Thyroid function and effect of aging in combined hetero/homozygous mice deficient in thyroid hormone receptors α and β genes

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Abstract

The maintenance of thyroid hormone (TH) homeostasis is dependent on the synthesis and secretion of TH regulated by TSH. This is achieved, in turn, by the negative feedback of TH on TSH secretion and synthesis, which requires the interaction with TH receptors (TRs). Derived by alternative splicing of two gene transcription products, three TRs (TR β 1, TR β 2 and TR α 1) interact with TH while another, TR α 2, binds to DNA but not to TH. In this study we compare the results of thyroid function tests in mice with deletions of the TR α and TR β genes alone and present novel data on mice that are double homozygous and combined heterozygous. Homozygous deletions of both the TR α and TR β in the same mouse (TR α o/o; TR β –/–) resulted in serum TSH values only slightly lower than those in athyreotic, Pax8 knockout mice. Whereas the absence of TR α alone does not cause resistance to TH, the absence of TR β in the presence of TR α results in a 205, 169, 544% increase in serum

thyroxine (T₄), triiodothyronine (T₃) and TSH concentrations respectively. However, in the absence of TR β , loss of one TR α allele can worsen the resistance to TH with a 243 and 307% increase in T₄ and T₃ respectively. Similarly, while the heterozygous mouse with a single TR β allele shows no alteration in thyroid function, the concomitant deletion of TR α brings about mild but significant resistance to TH. Furthermore, the severity of the resistance to TH was noted to decrease with age in parallel with the decrease in serum free T₄ values also seen in wild-type mice. These results demonstrate that (1) unliganded TR α or TR β are not absolutely necessary for the upregulation of TSH; (2) TR β but not TR α is sufficient for TH-mediated downregulation of TSH; and (3) TR α may partially substitute for TR β in mediating a partial TH-dependent TSH suppression.

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Introduction

Thyroid hormone (TH) is essential for normal growth and development as well as for the maintenance of metabolic activity in the adult. TH secretion by the thyroid gland is under the control of pituitary thyrotropin (TSH). An important regulator of TSH synthesis and secretion is the negative feedback of TH on TSH gene transcription. This is mediated by intranuclear TH receptors (TRs). Two TR genes have been identified, TR α and TR β , each generating two major proteins by alternative splicing. Three of these, α 1, β 1 and β 2, bind TH (Sap *et al.* 1986, Weinberger *et al.* 1986, Hodin *et al.* 1989, Lazar 1993). A fourth product, TR α 2, binds to thyroid hormone response elements but, due to a sequence difference at the ligand binding domain, it does not bind TH or function as a TR

proper (Mitsushashi *et al.* 1988). Additional, minor transcripts of the TR α gene that do not bind TR and may act as competitive inhibitors of TH action are $\Delta\alpha$ 1, $\Delta\alpha$ 2 (Chassande *et al.* 1997) and rev-erbA α (Spanjaard *et al.* 1994). Two additional TR β isoforms which are a result of alternative splicing have been described in most rat tissues (except testes) – TR β 3 which acts as a functional TR and TR β Δ3 that lacks the DNA binding domain but retains a ligand binding activity and has a potent dominant-negative effect (Williams 2000). The four principal TR proteins are expressed in the pituitary but their relative role in the regulation of the pituitary–thyroid axis has not been clearly defined. The study of this axis in mice deficient in one or more of the TR genes provides a means to determine the functional role of the individual receptors. Several knock-out mice with deletions of the TR α and TR β in various

combinations have been generated (Forrest *et al.* 1996b, Fraichard *et al.* 1997, Wikström *et al.* 1998, Gauthier *et al.* 1999, Göther *et al.* 1999). However, comparison of the serum TH and TSH levels in these mice as a means to assess the sensitivity of feedback regulation requires measurements using the same assays. This is particularly important for the determination of TSH concentration in mouse serum. In this report we describe the effect of deletion of one or more TRs and the relative importance of TR α and TR β in the regulation of the pituitary–thyroid axis in the mouse. We demonstrate that mice deficient in TR β become even more resistant to TH when one of the TR α alleles is also deleted. Furthermore, mice completely deficient in TR α , which by itself does not produce a TH-resistant phenotype, become TH resistant when one of the TR β genes is deleted. Our results suggest functional overlap of TR isoforms and dose dependency in the feedback inhibition of pituitary TSH.

Materials and Methods

Mice

Mice were weaned four weeks after birth and fed a rodent diet (No 5053; Lab Diet, Brentwood, MO, USA) containing 0.53 ppm iodine and were given tap water available *ad libitum*. They were housed, 3 to 5 mice per cage, in a controlled environment at 19 °C and under 12 h alternating darkness and artificial light cycles. All animal experiments were performed according to approved protocols at the University of Chicago by the Institutional Animal Care and Use Committee and the Ecole Normale Supérieure de Lyon.

Male mice were used exclusively because of previously reported sex differences in thyroid function tests within the same strain (Pohlenz *et al.* 1999). Mice were 39–140 days old at the time of blood sampling, with the following exceptions: (1) Pax8 $^{-/-}$ mice were 3 weeks old because, if untreated, survival declines rapidly after this age; (2) the effect of age was studied in male mice 2, 3, 6, 9, 12 and 20 weeks old. In mice aged 6 weeks and older, 300 μ l blood were obtained by retroorbital vein puncture under light methoxyflurane (Pitman Moore, Mundelein, IL, USA) anesthesia. Bleeding was generally carried out between 0900 h and 1200 h. Serum was separated by centrifugation and stored at -20 °C until analyzed.

The TR β knockout mice were produced by insertion of the LacZ–NeoR cassette downstream to the splice site of exon 4, eliminating the expression of the DNA and ligand binding domains of TR β 1 and TR β 2 (TR β $^{-/-}$) (Gauthier *et al.* 1999). The TR α knockout mice were produced by insertion of the LacZ–NeoR cassette downstream to exon 3 and replacing exons 5 through 7, thus effectively abolishing not only the generation of TR α 1 and TR α 2 transcripts but also that of TR $\Delta\alpha$ 1 or TR $\Delta\alpha$ 2 (TR α o/o) by removal of their transcription start point at

intron 7 (Gauthier *et al.* 2001). The gene sequence for rev-erbA α protein encoded by the opposite strands for the TR α (Spanjaard *et al.* 1994) remains intact. In both sets of mice the recombinant embryonic stem (ES) cells were derived from 129sv mice and were implanted into C57BL/6 recipient blastocysts. C57BL/6 mice were mated to each chimeric mouse and then back-crossed 3–4 times into the same strain, thereby diluting the 129sv background. Separate wild-type mice were maintained for the TR α o/o ($n=18$) and β $^{-/-}$ ($n=54$) mice. Wild-type mice were also produced from backgrounds of the Pax8 knockout mice (Pax8 $^{-/-}$) ($n=7$) and separate wild-type with the background of the SRC-1 knockout (SRC-1 $^{-/-}$) mice ($n=17$). The SRC-1 $^{-/-}$ and the Pax8 $^{-/-}$ mice were also produced with recombinant ES cells from 129Sv mice and were implanted into C57BL/6-derived blastocysts. Offspring were outbred into C57BL/6 mice at least 5 times to dilute the 129sv background. Thyroid function tests for each of these wild-type mice are reported separately, but there was no significant difference in the values among them. The serum samples from Pax8 $^{-/-}$ mice were provided by Drs Ahmed Mansouri and Peter Grüss (Mansouri *et al.* 1998) and those from SRC-1 $^{-/-}$ mice were from Drs Jianming Xu and Bert O'Malley (Weiss *et al.* 1999).

Induction of hypothyroidism and treatment with TH

TH deficiency was induced in 5 male mice of each type (wild-type, TR α o/o, and TR β $^{-/-}$) with a low iodine (LoI) diet containing 0.15% 5-propyl-2-thiouracil (PTU) as described (Weiss *et al.* 1997). On the 14th day of PTU treatment, TSH releasing hormone (TRH) stimulation tests were performed in these mice as well as in a similar number of mice from each genotype maintained on a normal diet. Blood samples were obtained from the tail vein before and 15 min after the i.p. administration of 0.275 μ g TRH. We had previously determined that the peak response of TSH to TRH is at 15 min (data not shown).

In a separate experiment, 15 mice from each group received the PTU/LoI diet and 15 mice from each group were placed on a normal diet. On the 11th day, mice of each genotype on the different diets were divided into 3 groups (5 mice per group). The mice fed the normal diet were given daily i.p. injections of vehicle, 0.2 or 0.8 μ g L-triiodothyronine (L-T $_3$)/100 g body weight for 4 days while the PTU-fed mice received daily i.p. injections of vehicle, 1.0 or 2.5 μ g L-T $_3$ /100 g body weight for 4 days with the PTU diet given throughout the L-T $_3$ and vehicle treatment period. On the morning of the 5th day, TRH stimulation tests were performed as described above. Serum T $_3$ concentrations achieved at different times after administration of L-T $_3$ were not different among the different genotypes of mice (Macchia *et al.* 2001).

Hormone measurements

Serum total thyroxine (TT₄) and T₃ (TT₃) concentrations were measured by coated tubes RIAs (DPC, Diagnostic Products, Los Angeles, CA, USA) using 25 and 50 μ l serum respectively. The sensitivities of these assays are 0.2 μ g T₄/dl (2.6 nmol/l) and 20 ng T₃/dl (0.3 nmol/l). The inter-assay coefficients of variation are 5.4, 4.2 and 3.6% at 3.8, 9.4 and 13.7 μ g/dl for T₄ and 7.7, 7.1 and 6.2% at 32, 53 and 110 ng/dl for T₃. Free T₄ (FT₄) and free T₃ (FT₃) were estimated by calculating the free indexes (FT₄I and FT₃I) using the respective total hormone values and the resin T₄ uptake ratio (Robin *et al.* 1971).

Serum TSH was measured in 50 μ l serum using a sensitive, heterologous, disequilibrium, double antibody precipitation RIA as described (Pohlenz *et al.* 1999). The sensitivity of this assay was 5–10 mU/l. The intra-assay and interassay coefficients of variation were respectively: 16 and 27% at 20 mU/l, 6.3 and 8.2% at 200 mU/l, 5.4 and 9.8% at 850 mU/l, 10 and 24% at 2000 mU/l. Samples containing more than 1000 and more than 10 000 mU TSH/l were diluted 10- to 100-fold, respectively, with '0' TSH mouse serum obtained from wild-type mice treated with a suppressive dose of T₄ (Pohlenz *et al.* 1999).

Data analysis

Values are reported as means $\pm$ standard deviation or standard error where indicated. *P* values were calculated by two-way ANOVA when comparing mice of different genotypes and treatment, and by the unpaired Student's *t*-test when comparisons were made within the same genotype, using the Statview 5.0 program (SAS Institute, Inc., Cary, NC, USA).

Results

Thyroid function tests were determined in mice lacking various combinations of the TR α and TR β genes (Table 1). Four wild-type substrains of C57BL/6 mice were also evaluated that corresponded to the TR α , TR β , Pax8 $^{-/-}$ and SRC-1 $^{-/-}$ mice; however there was no significant difference in any of the determinations among these 4 substrains of mice (Fig. 1). Data from the wild-type mice matched to the TR α and TR β knockout mice were combined into a single group since they were generated by backcrossing with the same strain (Table 1, wild-type). Elevation of TH levels without suppression of serum TSH is usually indicative of resistance to TH at the level of the thyrotropes. Mice completely deficient in TR α gene products (TR α o/o) showed no important differences in basal thyroid function tests compared with

the wild-type mice except for slightly but significantly lower serum T₄ concentrations. The lower mean FT₄I and FT₃I did not reach statistical significance. In contrast, there was a clear elevation in FT₄I (2.5-fold), FT₃I (1.6-fold) and TSH (5.4-fold) ($P < 0.0005$) in the homozygous TR β $^{-/-}$ mice, compatible with resistance to TH. The mice homozygous for the combined TR α and TR β deletions displayed higher TH levels even though the absence of TR α alone did not produce TH resistance. FT₄I, FT₃I, and TSH values were, respectively, 14, 124 and 822 times higher than those of wild-type mice. The elevation of TSH in the combined TR α /TR β knockout mice was only half that found in the athyreotic Pax8 gene knockout mice (Pohlenz *et al.* 1999) (Fig. 1).

Mice heterozygous for a deletion of only one allele of the TR β (TR β $^{-/+}$) or TR α (TR α o/+) genes have thyroid function tests that are not different from the wild-type mice (Forrest *et al.* 1996b, Gauthier *et al.* 1999). However, when one allele of the TR β is absent in combination with complete deletion of TR α (which alone causes no TH resistance), increases of 1.5-fold in FT₄I, 1.3-fold in FT₃I and 1.7-fold in TSH were observed compared with wild-type mice, suggestive of TH resistance (Table 2 and Fig. 1). Similarly, when one allele of the TR α was absent together with complete deletion of the TR β (TR α o/+;TR β $^{-/-}$), the mice displayed a further 1.7-, 1.8- and 3.6-fold increase in the FT₄I, FT₃I and TSH respectively compared with TR β $^{-/-}$ mice with preservation of both TR α alleles (Table 2 and Fig. 1). This suggests that TR α may act to attenuate the resistance to TH at the level of the pituitary.

FT₄I levels decrease with age in the TR α o/o, TR β $^{-/-}$ and in TR α o/o;TR β $^{-/-}$ mice as seen in wild-type mice (Fig. 2, Table 3). However results in the double knockout mouse are based on only two animals. The FT₄I levels at 2 weeks of age were consistently and significantly higher than at older ages. Wild-type and TR β $^{-/-}$ mice had higher FT₄I levels at 6 weeks compared with older mice at 20 weeks, whereas the TR α o/o mice were not different at these time points.

The responses of serum TSH to TRH in intact and TH-deprived mice are shown in Fig. 3. Mice fed a normal diet had robust TSH responses to TRH administration. Although all three genotypes (wild-type, TR α o/o, TR β $^{-/-}$) reached a similar peak TSH value, the percentage increment was significantly less for the TR β $^{-/-}$ compared with wild-type (only 4.1% $\pm$ 1.4 versus 69.5% $\pm$ 26.7, $P = 0.006$) or TR α o/o (80.8% $\pm$ 61.9, $P = 0.026$). However TH-deprived mice had no TSH response to TRH stimulation. The lack of a significant increase in TSH after TRH stimulation is contrary to the hyper-responsiveness seen in hypothyroid humans (Saberri & Utiger 1975).

The responses of serum TSH to TRH before and after the administration of two incremental doses of L-T₃ are shown in Fig. 4. In mice fed a normal diet, the higher dose

Table 1 Thyroid function tests in male TRα and TRβ knockout mice. Results are means ± standard deviation, numbers in parentheses=number of mice; numbers in square brackets=range of ages

Genotype	β1	β2	α1	α2	TT ₄ (μg/dl)	FT ₄ I (units)	TT ₃ (ng/dl)	FT ₃ I (units)	TSH (μU/ml)	Age (days)
αα/+	+	+	1/2	1/2	3.7 ± 1.3 (6)	5.4 ± 2.0 (6)	117 ± 18 (4) ^b	172 ± 26 (6) ^b	27 ± 46 (4)	75 [42–140]
αα/o	+	+	–	–	3.3 ± 0.5 (30) ^a	4.7 ± 0.7 (15)	96 ± 24 (11)	134 ± 28 (9)	27 ± 22 (31)	59 [39–78]
αα/o;β–/+	1/2	1/2	–	–	5.8 ± 0.9 (7) ^b	8.2 ± 2.0 (7) ^b	107 ± 28 (7) ^c	148 ± 25 (7)	42.5 ± 20 (7) ^c	75 [42–140]
β–/+	1/2	1/2	+	+	4.3 ± 0.7 (9)	5.9 ± 0.9 (9)	120 ± 12 (9)	162 ± 12 (9)	9.5 ± 8.0 (9)	75 [42–140]
β–/–	–	–	+	+	7.8 ± 2.0 (37) ^b	11.7 ± 4.9 (11) ^b	142 ± 43 (12) ^b	202 ± 74 (10) ^a	136 ± 116 (41) ^b	79 [46–124]
αα/+;β–/–	–	–	1/2	1/2	19.0 ± 6.6 (10) ^b	19.8 ± 7.8 (8) ^b	329 ± 137 (8) ^b	354 ± 156 (8) ^b	486 ± 150 (10) ^b	75 [42–140]
αα/o;β–/–	–	–	–	–	43.7 ± 8.2 (6) ^b	64.8 ± 13.9 (3) ^b	1527 ± 706 (5) ^b	2389 ± 1446 (3)	20560 ± 7895 (6) ^b	66 [21–110]
Pax8–/–	+	+	+	+	0.5 ± 0.2 (3) ^c	0.4 ± 0.2 (3) ^c	65 ± 32 (3) ^b	61 ± 32 (3) ^b	46380 ± 13869 (5) ^c	21 [21–25]
Wild-type ^d	+	+	+	+	3.8 ± 0.7 (46)	4.5 ± 1.2 (12)	84 ± 11 (13)	124 ± 32 (11)	25 ± 20 (54)	74 [43–123]

^aP<0.05, ^bP<0.005, ^cP<0.0005, compared with wild-type. ^d(α+/+,β+/+) wild-type mice.

Figure 1 Relationship of serum FT₄I and TSH in mice lacking one or more TRs (solid symbols; error bars are standard deviation). The shaded area indicates the 4 different wild-type mouse strains tested (open symbols). Wild-type mice were obtained by back-crossing into C57BL/6 mice with TR α o/o (1; $n=18$); TR β -/- (2; $n=54$); Pax8-/- (3; $n=7$); or SRC-1-/- (4; $n=18$) mice to obtain heterozygotes and then back-crossing to obtain wild-type mice. In each group male mice aged 39–140 days were used, except for the Pax8-/- mice which were 21 days of age due to decreased survival past that age.

of L-T₃ suppressed the TSH response to TRH by 50% in the wild-type compared with a 4.3% increase in the TR β -/- mice (2-way ANOVA, $P<0.001$) and a 78% decline in the TR α o/o mice ($P<0.001$). In TH-deprived mice, the higher dose of L-T₃ suppressed the TSH response by >90% in the wild-type, TR β -/- and TR α o/o mice. Collectively, these data indicate that the feedback regulation of the pituitary–thyroid axis of TR β -/- mice is resistant to TH and that the TR α o/o mice are more sensitive to TH.

Of interest is the paradoxical response of TSH to TRH (a 153% increase; 2-way ANOVA, $P=0.0010$) seen in TR β -/- mice on the lower dose of L-T₃ and fed a normal diet, as we reported previously in these mice (Macchia *et al.* 2001) and in humans homozygous for the TR β gene deletion (Refetoff *et al.* 1980). This paradoxical response to L-T₃ is not seen in TR β -/- mice fed a PTU/Lol diet.

Discussion

The relative serum levels of TH and TSH reflect the sensitivity of the pituitary to the feedback regulation of TSH by TH which is, in turn, dependent on the interaction of TH with TRs in the thyrotropes. Selective removal of one or more TR alleles would allow one to determine the role of each TR isoform and its relative importance in the mediation of TH action at the level of the pituitary gland. We present novel data on thyroid function of mice that are double heterozygous or homozygous in all combinations suggesting different and overlapping roles for TR α and TR β . Lack of TR β in the mouse has been shown to cause resistance to TH, i.e. elevated levels of T₄, T₃ and TSH (Forrest 1994, Forrest *et al.* 1996a, Weiss *et al.* 1997).

The upregulation of TSH is relatively independent of the presence of unliganded TR β and TR α genes as

Table 2 Statistical differences (P values) in the thyroid function tests of compound hetero/homozygous TR α and TR β knockout mice^a

Genotype	T ₄	FT ₄ I	T ₃	FT ₃ I	TSH
TR α o/o;TR β +/- vs TR α o/o;TR β +/-	<0.001	<0.001	0.016	0.003	0.020
TR α +/-;TR β -/- vs TR α o/o;TR β -/-	0.05	0.001	0.001	Not significant	Not significant

^aActual values are given in Table 1.

Figure 2 Effect of age on serum FT₄I levels in combined TRα0/o;TRβ−/− mice as compared with TRβ−/−, TRα0/o, and wild-type mice. Individual values are shown for serum FT₄I concentrations in mice of different ages.

Table 3 Effect of age on serum FT₄I values (means ± S.E.) in wild-type, TRα0/o, TRβ−/−, and combined TRα0/o;TRβ−/− mice. Numbers in parentheses=number of mice

Genotype	Age (weeks)					
	2	3	6	9	12	20
Wild-type	11.9 ± 0.9 (5)	9.3 ± 0.4 ^a (6)	7.9 ± 0.6 ^b (6)	8.4 ± 0.5 ^a (4)	5.6 ± 0.3 ^b (3)	5.7 ± 0.7 ^b (7)
TRα0/o	11.6 ± 0.9 (5)	9.9 ± 1.0 ^a (3)	6.8 ± 1.2 ^b (3)	7.6 ± 0.4 ^b (9)	4.2 ± 0.6 ^b (3)	7.2 ± 0.5 ^b (9)
TRβ−/−	60.5 ± 3.2 (5)	30.3 ± 3.6 ^b (6)	19.6 ± 1.7 ^b (6)	19.2 ± 4.3 ^b (4)	6.4 ± 0.9 ^b (3)	10.4 ± 1.1 ^b (7)
TRα0/o;TRβ−/−	Not done	36.9 ± 4.6 (6)	38.9 ± 10.0 (4)	Not done	Not done	5.74; 3.90*

^a*P*<0.05, ^b*P*<0.001, compared with 2-week value of the same genotype.
*Only 2 animals were available from this genotype at this age.

Figure 3 Serum TSH response to TRH. Groups of different mice maintained on a normal diet (right panel) or a diet containing low iodine and PTU (PTU/LoI, left panel) were monitored before (open bars) and 15 min after (shaded bars) TRH stimulation. Individual values are also shown. Mice were studied under PTU/LoI treatment in order to bring baseline values of TSH to the same range in all the genotypes studied. Brackets above bars indicate the *P* values between 0 min and 15 min post TRH injection. NS, not significant, **P* < 0.001. Note the lack of stimulation with TRH in the TH-deprived mice and the difference in the TSH scales in the two panels.

evidenced by the high TSH values attained in mice devoid of these two receptors. This suggests that TRs are involved mainly in the transcriptional repression of TSH and not in TSH gene activation. In fact, the serum TSH values in the completely TR-deficient mice (*TR α o/o*; *TR β -/-*) are not substantially lower than those measured in athyreotic, *Pax8-/-* mice. Relative to values in the wild-type mice, mean TSH levels in TR-deficient mice were 822-fold higher as compared with 1855-fold higher in *Pax8-/-* mice. Since TSH is downregulated by TH, it is the absence of TH that results in increased transcription of TSH mRNA. This has been demonstrated in wild-type hypothyroid mice in which there was an increase in both α subunit and TSH β mRNAs (Chin *et al.* 1985, Gurr *et al.* 1986). This effect appears to require the stimulation by TSH releasing hormone (Abel *et al.* 1999, Shibusawa *et al.* 2000).

Eliminating only one *TR β* allele is not sufficient to produce a phenotype distinct from the wild-type mouse. Furthermore, the absence of *TR α* in the presence of *TR β* does not produce the TH resistant phenotype. However, the absence of only one *TR β* allele in *TR α* -deficient mice results in significant TH resistance. Therefore, the presence of *TR α* compensates for haploinsufficiency resulting from the lack of one *TR β* allele. In other words, *TR α* can partially substitute for *TR β* .

In addition to *TR β 1* and *TR β 2*, a third isoform, *TR β 3*, has been described in rat which could potentially mediate

Figure 4 Effect of TH on the TSH response to TRH. Groups of mice from the 3 phenotypes were treated for 4 days with 2 different doses of *L*-T₃ or with the vehicle only (0). The upper panels show the results for mice fed a normal diet and the bottom panels show results for mice fed a PTU/LoI diet. Data shown are serum TSH values 15 min after TRH administration. The number of mice in each group is indicated within the bars. **P* < 0.001 compared with vehicle for each treatment. Note the paradoxical increase in TSH in the *TR β -/-* mice on a normal diet and receiving 0.2 μ g *L*-T₃/100 g body weight (BW) and the greater suppressive effect of 0.8 μ g *L*-T₃/100 g BW in the *TR α o/o* mice on a normal diet and of 1.0 μ g *L*-T₃/100 g BW in the *TR α o/o* mice on a PTU/LoI diet.

the effects of TH (Williams 2000). This isoform, however, is unlikely to be involved in the TR β knockout mouse, as these mice are generated by removal of exons 4 and 5 (Gauthier *et al.* 1999). These exons are shared by TR β 1, TR β 2, TR β 3 as well as the truncated TR isoforms (TR $\beta\Delta$ 1, TR $\beta\Delta$ 2 and TR $\beta\Delta$ 3). Direct measurement of TR β 3 has not been performed because TR β 3 has not been demonstrated in mouse and sequences are not available for gene amplification. Similarly, in the TR α o/o mouse one needs to be assured that there are no other TR α isoforms expressed in the pituitary that could mediate TH action. The construction of the TR α o/o mouse would not allow for any other known TR α isoforms to be formed (Gauthier *et al.* 2001). Furthermore, whereas TR α 1 and TR α 2 could be detected by RT-PCR in pituitaries of wild-type mice, these isoforms could not be detected in the pituitaries of TR α o/o mice (authors' personal observations).

Differences in the action of TR β and TR α can be due to different levels of expression of the isoforms in the pituitary. TR β is more abundant in the pituitary than TR α in human (Falcone *et al.* 1992) and rat (Hodin *et al.* 1989), which explains the increased magnitude of TH resistance seen in TR β -/- compared with the TR α o/o mice. Data suggesting higher affinity of the T₃-ligand for the TR β than for the TR α 1 isoform may play a role (Schueler *et al.* 1990). Finally, the specificity of interaction of the TR α and TR β with other elements of the transcriptional machinery may be responsible for a dominant role of TR β on TSH regulation. We have previously shown that SRC-1 knockout mice have TH and TSH levels consistent with resistance which is of a milder degree compared with TR β -/- mice (Weiss *et al.* 1999). Although there is no *in vitro* evidence to suggest which TR isoforms interact preferentially with SRC-1, it is possible that isoform-specific recruitment of cofactors is important in the TH-mediated regulation of TSH expression. TR β 2 has impaired functional interactions with corepressors (Hollenberg *et al.* 1996) which may be due to interaction of the amino terminus with coactivators (Oberste-Berghaus *et al.* 2000) or interaction with other corepressors (Yang *et al.* 1999).

The observation that hypothyroid mice, unlike humans, do not hyper-respond to TRH stimulation may reflect the physiological difference between the species and could possibly be due to decreased stored TSH in the pituitary glands of hypothyroid mice. However, as in humans (Refetoff *et al.* 1980), mice deficient in TR β show a paradoxical increase in the TSH response to TRH with low suppressive doses of L-T₃. This was not observed when mice were pretreated with PTU, probably due to depletion of pituitary reserves during hypothyroidism.

A significant inverse relationship between age and serum TH levels has been reported in mice (Eleftheriou 1975, Mobley & Dubuc 1978). This appears to be independent of feedback by TH since it was also observed

in the absence of both TR α and TR β . However, we were able to follow only two mice with the combined TR α o/o;TR β -/- genotype. The results reported herein support casual observations that the hormonal resistance in subjects with resistance to TH seems to improve with age (Refetoff *et al.* 1993). This reflects the tendency of T₄ levels to decrease with age in normal subjects as well as in patients with resistance to TH (Weiss & Refetoff 2000).

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