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The Regulation of the Thyroid-stimulating Hormone of the Anterior Pituitary Gland by Thyroid Hormone and by 9-*Cis*-Retinoic Acid

Thyroid-stimulating hormone, TSH, of the anterior pituitary gland is regulated by the binding of the thyroid hormone-activated thyroid receptor to the TSH gene at the same time as the binding of the 9-cis-retinoic acid-activated retinoid X receptor to the same gene. Both interactions, separately or simultaneously, can suppress and thus regulate the expression of the TSH gene.

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An association between vitamin A and thyroid hormones has been known since the 1940s.[1] Investigators observed an increase in thyroid hormones in the serum of vitamin A–depleted rats[2] and, conversely, a significant decrease in the serum thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (T_4) in rats fed excess vitamin A.[3] The anterior pituitary gland was implicated because vitamin A–deficient rats also showed increased serum levels of the anterior pituitary thyroid–stimulating hormone (TSH).[4] Sherman et al.[5] recently found that the

association between vitamin A and the pituitary-thyroid axis also applied to humans. A group of 27 patients with cutaneous T-cell lymphoma was treated with a synthetic retinoid (bexarotene) that specifically binds to the retinoid X receptor (RXR). An unintended side effect of this treatment was a twofold decrease in the patients' serum free thyroxine level and a 40-fold decrease in their serum TSH level, with symptoms of hypothyroidism appearing in 19 of the patients.

The anterior pituitary hormone TSH consists of two noncovalently bound glycoprotein subunits—TSHα, common to all anterior pituitary hormones, and TSHβ, unique to the thyrotropic cells of the anterior pituitary. Secretion of TSH into the circulation stimulates the thyroid gland to secrete T_3 and T_4. Conversely, T_3 and T_4 regulate the output of TSH by feedback inhibition of the transcription of the *TSHα* and *TSHβ* genes.[6] The level of thyroxine in the serum is therefore kept constant; increases in T_3 and T_4 inhibit TSH production, whereas decreases relieve this inhibition, allowing TSH to stimulate the thyroid.

The inhibition of TSH formation occurs through action of the thyroid receptor (TR), a nuclear protein belonging to the family of nuclear receptors that include the retinoid, vitamin D, and peroxisome proliferator–activated receptor. T_3, when bound to the TR monomer, which occupies a TR element half-site on the promoter DNA of the *TSHβ* gene, suppresses transcription of the gene. Recent work suggested that TR acted not as a monomer, but as a heterodimer, binding to DNA together with liganded RXR.[7]

RXR occurs in the form of three isomers: RXRα, RXRβ, and RXRγ. Whereas the α and β isoforms are widely distributed throughout the mammalian organism, the occurrence of RXRγ is restricted to skeletal muscle and brain; it is particularly abundant in the thyrotrope cells of the anterior pituitary gland.[8] The increase in serum thyroid hormones and serum TSH observed when rats were made vitamin A–deficient[3] led to the hypothesis that 9-*cis*-retinoic acid (9-*cis*-RA), the known ligand of RXR, is involved in the suppression of TSH expression. Thus, Breen et al.[9] showed that vitamin A–deficient rats expressed increased levels of the TSHβ subunit of TSH. The case was clinched by Haugen et al.[8] in the following way.

RXRγ exists in two isoforms—RXRγ1 and RXRγ2— that are generated by alternate splicing of the RXRγ mRNA.[10] Haugen et al.[8] used TtT-97 cells in culture, derived from an anterior pituitary thyrotrope tumor. They determined by Northern blot analysis that RXRγ1 mRNA was expressed exclusively in the thyrotrope TtT-97 cells, whereas RXRγ2 appeared in other anterior pituitary cells, such as somatomammotropes (GH3 cells), but was lacking in the TtT-97 cells.
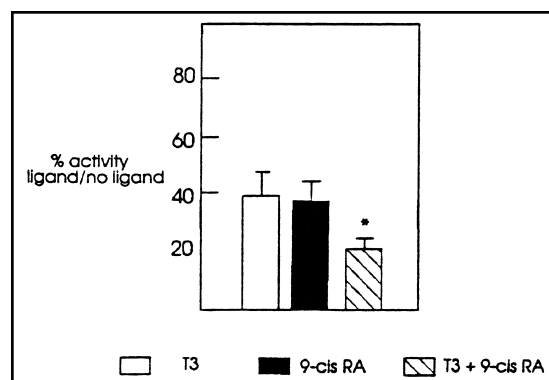

Figure 1. Murine TSHβ promoter activity in TtT-97 cells in the presence of T3 or 9-*cis*-RA. Mouse TSHβ (-390 to $+40$) promotor-luciferase reporter DNA was transfected into TtT-97 cells. These were incubated in the absence or presence of T3, 9-*cis*-RA, or both. Results are expressed as percent promoter activity in the presence of ligand versus no ligand. Asterisk denoted a significant difference ($P < 0.05$) between the T3/9-*cis*-RA group and the other two groups (from reference 8, with permission).

By transfecting a TSHβ promoter DNA attached to a luciferase reporter DNA into TtT-97 cells, the authors[8] found that the reporter indicated a 60% suppression of its activity in the presence of the thyroid hormone T_3, a 60% suppression in the presence of 9-*cis*-RA, but an 80% suppression by a combination of T_3 and 9-*cis*-RA (Figure 1). This important experiment showed that T_3 became attached to TR at the same time as 9-*cis*-RA became attached to RXRγ1; both receptors were residing on the TSHβ promoter. The result was the strong suppression of TSHβ expression in the presence of T_3 and 9-*cis*-RA (Figure 2). For a control experiment, the authors[8] used pituitary somatomammotrope cells (GH3 cells) that respond to T_3, but lack RXRγ1; transfecting the TSHβ promoter–luciferase reporter DNA into these cells showed a response of the reporter to T_3 (60% suppression), but not to 9-*cis*-RA.

Deletion mapping placed the 9-*cis*-RA-mediated suppression on the TSHβ promoter between -200 and -149 bp, similar to the findings of Breen et al.,[6] who also determined that the T_3-responsive elements reside between -15 and $+9$ bp.

A more recent report by Brown et al.[10] described the effect of disruption of the *RXRγ* gene on the response of the pituitary-thyroid axis. The RXRγ knockout mice were produced according to Krezel et al.[11] in the following way. An embryonic stem cell–derived genomic library was screened with a mouse RXRγ full-length DNA probe. A 2.5-kb fragment harboring the RXRγ DNA binding domain in the *RXRγ* gene was replaced by a PGK-NED-poly(A)+ cassette. The resulting DNA was electroporated into embryonic stem cells. Clones were prepared and injected into mouse blastocysts. From the litters obtained, homozygous mice were produced by
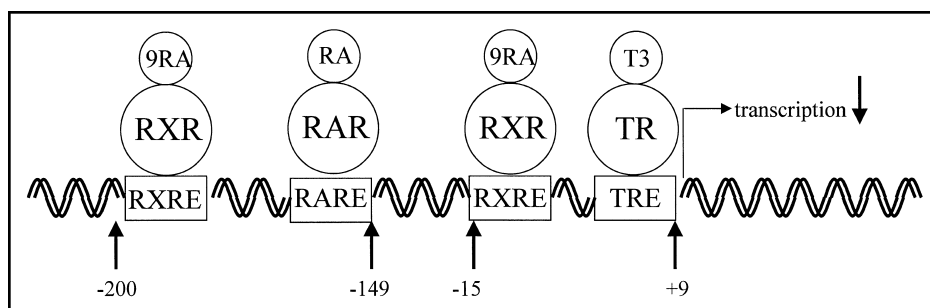
Figure 2. Hypothetical and simplified diagram of the promoter region of the *TSHβ* gene. 9RA = 9-*cis*-RA, RXR = RXRγ1, RXRE = retinoid X–receptor element, RA = retinoic acid, RAR = retinoic acid receptor, RARE = retinoic acid receptor element, T3 = triiodothyronine, TR = thyroid receptor, TRE = thyroid receptor element. Numbers denote nucleotide base pairs from transcription start site. Downward arrow denotes suppression of transcription.

heterozygous matings. These mice were phenotypically normal in their viability and fertility, but failed to express the *RXRγ* gene in their pituitary glands.[12] Using these RXRγ knockout mice, Brown et al.[10] showed complete absence of both RXRγ1 and RXRγ2 mRNA, normal levels of RXRα and RXRβ mRNA, and slightly higher TSHβ mRNA levels. Serum TSH concentration was also somewhat higher in the RXRγ$^{-/-}$ mice. Wild-type and RXRγ$^{-/-}$ mice were made hyperthyroidic by giving them increasing amounts of T_3 in their drinking water. The wild-type mice, by the normal process of feedback inhibition of TSH secretion in response to the increased serum T_3, showed severe suppression of their serum TSH level; serum TSH at the higher T_3 intakes completely disappeared (Figure 3). In the RXRγ$^{-/-}$ mice, administration of T_3 also resulted in its suppression, but to a much lesser degree than in the mutants; it was never completely suppressed, even at high T_3 intakes (Figure 3). This result indicated that in the wild-type mice both the TR and the RXRγ, acting on the TSH promoter, responded with suppression of TSH. In the RXRγ$^{-/-}$

Figure 3. T3 suppression test in littermate mice. Serum from wild-type (open bars) and RXRγ$^{-/-}$ (filled bars) mice was collected after 10 days of treatment with different levels of T3 in the drinking water. TSH levels at each treatment dose are shown. "A" denotes a significant difference, $P < 0.05$ (from reference 10, with permission).

mice, the TR on the TSH promoter, which was intact, responded to the increased T_3. However, the lack of RXRγ attenuated this response, because there was no receptor to which 9-*cis*-RA could bind.

In an inverse experiment, hypothyroid mice were prepared by ablation of the thyroid gland through injection of radioactive iodine. Four months of this treatment resulted in a radical (35-fold) decrease in serum T_4 in the wild-type mice, and a much lesser decrease in the mutant mice (sixfold). As in the hyperthyroid experiment, the serum TSH levels of both the wild-type and the RXRγ$^{-/-}$ mice responded; the serum level of the wild-type TSH rose 40-fold from the level of untreated controls. The TSH of the mutants showed higher levels than the normothyroid controls, despite the mutants' higher serum T_4 compared with wild-type controls. One would have expected the higher serum T_4 to suppress the TSH level, but in absence of RXRγ the response of the pituitary hormone was diminished. Again, this experiment indicated attenuation of the TSH response in absence of the RXRγ receptor, which prevents an input of the retinoid through its receptor.

The authors,[10] by means of whole-animal indirect calorimetric measurements, determined that the RXRγ$^{-/-}$ mice had a metabolic rate 37% higher than their wild-type littermates; this may be the case because the RXRγ$^{-/-}$ mice had increased levels of serum T_4.

In conclusion, the regulation of the anterior pituitary hormone TSH depends on two factors: the binding of the TR, which is activated by T_3 and T_4, to the *TSHβ* gene, and the binding of the RXRγ1, which is activated by 9-*cis*-RA, to the same gene. Each interaction alone and both interactions simultaneously inhibit expression of TSHβ mRNA and TSH hormone production. In this way, there occurs not only the feedback inhibition of serum TSH by serum T_3 and serum T_4, but also inhibition by vitamin A. The physiologic significance of this unique inhibitory action by vitamin A on a hormone remains unclear.
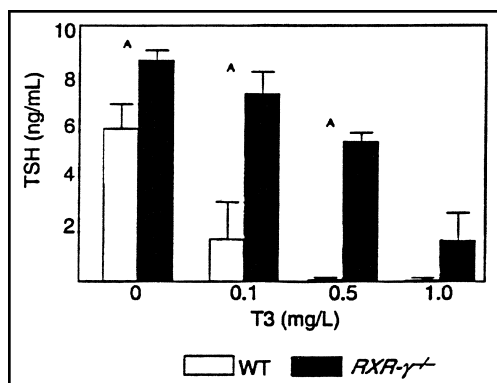
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