maldevelopment (less sarcoplasm in the thin and long cell, transverse striations development delay and widened Henle’s fissures) may be closely associated with abnormal TR expressions. TRα1 and TRβ1 are functional receptors of thyroid hormones in the myocardium, and downregulated TRα1 and TRβ1 expression may lead to abnormal expression of multiple genes regulated by thyroid hormones, thus resulting in a series of electrophysiological abnormalities of the heart and myocardial functional injury. In addition, thyroid hormones are crucial for myocardial development and they may promote myocardial cell survival. Lack of thyroid hormones in the embryonic stage may induce myocardial cell apoptosis and downregulate the mRNA expressions of functional thyroid hormones receptors (Alisi et al. 2005, Verga et al. 2006).

At an early stage of the experiment, we wanted to establish a neonatal hyperthyroidism model to monitor the influences of hyperthyroid on myocardial TR expressions from birth through adulthood, but the mortality of pregnant and neonate rat were so high that adequate samples cannot be obtained. Considering hyperthyroidism is more common in adults than in children, we had to alter the experiment design. We had to give up the neonate hyperthyroidism model and establish an adult hyperthyroidism model to detect the effect of thyroid hormone on the adult hyperthyroid rat myocardium. In the future, we
plan to study TR’s differential expressions in hyperthyroid developmental myocardium on the basis of this article.

In summary, the present study demonstrated that abnormal thyroid hormone concentrations caused abnormal TR mRNA expressions in the myocardium. During hyperthyroidism, high thyroid hormone level may influence the transcription level of TR and lead to faster HR, and a hyperkinetic state of the heart by influencing the expressions of thyroid hormone-regulated genes. Hence, a long-term exposure to high thyroid hormone level will lead to heart failure. During CH, TR mRNA expression peak appeared late and abnormal expression changes of TRs occurred in the myocardium, which resulted in maldevelopment of myocardial cells and conduction bundles, irreversible damage to myoccardial structure and function, and ultimately hypothyroid heart disease. Therefore, in the presence of thyroid dysfunction, abnormal expressions of TR subtypes in the myocardium are closely associated with the pathogenesis of heart lesions. The present study not only deepened the understanding of pathogenesis of thyroid dysfunction caused heart lesions, but also may guide gene therapy and the selective application of various TR subtype antagonists for such heart lesions.

Acknowledgements

This research was supported by Natural Science Foundation of Tianjin (033605711), Science and Technology Development Program of Tianjin (05YFGDSF02700) and Chinese People’s Armed Police Force Foundation (wy2006-4). We are grateful to Jason Tam, Miaojun, and Miaojie for English revision. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Dillmann WH 1990 Biochemical basis of thyroid hormone action in the heart. American Journal of Medicine 88 626–630.


Dillmann WH 2002 Cellular action of thyroid hormone on the heart. Thyroid 12 447–452.

Forrest D, Erway LC, Ng L, Akschuler R & Curran T 1996a Thyroid hormone receptor beta is essential for development of auditory function. Nature Genetics 13 354–357.


Sussman MA 2001 When the thyroid speaks, the heart listens. *Circulation Research* **89** 557–559.


Tagami T, Kopp P, Johnson W, Arseven OK & Jameson JL 1998 The thyroid hormone receptor variant alpha2 is a weak antagonist because it is deficient in interactions with nuclear receptor corepressors. *Endocrinology* **139** 2535–2544.


Received in final form 9 September 2007
Accepted 19 September 2007
Made available online as an Accepted Preprint 19 September 2007