Thyroid hormone and cardiac function in mice deficient in thyroid hormone receptor-α or -β: an echocardiographic study

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Thyroid hormone (TH) exerts a profound effect on the chronotropic and ionotropic function of the heart (25, 28). TH deficiency results in systolic and diastolic dysfunction with reduction in heart rate and force of contraction and an increase in cardiac relaxation (18, 23).

The molecular basis for the negative ionotropic effect seen in hypothyroidism, namely the bradycardia, is related to decreased expression of the hyperpolarization-activated, cyclic nucleotide-gated ion channels (2 or 4), which are specific targets of the TH receptor (TRα) gene (19).

TH action is mediated by its interaction with specific nuclear TRs functioning as ligand-dependent transcription factors that modulate the expression of target genes (30, 33). The two TR genes (TRα and TRβ) have substantial structural and sequence similarities. Each generates multiple TR proteins by alternative splicing (α1 and α3; β1, β2, and β3) or alternative start sites (α3, β2, revErb). TRα2 binds to DNA, but, due to a sequence difference at the ligand-binding site, it does not bind TH and thus does not function as a TR proper (35). The relative expression of TR genes and the distribution of their products vary among tissues and during different stages of development (20, 31, 46). Furthermore, an internal promoter, located within intron 7 of the TRα gene, is responsible for the expression, in mice, of truncated isoforms of TRα1 and TRα2, (TRα01 and TRα02) containing the carboxy-terminal segment of the molecule. These additional products of the TRα gene may play a role in downregulation of transcriptional activity (5, 17, 40). TRα1 and TRα2 are the major isoforms of TR expressed in the heart (24).

Although TRβ1 is expressed at low levels (10), TRβ2 is also expressed (43). The use of mice with disruption of either the TRβ or TRα gene has led to the conclusion that the effect of L-triiodothyronine (L-T3) on the heart is mediated predominantly by TRα (19, 21, 22, 38, 48). These studies have primarily examined the effect of TH deprivation and have not examined the effect of long-term TH excess.

The purpose of the present study was to examine the contribution that both TRβ and TRα make in the presence of TH withdrawal and TH treatment on the chronotropic and ionotropic effect on the heart in vivo as determined by echocardiographic parameters. Echocardio-
cardiomyocytes uniquely allows the study of sequential changes in TH action over time in the same animals. We have confirmed that chronotropic effects of TH on the heart are primarily TRα dependent. Furthermore, we have demonstrated that an intact animal the action of TH on the heart is both TRβ and TRα dependent.

**METHODS**

**Mice.** Mice were weaned on the 4th wk after birth and fed a rodent diet (no. 5053: Lab Diet, Brentwood, MO) containing 0.53 ppm iodine and were given tap water ad libitum. They were housed three to five mice per cage in an environment of controlled 19°C temperature and 12-h alternating dark-artificial light cycles. All animal experiments were performed according to approved protocols at the University of Chicago by the Institutional Animal Care and Use Committee.

Male mice were 50–90 days old at the time of initial blood sampling. Three hundred microliters of blood were obtained by retroorbital vein puncture under light methoxyflurane (Pitman Moore, Mundelein, IL) anesthesia. Bleeding was generally done between 0900 and 1200. Serum was separated by centrifugation and stored at −20°C until analyzed.

The TRβ knockout mice were produced by insertion of the LacZ-NeoR cassette downstream of exon 3 and replacing exons 5–7, thus effectively abolishing not only the generation of full TRα1 and TRα2 transcripts but also that of TRα1α2 (TRα0/0) by removal of the transcription start point at intron 7 (17). The gene sequence for rev-erbα-α protein encoded by the opposite strands for the TRα (45) remains intact. In both sets of mice, the recombinant ES cells were derived from 129sv mice and were implanted into C57BL/6 recipient blastocysts. C57BL/6 mice were mated to each chimeric mouse and then back-crossed three to four times into the same strain, thereby diluting the 129sv background. The TRα0/0 and TRβ−/− mice were crossed for greater than five generations to select wild-type mice that had similar backgrounds.

**Induction of hypothyroidism and treatment with TH.** TH deficiency was induced in 10 male mice of each type (wild-type, TRα0/0, and TRβ−/−) with a low-iodine diet containing 0.15% 5-propyl-2-thiouracil (PTU) for 4 wk. Blood samples were obtained from the retroorbital vein after recording of the atria and the valves, blotted of excess fluid, and were weighed on a Mettler Balance (model no. AG245) with an accuracy of ± 0.01 mg.

**Electrocardiograms.** For animal preparation, 30 mice were studied, including 10 wild-type, 10 TRβ−/−, and 10 TRα0/0 mice, as described.

Before acquisition of cardiac ultrasound recordings, anesthesia was induced by administering chloral hydrate (500 mg/5 ml; Pennex Pharmaceutical) intraperitoneally in a 4% solution in PBS at a dose of 0.4 mg/g mouse. Animals were then secured to a custom-made waterbed in a shallow left lateral decubitus position to facilitate imaging. The bed was connected to a circulating water bath set at 40°C to prevent hypothermia. The actual bed temperature was maintained at 38°C throughout the experiment, and every effort was made to maintain constant body temperature, but the application of gel for echocardiography was an unavoidable variable. Transsthoracic echocardiography was performed three times during the experiment. Baseline recordings were obtained at 8 wk of age, after 4 wk with PTU treatment (as described, at 12 wk of age), and after 4 wk of l-T4 treatment (as described, at 16 wk of age).

**Data acquisition.** Cardiac ultrasound imaging was performed using a high-frequency 15-MHz linear transducer (Sonos 5500; Agilent, Andover, MA) at a maximum frame rate of 120 frames/s. Parasternal long- and short-axis views were obtained after adjusting gain settings for optimal epicardial and endocardial wall visualization. From the short-axis view, left ventricular (LV) M-mode tracings were obtained. From an unconventional, more superior parasternal long-axis view, ascending aortic two-dimensionally targeted M-modes were recorded. Ascending aortic pulse wave Doppler velocities were obtained from the suprasternal window by means of a pediatric short focal length, 12-MHz phased array transducer (Sonos 5500; Agilent Technologies). To improve image quality, an acoustic coupling gel standoff was mounted to the probe. This resulted in a 1- to 1.5-cm standoff between the transducer and the chest wall, enabling the transducer to work at its ideal focal length. To further improve quality by decreasing artifacts, the gel (Aquasonic 100; Parker, Orange, NJ) was centrifuged at 2,000 g to remove air bubbles.

Echocardiographic loops of ≥20 cardiac cycles containing the two-dimensional data, M-mode tracings, and Doppler velocity panels were stored digitally on magneto-optical disk for off-line analysis.

**Measurements.** From the short-axis view, epicardial and endocardial LV areas were measured offline at end systole and end diastole. Images were considered adequate for measurement when >75% of the epicardial and endocardial contour could be adequately visualized. In accordance with American Society of Echocardiography recommendations, the short-axis endocardial border was traced on the innermost endocardial edge, whereas the epicardial border was traced along the first bright pixel immediately adjacent to the darker myocardium (Fig. 1B). The LV length, defined as the distance between the apex and the midmural annulus, was obtained from the parasternal long-axis views in which the mitral annular plane and the apex were well defined (Fig. 1A). LV measurements were made from at least three cardiac cycles, at both end systole and end diastole.
LV mass was calculated using the formula

\[
\text{2-D area-length method LV mass} = [1.05 (5/6 A_1(L + t) - 5/6 A_2L)]
\]

where 1.05 is the specific gravity of muscle, \(A_1\) and \(A_2\) are the epicardial and endocardial parasternal short-axis area, respectively, \(L\) is the parasternal long-axis length, and \(t\) is the wall thickness calculated from \(A_1\) and \(A_2\) (Fig. 1).

Two-dimensionally targeted M-mode echocardiographic images were obtained at the level of the papillary muscles from the parasternal short-axis view and recorded at a speed of 150 cm/s (Fig. 2). LV internal diameters and wall thickness (leading edge to trailing edge) were obtained at end systole and end diastole from cross-sectional short-axis views. Heart rate was measured, and shortening fraction (SF), the echocardiographic equivalent of ejection fraction, was calculated from these tracings by using the formula

\[
\%SF = \left| \frac{LVedd - LVeds}{LVedd} \right| \times 100
\]

where \(LVedd\) and \(LVeds\) are the LV internal diameter at end diastole and end systole, respectively.

For both two-dimensional and M-mode calculations, LV end-diastolic measurements were obtained at the peak of the R wave, whereas end-systolic measurements were obtained at the time of minimal chamber area.

Aortic stroke volume (\(SV_{ao}\)) was calculated from pulse wave aortic Doppler recordings and measurements of the proximal ascending aortic diameter (Fig. 3). Because of the pulsatile nature of the cardiovascular system, velocities are not constant throughout the cardiac cycle, thereby requiring temporal integration of the Doppler velocities known as the time velocity integral (TVI). The cross-sectional area of the aorta (\(CSA_{ao}\)) was calculated assuming a constant circular orifice throughout the cardiac systole by using the formula

\[
CSA_{ao} = \left( \frac{AO \text{ diameter}}{2} \right)^2 \times \pi
\]

\[
SV_{ao} = CSA_{ao} \times TVI_{ao}
\]

\[
CO_{ao} = SV_{ao} \times HR
\]

\[
CI = \frac{CO_{ao}}{\text{body wt}}
\]

where \(CO\) is cardiac output, \(CI\) is cardiac index, and \(HR\) is heart rate.

Data analysis. Values are reported as means ± SD. \(P\) values were calculated by two-way ANOVA when mice of different genotypes and treatment were compared and by the Student’s \(t\)-test when comparisons were made within the same genotype with the Statview 5.0 program (SAS Institute, Cary, NC).

Fig. 1. Representative echocardiograms used for measurement of left ventricular (LV) mass by use of the area-length method. A: in the parasternal long-axis view, the ventricular length (L) is measured from the endocardial apex to the mitral valve annulus (MVA). B: in the short-axis view, the LV area is traced at the level of the papillary muscles (arrow). The tip of the papillary muscles was used as a landmark reference.

Fig. 2. Representative echocardiogram used for calculation of shortening fraction (%SF). This is an LV 2-dimensionally targeted M-mode tracing at midcavity level, where LV end-diastolic (LVedd) and end-systolic (LVesd) dimensions are measured and used for calculating %SF.

Fig. 3. Two-dimensionally targeted M-mode tracing of proximal ascending aorta (A) and aortic Doppler velocity profile (B). AO, aorta; LA, left atrium.
RESULTS

Thyroid function tests and body weights. The thyroid function tests before and after treatment with PTU and L-T4 are shown in Table 1. Determination of the effect of TH on heart physiology could not be done with baseline measurements alone, because the mice had different serum concentrations of T₄ and TSH. Therefore, mice of all genotypes were made deficient in TH by PTU treatment for 4 wk. This treatment normalized the serum T₄ levels to <0.02 µg/dl in all groups, whereas the TSH concentration increased 340-, 48-, and 149-fold in the wild-type, TRα₀₀₀, and TRβ⁻/⁻ mice, respectively. L-T₄ treatment suppressed the TSH in all genotypes to <20 mU/l, whereas serum T₄ levels were similar in wild-type and TRα₀₀₀ mice, TRβ⁻/⁻ mice had slightly higher concentrations.

At baseline, TRβ⁻/⁻ mice had markedly elevated serum T₄ and TSH, consistent with their state of resistance to TH. Four-week treatment with PTU resulted in a decrease of serum T₄ levels below the limit of detection with a concomitant increase in TSH. In the TRα₀₀₀ mice, the serum TSH did not reach the level attained in the wild-type or TRβ⁻/⁻ mice. Treatment with L-T₄ suppressed the TSH in all mice, and although the serum T₄ concentration attained in the TRβ⁻/⁻ mice was not different from that in wild-type mice, it was slightly higher in the TRα₀₀₀ mice (P < 0.05). There were no significant differences in body weight at baseline for any of the three genotypes. Mice treated with PTU for 4 wk did not gain weight during this time. However, after 4 wk of L-T₄ treatment, body weight increased by 26, 13, and 32% in wild-type, TRα₀₀₀, and TRβ⁻/⁻ mice, respectively, compared with baseline weights.

Effect of TH on heart rate. Heart rate in these mice determined during the echocardiographic analysis demonstrated resting tachycardia in untreated TRβ⁻/⁻ mice (415 ± 10 beats/min) relative to the untreated wild-type mice (372 ± 10 beats/min) and TRα₀₀₀ mice (407 ± 10), likely reflective of the higher baseline T₄ levels in these mice. The difference in basal heart rate between wild-type and TRα₀₀₀ mice was not significant. During TH deprivation, heart rates significantly decreased in both wild-type (49 ± 6%) and TRβ⁻/⁻ (39 ± 6%) mice and changed only a small amount in the TRα₀₀₀ mice (5 ± 6%). Although TH treatment resulted in an increase in the wild-type mice (43 ± 6%), there was only minimal change in the TRβ⁻/⁻ (5 ± 2%), yet a moderate increase in the TRα₀₀₀ (21 ± 6%) compared with wild-type mice (Fig. 4).

Echocardiographic analyses. The SF, equivalent to the ejection fraction, was significantly reduced in the hypothyroid wild-type mice (25.6 ± 2.3%) compared with pretreatment baseline (38.4 ± 2.3). Although there was a trend toward having decreased SF in TRβ⁻/⁻ and TRα₀₀₀ mice, it was not significantly different from that of wild-type mice (Fig. 5, A-C). There were no significant changes in SF with L-T₄ treatment in the different groups compared with baseline or compared with the hypothyroid state. Preload CO (Fig. 5, D-F) and CI (Fig. 5, G-I) decreased by 31 ± 8% and 38 ± 2% compared with baseline, respectively, and TRβ⁻/⁻ mice had a trend toward having decreased SF in the hypothyroid state. Preload CO (Fig. 5, D-F) and CI (Fig. 5, G-I) decreased by 31 ± 8% and 38 ± 2% compared with baseline, respectively, and TRβ⁻/⁻ mice had a trend toward having decreased SF in the hypothyroid state.
33 ± 10%, respectively, in hypothyroid mice and increased by 69 ± 10% and 35 ± 8%, respectively, in hyperthyroid wild-type mice. Neither the TR β⁻/⁻ nor the TR α⁰/⁰ mice had a response in CO or CI to TH deprivation, and although the TR α⁰/⁰ mice had a slight increase in CI and CO in response to TH treatment, it was not as robust as that seen in the wild-type mice (Fig. 5).

Effect of TH on LV mass. LV mass was determined by area- to length-based estimates via transthoracic echocardiographic measurements in diastole (ALd) and systole (ALs). Although there was no significant difference in LV mass between baseline and hypothyroid mice in any genotype, L-T4 treatment resulted in an increase in the ALd of wild-type (0.079 ± 0.001 to 0.126 ± 0.016 g, \( P < 0.05 \)) and TR α⁰/⁰ mice (0.079 ± 0.012 to 0.114 ± 0.021 g, \( P < 0.05 \)) but less so in the TR β⁻/⁻ mice (0.085 ± 0.011 to 0.089 ± 0.009 g, \( P > 0.05 \)) (Fig. 6). Similar changes were seen in ALs and in ALs index and ALd index when corrected for body weight. At autopsy, upon completion of the L-T4 treatment arm of the experiment the LV weight (mg) per gram of mouse correlated with the echocardiographic measurements (3.9 vs. 4.2 mg/g, respectively, for the wild-type mice; 3.4 vs. 3.5 mg/g, respectively, for the TR β⁻/⁻ mice; and 4.0 vs. 4.0 mg/g, respectively, for the TR α⁰/⁰ mice.)

DISCUSSION

The effect of TH on the heart is both intrinsic and extrinsic. Studies demonstrating effects of TH on isolated cardiac muscle (19, 34) or perfused intact hearts (37) give insight into the intrinsic effects of TH on cardiac function, but noninvasive assessment of cardiovascular function has not been well reported in vivo. Technological advances, such as smaller probes and the creation of high-frequency linear transducers, have...
allowed accurate echocardiographic analysis of small mice and enabled one to obtain in vivo measurements of cardiac function (8, 11, 13–15, 36). Echocardiographic measurements can accurately determine dynamic changes in LV mass (8). Furthermore, studies using mice with deletion of either of the TRs allows one to delineate their contribution to TH action. This study demonstrates the role that TRβ and TRα play in TH action in the heart. Whereas TRα1 and TRα2 are the major TR isoforms expressed in the heart, it is not surprising that wild-type and TRβ−/− mice had a decreased heart rate in response to TH deprivation, whereas the TRα0/0 mice were unresponsive (32, 48). Whereas all three genotypes demonstrated an increase in heart rate in response to TH treatment, there was a blunted response in both the TRα0/0 and TRβ−/− mice, indicating that TRβ also plays a role in the increase in heart rate associated with TH treatment. This study does not indicate whether the TRβ responsiveness is a direct effect on the heart or an indirect effect on sympathomimetic factors, which, in turn, increase heart rate.

Contrary to the heart rate data presented in this paper, two previous reports, including one from our laboratory (19, 32), have demonstrated baseline bradycardia in the TRα0/0 mice. Variables that may account for this discrepancy include 1) the method of anesthesia, 2) the number of mice analyzed, and 3) the body temperature of the animal at the time of analyses. Although Gloss et al. (19) used a ketamine-xylazine cocktail, Macchia et al. (32) used chloral hydrate as we did in the present report. Although Gloss et al. analyzed six animals and reported a difference in heart rate, Macchia et al. required 43 wild-type mice to show significant differences, due to the variability in heart rate. Furthermore, our measurement of heart rate was done during simultaneous echocardiographic analysis, and although every effort was attempted to maintain euthermia in the mice, there may have been differences in body temperature to account for the differences in heart rate at baseline.

TRα1 knockout mice had an average heart rate 20% lower than that of wild-type mice, with prolonged QRS and QT durations at baseline and with TH treatment, suggesting that TRβ can also affect heart rate (49). Transgenic mice with myocardium-specific expression of a mutant TRβ (Δ377T) demonstrated heart failure in vitro but not in vivo, suggesting that diminished performance in these mice may be compensated for by other mechanisms in vivo (38).

Change in %SF was limited to the wild-type mice. Although there was a trend toward the TRβ−/− mice having decreased %SF with TH deprivation and an increase with TH treatment, this did not reach significance. Furthermore, CO and CI were increased in response to TH treatment and were decreased in response to TH withdrawal in the wild-type mice, and these changes were blunted in both the TRβ−/− and TRα0/0 mice. Therefore, whereas TRα seems to be more important than TRβ for the chronotropic effects of TH on cardiac function, both TRα and TRβ are necessary for the ionotropic effects. In humans, hyperthyroidism results in an increase in echocardiographic indexes of myocardial contractility (12, 39). Whereas normal CO in humans is 4.0–6.0 l/min, it is >7.0 and <4.0 l/min in hyperthyroid and hypothyroid humans, respectively.

Hyperthyroidism has been reported to result in increased cardiac mass in both humans (7) and mice (4, 26). The mechanism of increased cardiac mass may be related to the local activation of the renin-angiotensin system in the heart in response to TH that can be reversed with angiotensin-converting enzyme inhibitors (1, 3, 29). In addition, the TH-induced increase in heart size may be due, in part, to coordinated capillary and myocardial growth that is TH dependent (6, 9, 47). The absence of TRβ prevented the TH-mediated increase in cardiac mass, whereas the absence of TRα did not. TRβ may be the important TR for the generation of tissue angiotensin-converting enzyme. In our mice, the absolute T4 concentrations were higher in the TRβ−/− mice compared with the TRα0/0 mice only. This difference may partially account for the increase in LV mass seen in TRβ−/− mice but cannot explain the difference compared with wild-type mice.

TRα is the predominant isoform in the heart. We have shown that TRα is required for the changes in heart rate seen with TH deprivation or treatment. However, the TH-induced increase in cardiac mass is TRβ dependent. Taken together, these data suggest...
that TRα and TRβ play different roles in the physiology of TH action on the heart. Furthermore, TRβ may play a more important role in indirect effect of TH on the heart.

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