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Thyroid Hormone Resistance in the Heart: Role of the Thyroid Hormone Receptor β Isoform

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Several cardiac genes possess thyroid hormone (TH) response elements regulated by TH receptors. Mutation in TR-β gene causes the human syndrome of resistance to TH, which is characterized by elevated serum concentration of T_4 and T_3 and variable degrees of insensitivity to TH. It is unclear, however, whether a mutant TR-β could function as a dominant negative in the heart when expressed from the endogenous locus. A well-described resistance to TH (Δ337T) was either introduced into germline of mice (KI-mut) or expressed as a transgene in the heart using a cardiac-specific promoter (KS-mut). Mice were studied at baseline, after 5-propyl-2-thiouracil (PTU) or after PTU and T_3 treatment (PTU + T_3). PTU + T_3 treatment significantly increased left ventricular mass in all groups compared with baseline measurements, although the increase in left ventricular mass was significantly less in KI-mut animals. Baseline heart rates (HRs) were similar in wild-type (WT) and KI-mut but were lower in KS-mut animals. After TH deprivation (PTU), HR decreased in WT and KI-mut animals; similarly, HR increased in WT and KI-mut after PTU + T_3. In contrast, HR in KS-mut animals did not change after either treatment. Except for cardiac hypertrophy, the presence of a germline TR-β mutation had surprisingly little effect on cardiac function. (*Endocrinology* 145: 1625–1633, 2004)

THYROID HORMONE (TH) has profound effects on the heart and vascular system. Many of the clinical manifestations of hyperthyroidism are due to the ability of THs to alter cardiovascular hemodynamics (1). Although both T_4 and T_3 bind to TH receptors (TRs), T_3 is thought to be the biologically relevant TH molecule in cardiac myocytes, as in other cells, due to its higher receptor affinity. Various cardiac proteins, such as α-myosin heavy chain (MHC), sarcoplasmic reticulum Ca^{2+}-ATPase, Na^+/K^+-ATPase, voltage-gated potassium channels and β1 adrenergic receptors are positively regulated by THs (2–5). Others, such as β-MHC, phospholamban, and Na^+/Ca^+ exchanger are negatively regulated by THs (2, 3). Most of genes regulated by THs possess TH response elements in their promoter region and are regulated by both unliganded and liganded TRs. In addition to direct effects, T_3 has indirect effects as well on the cardiovascular system, the most important being to decrease peripheral vascular resistance (1).

The two TR genes, TR-α and TR-β, have important structural and sequence similarities and generate, by alternative splicing, at least four active forms of the receptor α-1, β-1, β-2, and β-3. The relative expression of TR isoforms varies among tissues (6, 7). TR-α1 is the major TR isoform expressed in the heart (8), although TR-β1 is expressed at lower levels. Data obtained from mice with disruption of either TR-α or TR-β gene have led to the conclusion that the effect of T_3 on the heart is mediated predominantly by TR-α (9–12). The physiological relevance of TR knockout models, however, is unclear because TR functions both in the absence and presence of ligand and dominant-negative mutations of the TR may have unpredictable effects on gene expression.

Patients with hyperthyroidism have increased left ventricular (LV) systolic and diastolic contractile function, a finding consistent with changes in the expression of contractile and calcium-regulatory proteins, as described previously (13, 14). Administration of β-adrenergic receptor antagonist to patients with hyperthyroidism slows the HR but does not alter systolic or diastolic performance, confirming that THs act directly on cardiac muscle (15).

Mutations in the β isoform of TR are associated with the human syndrome of resistance to TH (RTH), which is characterized by elevated serum concentration of T_4 and T_3 and variable degrees of peripheral tissue insensitivity to TH (16). Despite peripheral tissue resistance, tachycardia is frequently observed in patients with RTH, suggesting that the heart may not be resistant to the actions of TH (17). A recent study of patients with RTH, which evaluated their cardiac function in some detail, also found relatively little evidence for significant cardiac resistance in this disorder (18). In contrast, our laboratory has shown that the cardiac-specific expression of a RTH mutant TR-β (Δ337T) induced a significant hypothyroid phenotype in the heart in the presence of normal TH serum concentrations (19). The Δ337T mutant TR is

Abbreviations: dP/dT, Pressure derivatives; EDD, LV end-diastolic; ESD, LV end-systolic; HR, heart rate; KI-mut, germline heterozygous mutation for TR-β (Δ337T); LV, left ventricular; MHC, myosin heavy chain; MHC-mut, mice with cardiac-specific expression of a mutant TR (Δ337T); PTU, 5-propyl-2-thiouracil; PWT, posterior wall thickness; RNase, ribonuclease; RPA, RNase protection assay; RTH, resistance to TH; TH, thyroid hormone; TR, TH receptor; WT, wild-type.

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a potent dominant-negative inhibitor of wild-type (WT) TR function both *in vitro* and *in vivo* (20, 21). Given that TR-β is normally expressed at lower levels in the heart *vs.* TR-α, this model does not reproduce TR expression levels found in patients with RTH.

To try to clarify the cardiac response to the elevated TH levels found in the syndrome of resistance to TH, a new transgenic animal model for the generalized form of this syndrome was studied. In this model, the Δ337T mutation was introduced into the TR-β locus using homologous recombination (22). We demonstrate that germline expression of a mutant TR-β had minimal, if any, effects on the cardiovascular system *vs.* a model in which this same mutation was targeted to the heart.

Materials and Methods

Animal descriptions

All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at The University of Chicago.

Two different transgenic mice expressing a mutant TR (TR-β Δ337T) were used. This mutation was found in a family (Kindred S) with TH resistance (23). The MHC-α promoter was used to direct cardiac-specific overexpression of the mutant TR (MHC-mut, 19). In contrast, this same mutation was introduced in the germline of mice by homologous recombination (KI-mut, 22). All KI-mut mice used were heterozygous animals containing one WT and one mutant allele. All mice used in these studies were from the same mixed genetic background (C57BL6/129Svj), and littermate controls were used in all experiments. As demonstrated previously, there was no change in TR-α expression in these lines relative to WT animals (19, 22).

The same animals had their cardiac function evaluated by echocardiographic imaging at three time points: baseline (12 wk of age), after hypothyroidism and after T_3 treatment. From these groups, three to five animals were also evaluated by *in situ* hemodynamics before they were killed. Hypothyroidism was induced by feeding the animals a low-iodine diet (Harlan Teklad Co., Madison, WI) containing 0.15% 5-propyl-2-thiouracil (PTU) for 3 wk after baseline evaluation. The same mice were rendered thyrotoxic by daily sc injections of T_3 (10 μg/100 g body weight) for 3 wk while receiving the PTU diet. At these same times, blood samples were obtained from the tail vein for hormone determinations. Total T_4 and T_3 (T_4 or T_3 kit, ICN Pharmaceuticals, Costa Mesa, CA) were measured in duplicate, in the same assay.

Echocardiographic imaging

Immediately before performing the echocardiographic study, animals were anesthetized by administrating isoflurane mixed with O_2 inducing at 2.5% and maintaining anesthesia at 1–1.5%. Subsequently, mice received 1–1.5% isoflurane through a nose cone as needed to maintain sedation while spontaneously breathing. Once anesthetized, mice were secured to a custom-made waterbed in the left lateral position to prevent hypothermia and facilitate imaging. Transthoracic echocardiography was performed three times during the experiment: at baseline, after hypothyroidism induction by a PTU diet, and after T_3 treatment (as described earlier). Electrocardiographic monitoring was performed continuously during the cardiac imaging procedure. The anterior chest was shaved to facilitate ultrasound imaging. After the echocardiographic study, the anesthetic was discontinued, and mice were returned to their cages for recovery.

Cardiac ultrasound imaging was performed using a high-frequency 15-MHz linear transducer (Sonos 5500; Agilent Technologies, Andover, MA). Parasternal long- and short-axis views were obtained after adjusting gain settings for optimal epicardial and endocardial wall visualization. Two-dimensionally targeted M-mode echocardiograms were obtained from the short-axis at the tip of the papillary muscle. From an unconventional, more superior parasternal long axis view, ascending aortic two-dimensionally targeted M-modes were recorded immediately distal to the aortic valve. Ascending aortic pulse wave Doppler velocities were obtained from a suprasternal window using a pediatric short focal length, 12-MHz phased array transducer (Sonos 5500; Agilent Technologies). To improve image quality, an acoustic coupling gel standoff was attached to the transducer. 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 minimize artifacts caused by air bubbles in the gel, it (Aquasonic 100; Parker, Orange, NJ) was centrifuged at $2000 \times g$. All echocardiographic data were recorded to a magnetic optical disk for off-line analysis (Fig. 1).

Measurements

The LV septal wall thickness and posterior wall thickness (PWT) as well as LV end-diastolic (EDD) dimensions were measured at the peak of the electrocardiogram R-wave. LV end-systolic (ESD) dimensions were measured at the time of minimal cavity dimensions. Fractional shortening was computed as $(EDD - ESD)/(EDD) \times 100$. LV mass was estimated using the recently validated two-dimensional area-length formula wherein LV mass = $[1.05 \times [5/6\ A_1\ (L + t) - 5/6\ A_2 L]$, where 1.05 is the specific gravity of muscle, A1 and A2 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 A1 and A2. All measurements were performed in a blinded manner, and the average of three measurements is reported.

Continuous-wave aortic Doppler recordings were obtained, with two-dimensional guidance, from a right supraclavicular view using a 12-MHz phased-array transducer (Hewlett-Packard, Palo Alto, CA). Peak aortic velocity and velocity time integral were determined. The

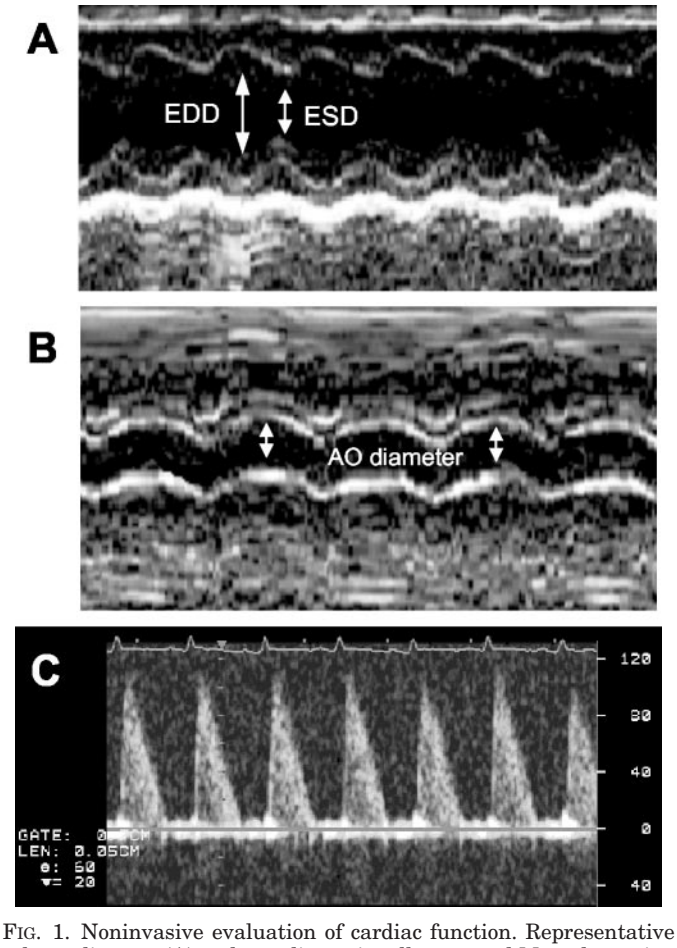

FIG. 1. Noninvasive evaluation of cardiac function. Representative echocardiogram (A) and two-dimensionally targeted M-mode tracing of proximal ascending aorta (B) and aortic Doppler velocity profile (C) of a WT animal. AO, Aorta.

average of three determinations was used. LV stroke volume was calculated as the product of the velocity time integral (AoVTI) of the aortic Doppler recording and the aortic cross-sectional area (LVOT area). The aortic diameter (D) was measured using M-mode echocardiography at the level of the proximal ascending aorta, and aortic cross-sectional area was computed as $(D/2)^2 \times \pi$. Cardiac output was calculated as stroke volume times HR and then indexed to body weight. All the procedures were described earlier in details (24).

In situ hemodynamics

Mice were anesthetized with isoflurane inhaled in a closed chamber and intubated with an 18-gauge angiocath tube. Surgical anesthesia was maintained using 0.5% isoflurane delivered through a vaporizer with a mixture of 100% oxygen connected in series to a rodent ventilator with the stroke volume set at 0.2–0.4 ml/min and a respiration rate of 125 breaths/min. The right carotid artery was instrumented with an ultraminiature pressure transducer (1.4 French; Millar Instruments, Houston, TX) and advanced into the aortic arch for measurement of systolic, diastolic, and mean blood pressure. Subsequent to arterial blood pressure measurements, the transducer was advanced into the LV and baseline intraventricular measurements were obtained. The mice were also subjected to an inotropic challenge by a bolus injection of dobutamine (200 μl; 37 μM) into the femoral vein and measurement of the intraventricular contractile response. After the hemodynamic recordings, the catheter was withdrawn and the animals received an overdose of isoflurane (2.0%), and the heart was explanted and frozen for total RNA extraction.

Ribonuclease protection assay

Total heart RNA was extracted from mouse ventricles using a commercial kit (TRIZOL, BRL, Scotland, UK). Samples of total RNA were then stored in −70 C until assayed. MHC-α and MHC-β gene expression was evaluated by RPA [ribonuclease (RNase) protection assay] with the use of specific ^{32}P-labeled 3′ MHC-β riboprobe. This probe was obtained by RT-PCR of mouse heart mRNA with the use of primers to the 3′ coding sequence and untranslated region of MHC-β. The following primers were used MHC-β 5′-GCCAACACCAACCTGT-CCAAGTTC-3′ and 5′-TGCAAAGGCTCCAGGTCTGAGGGC-3′, generating a 206-bp product for MHC-α and 307-bp product for MHC-β, as described previously (19). A cyclophilin riboprobe was added to all samples to control RNA quality and quantity. Both probes were evaluated with 10 μg of yeast tRNA in the presence of RNase A and T1 following the RPA kit (RPA III, Ambion, Austin, TX) protocol. Radiolabeled antisense β-MHC (0.5×10^5 cpm/sample) and cyclophilin (1×10^4 cpm/sample) probes were mixed with 10 μg of total ventricle RNA. Samples were hybridized for 18 h at 42 C. Then, RNase A and T1 were added. Analysis of protect fragments was made in 8 M urea/5% polyacrylamide gels, which were dried and exposed to Kodak (Rochester, NY) XAR-5 film in cassettes with intensifying screens at −70 C. Bands corresponding to protected fragments were quantified by densitometry (NIH Image, NIH, Bethesda, MD).

Statistical analysis

Data are reported as means ± SEM. One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test was employed for assessment of significance when comparisons were made within the same genotype. Two-way ANOVA was employed when mice of different genotypes and treatment were compared. Paired *t* test was used to analyze transgenic animals *vs.* WT at the same time point in Fig. 2 (GraphPad Prism, GraphPad Software, Inc., San Diego, CA). Differences were considered to be significant at $P < 0.05$.

Results

Effect of treatment on body weight and TH levels

At the baseline evaluation (12 wk of age), no intergroup differences were noted in total body weight (Table 1). During treatment with PTU, no significant change in body weight was noted in any group. After 3 wk of T_3 treatment, body weight increased by 25, 24, and 10% in WT, MHC-mut, and KI-mut, respectively, compared with baseline weights (Table 1). These increases in body weight were statistically significant in the WT and MHC-mut groups *vs.* their corresponding baseline measures but did not reach statistical significance in KI-mut animals. Moreover, KI-mut animals weighed less than WT after T_3 treatment.

At baseline, there was no difference between the T_4 levels of MHC-mut and WT animals, whereas KI-mut animals demonstrated higher T_4 levels (77% higher than WT animals, $P < 0.01$). These results are consistent with the germline expression of the mutant TR in KI-mut animals, which alters the set point for TH production, *vs.* the cardiac-specific expression of TR mutant in the MHC-mut animals, which does not alter the set point (19, 22). Because higher TH levels in the KI-mut animals could affect cardiac function, we next treated all groups with PTU to reduce endogenous TH production and then replaced animals with supraphysiological T_3 to induce a similar degree of elevation in T_3 levels. After replacement, all animals presented with very high T_3 levels, which were not significantly different among the groups (267–283 ng/dl).

Echocardiographic measurement of cardiac function in mice expressing mutant TRs

We explored the effect of the mutant TR transgene on cardiac physiology at baseline, after PTU and after PTU + T_3 treatment using noninvasive echocardiography. At the initial evaluation (baseline) and after PTU treatment, differences in LV mass and measures of LV size were not found among the groups, with the exception of a greater LV mass and PWT in the MHC-mut animals during the PTU diet (Table 2). Treatment with PTU + T_3 significantly increased LV mass in all groups compared with their respective baseline values (Table 2). The increase in LV mass, however, was least in the KI-mut animals (WT 52%, MHC-mut 44%, and KI-mut 32%) and was significantly less than the increase observed in WT animals treated in the same way. ESD and EDD were also significantly increased in all groups after PTU + T_3 treatment, perhaps reflecting an increase in blood volume. In summary under basal conditions, MHC-mut animals displayed increases in both LV mass and PWT compared with WT animals. In contrast, KI-mut animals were similar to WT animals in these parameters.

Under basal conditions, MHC-mut animals had a marked decrease in HR compared with WT animals (Fig. 2A). In contrast, the HR of KI-mut animals was the same as WT animals although T_3 levels were higher in the former group. In response to either PTU or PTU + T_3 treatment, the HR of MHC-mutant animals changed minimally in comparison to either WT or KI-mut animals. When TH levels were similar (PTU + T_3), there was no significant difference in HR between KI-mut and WT animals. Both shortening fraction and stroke volume were increased in MHC-mut animals (Fig. 2, B and C, respectively), which more than compensated for the decreased HR, resulting in a statistically significant increase in cardiac output in MHC-mut animals in the hypothyroid state compared with WT animals (Fig. 2D). After PTU + T_3 treatment, however, the increase in stroke volume was not

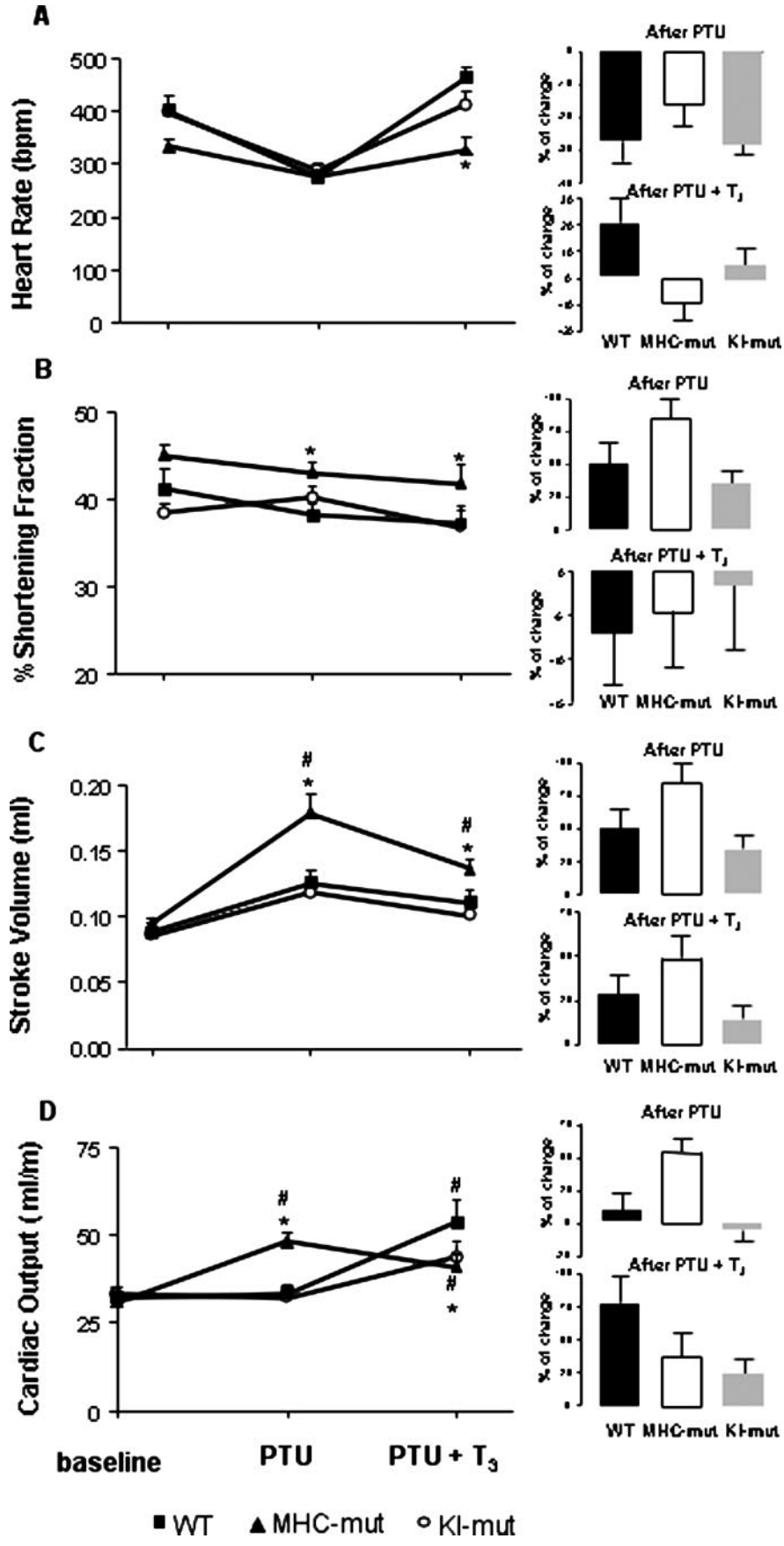

FIG. 2. Echocardiographic measurements in WT, MHC-mut, and KI-mut mice at baseline, after PTU treatment and after PTU + T_3 treatment. HR (A), shortening fraction (B), stroke volume (C), and cardiac output (D). Next to each *line*, a bar graph summarizing percentage of change in measures relative to baseline determination. *, $P < 0.05$ (or less) *vs.* WT of the same thyroidal state. #, $P < 0.05$ (or less) compared with baseline of same genotype. n = 9–15 animals/group. Statistical analyses were performed using absolute values. Data are reported as means ± SEM.

TABLE 1. Body weights and thyroid hormone levels

Genotype	Baseline		PTU		PTU + T_3	
	BW (g)	T_4 (μg/dl)	BW (g)	T_4 (μg/dl)	BW (g)	T_3 (ng/dl)
Wild type	25.9 ± 1.5	3.5 ± 0.24	27.2 ± 1.5	<1	32.5 ± 1.5[a]	273 ± 65
MHC-mut	25.6 ± 0.7	3.1 ± 0.18	25.6 ± 0.8	<1	31.9 ± 0.9[a]	267 ± 20
KI-mut	25.2 ± 1.1	6.2 ± 0.11[b]	24.5 ± 0.7	<1	27.8 ± 1.4[b]	283 ± 40

Values are means ± SEM.
[a] $P < 0.05$ (or less) compared with baseline of same genotype. n = 9–15 animals/group. [b] $P < 0.05$ (or less) *vs.* wild type of the same thyroidal state.

TABLE 2. M-mode and Doppler echocardiographic data

	Wild type			MHC-mut			KI-mut		
	Baseline	PTU	PTU + T_3	Baseline	PTU	PTU + T_3	Baseline	PTU	PTU + T_3
LV (mg)	92 ± 4.3	92 ± 3.2	140 ± 5.1[a]	98 ± 2.5	102 ± 3.6[b]	141 ± 6.2[a]	92 ± 4.2	89 ± 2.7	122 ± 4.3[a,b]
EDD (mm)	3.84 ± 0.2	3.92 ± 0.1	4.36 ± 0.1[a]	3.84 ± 1.1	3.94 ± 0.8	4.52 ± 0.5[a]	3.85 ± 0.1	3.94 ± 0.1	4.25 ± 0.1[a]
ESD (mm)	2.36 ± 0.2	2.43 ± 0.1	2.80 ± 0.2[a]	2.16 ± 0.1	2.25 ± 0.0	2.61 ± 0.0[a]	2.34 ± 0.1	2.33 ± 0.1	2.76 ± 0.1[a]
PWT(d) (mm)	0.84 ± 0.1	0.83 ± 0.1	1.05 ± 0.1[a]	1.07 ± 0.5[b]	1.01 ± 0.1[b]	1.13 ± 0.1	0.84 ± 0.1	0.83 ± 0.1	0.95 ± 0.3[a]

Values are means ± SEM.
[a] $P < 0.05$ (or less) compared with baseline of same genotype. n = 9–15 animals/group. [b] $P < 0.05$ (or less) *vs.* wild-type animals of the same thyroidal state.

sufficient to compensate for the impaired HR response in the MHC-mut animals and cardiac output was reduced compared with WT animals. Importantly with regard to these parameters, WT and KI-mut animals were similar in both the basal state and after PTU treatment. The increase in cardiac output in KI-mut animals after T_3 treatment was reduced relative to WT animals, but this did not achieve statistical significance (Fig. 2D). When cardiac output was corrected for body weight the same results were observed (data not shown).

Hemodynamic measurement of cardiac function in mice expressing mutant TRs

We next explored the effect of the mutant transgene on cardiac contractility by *in situ* hemodynamics before and after dobutamine stimulation. Figure 3 displays HR (panel A), +dP/dT (pressure derivatives) (panel B), and −dP/dT (panel C), LV pressure (panel D) from WT, MHC-mut, and KI-mut animals without treatment, after PTU diet and after PTU + T_3 treatment. Consistent with the echocardiographic data, MHC-mut animals had markedly decreased HR (Fig. 3A), which did not respond to either hypothyroidism or thyrotoxicosis. Both systolic (+dP/dT) and diastolic (−dP/dT) pressure derivatives were also impaired (Fig. 3, B and C, respectively) and, importantly, did not respond to T_3 treatment. MHC-mut animals generated less LV pressure, but this was only apparent after PTU + T_3 treatment. Interestingly, there were no significant differences between WT and KI-mut animals in any of these parameters.

In response to dobutamine administration, MHC-mut mice displayed smaller increases in HR, +dP/dT, and −dP/dT than either WT or KI-mut animals at baseline. This was especially true of both the +dP/dT and −dP/dT measures. After PTU + T_3 treatment, responses to dobutamine were improved but were still quantitatively less than those observed in either WT or KI-mut animals. Dobutamine responses in KI-mut animals were similar to those found in WT animals.

Cardiac gene expression in mice expressing mutant TRs

Expression of MHC isoforms was determined in WT, MHC-mut, and KI-mut mice under basal conditions and after treatment with either PTU or PTU + T_3. Both α-MHC and β-MHC were determined in a single RNase protection assay of cardiac total RNA. WT animals, as expected, showed an increase in MHC-β and a corresponding decrease in MHC-α expression mRNA expression after PTU treatment. These changes in MHC isoform expression were reversed by the addition of T_3 to the PTU treatment (PTU + T_3 group, Fig. 4). MHC-mut animals, in contrast, expressed MHC-β at high levels regardless of the thyroidal status. These data suggested that cardiac tissue was hypothyroid in each of these conditions. Notably, KI-mut animals expressed MHC isoforms in a pattern identical with WT animals, suggesting that germline expression of the TR-β mutant had no measurable effect on cardiac TH-responsive gene expression.

Discussion

We have previously demonstrated that cardiac-restricted expression of a mutant TR-β (KS, Δ337T) resulted in cardiac hypothyroidism regardless of the circulating TH levels (19). In these mice, the α-MHC promoter was used to direct cardiac expression. When cardiac function was evaluated *ex vivo*, marked systolic and diastolic dysfunction were found presumably due to cardiac tissue hypothyroidism. In contrast, cardiac function was preserved in these animals when tested *in vivo*, suggesting that changes in either autonomic tone, vascular resistance, and/or circulating blood volume compensated for the cardiac defect. In another animal model (25), the same mutant TR was driven by a β-actin promoter, which is ubiquitously expressed. Those animals also showed an altered cardiac phenotype with decreased MHC-α expression. Unfortunately, a limitation of this model is that a mutant TR-β was overexpressed in the heart relative to endogenous TR-β expression. To determine whether allelic expression of the same mutant TR-β caused a similar pheno-

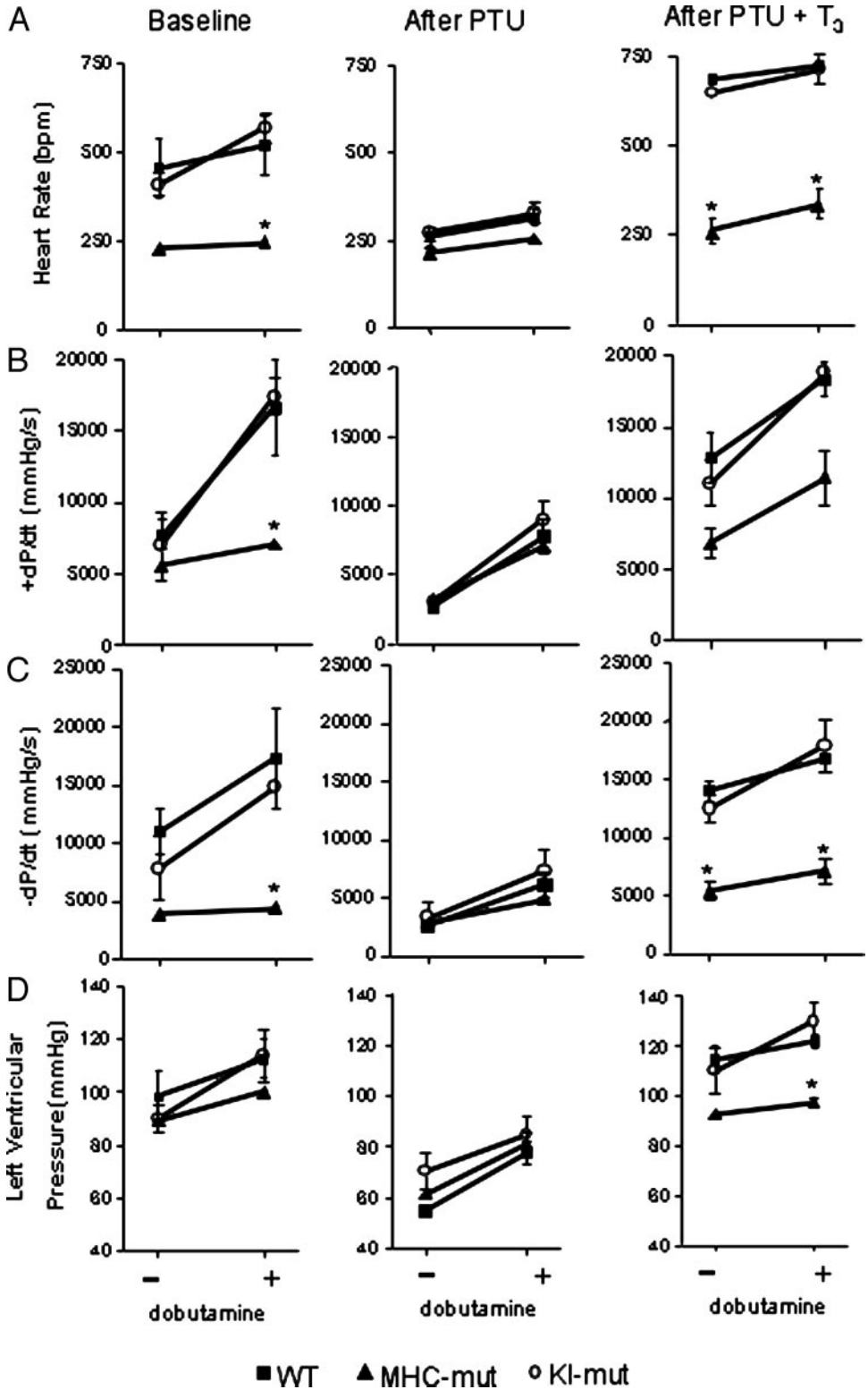

FIG. 3. Hemodynamics measurements before and after dobutamine stimulation in WT, MHC-mut, and KI-mut mice at baseline, after PTU treatment and after PTU + T_3 treatment. *, $P < 0.05$ (or less) *vs.* WT of the same thyroidal state. n = 3–5 animals/group. Data are reported as means ± SEM. The group KI-mut after PTU + T_3 has n = 2 and was reported as means ± (maximum and minimum).

type of cardiac hypothyroidism in mice, Δ337T knock-in mice (KI-mut) were studied. The use of echocardiography allowed for sequential study of cardiac function in mice after peripheral TH levels were altered.

In humans and rodents, cardiac mass increases as an effect of TH treatment (26–28). Previous investigators have suggested that this was not a direct effect of TH on the heart but rather due to a change in sympathetic tone mediated by TH (28). Alternatively, other investigators have suggested that TH-induced cardiac hypertrophy occurs due to local activation of renin-angiotensin system in the heart (29–31). Interestingly, a recent paper from Weiss *et al.* (24), together with

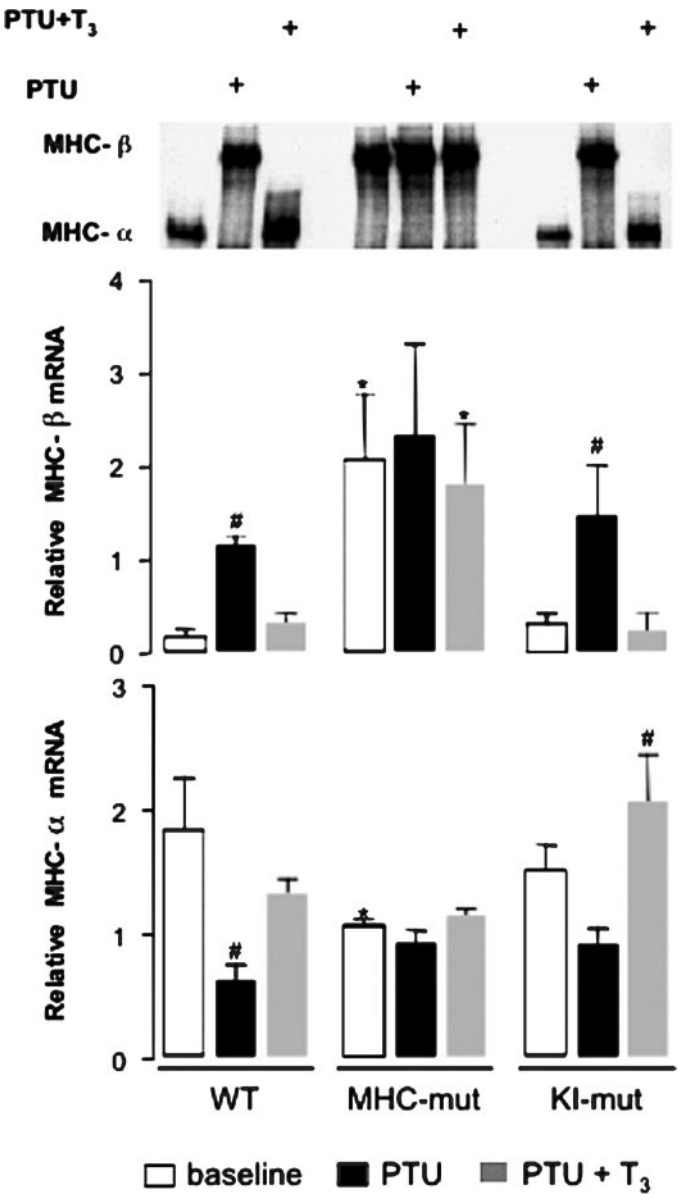

FIG. 4. Relative MHC isoform expression (corrected for cyclophilin) in WT, MHC-mut, and KI-mut mice at baseline, after PTU treatment and after PTU + T_3 treatment. MHC-mut, mice with cardiac-specific expression of a mutant TR(Δ337T); KI-mut, germline heterozygous mutation for TR-β (Δ337T). *, $P < 0.05$ (or less) *vs.* WT of the same thyroidal state. #, $P < 0.05$ (or less) compared with baseline of same genotype. This is a representative protection assay performed on 6–10 animals/group at least five separate times. Data are reported as means ± SEM.

other papers using TR-β and TR-α knockout mice, demonstrate that generalized absence of TR-β prevented TH cardiac hypertrophy, whereas the generalized absence of TR-α did not prevent hypertrophy (10, 32, 33).

The results in TR-β knockout animals differ from those observed in animals with cardiac-restricted expression of a mutant TR (Δ337T). As shown in Table 2, cardiac hypertrophy in response to TH treatment was similar in WT and MHC-mut animals. In contrast, cardiac hypertrophy in response to TH treatment was impaired in KI-mut mice when compared with WT mice. These data suggest that TH-dependent cardiac hypertrophy may be caused by an indirect action of TR-β because there is generalized expression of the mutant TR in KI-mut animals and cardiac-restricted expression of the same mutant TR in MHC-mut animals. It is possible, for example, that the autonomic tone of the heart and/or peripheral circulation may be altered in the KI-mut animals—due to generalized expression of a mutant TR-β—leading to less cardiac hypertrophy. Alternatively, regional differences in cardiac expression of TR-β in MHC-mut and KI-mut animals could explain these data, given that expression by the α-MHC promoter may not be qualitatively equivalent to expression by the endogenous TR-β locus in the heart. However, the normal degree of cardiac hypertrophy in MHC-mut animals in the setting of significant cardiac hypothyroidism, makes this latter explanation much less likely (see below).

Homozygous deletion of the TR-β gene is associated with increased basal HRs and a blunted HR response to T_3 (10). The increase in basal HR in these animals is likely due to increased TH levels acting on the remaining TR-α1. After T_3 treatment, the increase in HR relative to basal levels is less in TR-$\beta^{-/-}$ *vs.* WT animals, but the absolute HR is not significantly different from WT animals. In contrast, TR-$\alpha1^{-/-}$ mice are bradycardic, with HRs 20% less than WT mice, and their electrocardiogram time intervals are increased (33). Here we show that, like TR-$\alpha1^{-/-}$ mice, cardiac-specific expression of the mutant TR causes significant bradycardia (see Fig. 2). In fact, HRs in MHC-mut mice were similar to hypothyroid WT mice regardless of the thyroidal status. This is likely due to dominant-negative inhibition of TR-α1 by the TR-β mutant based on results observed in knockout animals. Interestingly, bradycardia was not observed in KI-mut animals, which express the same mutant TR-β. Although this could be due to regional differences in TR-β expression, it is more likely due to the relative expression of mutant TR-β in these models. In summary, available data support the concept that HR is governed, at least in part, by the direct action of TR-α1 in the heart. Importantly, cardiac TR-β appears to play no significant role in regulation of HR.

Even though HR was markedly depressed in MHC-mut animals, cardiac output was maintained *in vivo* due to a significant increase in stroke volume (Fig. 2). This was most notable after PTU treatment but was also observed after PTU + T_3 treatment. In the former condition, cardiac output was actually higher than WT animals, whereas in the latter condition it was less than control. Clearly, this is an *in vivo* adaptation to poor cardiac function in the MHC-mut animals. Figure 3 demonstrates that pressure development and pressure derivatives were both impaired in these mice with cardiac-specific expression of TR-β. Importantly, KI-mutant animals had virtually identical cardiac function as control animals with the exception of a slightly (but not significantly) lower cardiac output than WT animals after PTU + T_3 treatment (Fig. 2). These slight differences were not observed when cardiac function was evaluated by an *in situ* method. Thus, by inference, TR-α1 appears to be the most important TR isoform in the heart; this is probably the result of its higher expression levels in the heart.

Cardiac-specific expression of the mutant receptor also

affected the ability of the ventricle to respond to dobutamine. Although HR and pressure derivatives significantly changed in WT mice after dobutamine treatment under basal conditions, these responses were absent in MHC-mut animals. These data suggest that adrenergic stimulation of the heart is TH dependent, as noted by other investigators (34, 35), and that a direct action of TR on the heart modulates this stimulation. As expected, these same parameters were depressed after PTU treatment and were much less responsive to dobutamine stimulation (Fig. 3). Under thyrotoxic conditions, HR, LV pressure, and pressure derivatives were all increased from the basal state in WT mice, but markedly lower in MHC-mut animals.

Although unexpected, there were no significant differences between WT and KI-mut animals in any of studied parameters or under any of the different thyroidal states. Thus, *in situ* hemodynamics data confirm echocardiographic measurements that demonstrate impaired cardiac function in MHC-mut animals and normal function in KI-mut animals.

TH may also alter HR through an indirect mechanism. In hyperthyroid patients, the renin-angiotensin-aldosterone system is activated and eritropoetin secretion is increased (36). Both effects contribute to the increase in total blood volume and cardiac preload. The effects of TH on peripheral circulation also play a central role in regulating cardiac performance by reducing systemic vascular resistance. This is believed to be an indirect effect due to an increased whole body and myocardial oxygen consumption, as well as increased tissue blood flow (37). In this paper, we try to minimize all indirect effects comparing animals of different genotypes with the same TH levels after PTU and PTU + T_3 treatment.

Another result that highlights the dominant-negative effect of mutated TR in the heart of MHC-mut animals is the hypothyroid pattern of MHC gene expression. As shown in Fig. 4, MHC-β mRNA levels were increased and MHC-α were decreased in MHC-mut mice regardless of the thyroidal status, indicating profound cardiac hypothyroidism in these animals. In contrast, KI-mut animals displayed a WT MHC gene expression pattern in response to changes in circulating TH levels. Clearly, because the same mutant TR is expressed in these animal models, the difference in MHC gene expression must be due to either qualitative or quantitative differences in mutant TR-β gene expression in the myocardium. Recently, another group had showed similar results using a different mutation (PV) introduced into germline of mice (38) confirming our findings.

TH resistance is caused by dominant-negative mutations of the TR-β gene. In this study, the Δ337T mutation was evaluated because it has been described in a consanguineous family with RTH (23, 39). Affected family members noted palpitations and dyspnea and these symptoms are frequent in patients with RTH (16, 20, 39). Given the differences in TR isoform expression, it has been suggested that the heart (TR-α1 predominant) is sensitive to the elevated TH levels in RTH patients. Although we did not note an increase HR in KI-mut mice under basal conditions, this may be a limitation of the mouse model because basal HRs are already quite elevated (>400 beats per minute).

These data support the concept that the heart of mice with RTH is sensitive to TH, although small differences with control mice were uncovered at very high circulating TH levels in KI-mut animals. Because the mutation is expressed from the TR-β locus, its regional and quantitative expression patterns in the mouse should be identical with patients harboring this same mutation. Except for cardiac hypertrophy, the presence of a germ line TR-β mutation had surprising little effect on cardiac structure and function.

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