Resistance to thyroid hormone, and peroxisome-proliferator-activated receptor γ resistance

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Abstract
Resistance to thyroid hormone (RTH) is usually inherited in a dominant fashion, and is characterized by elevated serum thyroid hormone levels and failure to suppress pituitary secretion of thyroid-stimulating hormone, with variable refractoriness to hormone action in peripheral tissues. Two major forms of the disorder are recognized: asymptomatic individuals with generalized resistance (GRTH) and patients with thyrotoxic features suggesting predominant pituitary resistance (PRTH). In over 100 families with GRTH or PRTH, we have identified heterozygous mutations in the thyroid hormone receptor β isoform (TRβ), which localize to three regions (amino acids 234–282, 310–353 and 429–461) of the hormone-binding domain of the receptor. The mutant receptors are transcriptionally impaired, due either to reduced ligand binding or to attenuated interaction with co-activators, and inhibit wild-type TR action in a dominant-negative manner. In the TRβ crystal structure, most RTH mutations cluster around the hormone-binding pocket, with receptor regions that mediate functions (DNA binding, dimerization, co-repressor recruitment) required for dominant-negative activity being devoid of natural mutations. The pathogenesis of variable tissue resistance is not fully understood, but may be related to the differing tissue distributions of TRα and TRβ, and to variable dominant-negative activity of mutant receptors on different target genes. The nuclear receptor peroxisome-proliferator-activated receptor γ (PPARγ) regulates adipogenesis and mediates the action of thiazolidinediones – novel antidiabetic agents which enhance tissue insulin sensitivity. The PPARγ gene was screened in 85 subjects with severe insulin resistance, and two different heterozygous receptor mutations (P467L and V290M) were identified in three affected individuals. The PPARγ mutants are markedly transcriptionally impaired due to altered ligand binding and co-activator recruitment. Analogous to RTH, they inhibit the function of wild-type PPARγ when co-expressed, and such dominant-negative inhibition is linked to their ability to

Key words: dominant-negative inhibition, insulin resistance, nuclear hormone receptor.

Abbreviations used: CREB, CAMP-response-element-binding protein; PPAR, peroxisome-proliferator-activated receptor; RTH, resistance to thyroid hormone; GRTH, generalized RTH; PRTH, selective pituitary RTH; RXR, retinoid X receptor; TR, thyroid hormone receptor; TRH, thyrotrophin-releasing hormone; TSH, thyroid-stimulating hormone; TZD, thiazolidinedione.

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silence basal gene transcription via aberrant interaction with co-repressors. In addition to insulin resistance, all three affected subjects developed Type II diabetes mellitus and hypertension at an unusually early age. Our findings provide compelling evidence that PPARγ is important in the control of insulin sensitivity, glucose homeostasis and blood pressure in humans. Future studies aim to elucidate the mechanism by which this receptor regulates insulin action and vascular tone.

**Introduction**

The nuclear receptor superfamily comprises proteins that mediate the action of known hormones, including steroids, vitamin D, thyroid hormone and retinoids. In addition, a growing number of ‘orphan’ receptors have been identified whose ligands and biological roles remain to be elucidated. One in ten of the most widely used drugs acts via nuclear receptors, attesting to the importance of these proteins as therapeutic targets. Nuclear receptors are ligand-inducible transcription factors, and are organized in functional domains which are highly conserved among family members: the N-terminal region often encodes an intrinsic transcription activation function (AF-1); a central DNA-binding domain mediates interactions with regulatory DNA sequences in target gene promoters; and the C-terminal domain mediates hormone binding and encompasses a powerful ligand-dependent transactivation function (AF-2). A subset of nuclear receptors, including the thyroid hormone receptor (TR), retinoic acid receptor and peroxisome-proliferator-activated receptor (PPAR), bind DNA as heterodimers with the retinoid X receptor (RXR). Recently, transcriptional cofactors which interact with nuclear receptors have been identified. Co-repressors [nuclear receptor co-repressor (NCoR), silencing mediator for retinoid and thyroid receptors (SMRT)] bind the unliganded receptor (TR, retinoic acid receptor) and mediate inhibition or silencing of basal gene transcription; conversely, a complex of co-activators [steroid receptor co-activator-1 (SRC-1)/activator for thyroid and retinoid receptors (ACTR)/transcriptional intermediary factor-2 (TIF-2) plus CREB (cAMP-response-element-binding protein)-binding protein (CBP) and p300/CREB-binding protein (pCAF)] interacts with hormone-bound receptor to mediate ligand-dependent transcriptional activation or inhibition.

**Resistance to thyroid hormone (RTH)**

Thyroid hormones regulate diverse processes, such as growth, myocardial contractility, differentiation of the nervous system and metabolic rate. The synthesis of thyroid hormones is controlled by hypothalamic thyrotrophin-releasing hormone (TRH) and by thyroid-stimulating hormone (TSH) from the pituitary; in turn, thyroxine and 3,3',5-tri-iodothyronine regulate TRH and TSH production as part of a negative feedback mechanism. The effects of thyroid hormones on cellular processes are accompanied and mediated by its actions on key target genes in different tissues. Thus the feedback effects of thyroid hormones on TSH secretion are mediated by inhibition of TSHα and TSHβ subunit gene expression. Examples of target genes that are induced by thyroid hormones include those encoding ‘malign’ enzyme and sex-hormone-binding globulin in the liver, myosin heavy chain and Na⁺/Ca²⁺-ATPase in the myocardium, and myelin basic protein in the brain. The effect of thyroid hormone on the transcription of these genes is mediated by proteins that are members of the nuclear receptor superfamily. In humans, two receptor genes (TRα and TRβ), on chromosomes 17 and 3 respectively, are alternatively spliced to generate three highly homologous receptor isoforms, TRα1, TRβ1 and TRβ2, with differing tissue distributions: TRα1 is most abundant in the central nervous system, myocardium and skeletal muscle, TRβ1 in liver and kidney, and TRβ2 in the pituitary and hypothalamus.

The syndrome of RTH is a dominantly inherited disorder characterized by elevated circulating levels of thyroid hormones, failure to suppress pituitary TSH secretion and variable peripheral refractoriness to hormone action [1]. Following the observation that RTH is linked to the TRβ gene locus, we have identified over 130 families with RTH, representing the largest worldwide cohort. We have described over 60 different heterozygous mutations, all localizing to the ligand-binding domain of TRβ [2,3].

Our studies of the properties of mutant TRβ proteins from subjects with RTH have provided important insights into structure–function relationships in the receptor. Consonant with their location, the mutant proteins exhibit impaired ligand-binding and transcription regulatory properties [3,4]. More recently, we have identified RTH mutants which fail to regulate transcription, despite preserved hormone- and DNA-binding
properties [3, 5]. We have shown that these unusual mutations involve residues that are critical for co-activator recruitment by the TR and, in conjunction with the use of additional artificial site-directed receptor mutants [6], we have mapped part of the receptor/co-activator interface. In keeping with the dominant inheritance and heterozygous nature of receptor defects in RTH, the mutant receptors inhibit the action of wild-type TR in a dominant-negative manner [7]. Clinical and genetic data from two unusual RTH kindreds has provided evidence in favour of such dominant-negative interactions in vivo. Affected individuals from a kindred with recessively inherited RTH were shown to be homozygous for a complete deletion of both TRβ receptor alleles; significantly, obligate heterozygotes in this family, harbouring a deletion of one TRβ allele, were completely normal [8]. Conversely, in another kindred, a single individual who was homozygous for two mutant TRβ alleles exhibited severe resistance, presumably reflecting the marked inhibitory effects of two dominant-negative mutant receptors [9]. In keeping with the hypothesis that mutant TR–RXR complexes compete with wild-type receptor complexes at binding sites in target genes, we have shown that the dominant-negative action of RTH mutants is abolished by artificial mutations that disrupt their DNA binding or dimerization with RXR [4]. A further attribute that is preserved in RTH mutants is their ability to silence basal gene transcription via co-repressor recruitment. Indeed, we have shown that some RTH mutants show enhanced co-repressor binding, and that mutational disruption of such an interaction abolishes their dominant-negative activity [10]. Our functional studies have culminated in the mapping of RTH mutants on the crystal structure of the TRβ ligand-binding domain. The RTH mutations all localize to three areas around the hydrophobic ligand-binding pocket. In keeping with our functional studies, receptor regions involved in DNA binding, dimerization and co-repressor interaction are devoid of natural mutations [3]. We then studied a novel RTH mutant, in an unusual location outside the three mutation clusters, and showed that it was selectively impaired for co-repressor release and negative regulation of the TRH and TSH genes [11], suggesting that perturbation of these functions might be the minimum receptor abnormalities required to cause RTH.

The clinical symptoms in RTH are variable: many affected individuals are asymptomatic and deemed to have both pituitary and peripheral, or generalized, RTH (GRTH); however, a subset exhibit thyrotoxic features, such as failure to thrive in childhood, or cardiac and sympathetic symptoms in adults, suggesting selective pituitary RTH (PRTH). Our molecular genetic analyses suggest that both GRTH and PRTH are associated with mutations in the TRβ gene, indicating that the two disorders represent phenotypic variants of a single genetic entity [2].

Overall, our studies suggest that the ability to exert a dominant-negative effect within the pituitary—thyroid axis is a key property of mutant proteins, and generates the characteristic biochemical abnormality that leads to the detection of RTH. On this background, variable tissue resistance to thyroid hormone is observed both in different patients and even within a single individual. These observations may be partly explicable on the basis of the differing tissue distributions of the TRα and TRβ receptor isoforms. The liver and pituitary express predominantly TRα1 and TRβ2 respectively, whereas TRα1 is the major species detected in myocardium. Therefore mutations in the TRβ gene in RTH are associated with pituitary and liver resistance, as exemplified by normal serum sex-hormone-binding globulin and non-suppressed TSH levels seen in patients, while the tachycardia seen in many RTH cases may represent retention of cardiac sensitivity to elevated thyroid hormones acting via a normal TRα.

**PPARγ resistance**

PPARγ, an orphan member of the nuclear hormone receptor family, was first characterized as a transcription factor which regulates expression of the adipocyte P2 (αP2) gene [12] and induces murine preadipocyte differentiation [13]. The thiazolidinediones (TZDs) are a novel class of anti-diabetic agents which lower blood glucose by enhancing tissue sensitivity to insulin in vivo [14]. Most pharmaceutical companies have developed such compounds [Troglitazone, Rosiglitazone (BRL49653), Pioglitazone], which are effective in the treatment of Type II diabetes [15] and polycystic ovary syndrome [16], and some are now licensed in the U.S.A. and Europe. TZDs have been shown to bind PPARγ [17], and the rank order of their anti-hyperglycaemic potency mirrors their receptor ligand-binding affinities. The endogenous activator for PPARγ remains unclear, but prostaglandin J₂ [18], unsaturated fatty acids and eicosanoids [19] have been pro-
posed to be natural receptor ligands. PPARγ also regulates target gene transcription as a heterodimer with RXR, and this complex is activated synergistically by both TZDs and RXR-specific ligands [20].

Common disorders such as obesity, Type II diabetes and polycystic ovary syndrome are associated with moderate insulin resistance. Severe insulin resistance is rare, and is defined by the coexistence of extreme hyperinsulinaemia and the skin lesion acanthosis nigricans. The PPARγ gene was screened as a candidate gene in a cohort of 85 subjects with severe insulin resistance. Two different heterozygous, missense (P467L and V290M) mutations in the PPARγ ligand-binding domain were identified in three affected individuals, with autosomal dominant inheritance in one family [21]. Functional studies showed that both mutant receptors exhibited reduced ligand binding and co-activator recruitment, resulting in impaired transcriptional activity. These properties were consistent with crystallographic modelling which predicted that both mutations destabilize helix 12 at the receptor C-terminus, which is involved in ligand binding and co-activator recruitment. In addition to insulin resistance and Type II diabetes, all three patients had also developed marked early-onset hypertension, which was unrelated to complications secondary to diabetes. Coupled with the knowledge that TZDs inhibit vascular endothelin secretion [22] and calcium channel activity [23], and up-regulate nitric oxide synthesis [24], this suggests that PPARγ plays an additional role in the regulation of blood pressure. The occurrence of severe pre-eclampsia in both pregnancies in our index case may also be highly significant, with the recent unexpected observation that murine PPARγ deficiency is embryonic lethal due to impaired trophoblast differentiation and placental vascularization [25]. Although none of the patients exhibited overt lipodystrophy on clinical examination, using magnetic resonance imaging we have recently found that some adipose tissue depots are attenuated.

Analogous to TRβ mutations in RTH, the natural PPARγ mutants inhibited the action of co-expressed wild-type receptors in a dominant-negative manner. In addition, we have shown that the natural mutants repress basal gene transcription. This property is particularly significant, since wild-type PPARγ (unlike TRβ or the retinoic acid receptor) does not repress basal gene transcription or recruit co-repressors when bound to DNA [26]. We have investigated the molecular basis for dominant-negative inhibition, and analysed an artificial dominant-negative PPARγ mutant in which conserved hydrophobic and charged residues in helix 12 were mutated to alanine [27]. This compound PPARγ mutant also inhibits basal gene transcription, recruits co-repressors and exhibits delayed ligand-dependent co-repressor release. When expressed in primary human preadipocytes by infection with recombinant adenovirus, this dominant-negative mutant blocked TZD-induced differentiation. This biological PPARγ antagonist can be used to elucidate the role of this receptor in other pathways.

Conclusions
Heterozygous, loss-of-function mutations in human TRβ and PPARγ genes are associated with the syndromes of RTH and severe insulin resistance respectively. In each case, the mutant receptors inhibit the function of their wild-type counterparts, and such dominant-negative inhibition is mediated by transcriptional silencing via aberrant co-repressor recruitment. It is tempting to speculate that homologous defects in nuclear receptor cofactors might be the cause of hormone (thyroid, insulin) resistance syndromes that are not due to receptor gene abnormalities.

References

Abstract

The binding sites of the oestrogen receptors α and β have a structural requirement for ligands with two oxygen atoms (one of which is phenolic) spaced 11–12 Å apart. They are open to many non-steroidal compounds, such as those from plants and the chemical industry. The importance of this interaction is the subject of current food safety research. This article examines the methods of assessment of oestrogenicity and their relationship to physiological events.

Introduction

The 20th century saw the development of appreciation and interest in the importance of oestrogens. They were first isolated in the late 1920s by Butendant [1] and Doisy et al. [2]. By the 1950s, synthetic oestrogens were being used in oral contraceptives. Although it was appreciated that they had a highly selective effect on specific organs (ovaries, uterus and breast), it was not until the pioneering experiments by Jensen et al. [3] in the early 1960s that a biochemical basis for their action was revealed (incidently, an important spin off of the ability to make radioactive compounds). Jensen’s discovery of a high-affinity oestrogen-binding protein, albeit in small amounts, which apparently migrated from an extranuclear compartment into the nucleus, also opened the window to the study of other nuclear receptors.

It was quickly realized that, prior to its activation by oestrogens, the oestrogen receptor (ER) was part of a larger protein complex [4]. Furthermore, ER and other steroid receptors substantially increased their affinity for binding to DNA following activation by their steroid ligands [5], thereby providing a rationale for how they could have specific effects on the transcription of...