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Update on Intrathyroidal Iodine Metabolism

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The thyroid concentrates iodide from the serum and oxidizes it at the apical membrane, attaching it to tyrosyl residues within thyroglobulin (Tg) to make diiodotyrosine and monoiodotyrosine. Major players in this process are Tg, thyroperoxidase (TPO), hydrogen peroxide, pendrin, and nicotinamide adenine dinucleotide phosphate (NADPH). Further action of TPO, hydrogen peroxide (H_2O_2), and iodinated Tg produce thyroxine (T_4) and triiodothyronine (T_3). Hormone-containing Tg is stored in the follicular lumen, then processed, most commonly by micropinocytosis. The lysosomal enzymes cathepsins B, L, and D are active in Tg proteolysis. Tg digestion leaves T_4 and T_3 intact, to be released from the cell, while the 3,5′-diiodotyrosine (DIT) and 3-iodotyrosine (MIT) are retained and deiodinated for recycling within the thyroid. Some areas of especially active recent research include: (1) the role of molecular chaperones in directing properly folded TPO and Tg to the apical membrane; (2) details of proteolytic pathways; (3) modulation of iodine metabolism, not only by thyrotropin (TSH) but by iodine supply and by feedback effects of Tg, glutathione, and inhibitory elements in the N-terminal region of Tg; and (4) details of Tg structure and iodotyrosyl coupling. Despite general agreement on the major steps in intrathyroidal iodine metabolism, new details of mechanisms are constantly being uncovered and are greatly improving understanding of the overall process.

Introduction

THE THYROID'S only clearly established function is to make its hormones, thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3). Iodine is an integral part of T_4 and T_3, contributing 65% and 59% to their respective molecular sizes. This article examines how the thyroid takes iodine from the circulation and returns it as hormone. Recent reviews have examined aspects of this topic (1–5). Also, in this issue of *Thyroid*, Shen et al. (6) describe the sodium iodide symporter (NIS), which transports iodide into the thyroid from the bloodstream. Here we focus on iodine once in the thyroid cell, with emphasis on recent experimental findings.

Figure 1 provides an overview of iodine metabolism, including thyroglobulin (Tg) iodination, hormone formation, hormone release, and factors that influence these processes. While the broad outlines have been known for some time, new details regularly appear in the literature, especially in the areas of cell trafficking and regulatory mechanisms.

Iodine's role within the thyroid cells is to provide raw material for hormone synthesis. To be useful, the iodide transported across the thyroid's basal membrane by NIS must be oxidized and attached to tyrosyl residues within Tg. The major actors in this process are thyroperoxidase (TPO), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide phosphate (NADPH), pendrin, cell trafficking proteins (the molecular chaperones), and Tg itself. Below we introduce these, and then describe how they collaborate to promote hormone formation.

Thyroglobulin

Tyrosyls within the peptide chains of this protein are the substrate for iodine attachment to produce the thyroid hormones. Tg synthesis in the endoplasmic reticulum is governed by thyroid specific transcription factors, particularly TTF-1, TTF-2, and Pax-8. These same transcription factors also control synthesis of TPO and the TSH-receptor. Of the 48 exons (7,8) of the Tg gene, exons 2, 18, 44, 45, and 48 encode the principal hormonogenic tyrosyl residues. The Tg gene appears to be a fusion of at least three distinct ancestral genes that code respectively for the N-terminal portion of 1,190 residues, a C-terminus of 541 residues, and a bridging midportion. The N-terminal portion has extensive internal homology, and shares a 5-residue motif with other proteins including saxiphilin and nidogens. The C-terminal region has a 28% homology with acetylcholinesterase (9).

After synthesis, the nascent Tg peptide chains initially aggregate, then separate into monomers that fold and form stable dimers (4,10,11). This process is aided by molecular chaperones, which next escort Tg to the Golgi for glycosylation. Approximately 10% of the weight of Tg is carbohydrate, dis-

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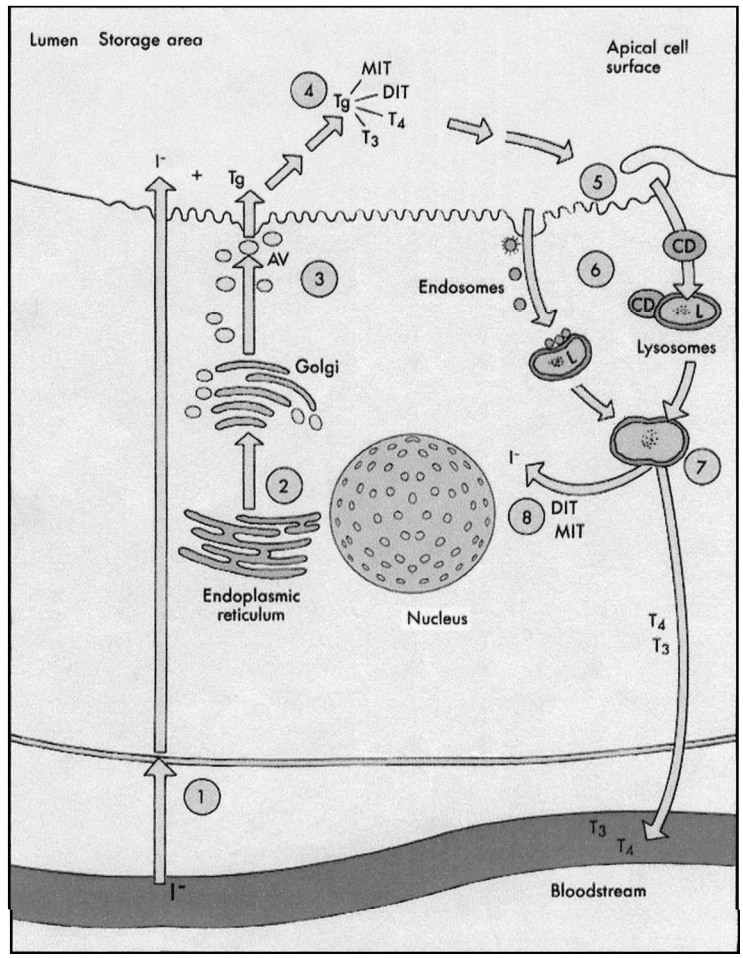

FIG. 1. Synthesis and secretion of hormone by the thyrocyte. (1) Iodide, concentrated at the basal cell membrane by a symporter (NIS), moves along its electrochemical gradient to the apical cell surface. (2) The polypeptide chain of Tg, synthesized on the surface of the endoplasmic reticulum, is translocated into the endoplasmic reticulum lumen. Carbohydrate chain synthesis is initiated as the polypeptide undergoes a series of conformational changes aided by folding enzymes and molecular chaperones. On formation of stable dimers, the nascent protein enters the Golgi where carbohydrate units are completed and sulfation occurs. (3) Newly formed Tg is transported to the apical surface in small apical vesicles (AV). (4) Iodination of Tg, and coupling of iodotyrosyl precursors to form thyroxine (T_4) and triiodothyronine (T_3) occur at the apical cell surface, mediated by thyroid peroxidase (TPO). (5) Tg retrieved by micropinocytosis passes first through the endosome system, where proteolysis and hormone release is initiated, then into lysosomes (L), where the process is completed. (6) Tg retrieved by bulk intake into colloid droplets (CD) passes directly into lysosomes. (7) T_4 and T_3 enter blood stream. (8) Iodide released from 3-iodotyrosine (DIT) and 3,5′-diiodotyrosine (MIT) is recirculated. (Adapted from Dunn AD, Dunn JT. In: Brody TM, Larner J, Minneman KP (eds) Human Pharmacology: Molecular to Clinical, 3rd edition, 1998, Mosby, St. Louis, p 535).

tributed mainly in two units. One of these contains mannose and N-acetyl glucosamine; the other, more complex, has several chains of N-acetyl glucosamine, galactose and fucose or sialic acid extending from a mannose core. The most common linkage is between N-acetyl glucosamine and an asparagine residue in Tg. Tg also contains phosphate in the complex carbohydrate unit and as phosphoserine and phosphotyrosine (12), and also sulfate, some in a chondroitin sulfate unit. In one study, sulfation of tyrosine in Tg correlated with hormone formation (13).

TPO

This enzyme at the apical membrane catalyzes the oxidation of iodide to an iodinating species that forms iodotyrosines and subsequently iodothyronines. The TPO gene codes for a 933-residue 103-kd protein (14). TPO has a 44% homology with myeloperoxidase that increases to 74% around the heme group located at histidine residue 407 (15). Gene transcription is controlled by TTF-1, TTF-2, and Pax-8, the same factors regulating Tg, NIS, and the TSH receptor. The nuclear p300 protein is a coactivator of Pax-8 in the TPO gene promoter (16). TPO is synthesized on polysomes, and glycosylated with mannose-rich oligosaccharide units in the Golgi. Proper folding of the molecule is essential for its activity, and only about 2% of the total TPO reaches the apical surface; the partially folded remainder is degraded by serine and cysteine proteases of the endoplasmic reticulum membrane (17). Calnexin and calreticulin are essential to initial folding (18).

H_2O_2

The thyroid couples the reduction of H_2O_2 by TPO with the oxidation of iodide to permit Tg iodination and hormone formation. Thus, H_2O_2 generation is a key control point in hormonogenesis. Its production requires both calcium and NADPH; the latter, in turn, derives from the action of NADPH oxidase on NADP. cDNAs for two NADPH oxidases code for peptides of 1,551 and 1,548 residues (19). These have a 43% homology with the first 500 residues of TPO. NADPH oxidase activity requires calcium, although generation of H_2O_2 from NADH also occurs and is only partially calcium dependent (20). Results in FRTL-5 cells suggest a negative feedback relationship between calcium and H_2O_2 (21), and calcium's regulation of NADPH oxidase reportedly involves its thiol groups (22).

The detailed molecular events in H_2O_2 generation are not established. In one scheme, NADPH oxidase produces oxygen, with subsequent conversion to superoxide (O_2^-), and then to H_2O_2 by superoxide dismutase (23). An alternate proposal argues for direct electron transfer from NADPH to oxygen (24).

In addition to its direct role in Tg iodination, H_2O_2 can affect thyroid cell metabolism in other ways. It may control attachment of heme to TPO at the apical membrane (25). Corvilain et al. (26) report that iodide stimulates H_2O_2 generation in thyroid slices in sheep, pig, bovine, and dog, but not consistently in horse and human, and the stimulation is greater in thyroids with lower iodine concentrations. This effect may help thyroids with lower iodine concentrations use iodine more effectively, but at the same time protect those with excess iodine from overproducing thyroid hormones.

Although H_2O_2 is essential to Tg iodination and other thyrocyte activities, it can also be toxic. Iodination within the cell is apparently avoided by controlling the intracellular availability of H_2O_2 through the action of glutathione peroxidase (27). Thioredoxin reductase, a 57-kd selenoprotein, has also been proposed as a means for the thyroid to detoxify H_2O_2 (28). TSH and H_2O_2 stimulate expression of peroxiredoxin 1, thus reducing H_2O_2-induced apoptosis in FRTL-5 cells, suggesting a protective mechanism (29).

Pendrin

This protein functions as an iodide/chloride transporter at the apical membrane. Genetic mutations lead to Pendred's syndrome (defective iodine organification, goiter, and congenital neurosensory deafness), hence the name. The gene, cloned by Everett et al. (30,31) in 1997 codes for a protein of 780 amino acids with high homology to several sulfate transporters. The protein's approximate molecular weight is 110 to 115 kd and it probably contains carbohydrate (32). Its expression in FRTL-5 cells was increased by low Tg concentrations (33).

Preparation for iodination

Tg, TPO, H_2O_2, and iodide are the essential components for iodination, which takes place at the apical membrane. Once iodide is transported across the basal membrane by NIS, it migrates, apparently freely, to the apical membrane, where pendrin probably positions it strategically for iodination. TPO and Tg must first undergo intracellular quality control to ensure proper folding and glycosylation before they are allowed to reach the apical membrane. Details of this process have been carefully studied by Kim and Arvan (4,10) and Kim et al. (11). First, proper folding of nascent peptides is controlled by endoplasmic reticulum enzymes including protein disulfide isomerase and peptidyl-prolyl isomerase. Several molecular chaperones, most notably calnexin, BIP, GRP94, ERP72, supervise Tg's subsequent maturation. Misfolded or misglycosylated Tg is rejected and degraded. The thyroid applies similar quality control mechanisms to TPO. Tg and TPO molecules that pass muster are transported in exocytotic vesicles to the apical membrane.

Iodide oxidation

Taurog (5) recently reviewed this topic in detail. Candidates for the iodinating intermediate include free radicals of iodine, the iodinium ion (I^+), and hypoiodite (OI^-). In the first of these proposed mechanisms, TPO-bound free radicals of both iodine and tyrosine are produced by oxidation to form iodotyrosines. The scheme with I^+, drawn from spectral change studies, suggests initial oxidation of TPO and then oxidation of I^- to I^+, followed by iodination of tyrosine. The third proposal also involves a two-electron change to produce hypoiodite and subsequent tyrosine iodination. The correct scheme from these choices is not yet established.

Tg iodination

Oxidized iodide attaches to tyrosyls within the Tg polypeptide chain, producing either 3-iodotyrosine (MIT) or 3,5′-diiodotyrosine (DIT). The human Tg dimer contains 132 tyrosyl residues, but they are not equally susceptible to iodination. Detailed mapping with incremental iodination of low-iodine hTg has identified favored sites at tyrosyls 2554, 130, 685, 847, 1447, and 5, in that approximate order. (The numbering of amino acids in hTg may vary by one or two residues in different reports reflecting slight species differences and recent refinements in the cDNA sequencing [34]). Three consensus sequences favor iodination: Asp-Glu-Tyr, Ser/Thr-Tyr-Ser, and Glu-X-Tyr, with some variation among species.

Hormone formation

After DIT and MIT form, two residues of DIT couple to make T_4, or one DIT and one MIT make T_3, all still within the Tg molecule. In this reaction, further oxidation, mediated by TPO and H_2O_2, produces an iodophenyl free radical, leaving T_4 or T_3 at the acceptor site, and dehydroalanine at the donor position. For hTg, the preferred sites for Tg formation are residue 5, 2554, and 2747, accounting respectively for 36%, 22%, and 15% of Tg's total T_4 in a typical study (35). Other animal species show some variation, but residues 5 and 2554 are consistently the most important. The sequence Asp/Glu-Tyr is the most common one for T_4 formation. Tyrosyl 130 is the outer ring donor for the most important T_4 site at residue 5 (36). The iodine content of mature Tg varies from 0.1% to 1.0% of its weight, or about 5 to 50 atoms iodine per 660 kd. A typical molecule, under conditions of normal iodine supply and thyroid activity, contains about 2.5 residues of T_4, 0.7 of T_3, 4.5 of DIT, and 5 of MIT.

Other iodination effects

In addition to hormone formation, Tg iodination is associated with fairly discrete cleavages in the Tg polypeptide chain (37,38). Chemically reduced hTg contains N-terminal 26-kd and 18-kd peptides, the latter increasing and the former decreasing with progressive iodination. These N-terminal cleavages contain the most important hormonogenic site of Tg at residue 5 as well as its donor at residue 130. Similar cleavages are found in all animal species examined. TSH stimulation enhances these cleavages. Their physiological significance is not established; perhaps they make the N-terminal area more susceptible to subsequent proteolytic degradation to release hormone.

Orderly formation of thyroid hormone depends on the proper amount of iodine available. Excess iodine permits formation of iodotyrosines, but inhibits hormonogenesis by tying up the TPO-iodinating species and diverting it from further iodination of iodotyrosyls to form thyroid hormones.

Iodinated lipids

Iodinated lipids also occur in the thyroid, especially after high doses of iodide. One of these, 2-iodohexadecanal increases linearly with iodine addition (39,40). It inhibits NADPH oxidase, and lipid iodination appears to decrease H_2O_2 production, and may thus retard Tg iodination as well.

Iodine storage

The mature iodinated Tg molecule is secreted into the follicular lumen, where it comprises most of the colloid. In this form, about one-third of Tg's iodine is in T_4 and T_3, the remainder being in the inactive precursors, DIT and MIT. Colloid represents a convenient storage form for both thyroid hormone and iodine. Tg can exist in two forms in the colloid. One is the soluble 19S 660 kd dimer and its tetramer. The other is a dense insoluble material (i-Tg) reaching a concentration near 600 mg/mL, held together by bonds of disulfide, tyrosine, and glutamyl-lysine crosslinks and containing virtually no thyroid hormone (41). The human thyroid has about 34% of its Tg in this form (42). This i-Tg is fairly resistent to the thyroid's usual proteolytic enzymes, but its iodide may be released by reactive oxidative species present within the thyroid (43,44).

Tg breakdown and hormone release

Further processing of Tg normally requires its reentry into the thyroid cell. In most species, including man, this occurs by micropinocytosis. Coated pits form at the apical surface and Tg is internalized into small vesicles that rapidly fuse with early endosomes (45). The process may be initiated by receptor mediated- or nonselective fluid phase endocytosis or perhaps by both, depending on Tg's ultimate fate. Once internalized, Tg follows one of at least three possible pathways. The most important of these results in its proteolytic breakdown and hormone release. Rousset and coworkers (46,47) used reconstructed porcine thyroid follicles and a combination of radioactive and immunogold labeling techniques together with intracellular markers to follow resorbed Tg from its first intracellular appearance within coated vesicles through its travels in the endosomal system and finally into lysosomes. Tg's gradual loss of immunogenicity begins within the endosomal system, presumably reflecting initiation of its proteolytic destruction. Endosomes are known to contain lysosomal enzymes in an acid environment conducive to their activity. Earlier studies by this group had characterized high molecular weight Tg within lysosomes as low in iodide, lacking hormone, and containing multiple peptide fragments held together by disulfide bonds (48). Evidently, selective removal of hormone and its iodinated precursors occurs prior to the destruction of Tg's peptide backbone.

Once iodoamino acids are removed from Tg they rapidly leave the lysosomal compartment. MIT and DIT are deiodinated by an iodotyrosine-specific deiodinase present in thyroid tissue and their released iodide becomes an important source of iodine for hormone synthesis (49). After its passage from lysosomes, an undetermined fraction of T_4 is first deiodinated to T_3 before leaving the cell by a thyroidal 5′-deiodinase similar to that found in peripheral tissue (50). The mechanism by which the hormones exit the thyrocyte is unknown but recent evidence suggests that export of T_3 may involve a carrier system that is saturable, stereospecific and verapamil sensitive (51). The endocytotic mechanism that initiates the proteolytic pathway has not been defined. The high concentration of Tg in the colloid (100–600 mg/mL) suggests that a high-affinity receptor may be unnecessary for efficient Tg retrieval. Despite intensive research over the past several decades, there is no compelling evidence that such a receptor exists.

Selective enzyme inhibition studies indicate that the lysosomal enzymes cathepsins B and L, and to a lesser extent cathepsin D, may be important in the early steps of Tg proteolysis (52). Isolated cathepsins B, D, and L can each cleave off hormone-enriched fragments from both the N and C termini of Tg *in vitro*. Because each does so in a distinctive pattern they may work in synergy *in vivo* (53). The combined actions of cathepsin B and lysosomal dipeptidase I can release T_4 from Tg's most important hormonogenic site at residue 5 (54).

Recent evidence suggests that Tg proteolysis may not be confined to the endosome-lysosomal system. Limited proteolysis of Tg and release of T_3 can occur extracellularly in cultured porcine thyrocytes (55,56). Several cysteine proteases, may be involved, notably cathepsins B, L and K, which are secreted in active form to the cell surface in this *in vitro* system. The physiological importance of this pathway remains to be determined.

Not all internalized Tg undergoes proteolysis. Some is recycled back to the follicular lumen, apparently by a selective process targeting immature Tg molecules. Follicular Tg is heterogenous in its iodine and carbohydrate composition and a close correlation exists between iodine-poor and incompletely glycosylated species. The existence of receptors capable of recognizing exposed carbohydrate moieties would therefore provide a means of selecting for immature molecules. Kostrouch et al. (57) found that reconstructed porcine thyroid follicles initially internalized bovine serum albumin (BSA) and Tg at similar rates, but the subsequent distribution of the two proteins suggested that some Tg was returned to the follicular lumen. In related studies Miquelis et al. (58) found that thyroid follicle fragments took up glycosylated derivatives of BSA from the incubation medium but returned only those with exposed N-acetyl-glucosamine.

Cells of the same follicle system internalized ovomucoid, a glycoprotein with exposed N-acetylglucosamine residues, then sequestered it into the Golgi where it incorporated galactose. The same group had previously obtained evidence for an N-acetylglucosamine-specific receptor in thyroid membranes (59) using ^{125}I-labeled N-acetylglucosamine BSA as a probe. Binding was highest at an acid pH, which would suggest its association with Tg retained within the endosome/lysosomal pathway. Binding required calcium ions and exhibited positive cooperativity. It was inhibited by Tg and to a greater extent by asialoagalactoside-Tg with exposed N-acetylglucosamine sites. The receptor was located at or near the apical cell surface by cytochemical techniques. Additional studies identified a peptide determinant within the amino terminal domain of human Tg residing between ser^{789} and met^{1172} (60). It was suggested that this N-acetylglucosamine receptor selectively binds immature Tg molecules and redirects them into the Golgi for further processing before being recycled back into the follicular lumen. The putative N-acetylglucosamine receptor has thus far eluded isolation and identification. Recent studies suggest that protein disulfide isomerase, an enzyme believed to contribute to the maturation of Tg in the endoplasmic reticulum, has many of the properties attributed to the N-acetylglucosamine receptor and could itself mediate Tg recycling (61). This protein is secreted by FRTL-5 cells and becomes associated with the plasma membrane. At an acid pH, the binding of Tg in FRTL-5 cells is selectively inhibited by antibodies to protein disulfide isomerase. The isomerase preferentially binds immature low-iodine Tg, and the Tg binding domain corresponds to that identified earlier on the N-acetylglucosamine protein receptor.

Indirect evidence suggests an asialoprotein receptor could also mediate Tg recycling. Consiglio et al. (62) originally described Tg binding to thyroid membranes that was optimal at an acid pH, and showed a preference for asialo- and iodine-poor Tg. An asialoglycoprotein receptor was eventually identified in thyroid cells (63) and found to be closely related to the corresponding receptor in liver cells. Characteristics relative to its possible role in Tg recycling in the thyrocyte include: its localization on the apical cell membrane of FRL thyroid cells (64), its TSH-dependent expression in PCC13 rat thyroid cells (64), and the ability of a recombinant protein corresponding to its carbohydrate binding to bind asialo-Tg (65). The recombinant protein specifically recognizes an amino terminal fragment of Tg (66). A surprising recent report, however, suggests this receptor could have the seemingly unrelated function of suppressing thyroid specific gene expression (67).

In a third intracellular pathway in thyroid cells, internalized Tg is transported intact from the apical to the basolateral cell membrane within vesicles and subsequently released into the bloodstream (68). This process, termed transcytosis, is stimulated by TSH and contributes to the pool of circulating Tg. Recent evidence suggests that transcytosis is receptor mediated. Megalin, a membrane protein of the low-density lipid receptor family, is expressed on the FRTL-5 cell surface in a TSH-dependent manner (69). Tg binding to megalin in FRTL-5 cells was saturable and of high affinity. Megalin inhibitors not only reduce Tg's binding but partially depress its endocytosis. In polarized FRTL-5 cells incubated in bicameral chambers, megalin-mediated endocytosis does not lead to the proteolytic pathway as it does in other cells but rather is associated with basolateral release of intact Tg, and thus, through the transcytosis pathway (70). It was suggested that the function of the pathway might be to restrict the release of thyroid hormone under conditions of intense TSH stimulation.

Modulators of iodine metabolism

TSH stimulates most aspects of intrathyroidal iodine metabolism, covered in many reviews (e.g., Dunn and Dunn [1], Dunn [2], and Taurog [5]). These effects include: increased production of Tg, TPO, and NIS, through stimulation of transcription factors; increased production of hydrogen peroxide from increased NADPH oxidase; modification of TPO distribution in vesicles (71); increased T_3 formation relative to that of T_4; an altered distribution of iodine and T_4 among hormonogenic tyrosyls; iodine-associated cleavage of peptide bonds; formation of colloid droplets; and enhanced activities of cathepsins B and L, but not of cathepsin D in lysosomes. Overall, these interrelated effects increase iodine's uptake by the thyroid and its efficient return to the circulation as hormone.

Iodine supply is another factor that modulates intrathyroidal iodine metabolism. Iodine deficiency leads to increased TSH stimulation, increased iodine uptake, rapid iodine turnover, and enhanced production of T_3 relative to T_4. Iodine excess clogs the TPO-H_2O_2 iodinating mechanism and retards thyroid hormone synthesis on Tg. In experimental rats, iodine organification appeared to increase follicular size (72).

According to recent reports, follicular Tg can selectively alter expression of thyroid transcription factors in FRTL-5 cells, apparently by decreasing TTF-1 promoter binding to nuclear factors (73). This effect has been seen with TTF-1, TTF-2, and Pax-8, with suppression of NIS, TPO, Tg, and TSH-receptor genes. These findings suggest that Tg can influence iodination and hormone formation by regulating its own synthesis and that of other proteins involved in iodination (74). In a similar vein, saxiphilin inhibits several cysteine proteases, including cathepsins B and L, and contains the type I domain of Tg; this latter is found in many secreted and membrane proteins, including Tg, offering a possible means for the Tg molecule to depress its own degradation (75).

Various experimental studies suggest still other mechanisms that may affect intrathyroidal iodine metabolism. In FRTL-5 cells, decreased glutathione concentration lowered TTF-1 and Pax-8, reducing expression of Tg mRNA but not that of TPO (76). Lowered intracellular copper was accompanied by decreases in Pax-8 mRNA and in TPO expression, without effect on Tg, again in FRTL-5 cells (77). Studies with human thyroid cells in primary culture suggest that nitric oxide stimulates TPO activity (78).

These many studies describe a complex series of checks and balances that the thyroid uses to control the orderly utilization of iodine for hormone synthesis and release. The experiments need to be extended, with a constant eye on how they translate to the normal physiology of the intact thyroid.

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