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The laboratory and imaging approaches to thyroid disorders

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INTRODUCTION

The central role of the thyroid gland in controlling metabolism was recognized in the 19th century, but evaluation of the function of the thyroid remains an evolving science. Initial approaches to the assessment of thyroid function centered on measuring end-organ responses as biological markers of thyroid hormone actions. Development of in vitro competitive binding assay methods allowed the direct quantification of hormone levels in serum, and sensitive immunoassays have demonstrated the subtleties of pituitary and hypothalamic control of the thyroid. Abnormalities of hormone binding by serum proteins necessitated sensitive estimation of free hormone levels. With the detection of serum markers of autoimmune and malignant diseases of the thyroid gland, earlier diagnosis and improved monitoring of these conditions have been achieved, often with greater sensitivity than may be clinically relevant. Limitations to the measurement methods utilized exist, however, particularly when underlying assumptions about the comparability of patient and control specimens are invalid. Nonetheless, the clinician can now effectively confirm suspected diagnoses of thyroid dysfunction, cost-effectively screen asymptomatic populations for common diseases, and appropriately monitor the treatment of patients with disorders of the thyroid.

PHYSIOLOGY OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS

Excellent reviews and books provide detailed explorations of the physiology of the hypothalamic-pituitary-thyroid axis, and the reader is invited to delve into those worthwhile sources (1). For the purposes of this chapter, a brief review of the biosynthesis and transport of thyroid hormones and the regulation of thyroid function by the hypothalamic-pituitary complex will suffice (Figure 1.1).

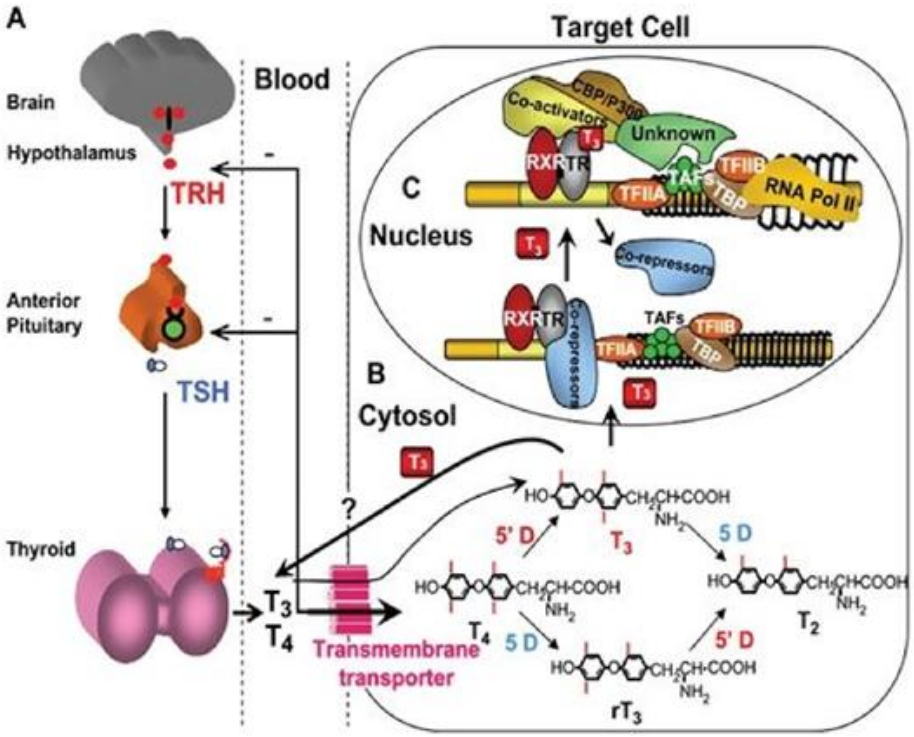

Figure 1.1 The hypothalamic pituitary thyroid axis. (From Refetoff S, Dumitrescu A. Best Pract Res Clin Endocrinol Metab. 2007;21:277–305. Used with permission.)

The synthesis of thyroxine (T_4) and triiodothyronine (T_3) begins with the active transport of iodide into the cell via a sodium-iodine symporter located in the basal membrane. Following oxidation by thyroid peroxidase, the iodide moiety is covalently attached to tyrosyl residues of thyroglobulin, and the resulting iodotyrosines are coupled and cleaved from thyroglobulin to form T_4 and T_3, normally in a 10:1 ratio. Thyroid hormone secretion requires endocytosis and degradation of iodinated thyroglobulin, followed by the release of T_4 and T_3 into the circulation. This process results in the total daily output of 80 to 100 μg of T_4. In contrast, only 20% of the circulating T_3 is produced by the thyroid, the remaining 80% is derived from the enzymatic outer-ring or 5¢-monodeiodination of T_4 in extrathyroidal tissues such as the liver, kidney, brain, muscle, and skin. Removal of the inner-ring or 5-iodine of T_4 forms the inactive metabolite reverse T_3 (rT_3). Other inactivating pathways for T_4 and T_3 include glucuronidation, sulfation, deamination, and cleavage. The normal daily fractional turnover rates for T_4 and T_3 are 10% and 75%, respectively.

In serum, at least 99.95% of T_4 and 99.5% of T_3 molecules are bound by the transport proteins thyroxine-binding globulin (TBG), transthyretin (thyroxine-binding prealbumin [TBPA]), and albumin. Although TBG is present in lower concentrations than either transthyretin or albumin, its greater affinity for thyroid hormones makes it the predominant serum carrier of T_4 and T_3. Variations in binding characteristics among normal and abnormal thyroid hormone-binding proteins are responsible for much of the

methodologic limitations in assays that attempt to measure concentrations of free T_4 and T_3. This large pool of protein-bound hormone provides a stable reservoir that maintains the supply of free, unbound hormone available for transport into the cells. Once within target cells, T_4 is further deiodinated to T_3, which in the nucleus binds to the thyroid hormone receptor, modulating the transcription of thyroid hormone-responsive genes and producing most of the clinical effects recognized as the metabolic effects of thyroid hormones.

The primary regulatory influence on thyroid gland function is the circulating level of thyrotropin (thyroid stimulating hormone, or TSH). Produced by thyrotroph cells of the anterior pituitary, TSH is a two-subunit glycoprotein, the specificity of which is conferred by its β-subunit; the α-subunit is structurally similar to that of follicle-stimulating hormone, luteinizing hormone, and human chorionic gonadotropin. Negative feedback by T_4 and T_3 influences TSH synthesis and release, as evidenced by a complex inverse relationship between the concentrations of TSH and free iodothyronine (2, 3). It is likely that each individual has a genetically determined set-point for this TSH/free T_4 relationship, based on twin studies (4, 5). TSH levels peak just before nocturnal sleep, and the nadir occurs in the late afternoon; this nocturnal surge is lost early in the course of nonthyroidal illness. TSH levels in various populations conform best to a log-Gaussian rather than Gaussian distribution (6). The hypothalamic tripeptide thyrotropin-releasing hormone (TRH) stimulates TSH secretion and modulates thyrotroph response to altered thyroid hormone levels. In conjunction with the suppressive effects of dopamine, corticosteroids, somatostatin, androgens, and endogenous opioids, TRH may be responsible for modulating the setpoint for the negative feedback loop that controls thyroid hormone levels. Hypothalamic production of TRH itself is regulated by circulating thyroid hormones, as well as by multiple central nervous system factors.

LABORATORY EVALUATION OF THYROID FUNCTION

Assays of thyroid hormones

TOTAL SERUM IODOTHYRONINE CONCENTRATIONS

When concentrations and binding affinities of thyroid hormone-binding proteins are normal, there exists at physiologic equilibrium a direct relationship between levels of total hormone and free hormone (7). Thus, measurement of total iodothyronine concentration can provide a reasonable surrogate for estimating the amount of free iodothyronine present. Either serum or plasma can be used to assay hormone concentrations, although serum is generally preferred. The most commonly employed technique for the determination of total T_4 (TT_4) and T_3 (TT_3) concentrations is competitive immunoassay, using either polyclonal or monoclonal "capture" antibodies directed against the specific iodothyronine. To ensure measurement of bound as well as free hormones, inhibitors of iodothyronine binding are added—e.g., 8-anilino-1-naphthalene sulfonic or salicylic acids for TBG and barbital for TBPA. These agents successfully dissociate the hormone from binding proteins without interfering with hormone binding to immunoglobulin.

Radioimmunoassay (RIA) depends upon measurement of the distribution of a tracer quantity of radiolabeled hormone that competes with the endogenous hormone in the patient's specimen for binding to a capture antibody. The higher the serum hormone

concentration, the lower the amount of radiolabel that binds to the antibody. Following the addition of a limited amount of capture antibody and the radiolabeled iodothyronine to be measured, the antibody-antigen complexes are separated from the serum. Separation techniques vary, including ammonium sulfate or second antibody precipitation. Newer methods that facilitate automated separation include attachment of the anti-T_4 antibody to a solid phase, such as the wall of the assay tube or magnetizable particles. The concentration of either TT_4 or TT_3 is then determined by comparison of the amount of antibody-bound radiolabel with a simultaneously derived standard curve. A fundamental assumption, therefore, is that there is no difference in the assay conditions (including protein binding and other constituents found in the serum) between the patient's sample and the control standards, an assumption that is often invalid.

Nonisotopic methods avoid reliance upon radioactive reagents and are now the most commonly used assays. The heterogeneous enzyme-linked immunosorbent assay (ELISA) incorporates enzymes, fluorescent, or chemiluminescent molecules that create a quantitative signal when interacting with a specific enzyme bound to the tracer hormone—e.g., alkaline phosphatase, horseradish peroxidase, or glucose-6-phosphate dehydrogenase. As in RIA, numerous physical and chemical approaches exist for separating signal bound to the anti-iodothyronine antibody from unbound signal. In contrast, homogeneous enzyme immunoassays do not require a separation step. Instead, the binding of the antibody to a tracer hormone directly affects the activity of the signal-generating enzyme bound to the tracer. Other technologies, such as liquid chromatograph-tandem mass spectroscopy (LC-MS/MS) have also been applied to provide greater specificity and less analytical interference (8).

Due to common alterations in serum TBG levels, TT_4 and TT_3 are generally not used as stand-alone tests in clinical practice, but are combined with direct measurements of TBG or TBG-binding capacity, which can then be used to calculate a Free Thyroxine Index (see below).

Reference ranges vary to some degree, but commonly cited ranges are 4.5–12.6 mcg/dL (58–160 nmol/L) for TT_4 and 80–180 ng/dL (1.2–2.7 nmol/L) for TT_3 (9). As developed by their manufacturers, these assay techniques have similar performance characteristics, although each may be affected by different sources of interference. TT_4 assays tend to be more reliable than TT_3 assays. For example, in a recent study, in which 11 TT_4 and 12 TT_3 assays were compared, with LC-MS/MS values as the reference serum concentrations, only 4/10 TT_4 assays and 4/11 TT_3 assays failed to agree to within 10% of the reference concentrations, with greater deviation seen with the TT_3 assay (10). Contributing factors to measurement error include qualitative differences between the protein constituents of sample diluents used for calibration and those found in patient sera, leading to differential dissociation of hormone from binding proteins.

DETERMINATION OF FREE T_4 AND T_3 CONCENTRATIONS

Because T_4 and T_3 are highly bound to serum proteins, alterations in either the levels of these proteins or their binding characteristics can significantly alter the concentration of total hormone. As it is the free hormone that is biologically active, however, techniques are required to permit either direct measurement or estimation of the serum free hormone levels. All methods that have been developed face the identical problem: distinguishing between the 3–4 orders of magnitude difference in the concentrations of the free and the