Cardiac Dysfunction Caused by Myocardium-Specific Expression of a Mutant Thyroid Hormone Receptor

Carmen Pazos-Moura,* E. Dale Abel,* Mary-Ellen Boers, Egberto Moura, Thomas G. Hampton, Jufeng Wang, James P. Morgan, Fredric E. Wondisford

Abstract—Thyroid hormone deficiency has profound effects on the cardiovascular system, resulting in decreased cardiac contractility, adrenergic responsiveness, and vascular volume and increased peripheral vascular resistance. To determine the importance of direct cardiac effects in the genesis of hypothyroid cardiac dysfunction, the cardiac myocyte was specifically targeted with a mutant thyroid hormone receptor (TR)-β (Δ337T-TR-β) driven by the α-myosin heavy chain (α-MHC) gene promoter. As a control in these experiments, a wild-type (Wt) TR-β was also targeted to the heart by using the same promoter. Transgenic mice expressing the mutant TR displayed an mRNA expression pattern consistent with cardiac hypothyroidism, even though their peripheral thyroid hormone levels were normal. When these animals were rendered hypothyroid or thyrotoxic, mRNA expression of MHC isoforms remained unchanged in the hearts of the Δ337T transgenic animals, in contrast to Wt controls or transgenic animals expressing Wt TR-β, which exhibited the expected changes in steady-state MHC mRNA levels. Studies in Langendorff heart preparations from mutant TR-β transgenic animals revealed evidence of heart failure with a significant reduction in +dp/dt, −dp/dt, and force-frequency responses compared with values in Wt controls and transgenic mice overexpressing the Wt TR-β. In contrast, in vivo measures of cardiac performance were similar between Wt and mutant animals, indicating that the diminished performance of the mutant transgenic heart in vitro was compensated for by other mechanisms in vivo. This is the first demonstration indicating that isolated cardiac hypothyroidism causes cardiac dysfunction in the absence of changes in the adrenergic or peripheral vascular system. (Circ Res. 2000;86:700-706.)

Key Words: thyroid hormone resistance ■ cardiac hypothyroidism ■ mice

Thyroid hormone deficiency has profound effects on cardiovascular function. In the heart, hypothyroidism induces systolic and diastolic dysfunction with reduction in the rate and force of cardiac contraction and a prolongation in cardiac relaxation.¹ ² Various cardiac proteins, such as α- and β-myosin heavy chain (α-MHC and β-MHC, respectively), sarcoplasmic reticulum Ca²⁺-ATPase, and voltage-gated potassium channels, which are important mediators of cardiac contractility, are regulated by thyroid hormone.³ ⁴ ⁵ The promoters of these genes possess thyroid hormone response elements (TREs), and their expression has been shown to be regulated by liganded and unliganded thyroid hormone receptors (TRs).

Nongenomic effects of thyroid hormone on cardiac contractility have also been described.⁶ ⁷ For example, thyroid hormone excess results in significant peripheral vasodilatation, which is believed to be a direct effect and not mediated by nuclear TR action.⁸ In contrast, reduced capillary red blood cell velocity,⁹ diminished blood volume,² ¹⁰ decreased adrenergic responsiveness,¹¹ and increased peripheral vascular resistance¹² occur in hypothyroidism. Therefore, the impaired cardiac function that occurs in hypothyroidism can be ascribed to direct effects on cardiomyocytes as well as to secondary effects on hemodynamic loading. Given the limitations of these in vivo observations, it has been difficult to determine the respective roles of direct versus indirect actions of thyroid hormone on the cardiovascular system.

Mutations in the β isoform of the TR are associated with the human syndrome of resistance to thyroid hormone (RTH), which is characterized by elevated serum concentrations of thyroid hormone and peripheral tissue insensitivity to thyroid hormone.¹³ Echocardiographic testing of patients with RTH has revealed evidence of increased cardiac contractility.¹⁴ However, these changes are less than would be expected given the degree of elevation of thyroid hormone concentrations. Thus, the blunted response suggests partial resistance of the heart to thyroid hormone in these subjects.¹⁴ ¹⁵ Furthermore, it is unclear to
what extent alterations in the peripheral circulation, which occur in response to the increased thyroid hormone levels, are contributing to these changes.

To clarify the specific contribution of TR-mediated thyroid hormone action on cardiac function, we developed a transgenic mouse model with cardiac-specific expression of a naturally occurring Δ337T mutant TR.16 This mutant receptor is a potent dominant-negative inhibitor of wild-type (Wt) TR function in vitro and in vivo.17–19 By targeting this receptor selectively to the hearts of transgenic mice, we were able to induce a hypothyroid phenotype in the heart in the presence of normal thyroid hormone serum concentrations and therefore study the direct cardiac effect of thyroid hormone, independent of effects on the adrenergic and vascular systems.

Materials and Methods

Transgenic mice overexpressing either Wt or Δ337T mutant16 TR-β, in the heart were generated. The murine α-MHC promoter was used to direct transgene expression selectively to cardiac myocytes.20 Wt or mutant human TR-β1 cDNA was cloned into a vector containing 5.5 kb of the murine α-MHC promoter and 0.6 kb of the human growth hormone poly(A) (gift of Dr J. Robbins, University of Cincinnati, Cincinnati, Ohio). Southern blotting was performed to identify transgenic animals containing a specific 2.4-kb BglII fragment with use of a human TR-β1 cDNA probe.

All studies were approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center. Transgenic animals have been followed up to 1 year, and no increase in morbidity or mortality was observed in either the MHC-mutant (MHC-Mt) or MHC-Wt strains. Hypothyroidism was induced by feeding animals a 0.15% propylthiouracil-containing diet (Harland Teklad, Co) for 4 weeks. Mice were rendered thyrotoxic by daily intraperitoneal injections of T3 (10 μg/100 g body wt) for 9 days. Blood was obtained from the tail vein for measurements of total T3 and T4 (T4 or T3 kit, ICN Pharmaceuticals).

Total RNA was extracted from heart, liver, kidney, skeletal muscle, and spleen by the phenol/guanidinium thiocyanate method. mRNA levels for mutant and Wt TR-β1 were determined by ribonuclease protection assay (RPA). Total RNA (20 μg) from each tissue was hybridized with a purified 32P-labeled antisense RNA probe, containing exon 6 and the surrounding intronic sequences from the mouse TR-β gene, and processed by use of an RPA kit (Ambion Inc). A mouse cyclophilin riboprobe was used to control for RNA quality and quantity.

α-MHC and β-MHC gene expression was also evaluated by RPA with the use of specific 32P-labeled 3' α- and β-MHC riboprobes. These probes were obtained by reverse transcription-polymerase chain reaction of mouse heart mRNA with the use of primers to the 3' coding sequence and untranslated region of α-MHC and β-MHC, respectively. The following primers were used: α-MHC 5'-GGAAGATTGAGCCGGCACCATTAG-3' and 5'-CTGCTGGA-GAGGTTATTCCTCG-3'; and β-MHC 5'-GCCAACACCAACTTGTCAGGTTC-3' and 5'-GTGAGGCTGCTCCATAGGGA-3', generating a 305- and 206-bp product for α-MHC and β-MHC, respectively.

Animals were anesthetized with ketamine and xylazine and instrumented, and ECG measurements were performed. A pressure transducer was then inserted through the mitral annulus into the left ventricular chamber, where peak left ventricular blood pressure, end-diastolic blood pressure, and peak negative pressure derivative (−dP/dt) were recorded.

Mice were heparinized, and the heart was rapidly excised and arrested in ice-cold buffer. The aorta was cannulated and perfused with Krebs-Henseleit buffer at a rate of ≈3 mL/min.22 A balloon connected to a pressure transducer at one end was inserted through the mitral annulus into the left ventricular chamber, and pressure measurements were performed. Indices were recorded at different pacing frequencies and after the administration of 0.03 μmol/L isoproterenol. Statistical differences were assessed by unpaired 2-tailed Student t test.

Results

Transgene Expression

Wt or mutant (Δ337T) human TR-β1 transgenes were specifically targeted to the cardiac myocyte by using the α-MHC gene promoter. Multiple transgenic founder lines were obtained from both constructs. One line overexpressing the Wt human TR-β1 (MHC-Wt, line 6) and 3 lines expressing the mutant human TR (MHC-Mt, lines 20, 23, and 30) were studied. As shown in Figure 1, a mouse TR-β exon 6 32P-labeled riboprobe was used to detect transgene expression in the myocardium and peripheral tissues. The region of the mouse TR-β used for this riboprobe is highly homologous with the same region of human TR-β. The left panel of Figure 1 demonstrates TR overexpression in the heart. The endogenous TR-β and Wt TR-β transgene mRNAs protect the full-length probe. Endogenous TR-β mRNA is difficult to appreciate at this exposure (lane 3), but a 4-fold increase in TR-β mRNA levels was noted in the MHC-Wt animal (lane 4). In contrast, mutant TR-β expression was detected as a smaller protected fragment because of the deletion of codon 337 (lanes 5 to 7). The degree of TR-β overexpression was similar between the MHC-Wt transgenic animals and 2 of the mutant lines (lines 20 and 23, ≈4-fold overexpression relative to endogenous TR-β mRNA levels). Mutant TR transgene expression was ≈60% lower in line 30, as assessed by PhosphorImager (Molecular Dynamics).

The right panel of Figure 1 demonstrates that expression of this transgene was cardiac specific. Data from a line 20 animal are shown, and similar data exist for the other lines. Mutant
TR-β was expressed only in the heart (lane 8) and not in the liver (lane 9), kidney (lane 10), spleen (lane 11), or skeletal muscle (lane 12). Endogenous mouse TR-β mRNA was easily detected in the liver (lane 9) and kidney (lane 10) and, to a lesser extent, in the heart (lane 8), which is consistent with previously published observations. Animals overexpressing either Wt or mutant TR-β had thyroid hormone levels that were indistinguishable from those of Wt animals (Figure 2).

**Analysis of MHC Gene Expression**

To determine the effect of TR overexpression on thyroid hormone signaling in the myocardium, MHC-Wt (line 6) and MHC-Mt (lines 20 and 23) mice were studied under euthyroid, hypothyroid, and thyrotoxic conditions. Figure 3 displays 2 RPA analyses of control (WT) mice and transgenic mice (MHC-Wt and MHC-Mt) using either a 3′ β-MHC (top panel) or 3′ α-MHC (bottom panel) 32P-labeled riboprobe. A 32P-labeled cyclophilin riboprobe was included as an internal control in both panels and simultaneously hybridized with the MHC riboprobe. In euthyroid WT animals, α-MHC mRNA is the predominant MHC species and is even more abundant during thyrotoxicosis. Conversely, β-MHC mRNA is the predominant species in hypothyroid WT animals. Table 1 displays the results of PhosphorImager (Molecular Dynamics) analysis of the MHC isoform mRNAs. Note that the α-MHC/β-MHC ratio in euthyroid WT animals is 16, reflecting the higher steady-state α-MHC and the much lower β-MHC mRNA levels at ambient thyroid hormone levels in adult mice. This ratio increases to 44 in thyrotoxic animals, primarily because of a decrease in β-MHC mRNA expression. In contrast, the ratio in hypothyroidism is 0.2, reflecting a moderate decrease in α-MHC mRNA levels and a marked induction in β-MHC mRNA levels.

Transgenic animals overexpressing WT TR-β in the heart displayed results qualitatively similar to those in WT animals in the various thyroid states studied. Quantitatively, there is a greater degree of downregulation of β-MHC expression in hyperthyroid MHC-Wt mice compared with hyperthyroid control mice, resulting in a greater α-MHC/β-MHC ratio (Table 1). Transgenic animals overexpressing the mutant TR-β exhibit changes in the expression of MHC isoforms that are consistent with the pattern associated with hypothyroidism regardless of their serum thyroid hormone levels. Table 1 indicates that these transgenic animals exhibit lower levels of α-MHC, and the expected induction of α-MHC and repression of β-MHC expression in thyrotoxicosis is not seen. Thus, the α-MHC/β-MHC ratios do not change appreciably and maintain a hypothyroid pattern. These data indicate that overexpression of the mutant TR-β, in the hearts of transgenic mice induces hypothyroidism in the myocardium regardless of ambient thyroid hormone levels.

**Cardiac Function in MHC-Mt Mice**

We next explored the effect of the mutant TR transgene on cardiac physiology. Figure 4 displays a representative ECG from a control (left) and a mutant TR (right) transgenic animal and the mean±SEM ECG intervals from 6 WT and 7
mutant transgenic animals (line 20). Note the widened complexes in the transgenic animals. All time intervals were longer in the transgenic animals than in the control animals. The PQ and PR intervals, which roughly represent the atrioventricular conduction time, are slightly but significantly prolonged (~33%). Importantly, increases were observed in the time required for ventricular depolarization, represented by the QRS complex (60% increase) and the ST interval, which represent the period of time between depolarization of ventricles and the period of rapid repolarization of ventricular muscle (79% increase).

To determine whether cardiac function was impaired in the mutant transgenic animals, cardiac contractility was measured in vivo and in isolated perfused heart preparations. Table 2 shows data from isolated heart preparations of Wt (control) animals and mutant and Wt TR-overexpressing transgenic animals. Mutant TR transgenic hearts from line 20 generated less left ventricular pressure and took longer to do so (+dP/dT) than either control hearts or Wt TR-overexpressing hearts. Diastolic relaxation was also impaired, as indicated by a 60% reduction in −dP/dT and an increase in the time constant of relaxation. Although both positive and negative dP/dT were impaired in the mutant TR transgenic hearts, the +dP/dT/−dP/dT ratio suggests that −dP/dT was affected more than +dP/dT. Similar studies performed in line 30 transgenic mice, which expressed 60% less of the mutant TR transgene than the other 2 lines, revealed an intermediate phenotype, with less impairment in cardiac contractility than that observed in the 2 lines with higher mutant transgene expression (data not shown). In studies of paced hearts, mutant TR transgenic hearts exhibited defects in both +dP/dT (Figure 5A) and −dP/dT (Figure 5B) at all pacing intervals, unlike either Wt or Wt TR-overexpressing animals. At the highest pacing interval, compared with Wt animals, MHC-Mt animals displayed a significant increase in EDP (Figure 5C). In contrast, compared with Wt animals, MHC-Wt animals had lower EDPs at all pacing intervals.

In response to isoproterenol administration, isolated hearts of MHC-Mt mice exhibited percent increases in left ventricular pressure, +dP/dT, and −dP/dT similar to those in control hearts. However, the absolute values of these parameters remained significantly lower in the MHC-Mt animals (Table 3). Compared with control mice, MHC-Wt mice responded to isoproterenol with a greater percent increase in left ventricular pressure but a smaller increase in both positive and negative dP/dT.

![Figure 4. Representative ECG and ECG time intervals in Wt and MHC-Mt transgenic mice. Animals were 2 to 3 months of age at the time of study. The Wt group contained 3 male and 3 female animals; the MHC-Mt group (line 20) contained 4 male and 3 female animals. No obvious sex-specific differences were found in either group. P values (unpaired 2-sided t test) are indicated.](image)

### Table 1. Relative α- and β-MHC mRNA Levels (Corrected for Cyclophilin) and α-MHC/β-MHC Ratios in Wt and Transgenic Animals With Cardiac-Specific Overexpression of Wt (MHC-Wt, Line 6) or Mutant TR-β (MHC-Mt, Lines 20 and 23)

<table>
<thead>
<tr>
<th>Line</th>
<th>Hypothyroid</th>
<th>Euthyroid</th>
<th>Thyrotoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α  β  α/β</td>
<td>α  β  α/β</td>
<td>α  β  α/β</td>
</tr>
<tr>
<td>Wt</td>
<td>0.6 3.4 0.2</td>
<td>3.2 0.2 16</td>
<td>3.1 0.1 44.0</td>
</tr>
<tr>
<td>MHC-Wt (line 6)</td>
<td>0.5 3.1 0.2</td>
<td>2.7 0.2 13.5</td>
<td>3.0 0.03 100.0</td>
</tr>
<tr>
<td>MHC-Mt (line 20)</td>
<td>0.6 3.8 0.2</td>
<td>1.2 2.4 0.5</td>
<td>1.1 2.0 0.6</td>
</tr>
<tr>
<td>MHC-Mt (line 23)</td>
<td>0.6 3.3 0.2</td>
<td>1.4 2.7 0.2</td>
<td>2.2 1.4 1.6</td>
</tr>
</tbody>
</table>

Relative α-MHC and β-MHC mRNA levels (α and β, respectively) and α-MHC/β-MHC (α/β) ratios are shown. mRNA levels were determined by using a PhosphorImager (Molecular Dynamics), which directly quantifies β emission from the RPA. Values shown are based on analysis of the α-MHC gel shown in Figure 3 and corrected for cyclophilin expression in each sample.

![Table 2. Isolated Heart Measurements in Wt Animals and in MHC-Mt and MHC-Wt TR-β-Overexpressing Transgenic Animals](image)
In contrast to the abnormal function in isolated hearts, cardiac performance in anesthetized mutant TR transgenic animals was similar to that of control animals, with no parameter being statistically different between the 2 groups (Table 4). There were trends toward lower systolic pressure and heart rate and higher EDP in the mutant TR animals.

**Discussion**

Myosin, a major protein of cardiac muscle, is composed of light and heavy chains. In euthyroid adult rodents, α-MHC is the predominant isoform expressed in cardiac myocytes, and the levels of the β isoform are negligible. In hypothyroidism, the β-MHC isoform, which has lower ATPase activity than the α-MHC isoform, becomes the predominant species. The α-MHC gene is transcriptionally regulated by TRs, which bind to a positive TRE in the promoter of the gene. Conversely, β-MHC gene expression is negatively regulated by thyroid hormone via mechanisms that remain unclear. Transgenic mice bearing the mutant TR in the heart revealed a pattern consistent with cardiac hypothyroidism, with suppression of α-MHC and a marked induction of the β-MHC isoform. Because circulating levels of thyroid hormones were normal, these findings are the likely result of dominant-negative interference of Wt TR function by the mutant TR.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Force-frequency relations in isolated hearts of Wt controls and MHC-Wt and MHC-Mt TR-overexpressing transgenic animals. Isolated perfused hearts were paced at the indicated frequencies, and $+\frac{dP}{dt}$ (A), $-\frac{dP}{dt}$ (B), and EDP (C) were measured. *$P<0.01$ vs Wt (control); # $P<0.05$ vs Wt (control) (unpaired 2-sided t test).

In contrast to the abnormal function in isolated hearts, cardiac performance in anesthetized mutant TR transgenic animals was similar to that of control animals, with no parameter being statistically different between the 2 groups (Table 4). There were trends toward lower systolic pressure and heart rate and higher EDP in the mutant TR animals.

**TABLE 3.** Isolated Heart Measurements in Wt Animals and in MHC-Mt and MHC-Wt TR-β-Overexpressing Transgenic Animals After Isoproterenol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wt (n=5)</th>
<th>MHC-Mt (n=6)</th>
<th>MHC-Wt (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP, mm Hg</td>
<td>70±4</td>
<td>42.0±8*</td>
<td>100.7±18†</td>
</tr>
<tr>
<td>Change, %</td>
<td>+35</td>
<td>+31</td>
<td>+73</td>
</tr>
<tr>
<td>+dP/dT, mm Hg/s</td>
<td>3734±150</td>
<td>1450±136*</td>
<td>3085±130†</td>
</tr>
<tr>
<td>Change, %</td>
<td>+68</td>
<td>+34</td>
<td>+49</td>
</tr>
<tr>
<td>−dP/dT, mm Hg/s</td>
<td>3114±253</td>
<td>1019±99*</td>
<td>2443±116*</td>
</tr>
<tr>
<td>Change, %</td>
<td>+100</td>
<td>+65</td>
<td>+60</td>
</tr>
<tr>
<td>+dP/dT−dP/dT</td>
<td>1.20±0.06</td>
<td>1.42±0.05*</td>
<td>1.26±0.02</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−14</td>
<td>−18</td>
<td>−7</td>
</tr>
<tr>
<td>t, ms</td>
<td>11.3±0.4</td>
<td>17.4±1.0*</td>
<td>13.8±0.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Percent increase due to isoproterenol is indicated below the parameter. Animals were 2 to 3 months of age, and each group contained an equal number of male and female animals except the Wt group (3 male, 2 female). MHC-Mt animals were from line 20, and MHC-Wt animals were from line 6. No obvious sex-specific differences were found in any group. *$P<0.01$ vs Wt (control); †$P<0.05$ vs Wt (control).

**TABLE 4.** In Vivo Blood Pressure Measurements in Wt and MHC-Mt TR-β Transgenic Animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type (n=6)</th>
<th>MHC-Mt (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP, mm Hg</td>
<td>97±9</td>
<td>80±6</td>
</tr>
<tr>
<td>DP, mm Hg</td>
<td>60±7</td>
<td>51±6</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>72±4</td>
<td>65±4</td>
</tr>
<tr>
<td>PSP, mm Hg</td>
<td>75±4</td>
<td>87±6</td>
</tr>
<tr>
<td>EDP, mm Hg</td>
<td>5±1</td>
<td>9±1</td>
</tr>
<tr>
<td>+dP/dT, mm Hg/s</td>
<td>2737±227</td>
<td>2770±354</td>
</tr>
<tr>
<td>−dP/dT, mm Hg/s</td>
<td>2554±245</td>
<td>2210±191</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>259±16</td>
<td>209±20</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SP indicates systolic pressure; DP, diastolic pressure; MAP, mean arterial pressure; PSP, peak systolic pressure; and HR, heart rate. SP and DP were measured in aortic root; other measurements were performed in left ventricle. Animals were 2 to 3 months of age. The Wt group contained 3 male and 3 female animals, and the MHC-Mt group contained 3 male and 2 female animals. No significant differences in any parameter nor any obvious sex-specific differences were found in either group.
hypothroid pattern of MHC gene expression in T$_3$-treated mutant transgenic mice indicates the presence of profound cardiac RTH. Hypothyroidism resulted in minimal changes in MHC gene expression in mutant transgenic mice, suggesting that the induction of β-MHC and the repression of α-MHC are nearly maximal at ambient thyroid hormone concentrations in euthyroid transgenic mice (Table 1). These data provide strong evidence of the role of the TR in regulating MHC gene expression in vivo.

Altered MHC expression may explain, in part, the impaired cardiac contractility observed in isolated perfused hearts. The activity of the myosin ATPases determines the rate of formation and dissolution of actin-myosin crossbridges and, ultimately, the velocity of muscle contraction. Myosin composed of β-MHC dimers has a markedly lower ATPase activity relative to myosin composed of α-MHC dimers, which leads to a decrement in the maximum velocity of contraction of cardiac muscle.26 It is also likely that impairment of other cardiac-specific genes regulated by thyroid hormone, such as sarcoplasmic reticulum Ca$^{2+}$-ATPase, are likely to be involved in the cardiac contractile dysfunction observed in mutant TR transgenic animals. Finally, decreased oxidative metabolism in the hypothryoid hearts of transgenic mice might lead to decreased ATP availability. Thus, findings of decreased cardiac contractility in the isolated hearts of our mutant TR transgenic mice support similar data obtained in the isolated perfused hearts of rats with systemic hypothyroidism.3

Our findings are novel compared with the work of Gloss et al.,18 who reported an altered cardiac phenotype in transgenic mice expressing the Δ337T-TR-β, mutant. In their study, expression of the mutant TR was driven by a β-actin promoter, which, in contrast to the present study, is ubiquitously expressed. Although these animals had apparently normal thyroid hormone levels, one cannot exclude the possibility that small differences exist in circulating or local tissue concentrations of thyroid hormones because of global expression of the mutant TR. Their animals showed a hypothryoid MHC mRNA expression pattern and a delay in the contraction and relaxation of isolated papillary muscles without a change in the maximum active tension. The present study provides new data on isovolumic contractile performance in isolated perfused hearts under baseline conditions and in response to changes in workload. In the present study, we have demonstrated that isolated cardiac RTH clearly results in global cardiac dysfunction.

The preserved in vivo contractile performance relative to Wt mice contrasts with the unambiguous demonstration of contractile dysfunction in isolated perfused hearts. There are a number of possible explanations for this observation. First, it is possible that compensatory in vivo mechanisms that ultimately result in improved cardiac performance exist. One possibility is increased adrenergic activity in the mutant transgenic mice. Although we did not directly measure adrenergic activation in the present study, our observation that administration of isoproterenol increases contractile performance of the transgenic isolated hearts provides some evidence that this could be a plausible mechanism. Sympathoadrenal activation is an important compensatory response in vivo that seeks to maintain cardiac output in the face of primary ventricular dysfunction. A second possibility stems from the fact that the assessment of in vivo cardiac contractility was performed in anesthetized mice. The effect of anesthesia is evident in the fact that the heart rates observed in Wt mice were significantly lower than values obtained in awake animals.27 Therefore, the cardiodepressant effect of anesthesia in Wt and transgenic mice could attenuate any differences that exist. In fact, some of the in vivo measurements tended to be abnormal in transgenic mice (increased EDP [P<0.06] and decreased heart rate [P<0.07]). It is possible that impaired ventricular performance would be detected if measurements were made during exercise, which would increase cardiac work. This is supported by the observation that the isolated perfused hearts of mutant transgenic animals demonstrated impairment in both systolic and diastolic function with cardiac pacing (Figure 5).

Changes in the ECG of mutant TR transgenic animals resemble those found in hypothyroid patients.28 Prolongation of ECG intervals indicate reduced velocity of conduction of the action potential diffusely throughout the contracting myocardial cells. The mechanism may be related to abnormal ion channel expression, because altered mRNA levels of voltage-gated potassium channels have been reported in experimental hypothyroidism.3 Careful analyses of ECG changes have been performed in transgenic mice lacking TR-α129 and TR-β and in TR-β and TR-α1 double-knockout mice.30 The deletion of TR-β is associated with increased basal heart rates but with a blunted heart rate response to T$_3$.30 In contrast, TR-α, knockout mice are bradycardic, with heart rates that are 20% less than heart rates in Wt control mice. Heart rates were also decreased by 20% in our model, which suggests that the mutant TR-β transgene blocked TR-α-mediated mechanisms that govern heart rate. The prolongation in the QRS complex of 65% and in the QT interval of 55% (versus control mice) seen in our transgenic mice is much greater than that reported in TR-α1/β and TR-α1 knockout mice. The difference in these models suggests that dominant-negative inhibition of thyroid hormone receptor action in the heart results in a different phenotype than is observed in the absence of all TRs in the heart. The molecular basis for this difference may be repression of cardiac gene expression by the mutant TR versus a lack of thyroid hormone activation in the TR knockout animals.

The present study indicates that the impairment of thyroid hormone action selectively in the heart leads to left ventricular dysfunction. Our model provides mechanistic insight into the observation of the relative impairment in cardiac contractility seen in many patients with RTH.14 This model has provided a more complete assessment of the role of thyroid hormone deficiency on cardiac function in the absence of peripheral vascular changes that occur in hypothyroidism.

Acknowledgments

This study was supported by National Institutes of Health grants to Dr Abel (DK-02485) and Dr Wondisford (DK-49126 and DK-50564). Drs Pazos-Moura and Moura were recipients of a National Council for Research Development (CNPq) grant from the government of Brazil. Dr Abel was the recipient of a Faculty Development
Award from the Robert Wood Johnson Foundation and of an Eleanor and Miles Shore Fellowship (Harvard Medical School).

References


17. Kahaly GJ, Chatterjee VKK. Cardiac investigation in resistance to thyroid hormone. In: Program of the 4th International Workshop on Resistance to Thyroid Hormone; May 23–25, 1990; Sao Paulo, Brazil. Abstract.


Cardiac Dysfunction Caused by Myocardium-Specific Expression of a Mutant Thyroid Hormone Receptor
Carmen Pazos-Moura, E. Dale Abel, Mary-Ellen Boers, Egberto Moura, Thomas G. Hampton, Jufenf Wang, James P. Morgan and Fredric E. Wondisford

Circ Res. 2000;86:700-706
doi: 10.1161/01.RES.86.6.700

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/86/6/700

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/