Thyroid hormones induce unique and potentially beneficial changes in cardiac myocyte shape in hypertensive rats near heart failure

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IT HAS BEEN WIDELY RECOGNIZED for years that pathological hypertrophies leading to congestive heart failure (CHF) produce a gene program similar to that observed during fetal development. Two interventions are known to reverse expression of the fetal gene program (FGP), exercise and thyroid hormones (THs; Refs. 16, 19, 22). Although it is now generally believed that exercise is beneficial for individuals with heart disease (21), it is not clear whether THs can be given in a manner that safely improves heart function and promotes beneficial remodeling. A distinct possibility is that the additional overloading stimulus from TH supplementation may actually induce or accelerate progression to heart failure in individuals with preexisting heart disease. The purpose of this study was to determine the effects of thyroid treatment of rats with hypertension and/or heart failure on left ventricular (LV) remodeling and function. Female rats with spontaneously hypertensive heart failure (SHHF) were treated for 1 mo just before the onset of symptomatic heart failure. Although this model does not appear to display overt thyroid dysfunction based on plasma hormone levels, there is distinct reexpression of the FGP (4). Based on a preliminary experiment to determine the approximate dose that produced signs of hyperthyroidism (e.g., we treated SHHF rats with several low doses of THs and monitored body temperature and heart rate changes), three different TH doses were used in the present experiment. The high dose produced signs of overt hyperthyroidism, whereas the low dose produced no significant changes in heart function or mass. However, the middle and high doses produced a unique, never-before-observed pattern of myocyte remodeling that appears to be beneficial.

MATERIALS AND METHODS

Experimental design. Lean female SHHF rats (21 mo of age) purchased from Charles River Laboratories (Indianapolis, IN) and divided into four experimental groups as follows: 1) untreated, 2) 0.05% TH treated, 3) 0.1% TH treated, and 4) 0.2% TH treated. Treated groups were fed ground Purina Rat Chow that contains the indicated percentage of thyroid powder (Sigma T-5146; contains both 3,5,3’-triiodothyronine and thyroxine) for 30 days and were provided water ad libitum. Age-matched Wistar-Furth (WF; Charles River Laboratories; Wilmington, MA) rats were used as normotensive controls. Animals were housed two per cage and kept on a 12:12-h light-dark cycle. At termination, cardiac function and remodeling were assessed by echocardiography, catheterization, and myocyte isolation from each animal in the study. Western blots were used to determine TH receptor protein and myosin isoform expression. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Echocardiographic measurements. In terminal experiments, rats were anesthetized using isoflurane (1–1.5%). LV dimensions in systole and diastole as well as ejection fraction (EF) and fractional shortening (FS) were measured from M-mode images using an HP
Sonos 2000 imaging system (with a 7.5 MHz transducer) as described previously (23).

**Hemodynamic measurements.** LV hemodynamics were obtained by catheterization of the right common carotid artery using a Millar Mikro-tip catheter (Millar Instruments; Houston, TX) as described previously (24). Measurements were recorded using a digital acquisition system (model HPA 410a; MicroMed; Louisville, KY).

**Myocyte isolation and morphometry.** Animals were administered heparin, and hearts were quickly removed, trimmed, blotted, and weighed. Isolated myocytes were prepared and fixed in 2.0% glutaraldehyde, and dimensions were determined as described previously (11). Briefly, cell volume (V) was measured with a Coulter Channelizer system (model Z2; Coulter Electronics; Miami, FL), myocyte length (L) was determined via microscopy, and cross-sectional area was calculated as V/L. Based on the formula for an elliptical cylinder, which most closely approximates myocyte shape, myocyte major and minor diameters were determined in the following manner: major diameter (A) was determined by tracing isolated myocytes (A = profile area/L). Minor diameter (B) was subsequently determined from the formula for V relative to an elliptical cylinder (V = πA/4 * B) and by substituting the Coulter values for V (9). To prevent compression of myocytes, two coverslips were used as spacers to support a central coverslip overlying the cell suspension. It has been clearly demonstrated that the shape of undamaged, intact myocytes is not altered by the cell isolation procedure (11). However, all data from a given animal were excluded from analysis if isolated cell preparations were of poor quality (e.g., less than ~60% rod cells).

**Western blot analysis.** Hearts were removed, snap-frozen in liquid nitrogen, and stored at ~80°C until the time of analysis. Myocardial samples were powdered in liquid nitrogen, and tissue was homogenized in lysis buffer (50 mM Tris at pH 7.4, 1% SDS, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, 1 mM NaF, and a protease cocktail inhibitor; Calbiochem; Darmstadt, Germany) by sonication. Protein concentrations were quantified using the bicinchoninic acid protein assay method (Pierce Biotechnology; Rockford, IL). For each sample, 30 µg of protein were separated by SDS-PAGE (12%) and transferred onto polyvinylidene difluoride (PVDF) membranes. Blots were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween (10 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% Tween) and incubated with a monoclonal antibody (1:1,000 dilution) to recognize TH receptor α-1 (TRα-1; Affinity Bioreagents; Golden, CO), α-myosin heavy chain [A4.951; American Type Culture Collection (ATCC)], β-myosin heavy chain (BA-G5; ATCC), SERCA2 (Santa Cruz Biotechnology; Santa Cruz, CA), phospho-phospholamban (serine 16), and phospholamban (Upstate Cell Signaling Solutions; Waltham, MA). Resultant bands were visualized using enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech; Piscataway, NJ). Detection and quantification were done using a VersaDoc Imaging System (model 3000; Bio-Rad; Hercules, CA).

**Statistical analysis.** All data are reported as means (SD) and are compared using one-way ANOVA. In addition, a Bonferroni post hoc test was used to examine significant differences observed with ANOVA. A value of P < 0.05 was considered statistically significant.

**RESULTS**

Average body weights, heart weights, and heart weight-to-body weight ratios are shown in Table 1. As indicated by the table, all SHHF groups including TH-treated animals had significant cardiac hypertrophy compared with the normotensive controls. There were no significant differences in body mass between any animal groups. Heart mass increased in a dose-related manner in TH-treated SHHF rats and reached statistical significance in the high-dose group.

Table 2 shows the average hemodynamic data for normotensive controls, untreated SHHF rats, and all TH-treatment groups. Heart rate was similar in all rat groups except the high-dose SHHF group, which displayed significant tachycardia. LV systolic blood pressure was not significantly different between untreated SHHF rats and control animals (it is typical for blood pressure to decline in SHHF rats as they approach failure). Compared with normal control animals, LV end-systolic pressure tended to be higher in all TH-treated SHHF groups but reached significance in only the 0.1% TH group. Contractility (±dP/dt) tended to be reduced in untreated SHHF vs. control rats, and TH treatment increased the values in the highest dose group toward that of the control group values. Compared with controls, LV systolic wall stress was increased in untreated SHHF rats but was normalized by the middle and high doses of TH.

**Echocardiographic data** are summarized in Table 3. Compared with controls, LV chamber diameters during systole and diastole were significantly increased in untreated SHHF animals. TH treatment tended to reduce systolic and diastolic chamber diameter toward normal values with the higher doses, but this did not reach statistical significance. There was a significant increase in LV wall thickness during systole and diastole in SHHF rats treated with the middle and high TH doses. These changes with the middle and high TH doses resulted in a dramatic ~60% reduction in the systolic chamber diameter-to-wall thickness ratio. EF and FS were significantly depressed in untreated SHHF animals compared with control rats, and TH treatment tended to normalize these values with the middle TH dose being the most effective.

Changes in LV myocyte dimensions are shown in Table 4. As expected, LV myocyte dimensions were significantly increased in all SHHF rat groups compared with control animals. Surprisingly, there was no additional increase in myocyte length in any TH treatment group. There was a tendency for myocyte volume and cross-sectional area to increase in the 0.1 and 0.2% treatment groups, but this did not reach significance. The A and B transverse dimensions were also examined in LV myocytes. Both dimensions tended to be increased in untreated SHHF rats compared with control animals. TH treatment tended to reduce the A dimension and increase the B dimension and thereby resulted in a progressive reduction in the A/B ratio with increasing dose.

TRα-1 expression tended to be reduced in untreated SHHF rats compared with control animals (Fig. 1). There was also a tendency for TH treatment to partially reverse the decreased expression of this receptor. None of these differences, however, were statistically significant. In untreated SHHF rats, α-myosin heavy chain levels were reduced and β-myosin...
Table 2. Hemodynamic data

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>LVP, mmHg</th>
<th>+dP/dt, mmHg/s</th>
<th>−dP/dt, mmHg/s</th>
<th>Wall Stress, kdyn/cm²</th>
<th>Heart Rate, beats/min</th>
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</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>162 (SD 17)</td>
<td>10,374 (SD 1,600)</td>
<td>9,216 (SD 1,538)</td>
<td>87 (SD 17)</td>
<td>376 (SD 41)</td>
</tr>
<tr>
<td>SHHF rats</td>
<td>0.05% TH</td>
<td>170 (SD 35)</td>
<td>8,312 (SD 1,798)</td>
<td>5,997 (SD 1,742)</td>
<td>169 (SD 65)</td>
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<tr>
<td></td>
<td>0.1% TH</td>
<td>197 (SD 31)</td>
<td>9,277 (SD 1,548)</td>
<td>7,302 (SD 1,105)</td>
<td>157 (SD 63)</td>
</tr>
<tr>
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<td>0.2% TH</td>
<td>220 (SD 46)*</td>
<td>10,388 (SD 2,365)</td>
<td>7,999 (SD 2,091)</td>
<td>105 (SD 36)*</td>
</tr>
<tr>
<td></td>
<td>0.0% TH</td>
<td>191 (SD 31)</td>
<td>10,775 (SD 2,039)*</td>
<td>8,812 (SD 1,362)*</td>
<td>104 (SD 23)*</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of rats identical to Table 1. LVP, left ventricular end-systolic pressure; +dP/dt, maximal rate of pressure development; −dP/dt, maximal rate of pressure decline; *P ≤ 0.05 vs. SHHF untreated rats; †P ≤ 0.05 vs. control animals.

Table 3. Echocardiographic data

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>LVIDd, mm</th>
<th>LVIDs, mm</th>
<th>PWTd, mm</th>
<th>PWTs, mm</th>
<th>Ejection Fraction, %</th>
<th>Fractional Shortening, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>6.1 (SD 0.3)</td>
<td>3.2 (SD 0.3)</td>
<td>2.5 (SD 0.5)</td>
<td>3.2 (SD 0.4)</td>
<td>84 (SD 3)</td>
<td>48 (SD 3)</td>
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<tr>
<td>SHHF rats</td>
<td>7.6 (SD 0.8)†</td>
<td>5.0 (SD 0.9)†</td>
<td>2.7 (SD 0.8)</td>
<td>3.6 (SD 0.6)</td>
<td>69 (SD 9)†</td>
<td>35 (SD 6)†</td>
</tr>
<tr>
<td></td>
<td>7.3 (SD 0.9)†</td>
<td>4.2 (SD 0.9)†</td>
<td>3.1 (SD 0.7)</td>
<td>4.1 (SD 0.5)</td>
<td>78 (SD 12)</td>
<td>42 (SD 9)</td>
</tr>
<tr>
<td></td>
<td>7.3 (SD 0.8)†</td>
<td>3.7 (SD 0.9)†</td>
<td>3.8 (SD 0.5)*‡</td>
<td>4.8 (SD 0.4)*‡</td>
<td>85 (SD 6)*</td>
<td>50 (SD 9)*</td>
</tr>
<tr>
<td></td>
<td>6.5 (SD 0.8)</td>
<td>3.9 (SD 0.6)</td>
<td>3.7 (SD 0.5)*†</td>
<td>4.6 (SD 0.4)*†</td>
<td>77 (SD 5)</td>
<td>41 (SD 5)</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of rats identical to Table 1. LVIDd and LVIDs, left ventricular diastolic and systolic internal diameter, respectively; PWTd and PWTs, diastolic and systolic posterior wall thickness, respectively; *P ≤ 0.05 vs. SHHF untreated rats; †P ≤ 0.05 vs. control animals.
We have previously shown (9) that the axis of the major transverse diameter runs predominately in a circumferential direction and increases in progressive chamber dilation associated with CHF, whereas the axis of the minor transverse diameter runs predominately in a transmural direction and is associated with changes in wall thickness. Compared with controls, LV myocytes from untreated SHHF rats showed an increase in major transverse diameter and length, which suggests again that growth in these two dimensions was likely responsible for progressive chamber dilation that led to CHF. With increasing TH dose in treated SHHF rats, the major transverse diameter declined and the minor transverse diameter increased. These changes were reflected in the echocardiography data by a reduction in chamber diameter and an increase in wall thickness. These changes in chamber dimensions due to altered myocyte transverse shape were responsible for the reduction in LV systolic wall stress as predicted by the Laplace equation (e.g., changes in wall stress are directly proportional to pressure and chamber diameter and inversely proportional to wall thickness). It is well known that TH promotes myocyte hypertrophy, so it was never anticipated that reverse remodeling might occur. Consequently, our finding that heart weight tended to increase with treatment was not a surprise.

We have noted previously (8) that regardless of the etiology of CHF, progressive dilatation during the transition to failure is characterized by myocyte lengthening without significant alterations in myocyte width. Myocyte remodeling resulting from normal physiological growth and TH treatment leads to proportional growth in myocyte length and width, which reflects proportional growth of chamber diameter and wall thickness (2, 10). This pattern of myocyte growth maintains stable wall stress in the absence of LV pressure changes. It is not clear at this time whether the critical defect in myocyte remodeling in progression to CHF is due solely to excessive myocyte lengthening or to impaired transverse growth. It is possible that myocytes are responding normally to increased preload by adding new series sarcomeres, whereas the normal check on this system, balanced myocyte transverse growth, is where the true dysfunction lies. Published results from hyper- and hypothyroid rats (3, 17) and the results of the present experiments suggest that THs may play an important role in the balanced regulation of myocyte shape as it relates to normalization of wall stress. Until now, it was not clear whether myocytes retained the ability to beneficially remodel transverse shape during the transition period to heart failure, which is characterized by an absence of transverse growth and excessive cell lengthening. Interestingly, there was no change in myocyte shape with the low-dose TH despite reversal of the myosin isoform abnormality (e.g., marker of FGP). This suggests that signaling related to myocyte growth could be independent of changes in the FGP.

Compared with untreated SHHF rats, LV systolic blood pressure tended to be higher with all TH doses. However, it should be noted that the LV pressures observed in TH-treated rats were in the range that we typically observe in pre failure SHHF rats (∼190–230 mmHg) that are a few months younger. In these experiments, LV pressure in untreated 22-mo-old SHHF rats was similar to controls. This decline in LV pressure is typical of SHHF rats approaching CHF. Consequently, we do not believe that TH treatment induced additional hyperten-
sion, but rather, TH treatment maintained LV pressure to the level found in slightly younger SHHF rats. Owing to the short duration of treatment and the limited supply of aged rats, we elected to have only one untreated SHHF rat group.

The idea that therapeutic administration of THs to animals or humans with CHF might be beneficial has been proposed by others. Gay et al. (7) demonstrated an improved inotropic effect in thyroxine-treated rats with myocardial infarction. Short- and medium-term improvements have been demonstrated in thyroid-treated patients with CHF (12). The potential risk of toxic effects from overdosing with THs, however, cannot be overstated. For this reason, some studies have examined TH analogs that may provide inotropic improvement without adverse chronotropic effects. Promising results have been reported in animal and human studies with the TH analog 3,5-diiodothyropropionic acid (20). It is not clear at this time whether THs or analogs will ultimately improve the condition of patients with CHF. Certainly, TH treatment of patients comparable to the SHHF rats used in this study (e.g., with sustained hypertension, LV dilatation, and no evidence of thyroid dysfunction based on blood hormone levels) would be ill advised at this time. The potential role of THs in the regulation of myocyte remodeling in heart disease merits additional investigation. Presently, potential signaling mechanisms by which THs alter myocyte shape are unknown.

GRANTS

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REFERENCES
