REVIEW

Do unliganded thyroid hormone receptors have physiological functions?

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Abstract

Thyroid hormone (TH) is required for the development of vertebrates and exerts numerous homeostatic functions in adults. TH acts through nuclear receptors which control the transcription of target genes. Unliganded and liganded thyroid hormone receptors (TRs) have been shown to exert opposite effects on the transcription of target genes *in vitro*. However, the occurrence of an aporeceptor activity *in vivo* and its potential physiological significance has not been clearly addressed. Several data generated using experimental hypothyroidism and thyrotoxicosis in wild type and TR knockout mice support the notion that apoTRs have an intrinsic activity in several tissues. ApoTRs, and in particular TR α 1, are predominant during the early stages of vertebrate development and must be turned into holoTRs for post-natal development to proceed normally. However, the absence of striking alterations of embryonic and fetal development in mice devoid of TRs indicates that apoTRs do not play a fundamental role. During development, as well as in adults, apoTRs rather appears as a system which increases the range of transcriptional responses to moderate variations of T₃.

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Introduction

Thyroid hormone (TH) has important roles in the metamorphosis of amphibians, in the postnatal development of birds and mammals, and in homeostasis of adults. The active form of TH, tri-iodothyronine (T₃), controls the transcription of target genes in many tissues. Several genes have been shown to be activated by T₃ [e.g. malic enzyme, 5' deiodinase (*DI*), myosin heavy chain α (*MHC α*), the K⁺ ionic channel gene (*HCN2*)], but a large number of genes are repressed in response to this hormone [thyrotrophin releasing hormone (*TRH*), thyroid stimulating hormone α and β (*TSH α* and *TSH β*), myosin heavy chain β (*MHC β*)]. Indeed, in liver, the majority of T₃-regulated genes are repressed in response to the hormone (Feng *et al.* 2000). T₃-dependent gene transcriptional regulation is mediated by thyroid hormone nuclear

receptors (TR), which are ligand-controlled transcription factors belonging to the superfamily of nuclear receptors. These receptors are encoded by two genes, *TR α* and *TR β* (Yen 2001) that, in addition to the receptors, also generate proteins devoid of DNA binding or T₃ binding, or both (Fig. 1, Table 1) (Chassande *et al.* 1997, 1999, Williams *et al.* 2000). The functions and mechanisms of action of these isoforms are largely unclear.

TRs associate with retinoid X receptors as heterodimers that bind to specific sites in the promoters of target genes (Glass 1994). They can adopt either the aporeceptor conformation (apoTR) when their ligand binding domain (LBD) is empty, or the holoreceptor conformation (holoTR) when the LBD is occupied by T₃. In physiological situations, TRs are in equilibrium between the apoTR and holoTR conformations, depending on the local concentration of T₃ and on

Figure 1 Structure of the *TRα* and *TRβ* loci and *TRα* and *TRβ* protein isoforms. Transcription initiation sites used to generate $\alpha1+\alpha2$, $\Delta\alpha1+\Delta\alpha2$, $\beta1$, $\beta2$, $\beta3+\Delta\beta3$ are represented by horizontal arrows anchored by vertical bars. Splice sites (sp) are represented by dashed lines. Translation AUG sites are represented by vertical arrows. DNA-binding domain (DBD)-encoding exons within the *TRα* and *TRβ* genes and DBD domains within the TR proteins are represented as closely cross-hatched boxes. LBD-encoding exons within the *TRα* and *TRβ* genes and LBD domains within the TR proteins are represented as widely cross-hatched boxes.

the affinity of TRs for T_3 . ApoTRs and holoTRs are themselves in rapid dynamic equilibrium between nucleoplasmic and DNA-bound forms. In addition, holoTRs are degraded by the proteasome pathway (Dace *et al.* 2000). As a consequence, a TH response element (TRE) within a T_3 target gene

may be either receptor-free or occupied by either the holoTR or the apoTR, the resulting effect on transcription depending on the concentration and proportion of holoTR and apoTR and on their respective intrinsic activities. Therefore, unlike membrane receptors or many nuclear receptors,

Table 1 Functionality of the DNA-binding and ligand-binding domains within the TR isoforms and susceptibility of these isoforms to mutations. RTH-associated mutations were found in human and are inheritable. Somatic mutations were found in human tissues (Lin *et al.* 1999, MacCabe *et al.* 1999, Kamiya *et al.* 2002, Puzianowska-Kuznicka *et al.* 2002). Knock-in mutations mimic RTH-associated mutations and were introduced in the mouse TR α or TR β genes

	DNA binding	T ₃ binding	RTH-associated mutations	Somatic point mutations	Knock-in mutations
TR α 1	+	+		+	+
TR α 2	+	–			
TR $\Delta\alpha$ 1	–	–		+	+
TR $\Delta\alpha$ 2	–	–			
TR β 1	+	+	+		+
TR β 2	+	+	+		+
TR β 3	+	+	+		+
TR $\Delta\beta$ 3	–	+	+		+

unliganded TRs are not silent, but compete for DNA binding with liganded receptors and exert a transcriptional activity that is the converse of that of holoreceptors. These properties confer on apoTRs a particular role in contributing to the transcriptional activity of T₃ target genes, in combination with holoTRs: one considerable difference between a system in which aporeceptors are silent and a system in which aporeceptors have an antagonistic activity is that the latter offers a much greater amplitude of response to variations in ligand concentration (Fig. 2).

It has been demonstrated *in vitro* that, in the absence of T₃, the LBD is tightly folded and binds corepressors, resulting in the repression of T₃-activated genes by chromatin deacetylation. Upon T₃ binding to the LBD pocket, the corepressor is released and the AF-2 domain becomes exposed and recruits coactivators, resulting in histone acetylation and chromatin loosening (Glass & Rosenfeld 2000, Hu & Lazar 2000). Despite abundant evidence for the role of corepressors and coactivators in TR activities *in vitro*, the mechanisms involved in T₃-independent (apoTR-mediated) and T₃-dependent (holoTR-mediated) transcriptional control *in vivo* are not yet fully understood. To date, scarce evidence has been presented for the role of coactivators in holoTR-mediated gene activation (Sadow *et al.* 2002). Transcriptional repression by holoTR does not seem to require the recruitment of corepressors (Becker *et al.* 2002), but rather that of coactivators (Weiss *et al.* 1999, 2002b), indicating that associ-

ation with the coactivator is determined by the conformation of the receptor induced by TR–T₃ interaction, and is independent of the sense of the transcriptional effect. The *in vivo* involvement of corepressors in apoTR function is better established. NcoR has been found to be associated with apoTR *in vivo* (Sachs *et al.* 2002). Mutations of the TR β gene resulting in delayed release of NcoR upon T₃ binding have been found to be associated with the syndrome of resistance to thyroid hormone (RTH) (Liu *et al.* 1998, Safer *et al.* 1998, Tagami *et al.* 1998). Finally, when a dominant negative form of NcoR is expressed in the liver of transgenic mice, the expression of T₃-activated genes is increased in the absence of T₃, because of the loss of apoTR function (Feng *et al.* 2001). Together, these data strongly suggest that corepressors are associated with unliganded TR *in vivo* and that T₃ binding causes the release of the corepressor. Therefore, as apoTR associates with corepressors, a potential role for apoTR could be to buffer corepressors. For instance, severe hypothyroidism, which generates increased amounts of apoTR, could result in the quenching of NcoR. This quenching could be responsible for the severe developmental abnormalities associated with severe hypothyroidism, reminiscent of the alterations caused by the absence of NcoR (Jepsen *et al.* 2000).

The question which is raised here is whether apoTRs have a physiological function. A prerequisite before analysing apoTR function is to identify when and where the unliganded TR is predominant. The literature provides data about the onset

of thyroid function and of receptor expression, which makes it possible to draw up a pattern of evolution of the apoTR/holoTR ratio during fetal life. In adults, data from experimental hypothyroidism and thyrotoxicosis enable the determination of the apoTR/holoTR ratio in several tissues. Other important clues are revealed by analysing the consequences of forcing the TRs into the aporeceptor conformation, either by generating severe hypothyroidism or by introducing point mutations into the receptors that prevent T_3 binding. These approaches will be used here to discuss the functions of unliganded TRs.

The proportions of holoTR and apoTR are determined by central production of TH and deiodinase activities

The proportion of apoTRs and holoTRs within a given cell depends on the local availability of T_3 . This availability depends on:

- (i) central production of thyroxine (T_4) and T_3
- (ii) permeation of T_4 and T_3 through the cell membrane
- (iii) local deiodination of T_4 to produce T_3
- (iv) intracellular buffering systems
- (v) mechanisms that control T_3 shuttling between the cytoplasm and the nucleus, and
- (vi) T_3 catabolism by 5 deiodination.

Among all these parameters, central production and local metabolism of T_4 and T_3 by deiodinases are well documented, whereas the other steps of TH metabolism are poorly understood. Central production of T_4 and T_3 is controlled by the hypothalamic paraventricular nucleus thyrotrophin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone (TSH). The local availability of T_3 is mainly determined by the local production of T_3 from its precursor T_4 via 5' deiodination and by inactivation of T_3 through 5 deiodination (for review see Bianco *et al.* 2002). Type I deiodinase catalyses the 5' deiodination of T_4 and the 5 deiodination of T_3 ; its expression is almost ubiquitous, but is not found in the central nervous system (CNS). Moreover, the expression of the type I deiodinase is low in the fetus. Type II deiodinase can catalyse the 5' deiodination and is mostly abundant in the brain, but also in the

Figure 2 Activity of a T_3 -dependent parameter as a function of holoTR/apoTR ratio: effect of an intrinsic aporeceptor activity. The ordinate represents the activity (P) of a T_3 -stimulated parameter (such as transcription, heart rate, etc.). The abscissa represents the holoTR/apoTR ratio (r). The theoretical relationship between P and r obeys the equation: $P = B + (r \times H) + (1-r) \times A$, where B is the baseline activity in the absence of any regulation by TR (such as in TR-knockout mice), A is the activity of apoTR and H the activity of holoTR. With the hypothesis of a silent aporeceptor ($A=0$), the theoretical curve is the continuous straight line. With the hypothesis of an intrinsic aporeceptor activity, the curve is the dashed line.

pituitary and in brown adipose tissue. Type III deiodinase catalyses the transformation of T_3 into 3,3'-di-iodothyronine and T_4 into reverse T_3 (rT_3), which do not bind to TRs. It is particularly abundant in fetal tissues and through premetamorphosis in amphibians, thereby preventing premature exposure of the embryos or larvae to high concentrations of TH, which would result in developmental alterations. It is also present in many tissues in adults, in particular in regions of the CNS where TRs are abundant, such as the hippocampus, the dentate nucleus and the cerebral cortex. Production of type III deiodinase is stimulated by hyperthyroidism and inhibited by hypothyroidism. Local variations in T_3 concentration can be determined by modifications in the activities of deiodinases, and both transcriptional and post-translational control of the expression of type I deiodinase can account for the variations in type I deiodinase activity.

Unliganded TRs are predominant at early stages of development

In amphibians, avians, rodents and human, TRs are expressed before the onset of thyroid function. In amphibian larvae, onset of expression of TR α occurs at stage 39 and expression of TR β at stage 46. T₃ is detected from stage 56 only, suggesting that TRs work as aporeceptors until TH production is switched on (Damjanovski *et al.* 2002). The functionality of the TR α receptors is demonstrated by the precocious induction of metamorphosis at stage 41 upon exogenous addition of T₄ (Sachs *et al.* 2000). The function of apoTR could be important in premetamorphic amphibians, and it would be particularly interesting to analyse the effect of TR gene inactivation in *Xenopus* to determine whether TR α has a function as a 'safety lock' to metamorphosis, in which case one would expect TR α gene knockout to result in precocious metamorphosis.

In chicken embryo, the thyroid gland is functional at embryonic day 9.5 and TH concentrations increase until hatching. However, T₃ and T₄ are present at low concentrations in the egg yolk and T₃ is released from the area opaca on the first day of development (E1) (Flamant & Samarut 1998), suggesting that T₃ might be available at the very beginning of chicken development. The TR α gene is detected from E1 and its expression is found in many tissues (brain, red blood cells, yolk sac) at E4, with a progressive increase until hatching (Forrest *et al.* 1990). The TR β gene is detected as early as at E6–E7 in the yolk sac and in the retina (Forrest *et al.* 1990; Sjöberg *et al.* 1992). It is therefore likely that, between day 1 and day 10, the receptors work mainly as aporeceptors, and are progressively turned into holoreceptors.

In mammals, the thyroid is fully functional at late stages of gestation (embryonic day 17 in rat, week 20 in human). Although THs are present early in the fetus, presumably through placental transfer from the mother, the combination of low amounts of type I deiodinase and large amounts of type III deiodinase in fetal tissues contributes to maintaining a low fetal concentration of T₃ (Burrow *et al.* 1994). During this period, TRs are expressed in significant quantities. In rat brain for instance, TR α 1 is detected from embryonic day 11.5 in the neural tube, and its level increases progressively until day 19.5 (Bradley *et al.* 1992). From these

data, we can extrapolate that, during the early fetal life, TRs are mainly present as apoTR, and are turned into holoR in postnatal animals, as TH concentrations increase. At least part of the TR must remain as aporeceptors to ensure a normal development, because thyrotoxicosis in fetus causes goitre, growth retardation, accelerated bone maturation, tachycardia and increased motility (Glinioer 2000). However, neither the inactivation of the TR α or TR β genes nor the inactivation of both genes has been reported to affect fetal development significantly, suggesting that apoTRs are not essential for the development of mammals. Instead, they should be considered as one component of a balanced system between apoTRs and holoTRs, which ensures optimized physiological functions.

ApoTR must be turned into holoTR for postnatal development to proceed normally

Perinatal hypothyroidism locks receptors in their aporeceptor conformation and compromises development

The perinatal period is characterized by a progressive increase in the serum concentration of TH, which reaches a peak at birth in humans (Burrow *et al.* 1994) and at 15 days in mice (Hadj-Sahraoui *et al.* 2000). However, it is the local increase in T₃ production that seems to account for the change in receptor occupancy within cells. A very nice description has been made of the role of type I deiodinase, T₃ and TRs in the onset of cochlear function during postnatal life. At 8 days after birth, there is a peak of type I deiodinase activity in the cochlea, associated with a peak in local T₃ concentration (Campos-Barros *et al.* 2000), whereas serum T₃ concentration remains low. This peak is correlated with the maturation of this organ, and the absence of TRs prevents its normal development (Rusch *et al.* 2001). As a consequence of increased TH concentration, during the perinatal period, a large proportion of the apoTR is turned into holoTR.

In some severe congenital or experimental TH deficiency states, the serum concentrations of T₄ and T₃ are dramatically reduced. Mice in which the *Pax8* gene has been inactivated are devoid of thyrocytes and hence cannot produce TH. These mice display very low concentrations of circulating

TH at a time (15 days postpartum) when the serum concentration of TH normally reaches a peak in wild-type mice and allows for the postnatal maturation of many organs such as cochlea, intestine and brain. Consequently, *Pax8*^{-/-} mice display developmental arrest and die within the first 2 weeks of life (Mansouri *et al.* 1998). When *Pax8*^{-/-} mice receive T₄ transiently during the second week, they are rescued, recover normal growth and can survive for months as adults in the absence of any further administration of T₄. Interestingly, the postnatal phenotype exhibited by deeply hypothyroid mice is qualitatively similar to that observed in mice lacking all TRs (slower growth, decreased mineralization of bones, smaller size of the spleen, impaired maturation of the intestine), but more severe (Flamant *et al.* 2002). In particular, TR double-knockout mice are viable, in contrast to *Pax8*^{-/-} mice. The stronger phenotype exhibited by *Pax8*^{-/-} mice as compared with TR double-knockout mice suggests two interpretations: (i) T₃ acts via TR α - and β -independent pathways and deprivation of TH affects functions other than those ensured by TRs; (ii) apoTRs exert a strong intrinsic activity that is extremely detrimental to postnatal development. This dilemma can be resolved by the following observations: (1) the very severe phenotype exhibited by *Pax8*^{-/-} mice is partially rescued in combined *Pax8*- and *TRa*-knockout animals (Flamant *et al.* 2002) and (2) knockout of the *TRa1* gene prevents alterations induced by hypothyroidism in the cerebellar structures of wild-type mice (Morte *et al.* 2002). In conclusion, the effects of the absence of TH can be attenuated by the removal of the TR α 1 receptor, meaning that TH deprivation induces a strong apoTR α 1 activity *in vivo* that is extremely detrimental to postnatal development. Thus the role of T₃ during the postnatal period in mammals might be to derepress the TR α 1 receptor.

Mutant receptors with impaired T₃ binding preclude normal development

Several naturally occurring or experimentally introduced mutations within the LBD of *TRa1* or *TR β* result in impaired T₃ binding, providing conditions of *in vivo* apoTR. In humans, mutations within the *TR β* LBD generate proteins with decreased affinity for T₃, which compete with the wild-type receptors for binding to DNA targets and

therefore act as dominant negative. Heterozygous patients display the family of syndromes collectively known as RTH (Refetoff 2001). The most common symptoms are increased T₄ and T₃ serum concentrations with normal or increased TSH, as the result of altered pituitary and hypothalamic feedback control of TH production. As a consequence of increased TH concentrations, *TRa1* is mostly in the holoR conformation, resulting in a thyrotoxic phenotype in tissues in which this receptor is the major mediator of T₃ action; tachycardia is an example of such a phenotype. Mice harbouring mutations in the LBD of *TR β* have been generated to provide experimental models to analyse the mechanisms of action of the dominant negative receptors, to provide better understanding of their tissue-specific effects and to offer the possibility of testing compounds aimed at restoring holoreceptor conformation of the mutant receptors and hence normal physiological activity. Mice with targeted mutations of the *TR β* gene have impaired growth and RTH (Kaneshige *et al.* 2000), in addition to impaired development of the cerebellum (Hashimoto *et al.* 2001) associated with a learning defect. Interestingly, these phenotypes are not observed in mice with a homozygous deletion of the *TR β* gene. The pattern of dominant negative *TR β* activity is related to the proportion of TR α and TR β in each tissue (Zhang *et al.* 2002). For instance in liver, where the TR β 1 is the major TR isoform and where *TR β* knock-in results in the abrogation of T₃ binding, the activities of T₃-activated genes are reduced in heterozygous or homozygous mutants, despite increased TH concentrations. In contrast, in heart where TR α 1 is the major TR isoform, the *MHC α* and *MHC β* genes are up- and downregulated respectively, in response to increased concentrations of TH.

The introduction of RTH syndrome mutations into the *TRa1* gene results in a very severe hypothyroid phenotype reminiscent of that observed for the *Pax8*^{-/-} mice – characterized, for example, by severe retardation of postnatal development (Tinnikov *et al.* 2002) or mortality (Kaneshige *et al.* 2001). Tissues such as bone, heart and the immune system, in which deletion of the TR α 1 receptor has been shown to produce alterations (Fraichard *et al.* 1997, Wilkstrom *et al.* 1998, Arpin *et al.* 2000, Gauthier *et al.* 2001), are severely affected by the *TRa* knock-in mutation. The predominant apoTR α 1 is partly turned into

holoTR α 1 when excess TH is provided to the *TRa* knock-in heterozygous mice, resulting in phenotypic rescue. Worthy of note is that the phenotypes of *TRa* knock-in mice are much more severe than those of the *TRa*-knockout, *TR β* -knockout, TR double-knockout or *TR β* knock-in mice. These observations further support the notion that a strong apoTR α 1 activity has a toxic effect on postnatal growth and development. The expression of apoTR β is clearly less detrimental to the overall development than is expression of apoTR α 1; however, the contributions of the different aporeceptor and holoreceptor subtypes to normal development are difficult to analyse. In cerebellum, where TR α 1, TR α 2, TR β 1 and TR β 2 have all been shown to be expressed (Strait *et al.* 1990; Bradley *et al.* 1992), the respective roles of TR α 1 and TR β aporeceptors are controversial. Hypothyroidism is known to affect cerebellar development and functions, and alterations in learning and movement have been reported in patients with the RTH syndrome. Remarkably, TR α - or TR β -knockout mice have not been reported to exhibit a significant cerebellum phenotype (Koibuchi & Chin 2000; Flamant & Samarut 2003). In contrast, the *TR β* RTH mutation leads to altered maturation of the cerebellum and impaired learning, suggesting that postnatal occupancy of TR β receptors by T₃ is critical for the correct development of this organ (Hashimoto *et al.* 2001). In contrast with these findings, Morte *et al.* (2002) have shown that the deleterious effects of hypothyroidism on cerebellar development could be rescued either by the deletion of the *TRa1* gene or by the administration of T₃, but not by the injection of the TR β agonist GC-1, suggesting that it is the occupancy of the TR α 1 receptor that is essential for the normal postnatal development of the cerebellum. Altogether, the above data suggest that maintaining either TR α 1 or TR β in the aporeceptor conformation is sufficient to block the maturation of the cerebellum or, put another way, all receptor subtypes must be derepressed by T₃ to permit progression of the maturation.

In conclusion, it is remarkable that, in amphibians in addition to chicken and mammals, the TR α 1 receptor is found in the aporeceptor conformation during a long period corresponding to prenatal or premetamorphosis stages. If this receptor is maintained under this conformation

during the perinatal period because TH concentrations remain low, or because it has a low affinity for T₃, developmental processes are severely compromised. Moreover, even though persistence of apoTR α 1 has the most deleterious effect, all receptors must be converted to the holoreceptor conformation during this period if normal development is to be ensured. However, the functions of apoTRs during the early stages of development remain unclear. The combined knockout of *TR* genes has not revealed any obvious alteration in prenatal development, suggesting that apoTRs do not have an essential role. However, a more careful examination of the fetal phenotype of *TR*-knockout mice has yet to be carried out. Indirect evidence for a role of apoTR in fetal life is provided by the analysis of mice in which *NcoR* has been deleted, resulting in altered apoTR activity. Erythrocyte precursors from *NcoR*^{-/-} mice display increased expression of carbonic anhydrase II, a gene that is activated by T₃ and is expected to be normally repressed by unliganded TR (Jepsen *et al.* 2000). Therefore the function of apoTR might be to block the transcription of T₃ target genes at a fetal/larval level before the increase in T₃ concentration, thereby maintaining a fetal/larval state in many tissues. It is likely however that, at least in mammals, redundant systems can substitute for TRs to exert this function.

Role of apoTRs in adults

Knock-in mouse models have not allowed for the analysis of apoTR function in adults, because all the phenotypes observed are developmental or could have a developmental cause. Most of the information about apoTR/holoTR ratios and aporeceptor function in adults has been provided by studies analysing the transcriptional effects of experimental hypothyroidism or thyrotoxicosis in adult tissues. This information allows for the determination of the percentage of receptor occupancy and, in combination with data from knockout mice, for the determination of the intrinsic apoTR activities in several tissues.

Role of ApoTRs in adult liver, heart and pituitary

Assuming that the transcriptional activity of a T₃-regulated gene is a linear function of the

(a)

(b)

(c)

(d)

(e)

percentage of TR occupancy, it is possible to determine the percentage of ligand-bound TRs in tissues of euthyroid animals *in vivo*, provided the activity of a cell-specific, T_3 -regulated gene can be measured: (i) in hypothyroid animals (activity at 0% occupancy), (ii) in thyrotoxic animals (activity at 100% occupancy) and (iii) in euthyroid animals. A straight line can be drawn between the extreme values corresponding to 0% and 100% occupancy (Fig. 3a) and the percentage of holoTR in euthyroid animals is determined by extrapolation of the activity plotted onto the linear graph.

It is also possible to determine whether apoTRs are silent or whether they have an intrinsic activity, provided the gene activity can be measured in the absence of TH and in the absence of TR. If this activity is different in receptor-free and in TH-deprived mice, this means that unliganded TRs do have an activity in the corresponding tissue. The opportunity to measure the baseline activity of T_3 -target genes is provided by the availability of mice devoid of all TRs.

Although receptor occupancy can be determined from data obtained in different animal models, I will focus here on data from mice, because they give access to the baseline activity of some genes in the absence of TRs, through *TR*-knockout mice. It must be pointed out that this treatment of the data offers only an approximation of reality, for at least two reasons: (i) the determination of apoTR and holoTR activities assumes that the pharmacological treatment used to generate hypothyroid or thyrotoxic animals leads to 0% or 100% occupation of the TRs respectively, which is an approximation; (ii) the 'activity' parameters measured are steady-state amounts of transcripts, or serum concentrations of hormones, which do not represent exactly the transcriptional activity of TRs, as these amounts also depend on mRNA stability, translation, secretion, etc.

In liver, the expression of several genes [*ME*, *Spot14* (Weiss *et al.* 1998), *DI* (Amma *et al.* 2001)] has been measured in hypothyroid, hyperthyroid and euthyroid mice. The amounts of each transcript measured in hypothyroid and thyrotoxic mice are plotted in Fig. 3b–d and straight lines have been drawn for each pair of data. The amount of each transcript measured in euthyroid mice has been plotted on the corresponding curve and the percentage of receptor occupancy has been determined on the abscissa (boxed numbers): extrapolated percentages are 35, 20 and 25% for *ME*, *Spot14* and *DI* respectively (Fig. 3a, b and c respectively), suggesting that, in physiological conditions, TRs are mostly in the apoR configuration in hepatocytes. This conclusion is consistent with biochemical analyses that have shown that, in rat liver, the percentage of occupancy of TRs is around 45% (Bianco *et al.* 2002). In another study, liver type I deiodinase activities were measured in hypothyroid and hyperthyroid wild-type and in *TR α 1*^{−/−}*TR β* ^{−/−} mice (Amma *et al.* 2001); the activity was found to be very similar in hypothyroid wild-type and in TR-free mice. Therefore, the basal transcription of the *DI* gene in the absence of TR is not modified by the presence of apoTR in hypothyroid mice, indicating that apoTRs have no intrinsic activity in these cells or at least in the control of *DI* promoter activity. As *TR β 1* is the predominant isoform in this organ (Zhang *et al.* 2002), we can conclude that *TR β 1* has no aporeceptor activity in liver. In conclusion, in liver, apoTRs are silent and TR activity in physiological conditions is low, as a result of a relatively low occupancy of the receptor. As a consequence, this organ has a high potential to respond to increased T_3 concentrations, and hence is a potential therapeutic target for TR agonists.

In heart, TH stimulates the transcription of the *MHC α* gene and represses the transcription of the

Figure 3 Determination of the fraction of occupied TRs. All graphs represent a T_3 -dependent activity as a function of the percentage of occupancy of the TRs. On each graph, two values of this activity, one corresponding to very low TH concentrations (very low T_3 or hypothyroid) and the other corresponding to saturating concentrations of T_3 (high T_3 or thyrotoxic), are plotted. The activity measured in euthyroid animals is plotted on the ordinate and the corresponding percentage of receptor occupancy is extrapolated on the abscissa (boxed numbers). (a) Theoretical graph representing the activity of a T_3 -activated (left) or T_3 -repressed (right) parameter as a function of receptor occupancy. (b) Amount of *Spot14* transcript (arbitrary units) as a function of receptor occupancy. (With permission from Weiss *et al.* 1998.) (c) Amount of malic enzyme transcript (arbitrary units) as a function of receptor occupancy. (With permission from Weiss *et al.* 1998.) (d) Activity of type I deiodinase (pmol l^{−1}/mg protein×min) as a function of receptor occupancy. (With permission from Amma *et al.* 2001.) (e) Serum concentration of TSH (ng/ml) as a function of receptor occupancy. (With permission from Weiss *et al.* 1997.)

MHC β gene, activates the transcription of the K^+ ionic channel gene, *HCN2*, and stimulates heart rate. The levels of expression of *MHC α* and *MHC β* genes in euthyroid mice have been found to be similar to those in thyrotoxic mice, suggesting that the percentage of holoTR in cardiomyocytes is close to 100% (Mansen *et al.* 2001). The knockout of the *TR α* gene leads to bradycardia (Johansson *et al.* 1999; Wilkstrom *et al.* 1998) and decreased expression of K^+ channels (Gloss *et al.* 2001), as the result of the depletion of holoTR. Mansen *et al.* (2001) have measured the expression of myosin heavy chain RNAs in wild-type hypothyroid and TR double-knockout mice, and found relative expression levels of 0.3 and 1 respectively for *MHC α* and 35 and 25 respectively for *MHC β* . As the levels of transcripts of *MHC α* and *MHC β* are different in conditions of low TH and in the absence of TRs, this indicates that apoTRs have an intrinsic activity in heart, resulting in transcriptional activation of *MHC β* , and repression of *MHC α* .

In pituitary, *TSH α* and *TSH β* genes are down-regulated by T_3 . In response to TH deprivation, the concentration of TSH is increased severalfold; in contrast, the administration of high doses of TH produces a modest reduction in TSH (Fig. 3e) (Weiss *et al.* 1997). One can conclude that, in physiological conditions, most of the TRs ($\sim 95\%$) are liganded in thyrotrope cells. The production of TSH in *Pax8^{-/-}* mice, which have very low TH concentrations and are deeply hypothyroid, is twice that in TR double-knockout mice (Weiss *et al.* 2002a), suggesting that TRs exert aporeceptor activity in these cells. Remarkably, *TR α 1* but not *TR β* seems to be responsible for aporeceptor-mediated activation of TSH (Sadow *et al.* 2002).

Fine-tuning of TR occupancy allows for physiological regulatory activity

Receptor occupancy can be modified within a given tissue, by physiological variations in T_3 concentrations, by pathological hypothyroidism or thyrotoxicosis, or by mutations within the receptors. One example of physiological variation of T_3 concentration is long-term adaptation to cold exposure. In response to 24 h exposure to cold in wild-type mice, serum T_4 and T_3 concentrations are unchanged; however, in brown adipose tissue,

type II deiodinase activity is stimulated 10–20-fold, resulting in a local increase in T_3 concentration that leads to increased heat production, thereby limiting the amplitude of hypothermia (de Jesus *et al.* 2001). When *DII^{-/-}* mice are exposed to cold, the hypothermia is more severe because these mice cannot elicit T_3 -mediated heat production. In this situation, the availability of unoccupied TRs allows for adaptative responses to increases in T_3 concentration elicited by external stimuli. ApoTRs are present in many tissues, where they participate in the mechanism of gene expression. The combination of holoTRs with active apoTRs permits a larger amplitude of transcriptional responses to moderate variations in T_3 concentrations, as compared with silent apoTR. Physiological homeostasis depends on a precise balance between apoTRs and holoTRs, and excess TH leads to several metabolic and behavioural pathologies in adults (Braverman & Utiger 2000).

Conclusion and future directions in the investigation of apoTR function

ApoTRs participate in the fine-tuning of T_3 -target genes. The saturation of TRs by high concentrations of T_3 is not compatible with normal development and healthy adult life, suggesting that this tuning has an extreme importance. However, it is clear that apoTRs alone, as biochemical entities, do not have a fundamental physiological role. In other words, it is likely that, if one could substitute the natural apoTRs with silent receptors, which would bind DNA, but would not interfere with transcription, there would not be significant consequences for the physiology. To check this assumption properly, however, one would have to generate TRs devoid of their aporeceptor function. Considering that one of the best known functions of aporeceptors is their ability to recruit corepressors, an elegant approach to suppress aporeceptor activity would be to abrogate recruitment of corepressors by introducing point mutations into the D domain of the receptors by knock-in.

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