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 TRα1, 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.

Journal of Molecular Endocrinology (2003) 31, 9–20

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 (MHCα), 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 (TSHα and TSHβ), myosin heavy chain β (MHCβ)]. 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, TRα and TRβ (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 retinoic 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
the affinity of TRs for T3. 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 T3 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,

Figure 1 Structure of the TRα and TRβ loci and TRα and TRβ protein isoforms. Transcription initiation sites used to generate α1+α2, Δα1+Δα2, β1, β2, β3+Δβ3 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.
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 holo receptors. 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 association 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 TRβ 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/hoVoTR ratio during fetal life. In adults, data from experimental hypothyroidism and thyrotoxicosis enable the determination of the apoTR/hoVoTR 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 T3 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 T3. This availability depends on:

(i) central production of thyroxine (T4) and T3
(ii) permeation of T4 and T3 through the cell membrane
(iii) local deiodination of T4 to produce T3
(iv) intracellular buffering systems
(v) mechanisms that control T3 shuttling between the cytoplasm and the nucleus, and
(vi) T3 catabolism by 5 deiodination.

Among all these parameters, central production and local metabolism of T4 and T3 by deiodinases are well documented, whereas the other steps of TH metabolism are poorly understood. Central production of T4 and T3 is controlled by the hypothalamic paraventricular nucleus thyrotrophin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone (TSH). The local availability of T3 is mainly determined by the local production of T3 from its precursor T4 via 5’ deiodination and by inactivation of T3 through 5 deiodination (for review see Bianco et al. 2002). Type I deiodinase catalyses the 5’ deiodination of T4 and the 5 deiodination of T3; 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 pituitary and in brown adipose tissue. Type III deiodinase catalyses the transformation of T3 into 3,3’-di-iodothyronine and T4 into reverse T3 (rT3), 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 T3 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.

**Figure 2** Activity of a T3-dependent parameter as a function of holoTR/TH ratio: effect of an intrinsic aporeceptor activity. The ordinate represents the activity (P) of a T3-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.
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 TRα occurs at stage 39 and expression of TRβ 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 TRα 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 TRα has a function as a ‘safety lock’ to metamorphosis, in which case one would expect TRα 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 T₄ 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 TRα 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 TRβ gene is detected as early as at E6–E7 in the yolk sack 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, TRα1 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 (Glinoer 2000). However, neither the inactivation of the TRα or TRβ 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 characterised 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 holoR.

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, Paxδ−/− mice display developmental arrest and die within the first 2 weeks of life (Mansouri et al. 1998). When Paxδ−/− 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 Paxδ−/− mice. The stronger phenotype exhibited by Paxδ−/− mice as compared with TR double-knockout mice suggests two interpretations: (i) T3 acts via TRα- 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 Paxδ−/− mice is partially rescued in combined Paxδ- and TRα-knockout animals (Flamant et al. 2002) and (2) knockout of the TRα1 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 TRα1 receptor, meaning that TH deprivation induces a strong apoTRα1 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 TRα1 receptor.

**Mutant receptors with impaired T3 binding preclude normal development**

Several naturally occurring or experimentally introduced mutations within the LBD of TRα1 or TRβ result in impaired T3 binding, providing conditions of in vivo apoTR. In humans, mutations within the TRβ 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, TRα1 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 TRβ 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 TRβ 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 TRβ gene. The pattern of dominant negative TRβ activity is related to the proportion of TRα and TRβ in each tissue (Zhang et al. 2002). For instance in liver, where the TRβ1 is the major TR isoform and where TRβ 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 TRα1 is the major TR isoform, the MHCα and MHCβ genes are up- and downregulated respectively, in response to increased concentrations of TH.

The introduction of RTH syndrome mutations into the TRα1 gene results in a very severe hypothyroid phenotype reminiscent of that observed for the Paxδ−/− 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 TRα1 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 TRα knock-in mutation. The predominant apoTRα1 is partly turned into...
holoTRα1 when excess TH is provided to the TRα knock-in heterozygous mice, resulting in phenotypic rescue. Worthy of note is that the phenotypes of TRα knock-in mice are much more severe than those of the TRα-knockout, TRβ-KO, TR double-knockout or TRβ knock-in mice. These observations further support the notion that a strong apoTRα1 activity has a toxic effect on postnatal growth and development. The expression of apoTRβ is clearly less detrimental to the overall development than is expression of apoTRα1; however, the contributions of the different apo-receptor and holoreceptor subtypes to normal development are difficult to analyse. In cerebellum, where TRα1, TRα2, TRβ1 and TRβ2 have all been shown to be expressed (Strait et al. 1990; Bradley et al. 1992), the respective roles of TRα1 and TRβ 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, TRα- or TRβ-knockout mice have not been reported to exhibit a significant cerebellum phenotype (Koibuchi & Chin 2000; Flamant & Samarut 2003). In contrast, the TRβ RTH mutation leads to altered maturation of the cerebellum and impaired learning, suggesting that postnatal occupancy of TRβ 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 TRα1 gene or by the administration of T3, but not by the injection of the TRβ agonist GC-1, suggesting that it is the occupancy of the TRα1 receptor that is essential for the normal postnatal development of the cerebellum. Altogether, the above data suggest that maintaining either TRα1 or TRβ 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 TRα1 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 apoTRα1 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 Neor has been deleted, resulting in altered apoTR activity. Erythrocyte precursors from Neor−/− 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
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, T₃-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 T₃-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 TRα1⁻/⁻TRβ⁻/⁻ 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 TRβ1 is the predominant isoform in this organ (Zhang et al. 2002), we can conclude that TRβ1 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 T₃ concentrations, and hence is a potential therapeutic target for TR agonists.

In heart, TH stimulates the transcription of the MHCa gene and represses the transcription of the

**Figure 3** Determination of the fraction of occupied TRs. All graphs represent a T₃-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 T₃ or hypothyroid) and the other corresponding to saturating concentrations of T₃ (high T₃ 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 T₃-activated (left) or T₃-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 I⁻/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.)
The expression of MHCα and MHCβ 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 TRα 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 MHCα and 35 and 25 respectively for MHCβ. As the levels of transcripts of MHCα and MHCβ 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 MHCβ, and repression of MHCα.

In pituitary, TSHA and TSHβ genes are downregulated by T3. 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 (Weiss et al. 1997). One can conclude that, in physiological conditions, most of the TRs (≈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, TRα1 but not TRβ 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 T3 concentrations, by pathological hypothyroidism or thyrotoxicosis, or by mutations within the receptors. One example of physiological variation of T3 concentration is long-term adaptation to cold exposure. In response to 24 h exposure to cold in wild-type mice, serum T4 and T3 concentrations are unchanged; however, in brown adipose tissue, type II deiodinase activity is stimulated 10–20-fold, resulting in a local increase in T4 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 T3-mediated heat production. In this situation, the availability of unoccupied TRs allows for adaptive responses to increases in T3 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 T3 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 T3-target genes. The saturation of TRs by high concentrations of T3 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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Received 20 January 2003
Accepted 8 May 2003

Journal of Molecular Endocrinology (2003) 31, 9–20

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