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Thyroid Hormone Action on Liver, Heart, and Energy Expenditure in Thyroid Hormone Receptor β-Deficient Mice*

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ABSTRACT

Thyroid hormone (TH) responsive genes can be both positively and negatively regulated by TH through receptors (TR) α and β expressed in most body tissues. However, their relative roles in the regulation of specific gene expression remain unknown. The TRβ knockout mouse, which lacks both TRβ1 and TRβ2 isoforms, provides a model to examine the role of these receptors in mediating TH action. TRβ deficient (TRβ−/−) mice that show no compensatory increase in TRα, and wild-type (TRβ+/+) mice of the same strain were deprived of TH by feeding them a low iodine diet containing propylthiouracil, and were then treated with supraphysiological doses of L-T_3 (0.5, 5.5, and 25 μg/day/mouse).

TH deprivation alone increased the serum cholesterol concentration by 25% in TRβ+/+ mice and reduced it paradoxically by 23% in TRβ−/− mice. TH deprivation reduced the serum alkaline phosphatase (AP) concentration by 31% in TRβ+/+ mice but showed no change in the TRβ−/− mice. Treatment with L-T_3 (0.5 to 25 μg/mouse/day) caused a 57% decrease in serum cholesterol and a 231% increase in serum AP in the TRβ+/+ mice. The TRβ−/− mice were resistant to the L-T_3 induced changes in serum cholesterol and showed increase in AP only with the highest L-T_3 dose. Basal heart rate (HR) in TRβ−/− mice was higher than that of TRβ+/+ mice by 11%. HR and energy expenditure (EE) in both TRβ+/+ and TRβ−/− mice showed similar decreases (49 and 46%)and increases (49 and 41%) in response to TH deprivation and L-T_3 treatment, respectively. The effect of TH on the accumulation of messenger RNA (mRNA) of TH regulated liver genes was also examined. TH deprivation down regulated spot 14 (S14) mRNA and showed no change in malic enzyme (ME) mRNA in both TRβ+/+ and TRβ−/− mice. In contrast treatment with L-T_3 produced an increase in S14 and ME but no change in TRβ−/− mice.

From these results, it can be concluded that regulation of HR and EE are independent of TRβ. With the exception of serum cholesterol concentration and liver ME mRNA accumulation, all other markers of TH action examined during TH deprivation exhibited the expected responses in the absence of TRβ. Thus, as previously shown for serum TSH, TRβ is not absolutely necessary for some changes typical of hypothyroidism to occur. In contrast, except for HR and EE, the full manifestation of TH-mediated action required the presence of TRβ. (*Endocrinology* **139:** 4945–4952, 1998)

THYROID HORMONE RECEPTORS (TRs), located in cell nuclei, mediate the action of thyroid hormone (TH) by positive and negative regulation of TH responsive genes (1). Although the two genes that encode the related TRα and TRβ are differentially expressed, the two receptors often coexist in the same cell type (2–4). The relative contribution of the two TR gene products in mediating a particular TH response is poorly understood because of a paucity of *in vivo* functional information. *In vitro* DNA binding analyses and cell transfection functional assays have generally indicated that the three TR isoforms that bind TH (TRβ1, TRβ2, and TRα1) exhibit similar ligand dependent transcriptional activity (5). Some studies have generated conflicting results concerning the specific effect of TR isoforms in gene regulation. For example, while Lezoualc'h *et al.* (6) and Hollenberg *et al.* (7) proposed that TH-mediated suppression of TRH gene transcription is TRβ1 specific, Feng *et al.* (8) observed that the same effect was mediated by each of the three TR isoforms. Interpretation of these apparently contradictory data are complicated because they are derived from artificial systems using overexpression of transfected chimeric gene constructs that may not be faithful models of events occurring in the intact animal.

The TRβ knockout mouse, which does not express either TRβ1 or TRβ2 (9), provides the means to explore the relative contribution of the TRα and TRβ isoforms to the TH-mediated regulation of various physiologic processes in different tissues. We have previously demonstrated by the use of this mouse model that TRβ is not required for the up-regulation of TSH during TH deprivation, but enhances the sensitivity of TSH down-regulation and may be essential for the complete suppression of TSH (10). These mice display features of resistance to thyroid hormone (RTH) similar to those observed in humans with deletion of the TRβ gene (11–13) including deafness, resistance to TH-induced suppression of

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TSH, and goiter. Therefore, they provide a model for the detailed investigation of TH regulation in different tissues in the absence of one specific TR isoform, through hormonal manipulations that could not be carried out in humans.

To this purpose, we examined the effects of TH deprivation and treatment with incremental doses of L-T_3 on the liver, heart, and energy expenditure (EE) in homozygous, TRβ deficient (TRβ−/−) mice and compared them to those observed in wild-type (TRβ+/+) mice. Our data show that TH deficiency can reduce and L-T_3 increase HR and EE to the same extent in the TRβ−/− and TRβ+/+ mice. In contrast to the TRβ+/+ mouse, the serum cholesterol of the TRβ−/− mouse does not respond to L-T_3, and the increase in serum alkaline phosphatase (AP) was severely blunted. The effect of L-T_3 on the accumulation of liver mRNA derived from TH regulated genes [spot 14 (S14) and malic enzyme (ME)] was markedly reduced in the absence of the TRβ. In contrast, TH deprivation had effects on S14 and ME in TRβ−/− mice that were similar to those seen in TRβ+/+ mice. These results indicate that, in the absence of TH, regulation of TH responsive genes can occur independently of the TRβ, whereas TH-mediated effect of the same genes is augmented by the presence of TRβ.

Materials and Methods

Experimental animals and design

TRβ-deficient (knockout or TRβ−/−) mice have a targeted mutation that deletes a part of the coding exon 3 of the TRβ gene, preventing the synthesis of functional TRβ1 or TRβ2. The TRβ gene defect was maintained on a hybrid genetic background of parental C57B1/6J and 129/SV mouse strains (14). Heterozygous, TRβ −/+ mice, were interbred to generate litters containing homozygous TRβ−/− and TRβ+/+ progeny. TRβ−/− and TRβ+/+ were then selected and separately interbred, the latter serving as controls. Experiments were performed on mice derived from the second to fourth generation of interbreeding. The genotype of mice was confirmed by analysis of tail DNA as previously described (14). All studies were conducted in accord with NIH guidelines and approved by the Committee on Animal Care and Use at The University of Chicago.

Mice were weaned on the fourth week after birth and were fed Purina Rodent Chow (0.8 ppm Iodine) *ad libitum* and tap water. They were housed, 3 to 5 mice per cage, in a controlled environment of 19 C and 12 h alternating darkness and artificial light cycles.

All mice were 60–70 days old at the beginning of each experiment. Weights of TRβ+/+ and TRβ−/− mice overlapped and ranged from 16–21 g (female) and 17–24 g (male). TH deficiency was induced by feeding a low iodine (LoI) diet supplemented with 0.15% propylthiouracil (PTU) purchased from Harlan Teklad Co. (Madison, WI). Experiments were terminated by exsanguination through eye vein puncture under light methoxyflurane (Pitman Moore, Mundelein, IL) anesthesia. Serum was separated by centrifugation and stored at −20 C until analyzed in the same assay for each experiment.

TH deficiency was induced in TRβ−/− and TRβ+/+ mice by feeding the LoI/PTU diet for 14 days. On the eleventh day, groups of TRβ−/− and TRβ+/+ mice were treated for 4 days with 0 (the vehicle only), 0.5, 5.5, and 25 μg of L-T_3/mouse daily. These L-T_3 amounts correspond to 4, 40, and 200 times the daily replacement dose. Twelve to sixteen hours after the last injection, experiments were terminated by exsanguination. L-T_3 was given by ip injections in a total volume of 0.2 ml of PBS and 0.002% human serum albumin as a vehicle. A stock solution of L-T_3 (Sigma Chemical Co., St. Louis, MO) was prepared in water containing 4 mM NaOH and kept at 4 C, protected from light. The concentration of L-T_3 was confirmed by RIA (Diagnostic Products, Los Angeles, CA).

Serum measurements

Serum TSH was measured in 50 μl of serum using a sensitive, heterologous, disequilibrium double antibody precipitation RIA (10). The sensitivity of this assay is 0.02–0.04 ng of TSH equivalent per ml, depending on the rat–[^{125}I]TSH batch, with intraassay coefficients of variation of 12, 13, and 4% for TSH concentrations of 0.03, 0.7, and 2.4 ng/ml, respectively. T_4 was measured by a double antibody precipitation RIA (Diagnostic Products, Los Angeles, CA) modified to measure T_4 in 15 μl of serum with a sensitivity of 0.5 μg/dl (6.4 nmol/liter). Cholesterol and AP were measured each on 10 μl of serum using a clinical chemistry autoanalyzer that required an additional 40 μl of serum to prime the pump. Isozyme fractionation of AP was performed by Mayo Clinic Laboratories (Rochester, MN) on 2 ml of serum pools from several animals of each group.

Heart rate and energy expenditure

Heart rate was determined electrocardiographically under chloral hydrate anesthesia (4 mg per 10 g body weight, ip) using a Hewlett Packer Monitor/Terminal Model 78534AA with a chart speed of 25 mm per second. Energy expenditure (EE) was determined by measurement of change in body weight and food consumption over 4 days as previously described (15) EE was calculated using the formula:

$$EE(Kcal/day) = [\text{food consumption (g/day)} \times 4.058^{*} \times 0.8^{**}] \pm [\text{weight change (g/day)} \times 7^{***}]$$

Where, * is the caloric value of the food (in Kcal/g); ** is adjustment for 20% food wasted in litter as determined by bomb calorimetry; *** is caloric value of 1 g body weight change. Loss of weight is added and weight gain subtracted.

Liver tissue analyzes

A, T_3-binding activity of TR: Approximately 1 g of liver from mice given LoI/PTU diet, was mechanically homogenized (10 strokes) in a Dounce homogenizer (Iwaki Glass Ltd., Tokyo, Japan) using pestle B with a buffer [SMTD-phenylmethylsulfonyl fluoride (PMSF)] containing 0.32 M sucrose, 1 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM PMSF. Nuclei were pelleted by centrifugation at 1,000 × *g* for 10 min. The pellets were homogenized again and washed once with SMTD-PMSF. Triton X-100 was added to a final concentration of 1% and 0.5% for the first and second homogenizations, respectively. The pellets were washed 3 times with SMTD-PMSF without Triton X-100 and resuspended in SMTD-PMSF for the performance of T_3 binding assay. All procedures were performed at 4 C.

The T_3-binding assay was performed in the isolated liver nuclei as previously described (16). In brief, nuclei were incubated for 2 h at 22 C with increasing amount of [^{125}I] T_3 ($0.32–2.0 \times 10^{-9}$ M) with or without unlabeled T_3 (3×10^{-7} M) to determine nonspecific T_3 binding. Nuclei were then collected by centrifugation at 1,000 × *g* for 10 min. Because approximately 10–30% of nuclear T_3 receptor is released from the nuclei during centrifugation, the radioactivity in the supernatant was also counted after adsorption of free T_3 onto Dowex resin (Dowex 1–8 CL-200–400 mesh anion exchange resin, Bio-Rad Laboratories, Inc., Richmond, CA) as previously described (16). Receptor bound T_3 was the sum of the radioactivity in the precipitate and excluded from the resin. Affinity constant (K_a) and maximal T_3 binding capacity (MBC) of nuclei were determined by Scatchard analysis (17). DNA was measured by Burton's method (18). Sucrose, dithiothreitol, and Triton X-100 were purchased from Wako Pure Chemicals, Ltd. (Osaka, Japan), PMSF was from Sigma Chemical Co. (St. Louis, MO), [^{125}I] T_3 (specific activity, 81.45 tetraBq/mmol) was obtained from DuPont NEN (Boston, MA).

B, Determination of S14 and ME mRNA: Total RNA was extracted by the acid-guanidinium thiocyanate-phenol-chloroform method (19) from liver that was frozen 1 to 2 min after death and kept at −85 C. Aliquots of 15 μg of total RNA were denatured and fractionated by electrophoresis on 0.8% agarose gel then transferred onto GeneScreen Plus (DuPont NEN) using VacuGene (Pharmacia) and hybridized as previously described (20). Hybridization probes were prepared by labeling S14 (21), and ME (22) complementary DNAs (cDNAs) with [^{32}P] γ deoxycytidine triphosphate (specific activity, 111 tetraBq/mmol (DuPont NEN) using

the Random Primed DNA Labeling Kit (Boehringer Mannheim, Germany). The quantity of mRNA on radiographs was measured either by Molecular Imager (Bio-Rad Laboratories, Inc.) or by Fujix Bioimage Analyser (BAS 2000, Fuji Photo Film Co., Ltd., Tokyo, Japan). The hybridized membranes were reprobed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA to assure uniformity of RNA transfer. Data from the quantitation of GAPDH was used to correct the results of S14 and ME analyzes.

Statistics

Values are reported as mean ± SD *P* values were calculated by an ANOVA unpaired *t* test using the Statview 4.5 software (Abacus, Berkely, CA). *P* values ≥ 0.05 were considered not significant. Note that slight differences in number of animals in each group are due to loss of an animal, tissue or insufficient sample.

Results

Tests of thyroid function (T_4 and TSH)

As shown previously (9), the absence of TRβ reduced the sensitivity of the pituitary thyrotrophs to TH as indicated by the higher concentration of serum TSH in TRβ−/− mice, despite concomitant elevation of serum T_4 level. The untreated mice had at baseline, mean ± SD values of serum TSH of 0.19 ± 0.14 and 1.05 ± 0.51 ng/ml, respectively in TRβ+/+ and TRβ−/− mice, whereas T_4 levels were 4.34 ± 0.86 and 8.91 ± 2.83 μg/dl in TRβ+/+ and TRβ−/− mice, respectively (Table 1).

Fourteen days of a LoI/PTU diet, resulted in an expected decline of serum T_4 concentration equally in both TRβ+/+ and TRβ−/− mice (1.35 ± 0.21 and 1.34 ± 0.43 μg/dl, respectively), whereas the serum TSH concentration increased dramatically in both groups of mice being not significantly higher in the TRβ+/+ mice. Treatment with 0.5 μg L-T_3 for 4 days resulted in suppression of the serum TSH in TRβ +/+ (0.28 ± 0.01 ng/ml) and to a lesser degree in the TRβ−/− mice (0.50 ± 0.26 ng/ml, $P < 0.005$). Higher doses of L-T_3 (5.5 and 25 μg/mouse/day for 4 days suppressed the TSH to undetectable levels (<0.02 ng/ml) in the TRβ+/+ mice but was unable to do so in the TRβ−/− mice (Table 1).

Serum cholesterol and alkaline phosphatase

Cholesterol levels (Fig. 1, *upper panel*) were slightly but significantly higher in the untreated TRβ−/− mice compared with the TRβ+/+ mice (110 ± 17 *vs.* 98 ± 20 mg/dl, $P < 0.03$) despite the elevated serum T_4 level in the TRβ−/− mice. While TH deprivation resulted in a significant increase in serum cholesterol in TRβ+/+ mice (137 ± 48 mg/dl, $P < 0.02$), a paradoxical decrease in cholesterol (85 ± 8 mg/dl, $P < 0.0005$) was observed in TRβ−/− mice. Treatment with T_3 (0.5, 5.5, or 25 μg/mouse) resulted in a significant reduction in serum cholesterol in TRβ+/+ but not in the TRβ−/− mice.

There were no differences in the serum AP of TRβ+/+ and TRβ−/− mice at baseline before treatment (Fig. 1, *lower panel*). TH deprivation resulted in a decrease in serum AP concentration in TRβ+/+ mice from 154 ± 36 to 106 ± 24 IU/liter ($P < 0.03$). This was not observed in TRβ−/− mice. In TRβ+/+ mice, treatment with L-T_3 (0.5, 5.5 and 25 μg) produced a dose-dependent increase in the serum AP concentration (171 ± 28, 271 ± 72, and 351 ± 146 IU/liter, respectively). In TRβ−/− mice there was no significant change in the serum AP except with the highest L-T_3 dose of 25 μg/day (Fig. 1, *lower panel*)

The decrease in serum AP concentration in TRβ+/+ mice during TH deprivation was predominantly due to a decline in the liver isozyme, from 47% to 17% of the total AP (Table 2). In contrast, in the TRβ−/− mice, the liver isozyme remained unchanged and was 50 and 63% of the total AP prior and during TH deprivation, respectively. In both TRβ+/+ and TRβ−/− mice there was appearance of intestinal AP isozymes during TH deprivation accounting for 9% of the total.

Heart rate and energy expenditure

At baseline, before treatment, the HR of TRβ−/− mice was significantly higher than that of TRβ+/+ mice (533 ± 48 and 480 ± 46 beats/min $P < 0.005$; Fig. 2, *upper panel*). This increase in HR is consistent with the slightly higher serum T_4 observed in the TRβ−/− mice. TH deprivation resulted in a decreased HR in both TRβ+/+ and TRβ−/− mice (270 ± 65 and 349 ± 88, respectively) with obliteration of the significant difference between the two types of mice. L-T_3 treatment produced an increase in HR of equal magnitude in both TRβ+/+ and TRβ−/− mice (Fig. 2, *upper panel*).

There was no significant difference in the EE between TRβ +/+ and TRβ−/− mice at baseline, before treatment (0.444 ± 0.1 and 0.456 ± 0.088 Kcal/day/g BW, respectively). In both TRβ+/+ and TRβ−/− mice there was a similar and significant decrease in EE after 10 to 14 days of TH deprivation (0.226 ± 0.058 and 0.248 ± 0.057 Kcal/day/g BW, respectively). L-T_3 treatment produced a significant increase

TABLE 1. T_4 and TSH at baseline, during TH deprivation and T_3 treatment

	Total T_4 (μg/dl)		TSH (ng/ml)	
	TRβ +/+	TRβ −/−	TRβ +/+	TRβ −/−
Basal (untreated)	4.34 ± 0.86[a] (19)	8.91 ± 2.83[a,b] (29)	0.19 ± 0.14[a] (13)	1.05 ± 0.51[a,b](13)
LoI/PTU	1.35 ± 0.21 (4)	1.34 ± 0.43 (7)	17.80 ± 2.04 (4)	10.94 ± 4.80 (7)
LoI/PTU + 0.5 μg T_3	0.5 ± 0.07[e] (5)	1.22 ± 0.59[c] (6)	0.28 ± 0.01[e] (5)	0.50 ± 0.26[d,e] (6)
LoI/PTU + 5.5 μg T_3	<0.5[e] (5)	0.88 ± 0.23[d,f] (8)	<0.02[e] (5)	0.64 ± 0.39[d,e] (8)
LoI/PTU + 25 μg T_3	<0.5[e] (4)	0.93 ± 0.23[d,f] (8)	<0.02[e] (4)	0.23 ± 0.16[e,e] (8)

[a] Statistical differences between values obtained before treatment and TH deprivation by LoI/PTU diet for each mouse type ([a] $P < 0.0005$).
[b–d], Statistical differences between TRβ +/+ and TRβ −/− mice at baseline and with each treatment ([c] $P < 0.05$; [d] $P < 0.005$; [b] $P < 0.0005$).
[e] and [f] Statistical differences for each mouse type between each T_3 dose and no T_3 treatment while maintained on LoI/PTU diet ([f] $P < 0.05$; [e] $P < 0.0005$).
Numbers *in parentheses* are the numbers of animals in each group.

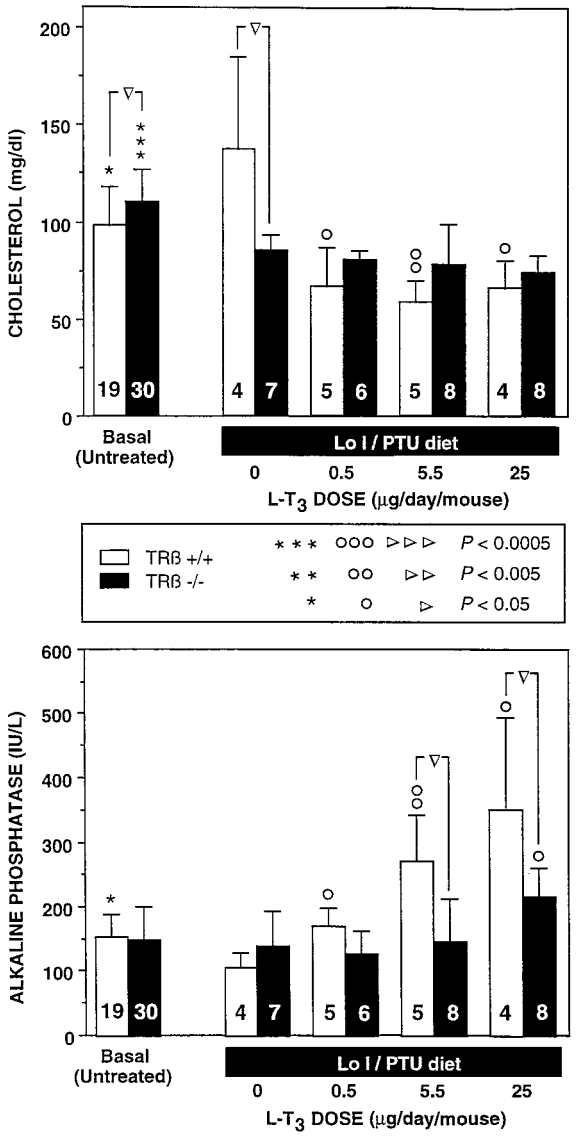

FIG. 1. Effect of TH deprivation and L-T_3 treatment on serum cholesterol (*upper panel*) and AP (*lower panel*) concentrations in TRβ+/+ and TRβ−/− mice. Determinations were performed on serum samples obtained at baseline, before treatment, and following a LoI/PTU diet fed for 14 days during which different doses of L-T_3 were given to groups of animals for the last 4 days on this regimen. Results are expressed as the mean ± SD, and the *numbers in the bars* are the number of mice in each group. The *asterisk* indicates statistical differences between values obtained before treatment and after TH deprivation by LoI/PTU diet for each mouse type. The *open circle* indicates statistical differences for each mouse type between each T_3 dose and no T_3 treatment while maintained on LoI/PTU diet. The *inverted triangle* (▽) indicates statistical differences between TRβ+/+ and TRβ−/− mice at baseline, before treatment, and with each treatment dose.

in EE, which was not different in both types of mice (Fig. 2, *lower panel*).

T_3 binding to liver nuclei

Results of T_3 binding activity of TR are shown in Fig. 3. The MBC in liver of TRβ+/+ was 72 fmol/100 μg DNA with a Ka of 7.1×10^9 M^{-1}. These values were almost identical to those reported in rat liver (23). In TRβ−/− mice the MBC was markedly reduced to 24% the TRβ+/+ mice and the Ka was slightly lower at 3.9×10^9 M^{-1}. Based on previous observations that TRα contributes 13% of the total TR activity in rat liver (2), the present data suggest that there is no compensatory increase in TRα in the liver of TRβ−/− mice.

TABLE 2. Serum isozymes of alkaline phosphatase (U/ml) before (basal) and during TH deprivation[a]

	Total	Liver	Bone	Intestine
TRβ +/+				
Basal	200	93	107	0
TH Deprivation	87	15	64	8
TRβ −/−				
Basal	163	81	82	0
TH Deprivation	135	85	38	12

[a] Measurements were done on pooled serum from mice in each group (N = 5 to 8).

Effect of L-T_3 on TH responsive genes in liver

S14 and ME are well characterized TH responsive genes the transcriptional regulation of which has been extensively studied (21, 22, 24). We determined the abundance of their mRNAs in the liver of TRβ+/+ and TRβ−/− mice by Northern blotting (Figs. 4 and 5). At baseline, before treatment, there was no difference between TRβ+/+ and TRβ−/− mice in the quantity of cytoplasmic S14 or ME mRNAs despite the elevated serum T_4 level in the TRβ−/− mice.

At baseline, before treatment, there was no difference in the amount of S14 mRNA accumulated in the livers of TRβ+/+ and TRβ−/− mice. In TRβ+/+ mice with TH deprivation, the mean decrement in the abundance of S14 mRNA of 2.0 arbitrary units (AU) (from 2.5 ± 1.1 at baseline to 0.5 ± 0.2 on LoI/PTU diet) was similar to that of 2.1 AU observed in TRβ−/− mice (from 3.5 ± 1.5 to 1.4 ± 1.1). Administration of L-T_3 to TH deprived mice increased the abundance of S14 mRNA in the TRβ+/+ mice but was without effect in the TRβ−/− mice even at the highest dose (Fig. 4, *upper panel*, and Fig. 5, *upper row*).

TH deprivation was without effect on the abundance of ME mRNA in either the TRβ+/+ mice or TRβ−/− mice, the decline in the former group not being statistically significant. Similar to the response of S14 to L-T_3 treatment, ME mRNA increased significantly in the TRβ+/+ mice but not in the TRβ−/− mice (Fig. 4, *lower panel*, and Fig. 5, *lower row*).

In summary, the absence of TRβ does not produce compensatory increase in the amount of TRα in liver nuclei. TRβ appears not to be necessary for the down-regulation of S14 mRNA during TH deprivation. On the other hand, the T_3 mediated up-regulation of S14 and ME mRNA requires the presence of TRβ.

Discussion

TH exerts its effects by interacting with a specific nuclear TRs that exist as three main isoforms: TRα1, TRβ1, and TRβ2 (5). These ligand-dependent nuclear transcription factors regulate target genes by binding to specific thyroid hormone response elements, either as homodimers or as heterodimers

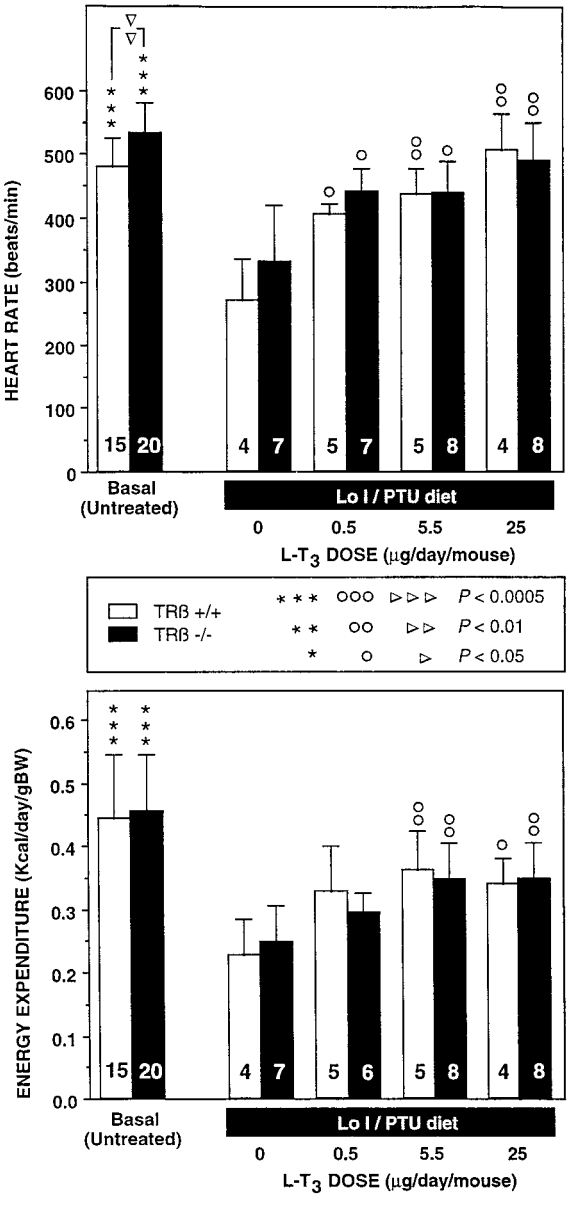

FIG. 2. Effect of TH deprivation and L-T_3 treatment on heart rate (*upper panel*) and energy expenditure (*lower panel*) in TRβ+/+ and TRβ−/− mice. Results are expressed as the mean ± SD and the numbers in the bars are the number of mice in each group. The *asterisk* indicates statistical differences between values obtained before treatment and after TH deprivation by LoI/PTU diet for each mouse type. The *open circle* indicates statistical differences for each mouse type between each T_3 dose and no T_3 treatment while maintained on LoI/PTU diet. The *inverted triangle* indicates statistical differences between TRβ+/+ and TRβ−/− mice at baseline, before treatment, and with each treatment dose.

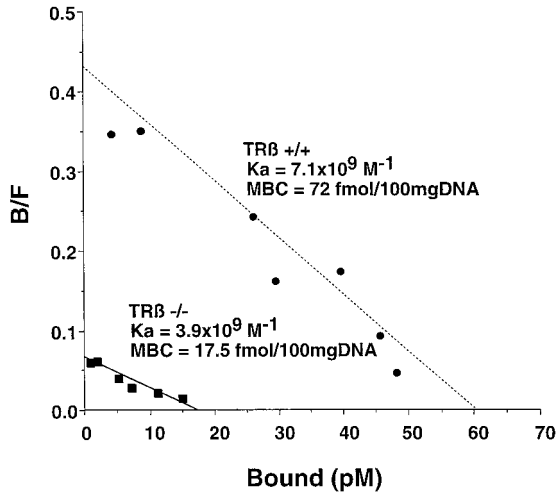

FIG. 3. T_3 binding to liver nuclei. Scatchard analysis of [^{125}I] T_3 binding to nuclei from TRβ+/+ and TRβ−/− mice. Ka, association constant. MBC, maximal T_3-binding capacity.

in association with retinoid X receptors (25, 26). Recent studies indicate that TRs mediate both TH-independent and TH-dependent transcriptional control, possibly through association with corepressors and coactivators (27–31).

Ample experimental evidence from *in vitro* studies indicates that unliganded TRs exert a silencing effect on genes regulated positively by TH (32–35) and it has been suggested that unliganded TRs have constitutively stimulating effect on genes regulated negatively by TH (6, 7, 36). That the former effect has physiological relevance and is dependent on the concentration of TR has been recently shown *in vivo* by the over expression of TRβ in mouse liver (37).

Because both TRα and TRβ genes are expressed in most tissues, either one or both can be implicated in the mediation of TH-dependent suppression (TSH and cholesterol) or stimulation (AP, EE, heart rate, S14, and ME) *in vivo*. Experiments were designed to determine the role of TRβ, the quantitative effect of total unliganded TR, and the ability of TH to regulate the above mentioned markers of TH action using TRβ−/− and TRβ+/+ mice. The targeted mutation in the mouse TRβ gene inactivates its function, but leaves the 5′ end of the coding reigon intact (9). In these TRβ−/− mice, some expression of the amino terminal fragment of the TRβ1, and possibly of the TRβ2 could be demonstrated by immunohistochemical analyzes of brain tissue (38). Although the physiological implication of this truncated peptide is unknown it does not bind to DNA or TH, and lacks the domains necessary for dimerization and interaction with corepressors and coactivators.

TH action on HR may be indirectly mediated by its effect on β (39) or α (40) adrenergic receptors, extranuclear effects on glucose metabolism and sodium transport, and nuclear effects by regulating transcription of TH responsive genes. Furthermore TH effect on HR may be mediated via the nervous tissue at the level of the cardiac pacemaker. Although TRα and TRβ are both expressed in heart (41), the relative contribution of each of these receptors to TH action in the heart is unknown. Observations of the influence of TH on the heart and EE in subjects from the single family with RTH due to TRβ gene deletion are not definitive but, as in other patients with RTH, there is a trend for increase in HR at baseline and variable effect on the basal metabolic rate (11, 42). Thus, the TRβ deficient mouse provided the opportunity to examine the role of TRβ on the regulation of HR and EE under conditions of TH deprivation and excess. The chronotropic effect of TH on the heart appears independent of the TRβ. Explanation of the observations noted here is consistent with

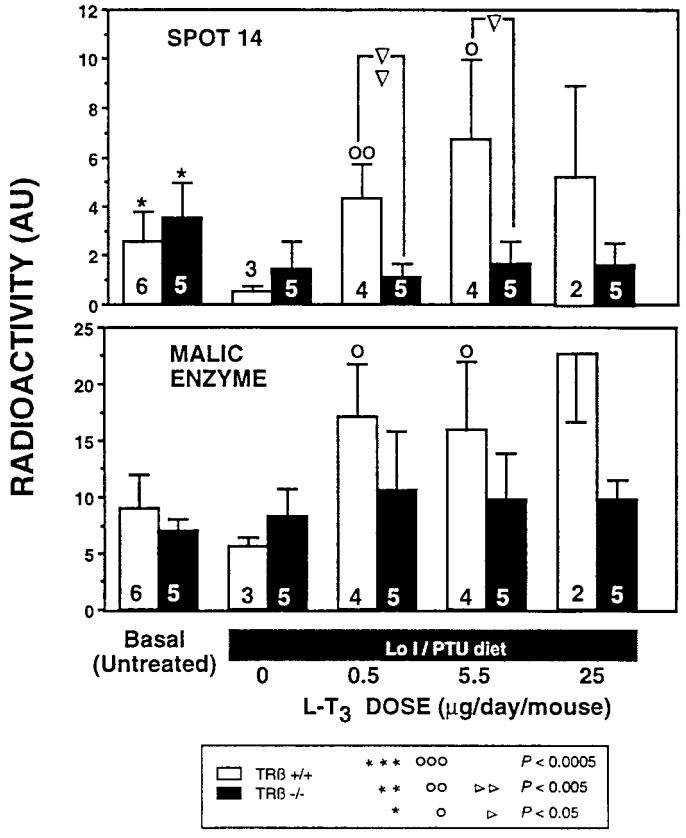

FIG. 4. Effect of TH deprivation and L-T_3 treatment on the abundance of liver mRNA of S14 (*upper panel*) and ME (*lower panel*) in TRβ+/+ and TRβ−/− mice. Results are expressed as mean arbitrary units (AU) ± SD, and the *numbers in the bars* are the number of mice in each group. Data were corrected for the amount of mRNA loaded and transferred onto the membrane by hybridization with a probe to GAPDH. The *asterisk* indicates statistical differences between values obtained before treatment and after TH deprivation by LoI/PTU diet for each mouse type. The *open circle* indicates statistical differences for each mouse type between each T_3 dose and no T_3 treatment while maintained on LoI/PTU diet. The *inverted triangle* indicates statistical differences between TRβ+/+ and TRβ−/− mice at baseline, before treatment, and with each treatment dose.

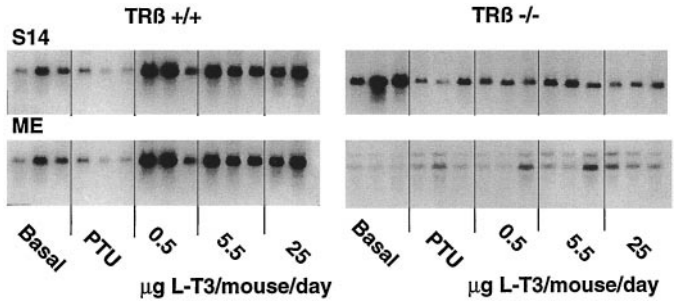

FIG. 5. Northern blot analysis of liver mRNAs from TRβ+/+ mice (*left panels*) and TRβ−/− mice (*right panels*) probed with [^{32}P] labeled S14 and ME cDNAs. Results of samples from three mice belonging to each treatment group are shown. Data from these and additional animals were quantitated and mean results are shown in Fig. 4.

TH action on the heart or nervous tissue controlling the pacing of the heart mediated by TRα. Recent demonstration that the mice lacking TRα1, have on the average HR lower than that of control animals, both before and after L-T_3 treatment, supports this hypothesis (43). The effect of TH on energy expenditure is no doubt multigenic. However, it appears that TRβ is not required to reduce the EE during TH deprivation and to produce an increase in EE in response to L-T_3 treatment. This is based on the observation that TH dependent changes in EE were not significantly different in TRβ +/+ and TRβ−/− mice and the finding of higher EE in TRβ−/− mice before treatment when their serum T_4 concentration was elevated.

We have previously reported that fibroblasts from subjects lacking the TRβ gene show no compensatory increase in TRα1 mRNA (44). Similarly, it has been shown that in TRβ−/− mice there is no obvious compensatory alteration in the levels of TRα1 or TRα2 mRNA in a variety of tissues including the liver (9). This was now confirmed at the protein level by the finding of 76% reduction in total nuclear T_3-binding capacity in liver of TRβ−/− mice that corresponds to the proportion of the TRβ isoform in liver nuclei of intact rats determined by isoform specific immunoprecipitation in the intact animal (2, 23). Thus, TRα mediated effect in the liver of TRβ−/− mouse reflects the contribution of this isoform in the normal TRβ+/+ mouse. However, we cannot fully exclude the possibility that blunting in ligand dependent and independent responses of some of the TH regulated markers may not be due to the overall reduction in the quantity of TR brought about by absence of TRβ.

Serum levels of cholesterol seem to be mainly regulated by TH action on the liver (37). Serum cholesterol changes in response to TH are similar to those of TSH in the TRβ+/+ mouse. Indeed, hypothyroidism increases serum cholesterol and treatment with excess L-T_3 results in a decrease. We have previously shown that up-regulation of TSH is independent of TRβ in this TRβ deficient mouse model (10), however this is not true for cholesterol. In fact, in the absence of TRβ there is a paradoxical decrease in serum cholesterol concentration. Since it has been demonstrated that TH stimulates the transcription of low-density lipoprotein receptor and cholesterol 7-α hydroxylase genes, thereby enhancing the removal of LDL and cholesterol (45, 46), differential action of TRβ and TRα on the expression of these genes should be determined in the future. TH is known to regulate serum AP concentration. In this study the observed changes in serum AP also appear to be mainly regulated by the effect of TH on liver (Table 2). In TRβ−/− mice, TH deprivation did not reduce the serum AP level and TH produced a minimal increase. While this suggests a TRβ mediated regulation of liver AP, it is interesting to note that modulation of the expression of the AP gene by TRα was demonstrated in TRα- null ES cells (47).

Transcription of S14 and ME genes is regulated by TH (21, 22, 24). This report demonstrates the differential roles of TRβ and TRα in the regulation of their expression. The suppression of S14 in the absence of ligand is not reduced by the absence of TRβ. In contrast, TH mediated accumulation of S14 and ME mRNA beyond baseline appears to be dependent only on TRβ.

This report demonstrates that S14 is an example of a TH responsive gene in the liver that maintains ligand independent suppression in the absence of TRβ. This is the first *in vivo*

demonstration of such effect. TH-mediated accumulation of S14 and ME mRNA in the presence of ligand excess appears to totally depend on the presence of TRβ. TH-mediated increase of serum AP was minimally dependent on TRβ.

TH action in tissues is therefore dependent to a variable degree on the presence of the TRβ. Whether the responses to TH observed in the absence of TRβ are due to interaction of TH with TRα or another yet unidentified TR, is unknown. Furthermore, because the total number of TRs is substantially decreased in the liver of TRβ−/− mice due to the lack of compensatory increase in TRα1, it is unclear to what extent the total amount of TR modulates the response of specific TH regulated genes. Nevertheless, based on data presented herein, TH regulated genes could be fully dependent, partially dependent or independent of TRβ. Serum cholesterol and liver ME mRNA fall under the first category because both up and down regulation was not observed in the absence of TRβ. The response of S14 mRNA was partially dependent on TRβ since down regulation during TH deprivation was not affected while up-regulation was blunted. The preservation of normal HR and EE responses to TH deprivation and excess indicated independence of TRβ.

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