Role of the Iodothyronine Deiodinases in the Physiology and Pathophysiology of Thyroid Hormone Action

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Abstract
Thyroxine (T4) is a prohormone and must be activated to 3,5,3’-triiodothyronine (T3) by either type 1 (D1) or type 2 (D2) selenodeiodinase. A third deiodinase (D3) inactivates T3 or T4 by removal of an inner ring iodine. These reactions require both a deiodinase enzyme and a cofactor, probably a thiol, to reduce the oxidized selenolyl group in the active center of each deiodinase. Thus, deiodination rates depend on both the enzyme and cofactor. The source of most of the circulating T3 is D1-mediated, while D2 provides nuclear receptor-bound hormone. Using sensitive and specific assays, it has become apparent that both D2 and D3 are widespread throughout vertebrate tissues. The complex interactions between the activating D2 and the inactivating D3 in tissues expressing these two enzymes determine the intracellular T3 concentration. This provides enormous flexibility for both developmental and tissue regeneration processes, allowing exquisite control of intracellular T3 concentrations. The endogenous factors regulating the activity of these enzymes, such as the hedgehog proteins, FoxO3, or the wnt/β catenin pathway together with the actions of thyroid hormone transporters, direct adjustments of nuclear receptor-bound T3 which in turn can control the balance between cellular proliferation and differentiation. Their actions provide dynamic flexibility to what appears on the surface to be a very static hormonal system.

Introduction
When dietary iodine is sufficient, the major product of the thyroid gland is thyroxine (T4) which is secreted at a rate of 10-fold that of 3,5,3’-triiodothyronine (T3) in humans. Thus, a rate-limiting step in thyroid hormone action is the conversion of the prohormone T4 to T3. This is the major role of two enzymes – type 1 and 2 iodothyronine deiodinases (D1 and D2, respectively) (fig. 1). While the half-life of T4 in humans is about 1 week, that of T3 is much shorter, <24 h, and its inactivation in peripheral tissues by removal of an inner ring iodine from T3 is primarily due to the action of type 3 iodothyronine deiodinase (D3). As discussed below, there are two important systems for maintaining T3 homeostasis in vertebrates. These systems maintain the synthesis and secre-
tion, and therefore the circulating concentration, of T4 at remarkably stable levels through the actions of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH). However, since T4 is a prohormone, the systems controlling its secretion rate depend largely on the effects of the thyroid hormone receptor-bound T3 derived from it. Thus, the iodothyronine deiodinases which control the formation and degradation of T3 are critical to the maintenance of the euthyroid status.

The key to this process is the actions of the deiodinases which are subspecialized in different tissues. Most of the T3 in the circulation is derived from the actions of D1 in the liver, kidney, and thyroid [1]. However, in the hypothalamus and pituitary thyrotroph, D2 permits the feedback loop to recognize the concentrations of T4 due to the fact that T4 is rapidly converted by D2 to T3 in those cells [2–4]. In the anterior pituitary nuclei, approximately one-half of the T3 bound to nuclear receptors is derived from circulating T3 whereas the remaining half comes from intrapituitary T4 to T3 conversion [1]. Recent studies have illustrated that D2 is highly expressed in thyrotrphs and its activity is regulated by T4 posttranslationally, per se [5]. The D3 content of thyrotrph tumor cells is quite low, indicating that the rate-limiting step in the action of T3 on TSH is at the level of its formation from T4 and together with that from the plasma balanced by its efflux from the nucleus and cytosol.

The major steps in the actions of T4 are initiated by the entry of T4 into the cell which is now recognized to be controlled by expression of various thyroid hormone transporters [6–8]. It must then be converted to T3 either in D1- or D2-expressing cells, allowing a dual source of T3 (systemic and local) to the hypothalamic-pituitary (HPT) axis. After transport into the cell, cytosolic T3 then enters the nuclei of cells in the HPT by yet to be defined pathways, and then binds to high-affinity chromatin-bound proteins, predominantly the thyroid hormone receptor [9]. Due to the rapid generation of T3 from T4 within these cells by D2, the cytosolic T3 concentration is higher and remains in a static disequilibrium with the serum T3 [1, 10, 11]. We now appreciate that D2 is expressed in a large number of tissues (albeit at low levels in some), including skeletal muscle, osteoblasts, pituitary cells, astroglia, endothelial cells, retina, cochlea, placenta, endothelial cells, and, perhaps, others [12].

The expression of D3 is especially important during fetal development. D3 is preferentially expressed in neurons, but in Siberian hamsters D3 also appears to be found in specialized glial cells lining the lower portions of the third ventricle of the hypothalamus, termed tanyocytes, where it is reciprocally regulated with D2 to main-
tain local control of the T3 concentration [13–15]. These interlocking pathways allow remarkably stable concentrations of T3 while also facilitating specific chronotropic and tissue-specific changes in T3 concentrations during periods when developmental or regenerative programs call for a transient increase or decrease in specific cells. This change cannot be accomplished by alterations in TSH since this would change the supply of thyroid hormone to the entire organism. Thus, the control of intracellular T3 concentration is local and requires D2 and D3.

One striking example of the cooperation of these two pathways occurs in iodine deficiency which represents an enormous challenge for terrestrial vertebrates. Much of the iodine initially in the earth’s crust has been eluted into the oceans by glaciation over many eons such that the soil content in many regions is inadequate to provide sufficient iodine in locally grown foodstuffs for the daily synthesis of sufficient T4. Under these circumstances, the HPT axis recognizes the deficiency of the prohormone T4 via its role as a substrate for D2 in the cells of the HPT axis. By an iterative process, D1-mediated T3 decreases but it is partly replenished by an increase in thyroidal T3 production by increased TSH secretion, as well as by increased efficiency of D2-mediated T3 formation. At the same time, the T3-dependent Dio3 gene transcription decreases, reducing neuronal D3 and prolonging the half-life of T3 [16]. This combination allows a decrease in the feedback suppression of TRH and TSH where the latter causes thyroid cell hyperplasia, and increases the sodium iodide symporter, thyroidal D1 and D2, thyroglobulin turnover, and the ratio of T3:T4 in thyroidal secretion. Thus, virtually all of these internal adjustments, including the downregulation of the T3-dependent D1 in the liver and kidney and increases in D2, permit conservation of whatever T4 can be secreted by the thyroid for the production of T3.

It is important to recall that the thyroid physiology of humans, especially those who are iodine-sufficient, differs considerably from that of rodents, the model animals for many of the experiments used to examine these adaptations. In humans, 80–90% of the T3 is formed by outer ring deiodination of T4. It is of interest in the context of this new journal that the first demonstration of T4 to T3 conversion in humans was provided by Dr. Rosalind Pitt-Rivers, a founding member of the European Thyroid Association [17]. In human thyroglobulin, the molar ratio of T4:T3 is about 15:1, with each 660,000 kDa molecule of this thyroidal protein containing approximately 3–4 residues of T4 while only about 1 in 5 contains a T3 residue [18]. While the expression of both D1 and D2 in the human thyroid cell increases T4 to T3 conversion such that the T4:T3 ratio is approximately 10:1 in thyroid secretion, this accounts for only a minor portion of circulating T3 in humans [19]. On the other hand, the thyroglobulin of the mouse and the rat have much lower T4:T3 ratios, about 3–4:1, and thus about half of the circulating T3 in these species derives directly from thyroid secretion [20]. This is especially important to note in the mouse in which a genetic inactivation of either or both D1 and D2 does not change serum T3 concentrations [21].

General Properties of the Iodothyronine Deiodinases

There are important common features of the three deiodinases which should be noted (table 1). They are all integral membrane proteins, although they are expressed in different cellular locations. D1 is found in the plasma membrane and D2 in the endoplasmic reticulum but both have their active centers in the cytosol, thus allowing access to cytosolic thiol cofactors [1, 22]. The precise location of D3 has been more difficult to discern, with the likely location being the plasma membrane but it is recycled through endosomes such that it may have actions on both extracellular and intracellular T3. This may be very important for the differential response of T4 activation (by D1 and D2) and T4 and T3 inactivation (by D3) to oxidative stress (see below) [23].

Another common feature of the deiodinases is that they belong to the small family of 25 selenoproteins in the human genome. They contain the rare amino acid selenocysteine (Sec) encoded by UGA in their active centers [24]. The much higher nucleophilicity of selenium (as opposed to the sulfur of cysteine) makes these enzymes 100-fold or more lower than that of the same protein containing cysteine due to the necessity to override the STOP codon function of UGA in order to cotranslationally insert selenocysteine [25, 26]. This process requires a specific stem loop structure in the 3′ untranslated region of the mRNA termed a ‘SECIS element’ (fig. 2) [26, 27]. In the following sections, some of the more important physiological systems which depend on the iodothyronine deiodinases are reviewed (table 2).
Thyroid Hormone Activation by D1 and D2

D1 is the likely source of most of the plasma T3 in humans and it was the first deiodinase to be cloned and studied intensively [1, 24]. Inhibition of D1 activity in the liver and kidney is commonly found in fasting or in illness, and a reduction in its function either through decreases in cofactor or T4 uptake, or an impairment of D1 transcription due to reduced T3, contributes to the reduction in serum T3 in sick patients [28]. This is often referred to as the 'low T3 syndrome'. Curiously, despite the fact that high D1 activity is expressed in the liver, kidney and thyroid, most of the T3 bound to the nuclear receptor in these tissues is derived from plasma T3. This is quite different from the situation in the pituitary and the cerebral cortex where most of the receptor-bound T3 derives from D2-mediated T4 to T3 conversion [1, 28, 29]. Another unique feature of D1 is that its activity is blocked by propylthiouracil (PTU) [30]. This is thought to occur through the competition of the thiol group of PTU with the endogenous thiols which are required for the reduction of the Se-I complex formed during D1-mediated iodothyronine deiodination [31]. D1 expression is, somewhat paradoxically, induced transcriptionally by T3 in liver and kidney and by thyroid receptor-stimulating immunoglobulins in thyroid, explaining why PTU is so effective in blocking T3 formation in patients with hyperthyroidism [32, 33]. Severe hyperthyroidism (thyroid storm) is a major indication for the use of high-dose PTU in adults due to its effects on D1-mediated T4 to T3 conversion in liver, kidney, and thyroid tissues.

The fact that D1 is a T3-dependent protein contributes to the adjustments that occur in the patterns of T4 to T3 conversion in hypothyroidism. Human D1 monodeiodin-
mates T4 in the outer ring forming T3, but is equally active in the inner ring monodeiodination of T4 producing reverse T3 (rT3), an inactive iodothyronine [34]. This is in sharp contrast to D2 which is exclusively an outer ring deiodinase. In hypothyroidism or in iodine deficiency, when T4 production is reduced, D1 activity is reduced while that of D2 is increased due to the fact that the T4-induced ubiquitination and proteasomal degradation of D2 is reduced. Thus, the half-life of cellular D2 is prolonged by the reduction in T4 [35, 36]. Since the efficiency of T4 to T3 conversion by D2 is double that of D1, the shifting of ratios in favor of D2 increases the efficiency of activation of whatever residual T4 can be formed. This facilitates T3 homeostasis in serum and in peripheral tissues [1].

Tissue-specific actions of D2 are especially important during development. One of the most impressive examples of this occurs in the development of the inner ear in which a sharp spike in the activity of D2 occurs at P6–8 in the mouse which is required for normal cochlear development (fig. 3) [37]. If D2 is genetically inactivated, the animals are deaf, illustrating the necessity of this deiodinase for development of normal hearing [38]. D2 is also required for the normal development and regeneration of skeletal muscle after injury [39]. In D2 knockout mice, development and regeneration after injury is markedly delayed. The increase in D2 in skeletal muscle requires an increase in FoxO3, which has been shown to specifically stimulate Dio2 gene transcription through a conserved FoxO3-binding sequence [39].

The functional significance of D2 was demonstrated in vitro in C2C12 cells and in a satellite cell-enriched primary culture of neonatal skeletal myocytes termed pp6 cells. In these in vitro models, the increase in D2 during differentiation increases conversion of T4 to T3 [40]. Increases in intracellular T4 to T3 conversion in D2-expressing tissues were confirmed in intact mice given injections of 125I-T4 and 131I-T3. In skeletal muscle, the ratio of T3 derived from T4 to that from serum was significantly higher in skeletal muscle than in serum, indicating the net production of 125I-T3 in those cells (fig. 4). This was also increased in skeletal muscle during the regeneration period after injury in parallel with increased D2 expression using the same techniques [40]. It does not occur in the D2KO mouse, confirming the physiological significance of D2 expression in skeletal muscle cells [41].

**Thyroid Hormone Inactivation by D3**

Type 3 deiodinase (D3) inactivates T3 and has significant actions during development and later in adult life. A major role for D3 is in the placenta, uterus, and the develop-
**Fig. 3.** A tissue-specific increase in D2 at P6–8 in the cochlear tissue increases the T3 content of that tissue without perturbing the circulating concentration of T3. 
[a] From Campos-Barros et al. [37]. b, c With permission and adapted from Campos-Barros et al. [37]. Copyright 2000 National Academy of Sciences, USA.

**Fig. 4.** Demonstration of the functional role of skeletal muscle D2 to provide intracellular $^{125}$IT3 from circulating $^{125}$IT4 analogous to the similar role of D2 in providing intracellular T3 to the central nervous system. The $^{131}$IT3 in all tissues is derived from circulating $^{131}$IT3. From Marsili et al. [40]. * p < 0.05 for difference from WT.
oping embryo/fetus protecting it from maternal T3 and T4. This allows the autonomous developmental program in the embryo and fetus to control the differentiation process. The increase in D3 during gestation may be enhanced by estradiol stimulation of the Dio3 promoter [42]. Particularly impressive is the presence of D3 in virtually every surface of the human fetus in contact with amniotic fluid, including the skin, alveolar cells, urothelium, and gastrointestinal tract [43]. The dramatic deleterious effects of D3 deficiency have been clearly demonstrated in the mouse D3 knockout model [44]. In these mice, the absence of D3 in the placenta and its marked reduction in the uterus allows free access of maternal thyroid hormone to the fetus and leads to impaired fertility and reprogramming of the HPT axis such that the postnatal D3 knockout mouse cannot maintain serum T4 concentrations at normal levels due to its insensitivity to the reduced concentrations of thyroid hormone. A similar but milder syndrome has been described in neonatal rats given exogenous thyroid hormone [45]. In the adult D3KO mouse, there is no response of TRH to hypothyroidism and the response of TSH to TRH is subnormal [46]. In addition, the thyroidal response to TSH is impaired and the thyroid markedly reduced in size in the D3KO mouse, suggesting that an intact HPT axis is required during development to establish the normal thyroidal responses to TSH [46].

Another striking example of the potency of D3 in humans is the syndrome of ‘consumptive’ hypothyroidism which can develop in human newborns with hepatic hemangiomas and even in adults with hepatic vascular tumors with high expression of D3 [1, 47, 48]. Hemangiomas are the most common tumors in infancy and are present in 5–10% of 1-year-olds. They have a characteristic growth pattern, growing rapidly in the first year of life then decreasing in size and involuting during later years. In general, they are highly localized but rarely may spread beyond the original site and involve the liver. The first patient identified with this syndrome was an infant with neonatal hypothyroidism who had not had neonatal screening. He was initially thought to have primary congenital hypothyroidism due to the absence of thyroid tissue [47]. Levothyroxine therapy was initiated with an initial TSH response but the TSH later increased to above 200 which could not be attributed to poor compliance. The presence of a hepatic hemangioma was diagnosed by MRI and the suspected excessive D3 expression by the hemangioma was later confirmed in a postmortem tissue sample [47]. A serum thyroglobulin concentration was >1,000 ng/ml, indicating the presence of adequate thyroid concentrations (0.3–6.2 μU/mm), serum thyroxine concentrations (6.8–13.3 μg/dl (88–171 nmol/l)), serum triiodothyronine concentrations (86–170 ng/dl (1.32–2.62 nmol/l)), and serum reverse triiodothyronine concentrations (10–50 ng/dl (0.15–0.77 nmol/l)). Each value is plotted relative to the normal range. Serum thyrotropin and reverse triiodothyronine concentrations are plotted on a logarithmic (base 10) scale. The infant was treated with intravenous (IV) infusions of both liothyronine and levothyroxine. The route of administration of levothyroxine was changed to nasojejunal on day 25 of hospitalization. From Huang et al. [47]. Copyright 2000 Massachusetts Medical Society.
Deiodinase Regulation of Thyroid Status

The infant required nearly 100 μg T3/day by infusion (about 3-fold the daily T3 production in an adult) in addition to oral levothyroxine of 50 μg/day, with subsequent normalization of serum T3 but no detectable serum T4. Serum rT3 was markedly elevated despite the absence of T4 (fig. 5). A similar adult patient was subsequently reported and a number of such patients have now been recognized. Further studies showed that even localized hemangiomas express high levels of D3, suggesting that this is part of the hemangiomaticous process. D3 is also highly expressed in human and mouse basal-cell carcinoma, the most frequent human malignancy. These tumors are typically induced by uncontrolled Sonic hedgehog (Shh) activity and subsequent studies have demonstrated the presence of a conserved Gli2-binding site which induces D3 expression in proliferating keratinocytes and in mouse and human basal-cell carcinomas. It is thought that T3 inactivation by D3 blocks cellular differentiation leading to continued proliferation of D3-expressing tumors. Knockdown of D3 or Shh in experimental basal-cell carcinomas results in a reduction in their proliferation rate and marked attenuation of growth in experimental tumors in nude mice.

Because of these syndromes and the expression of D3 in a number of different tumors, it has been referred to as an oncofetal protein. In experimental situations, the effect of D3 can be overcome either by suppression of its mRNA or by providing T3 to the cells in amounts in excess of that which can be inactivated by the D3 present.

IL-6 exposure and this was blocked by inhibition of the ERK pathway (U0126) or the MAPK pathway, which are induced by IL-6. N-acetylcysteine (NAC) does not block this response. From Wajner et al. [23]. * p < 0.05 for difference from WT.

Deiodinase Regulation of Thyroid Status

Fig. 6. Effects of IL-6 on D1 activity and in mRNA in HepG2 cells: a exposure to IL-6 for 24 h causes a dose-related decrease in outer ring deiodination by type 1 deiodinase despite an increase in total D1 activity in the same cells (b); c D1 mRNA was increased by IL-6 exposure and this was blocked by inhibition of the ERK pathway (U0126) or the MAPK pathway, which are induced by IL-6. N-acetylcysteine (NAC) does not block this response. From Wajner et al. [23]. * p < 0.05 for difference from WT.

Fig. 7. Effects of IL-6 on D2 or D3 activity in MSTO211 and MCF-7 cells, respectively: a IL-6 inhibits D2-mediated outer ring deiodination of T4 by intact MSTO211 cells despite an increase in total D2 activity in the same cells (c). In contrast, IL-6 increases T3 inactivation by intact MCF-7 cells (b) as expected from the increase in total D3 activity in the same cells (d). Both D2 and D3 mRNA were increased by IL-6 through a MAPK-dependent process (data not shown). From Wajner et al. [23]. * p < 0.05 for difference from control.
Recent studies have indicated that tyrosine kinase inhibitors, especially sunitinib, have an adverse effect on thyroid gland function possibly by reducing thyroid blood flow [50]. In addition, patients receiving sunitinib show increases in requirements for thyroid hormone over and above replacement doses and a decrease in the T3:rT3 ratio suggestive of increased D3 action [51]. These studies and the consumptive hypothyroidism reported in patients with hemangiomatous or other tumors of vascular origin illustrate the potency of this enzyme which causes inactivation of T3 and T4 at rates which exceed T4 and T3 production capacities even with a high TSH [47].

A less severe form of consumptive hypothyroidism probably accounts for the increased levothyroxine requirements during pregnancy and with oral contraceptives, since the Dio3 gene is transcriptionally stimulated by estradiol [42]. D3 activity has been identified in a number of malignancies, including gliomas, neuroblastomas, and colon carcinomas, all of which are derived from cells which are known or thought to be capable of D3 production in the embryo. In many of these an increase in Shh signaling is involved, while recent studies have shown that β-catenin is responsible for the D3 increases in adenomas and carcinomas of colonic epithelium [52].

Recent evidence also supports an element of consumptive hypothyroidism as a contributing cause of the 'low T3 syndrome' during human illness. Non-thyroidal illness syndrome (NTIS) is characterized by low T3 and elevated rT3, and is correlated with a lower hepatic D1, while D3 is markedly increased in both liver and skeletal muscle of critically ill patients [53, 54]. Insight into a potential mechanism for these disparate changes can be found in studies where the effects of the cytokine IL-6 on iodothyronine deiodination by intact human cells were found to differ between those expressing D1 or D2 (HepG2 and MSTO211 cells, respectively), and the human D3-expressing breast cancer cell line, MCF-7. IL-6 concentrations typical of those in the serum of sick patients caused an increase in reactive oxygen species (ROS) in all three cell lines which induced the transcription of deiodinase mRNA and protein in each of the cells through the MAPK/ERK pathways [23]. A striking result was that the increase in D1 and D2 activity in the cell lysates was not accompanied by increases in the 5′ deiodination of T4 by intact HepG2 and MSTO211 cells but the increase in cellular D3 was accurately reflected by an increase in T3 inactivation by intact MCF-7 cells (fig 6, 7) [23]. A similar result was obtained by inducing ROS by exposure to H2O2. A plausible explanation for these differences is that there is a greater sensitivity of D3 to the extracellular space as a source of thiol cofactor compared to that of D1 and D2 which depend predominantly on cytosolic thiols (fig. 8) [23]. In any case,
this is the first demonstration of the effect of a single event, an increase in cellular ROS, to suppress outer ring deiodinase activity while increasing that of D3-mediated inner ring deiodination, replicating the classical pattern of the reduced T3 and elevated rT3 in NTIS.

These clinically relevant aspects of deiodinase physiology illustrate the important role of these enzymes in the activation or inactivation of thyroid hormones. The local production of thyroid hormone is a highly regulated process during embryonic and fetal life, as well as during regeneration. A number of tissues, including skeletal muscle, skin, intestine, and the central nervous system, have been shown to co-express D2 and D3, and many studies are currently underway to define how the activation and inactivation by these deiodinases is regulated by factors such as hedgehog proteins, FoxO3, and wnt/β-catenin to modulate the intracellular concentration of T3 in specific cells without perturbing that of T4 or T3 in the circulation.

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References


36 Steinsapir J, Bianco AC, Buettner C, Harney J, Larsen PR: Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteosomal degradation and requires interaction with the enzyme’s active center. Endocrinology 2000;141:1127–1135.


