Syndromes of thyroid hormone resistance

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INTRODUCTION

The biological processes under the control of thyroid hormones (thyr-oxine: T4, and triiodothyronine: T3) include functional differentiation of the central nervous system, somatic growth, basal metabolic rate, muscular activity and myocardial contractility, as well as the secretion of TSH from the pituitary. Thyroid hormones mediate the large majority of their biological effects by changing the expression of specific genes in different tissues. Indeed, thyroid hormones may either induce the expression of genes, such as those encoding malic enzyme, sex hormone-binding globulin (SHBG), myelin basic protein, or inhibit the expression of others, such as those encoding TRH or TSH α- and β-subunits, through a negative feedback mechanism. Both stimulatory and inhibitory actions are achieved throughout receptor proteins that are members of the nuclear receptor superfamily of ligand-inducible transcription factors [25, 30].

In the present paper, the molecular defects of thyroid hormone receptor genes underlying the syndromes of resistance to thyroid hormone (RTH) will be reviewed. Particular emphasis will be given to the mutations of the gene encoding TRβ that is involved in the etiology of RTH, to the complex pathogenetic mechanisms causing the resistance and to the therapeutic approach to patients with such a rare disorder.

Key words: Thyroid hormone receptor (TR), Gene mutations, thyrotropin (TSH) secretion, Alpha and beta subunits of TSH, Thyrotropin-releasing hormone (TRH), Thyroid hormone resistance, Cofactors, Coactivators, Corepressors, TRIAC.
ETIOPATHOGENESIS OF THYROID HORMONE RESISTANCE (RTH)

RTH is biochemically characterized by high levels of circulating free thyroid hormones (FT4 and FT3) in the presence of measurable TSH concentrations. RTH presents with highly variable clinical features ranging from mental retardation and delayed bone maturation (generalized RTH: GRTH) to signs and symptoms of thyrotoxicosis (pituitary RTH: PRTH) [4]. Nonetheless, the great majority of RTH patients have been described as euthyroid, the distinctive feature being the presence of goiter.

More than 10 years ago, RTH was documented to be tightly linked to TRβ gene locus [29]. By using DNA amplification and sequencing techniques, about 130 different mutations in TRβ1 gene have now been identified in RTH patients belonging to more than 200 families, similar mutations occurring therefore in more than one family. The mutations cluster in 3 different “hot spots” (fig. 1) in the ligand-binding domain of the receptor, thus disrupting the ability of the receptor to bind T3 in almost all cases. Functional studies have definitively documented that RTH is caused by mutations in TRβ gene [1, 10, 22]. Interestingly, almost all patients with RTH express normal β-receptor from a single wild type allele and therefore resistance in these patients occurs because the mutant receptor inhibits the activity of the normal β- and α-receptors, a phenomenon referred to as dominant negative effects of mutant receptor [9, 22]. In order to exert their dominant negative effects, mutant receptors must retain normal dimerization and DNA binding properties [19]. For this reason, mutations in the receptor regions other than the 3 “hot spots” may elude discovery, as they lack dominant negative activity and are therefore clinically and biochemically silent. Moreover, heterozygote subjects with an inactivated TRβ gene are phenotypically normal, further supporting the idea that only the presence of a mutant receptor may impair the function of the normal receptors [28]. Several nonmutually exclusive models have been suggested to explain the mechanisms of dominant negative action of mutant receptors (table I) [19, 34].

Interestingly, in about one seventh of patients with clinical and biochemical phenotype of RTH no mutation could be found in TRβ gene. These data suggested the possible existence of an additional isoform of TRs (TRγ), but studies on knockout animals lacking both α and β isoforms demonstrate that no T3 binding may be found in any type of cell, thus excluding the presence of other TR isoforms [16]. Recently, Weiss and coworkers [32] have clearly documented that RTH may be caused by a deficient cofactor other than TR. Indeed, they studied mice deficient in the steroid receptor coactivator 1 (SRC-1) and showed that these animals are resistant to the action of thyroid hormones. In addition, RXRγ null mice show a biochemical phenotype consistent with RTH, further supporting the view that RXRγ plays an important in vivo role in the set point of pituitary-thyroid axis [7]. Therefore, patient with RTH and absence of TRβ mutation should be tested for mutation in SRC-1 and RXRγ genes. If the genes encoding for these cofactors show a normal nucleotide sequence, it should be considered that nonreceptor mechanisms may be disrupted to produce RTH in certain patients.

On this background, the variable clinical phenotype of patients with RTH may be due to variable degrees of peripheral resistance in different patients, as well as variable resistance in different tissues within a single individual. Table II summarizes the putative factors which may contribute to the variable phenotype of patients with RTH. The different tissue distributions of receptor isoforms may in part explain this variability. The liver and pituitary express predominantly TRβ1 and TRβ2 receptors respectively, whereas TRα1 is the major species detected in myocardium [20]. Therefore, mutations in the TRβ gene are likely to be associated with pituitary and liver resistance, as exemplified by non-suppressed TSH levels and normal SHBG [3], whilst the tachycardia often seen in RTH may represent retention of cardiac sensitivity to the action of elevated concentrations of circulating thyroid hormones, mediated by a normal α receptor. Another factor which may regulate the degree of tissue resistance is the relative expression of mutant versus normal alleles of the TRβ gene. Several studies have also showed that the dominant negative potential of mutant receptors can differ depending on the nature and configuration of TREs [10, 12, 35]. Finally, factors not strictly related to receptor mutation may also affect the clinical and biochemical phenotype of RTH. For example, an arginine to histidine mutation at codon 316 (R316H) was associated with normal thyroid function in one kindred [15], but in an unrelated family the same mutation was associated with abnormal thyroid function [1], suggesting that other variables in the pituitary-thyroid axis can modulate mutant receptor action (table II).

ROLE OF ALTERED THYROID HORMONE FEEDBACK MECHANISM IN RTH

The ability to exert a dominant negative effects within the hypothalamic-pituitary-thyroid axis is a key property of mutant TRβ proteins and generates the characteristic biochemical and clinical features that allow the detection of RTH [9]. In physiological conditions, TSH secre-
tion is in fact down-regulated by thyroid hormone binding to $\alpha$ and $\beta$ receptors. Many studies have been performed to define the mutual role of TR$\alpha$ and TR$\beta$ in in vivo regulation of TSH. TR$\beta$ knockout mice, lacking both TR$\beta$1 and TR$\beta$2 isoforms, provide an important model to this aim [13]. The biochemical phenotype of these knockout animals is similar to that observed in man with RTH [28]. If hypothyroidism is induced in these animals, TSH levels rapidly increase, reaching similar values in TR$\beta$–/– and in TR$\beta$+/+ mice, while the decline of serum TSH by treatment with both T3 and T4 was severely blunted in TR$\beta$–/– mice [31]. This would indicate that TR$\beta$ is not required for the up-regulation of TSH, whereas it enhances thyrotrope sensitivity to down-regulation, thus being essential for the complete suppression of TSH synthesis and secretion. The homozygous inactivation of TR$\alpha$1 and TR$\alpha$2 in mice leads these animals to die in less than 5 weeks [14]. The progressive hypothyroidism of central origin (TSH and circulating free thyroid hormones are lower than in normal animal), the growth arrest and the strong delay in maturation of small intestine, suggest crucial in vivo functions of thyroid hormones mediated by TR$\alpha$ during post-natal development, as well as a different regulation of the two TSH subunit genes by TR$\alpha$ isoforms [33]. The absence of any receptor (TR$\beta$ and TR$\alpha$) results in a much more severe impairment than that observed in either TR$\alpha$–/– or TR$\beta$–/– animals, suggesting that each TR isoforms mediates specific functions of T3, including those at the thyrotrone level [16].

In addition to knockout animal models, recent studies have thrown new light on the complex mechanism(s) of the feedback regulation of TSH secretion. The discovery of NCoR corepressor proteins has shown that these factors bind to the hinge region of TRs (CoR box, fig. 1) [18]. NCoR induce ligand-independent silencing of genes that contain positive TREs, but also play a role in the ligand-independent basal activation of genes negatively-regulated in response to T3 [26].

The presence of T3 is associated with the displacement of corepressor and recruitment of coactivator proteins, such as SCR-1. Thus, the remodeling of chromatin structure takes place throughout the process of histone acetylation and the transcriptional activity may start. To better understand how this mechanism is functioning, TR mutants in codons belonging to the hinge region have been investigated [24]. These mutants normally bind NCoR in the absence of T3, but

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<th>Possible mechanisms underlying the dominant negative effects of mutant TR$\beta$ on the normal TR$\beta$ and TR$\alpha$ receptors.</th>
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<td>a) Inactivation: formation of inactive homo- and/or heterodimers both in solution or on DNA can limit the amount of normal TR$\beta$ receptors and RXR in forming functional dimers</td>
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<td>b) Competition: mutant homo- and/or heterodimers can compete with normal dimers for binding DNA, thus altering the transcriptional process</td>
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<td>c) Titration: titration by protein-protein interaction can limit the amount of nuclear corepressors and coactivators involved in the ligand-mediated transcriptional activation</td>
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<td>d) Silencing: in the context of positively-regulated genes, mutant homodimers fail to dissociate with T3 and remain bound to the corepressors, thus silencing the transcriptional machinery. The opposite probably happens for negatively-regulated genes</td>
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dissociation of NCoR and recruitment of SCR-1 appear markedly impaired, except at very high T3 concentrations. This would suggest a novel mechanism for RTH whereby TR hinge mutants selectively affect T3 binding when complexed to DNA, and prevent corepressor dissociation from TRs. Altogether these findings suggest that the inadequate release of corepressor proteins, along with retained dimerization and DNA binding, are critical features for the dominant negative effects of mutant thyroid hormone receptors on both positively- and negatively-regulated genes. Thus, differential recruitment of cofactors may help explain TR isoform-specificity and phenotypic variations found in RTH syndromes, i.e. generalized and pituitary RTH [12, 23].

Finally, the majority of RTH patients have normal serum TSH levels, though they present with goiter and high concentrations of circulating free thyroid hormones. Furthermore, both FT3 and FT4 levels show an increase in response to endogenous TRH-stimulated TSH which is significantly higher than that observed in both normal subjects and in patients with simple goiter [6]. These observations prompted studies on the bioactivity of circulating TSH molecules. Indeed, in all RTH patients, serum biological activity to immunoreactivity ratio of circulating TSH molecules (TSH B/I) was enhanced as compared to controls [21]. Modifications of TSH glycosylated chains seem to account for the above finding [5]. Treatment with T3 or TRIAC was able to normalize TSH B/I, probably overcoming the dominant negative effects of mutant receptor both at the pituitary and hypothalamic level.

**TRIAC AS POTENTIAL THERAPY FOR PATIENTS WITH RTH**

One of the most important reasons for recognizing the presence of RTH in a given patient is that its management totally differs from that of other common forms of thyroid dysfunction. In the great majority of individuals with RTH, high circulating levels of thyroid hormones compensate the receptor defect. In this
condition, the patient is in an euthyroid state, generally with a goiter of variable size. Therefore, any therapeutic maneuvers aimed to reduce the high thyroid hormone levels, such as thyroid ablation or antithyroid drugs, should be avoided in these patients since they aggravate the hypometabolic state [6, 8]. In addition to individuals with reduced thyroid reserve, certain circumstances, such as hypercholesterolemia in adults or growth retardation in children, may warrant L-T4 administration. In particular, although a general criterion for treatment of infant with RTH is still lacking, in young children with growth and/or mental retardation, as well as in those with attention-deficit hyperactivity disorder, the administration of supraphysiological doses of L-T4 may be effective in overcoming the high degree of resistance in certain tissues. It is evident that such therapy needs careful monitoring by measuring not only TSH and free thyroid hormone levels, but also several indices of peripheral thyroid hormone action, such as the sex hormone-binding globulin, soluble interleukin 2 receptor, angiotensin converting enzyme, bone GLA protein, and others [6].

A more complex therapeutical approach is generally required in patients with signs and symptoms of thyrotoxicosis (PRTH). In fact, a reduction of TSH secretion and the consequent reduction of circulating thyroid hormone levels may be of benefit in these patients. Several drugs acting at various levels, such as corticosteroids, dopaminergic drugs, somatostatin analogs and L-T3, have been used for the treatment of PRTH, generally with poor beneficial effects. In view of a therapy for PRTH able to block pituitary secretion of TSH, but devoid to thyromimetic properties on peripheral tissues, we suggested the use of triiodothyroacetic acid (TRIAC) on an empirical basis [2]. TRIAC, as well as another thyroid hormone analog, the dextro-thyroxine [17], are effective in suppressing TSH secretion, thus leading to reduced thyroid hormone circulating levels and restoration of the euthyroid state in the majority of patients with PRTH [11]. In our experience, both the immunoreactivity and the biological activity of circulating TSH molecules, and consequently the free thyroid hormone levels, fell into the normal range in almost all patients during TRIAC treatment, thus leading to improvement of signs and symptoms of thyrotoxicosis [6]. Recently, the molecular basis for the therapeutic action of TRIAC has been provided by cotransfection studies using normal and mutant TRβ1 genes [27]. In this study, TRIAC appears to influence the activity of the only TR receptor involved in RTH, i.e. TRβ1. In fact, TRIAC, due to a higher affinity for TRβ1 than that displayed by T3, selectively increases TRβ1 function without affecting TRα1. Moreover, TRIAC is more effective than T3 in increasing the function of mutant TRβ1 and in overcoming its dominant negative effects. Future studies have to be planed in order to investigate the potential efficacy of TRIAC treatment in RTH patients presenting signs or symptoms of hypothyroidism, perhaps at doses higher than those employed in patients with PRTH. Lastly, in the presence of tachycardia one should consider, as additional or alternative therapy, the cardioselective β-adrenergic blockade which has been shown useful in almost all cases [6, 22].

REFERENCES


