Biochimica et Biophysica Acta 1830 (2013) 3893–3899

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Thyroid hormone receptor activity in the absence of ligand: Physiological and developmental implications ☆

Juan Bernal [a,*], Beatriz Morte [b]

[a] Instituto de Investigaciones Biomédicas "Alberto Sols," Consejo Superior de Investigaciones Científicas (CSIC), Universidad Autónoma de Madrid (UAM), Spain
[b] Center for Biomedical Research on Rare Diseases (CIBERER), Madrid, Spain

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 11 April 2012
Accepted 16 April 2012
Available online 24 April 2012

Keywords:
Thyroid hormones
Nuclear receptors
Metamorphosis
Development
Hypothyroidism
Aporeceptor

ABSTRACT

Background: The transcriptional activity of the thyroid hormone receptors is modulated by the ligand, T_3, but they have also activity as aporeceptors, in the unliganded state. Aporeceptor activity is thought to contribute to the severity of profound hypothyroidism. During development thyroid hormone receptors are expressed before onset of thyroid gland function and are present therefore in many tissues mainly as aporeceptors. The question we address is whether thyroid hormone aporeceptors are involved in physiological and/or developmental processes.
Scope of review: The scope of this article is to review the evidence for a role of thyroid hormone aporeceptors in physiology and development. Related to this topic is the activity of mutant receptors unable to bind hormone. These receptors usually have dominant negative activity. This review focuses on the wild type receptors, and does not discuss the properties of mutant receptors.
Major conclusions: Unliganded thyroid hormone receptors influence the timing and control certain aspects of amphibian pre-metamorphosis. In mammals they are likely to influence maturational processes in the brain and other organs before onset of thyroid gland function. Expression of types 2 and 3 deiodinases which control the local tissue concentration of T_3 regulates the fractional receptor occupancy and therefore the relative proportion of aporeceptors. This article is part of a Special Issue entitled Thyroid hormone signalling.

1. Introduction

The current model for the genomic actions of thyroid hormone contemplates that the nuclear thyroid hormone receptors, TRα and TRβ have transcriptional activity in the presence or absence of the ligand. This property of the TRs is due to their ability to bind to the regulatory regions of regulated genes in the presence and in the absence of the ligand. The unliganded receptors, or "aporeceptors," are not silent or devoid of activity, but rather may have transcriptional activity in the opposite direction as the liganded, "holoreceptor." If a gene is regulated positively by T_3, it would be repressed by the aporeceptor, and vice versa. This is usually observed in transactivation experiments in cultured cells where the activity of reporter genes are measured after receptor transfection. But the question is whether this property of TRs have a physiological relevance, implying that the different activities of the apo- and holo-receptors might be translated into different physiological functions.

In addition to their *in vitro* transcriptional properties, the idea that the unliganded TRs perform physiological functions derives from two main lines of evidence. One is the early realization that the absence of all forms of TRs in mice was not equivalent to the hormone deprivation leading to profound hypothyroidism, as would have been expected. The second line of evidence is related to the timing of receptor expression during development in several animal species in relation with the onset of thyroid secretion. The appearance of TRs in tissues before onset of secretion by the thyroid gland, may indicate that the receptors perform early developmental actions in the absence of the hormone.

The possible role of TR aporeceptors in physiology and development was specifically reviewed by Chassande [1]. He suggested that in a given physiological situation, with a fractional TR occupancy of 50-80% depending on the particular tissue, a fraction of the total receptor pool remains as aporeceptors, which would provide a greater amplitude of gene responses. Aporeceptors interact with the corepressors NCoR and SMRT [2] and may repress or enhance gene expression. In support of the Chassande hypothesis is the recent demonstration that the interaction of TR with NCoR determines the magnitude of the response and the sensitivity to T_3 in euthyroid animals [3,4]. Concerning development it was concluded [1] that there was no solid evidence to suggest a developmental role for TR

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* Corresponding author at: Instituto de Investigaciones Biomédicas, Arturo Duperier 4, 28029 Madrid, Spain. Tel.: +34 91 585 4447; fax: +34 91 585 4401.
E-mail addresses: jbernal@iib.uam.es, bmorte@iib.uam.es (J. Bernal).

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doi:10.1016/j.bbagen.2012.04.014

aporeceptors before onset of thyroid gland function. The main argument was that deletion of all forms of receptor does not result in obvious embryonic abnormalities. In the present review we will discuss more recent evidence supporting the view that unliganded TRs may have physiological and developmental actions, although in many instances it is very difficult to differentiate between the effects due to the intrinsic activity of the TR aporeceptor from those due to the lack of trans-activation or trans-repression in the absence of the hormone. Our concern here is to analyze the possible roles of unliganded wild type TRs. A detailed discussion on mutant receptors devoid of hormone binding and displaying dominant negative activity [5–7] is out of the scope of the present review.

2. Hypothyroidism

Thyroid hormones influence the maturation of many tissues, such as bone, intestine, and the central nervous system. Therefore, it came as a surprise that knocking out TRα1, TRβ, or all forms of TRs did not result in a phenotype comparable to hypothyroidism [8,9]. The most likely explanation was that the effects of hypothyroidism were due to the intrinsic activity of the unliganded TRs. This hypothesis was supported by *in vivo* studies: Morte et al. [10] showed that the effects of neonatal hypothyroidism on cerebellar development were prevented by TRα1 deletion. Hypothyroid TRα1-deficient mice showed essentially normal cerebellar structure whereas hypothyroid wild type mice showed delayed granular cell migration and defective Purkinje cell differentiation. In addition, Flamant et al. [11] also provided evidence for a general detrimental effect of unliganded TRs: In contrast to TR deletion, Pax8$^{-/-}$ mice, which suffer from profound congenital hypothyroidism, do not survive after weaning unless treated with thyroid hormones. Interestingly, knocking down TRα1, but not TRβ1, increases survival of the Pax8$^{-/-}$ mice, again indicating that the effects of hypothyroidism were mediated by the unliganded TRα1. This concept is also supported by the phenotypic effects, with hypothyroid features, of dominant mutant TRα1 in mice and man [5,12–14]. In target tissues hypothyroidism causes down regulation of genes positively regulated by thyroid hormone and up regulation of genes negatively regulated by thyroid hormone. Unliganded receptors are responsible for these changes in a subset of thyroid hormone responsive genes in the liver [15]. While *in vivo* repression of positively regulated genes by unliganded TRα1 is mediated by interaction with NCoR [3], the mechanisms for up regulation by unliganded TRs are not clear.

To our understanding there is no alternative hypothesis to the detrimental role of apo-receptors to explain why TR null animals do not show the profound deficiencies of hypothyroidism. Hypothyroidism is a pathological situation, and it is not possible from this line of evidence to conclude that unliganded TRs have a physiological role in addition to the amplification of hormonal responses suggested by Chassande [1].

3. Regulation of the thyroid axis

Hypothyroidism causes an increased production of hypothalamic TRH and pituitary TSH. Transient transfection experiments showed an increased transcription of TRH, and TSH subunit genes by unliganded TRs [16–18]. Therefore it was proposed that in hypothyroidism the increased TRH and TSH production were due in part by the lack of the inhibiting effects of thyroid hormone, and in part to the stimulation of transcription by the unliganded TRs. Indeed, the major TR isoform expressed in the pituitary, TRβ2, has intrinsic transcriptional activity that resides in its amino-terminal domain [19,20]. Results from TR knock out mice initially supported the view that unliganded TRs caused an increased TSH transcription in the absence of thyroid hormones. Thus, whereas TRα1 deletion induced a small decrease in TSH production, TRβ deletion increased TSH but only moderately in comparison to hypothyroidism. The difference between the effect of TRβ deletion and hypothyroidism could be perhaps explained by the stimulation of TSH transcription by the TRβ aporeceptor in hypothyroidism. However the combined TRα1 and TRβ deletion increased TSH to the same extent as in mice made hypothyroid by methyl-mercapto-imidazol administration [8]. These results indicate that the TSH increase in hypothyroidism is due to the lack of negative regulation of the TSH genes by T_3 repression, rather than to the effect of apo-receptor stimulation. A similar conclusion was reached by Weiss et al. [21] after detailed comparisons of serum TSH concentrations in mice with different combinations of TR deletions. These authors found that deletion of all forms of TRs led to only slightly lower concentrations of TSH than those found in athyreotic, Pax8 knock out mice. It was concluded that "unliganded TRα or TRβ are not absolutely necessary for the upregulation of TSH".

It is possible that differences between TR deficiency and hypothyroidism are due to increased TRH stimulation in the latter. Several lines of evidence indicate that unliganded TRβ1 is involved in the upregulation of TRH in hypothyroidism while TRβ2 is involved in T_3-dependent TRH repression [22,23]. Hypothyroidism increased TRH transcript density in the paraventricular nucleus of wild type mice and in mice lacking the TRβ2 isoform, but not in TRβ-null mice [22]. The conclusion was that TRβ1 is involved in the T_3-independent stimulation of TRH transcription. Studies involving the use of a TRH-luc reporter expressed in the hypothalamus [24] showed that unliganded TRα1 actually reduced TRH expression and suppressed T_3-induced TRH down regulation. The role of TRs on TRH promoter activity was also analyzed by knocking down individual TR isoforms by shRNA delivery to the hypothalamus [25]. It was found that deletion of TRβ1, but not TRβ2, decreased TRH promoter activity in the absence of T_3, in agreement with a positive effect of unliganded TRβ1 on TRH expression.

It may be concluded therefore that TRβ1 aporeceptor is directly involved in upregulation of TRH, and indirectly in the upregulation of TSH, after thyroid hormone deprivation.

4. Development

TRs are expressed before onset of thyroid function in several species, raising the question as to the developmental relevance of unliganded receptor functions. In tadpoles TRs are expressed during pre-metamorphosis at stage 38 or even earlier [26,27]. In rats the thyroid hormone receptor protein, measured by T_3 binding assays is present in brain from embryonic day 13.5–14, i.e. several days before onset of thyroid gland function at about E17.5 [28]. Also, the TRα1 mRNA is widely present in the rat brain from E14 onwards [29,30]. TRβ is also expressed before onset of thyroid gland function, and can be detected at E15.5 in the upper tegmental neuroepithelium [29]. In the human brain, the TRs are also expressed before onset of thyroid gland function [31,32]. Is it possible that the early expression of TRs results in apo-receptor functions? The presence of receptors during development before onset of thyroid gland function does not necessarily mean that the TRs are in the unliganded state, because thyroid hormone of maternal origin is present in the embryos [33–35]. With this reservation in mind we will next review the evidence for a role of aporeceptors in amphibian and mammalian development.

4.1. Amphibian metamorphosis

TRα and TRβ are expressed before metamorphosis during embryonic and larval stages (Fig. 1). TRβ, which is T_3-inducible, remains low during pre-metamorphosis, increases in parallel with T_3 concentrations during pro-metamorphosis, and peaks during the metamorphosis climax at stages 61–62, as T3 [27]. Half maximal concentrations of TRα are attained before the onset of thyroid gland activity [36]. The