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The Deiodinase Trio and Thyroid Hormone Signaling

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Abstract

Thyroid hormone signaling is customized in a time and cell-specific manner by the deiodinases, homodimeric thioredoxin fold containing selenoproteins. This ensures adequate T3 action in developing tissues, healthy adults and many disease states. D2 activates thyroid hormone by converting the pro-hormone T4 to T3, the biologically active thyroid hormone. D2 expression is tightly regulated by transcriptional mechanisms triggered by endogenous as well as environmental cues. There is also an on/off switch mechanism that controls D2 activity that is triggered by catalysis and functions via D2 ubiquitination/deubiquitination. D3 terminates thyroid hormone action by inactivation of both T4 and T3 molecules. Deiodinases play a role in thyroid hormone homeostasis, development, growth and metabolic control by affecting the intracellular levels of T3 and thus gene expression on a cell-specific basis. In many cases, tight control of these pathways by T3 is achieved with coordinated reciprocal changes in D2-mediated thyroid hormone activation D3-mediated thyroid hormone inactivation.

Keywords

Thyroid hormone; Deiodinase; Selenoprotein

1 Introduction

The thyroid gland takes up and concentrates iodide that is used to synthesize biologically active molecules known as thyroid hormones. Their biological activity and other intrinsic properties depend on the number as well as spatial localization of the iodide atoms within the molecule of thyroid hormone. Thyroxine (T4) is the main hormone produced by the thyroid gland. It has a relatively long half-life (~8 days) and is only minimally active. T3 (3,5,3'-triiodothyronine) is a fully active thyroid hormone; it has a much shorter half-life (~12 h), but is produced mostly outside the thyroid gland through deiodination of T4. In contrast, removal of the inner T4 or T3 ring iodine produces reverse T3 (3,3',5'-

triiodothyronine) or T2 (3,3'-T2), respectively, which are biologically inactive molecules. Deiodinases are enzymes that catalyze these activating and inactivating reactions. They are expressed across multiple organs and tissues, constituting enzymatic pathways that initiate or terminate thyroid hormone action. The deiodinases play a role in various aspects of vertebrate physiology such as development [1], TSH and TRH feedback regulation [2–4], and diseases states [5]. Please refer to recent reviews for a comprehensive discussion of this topic [6–8].

2 Fundamental Aspects of the Deiodinases

Types 1 and 2 deiodinases (D1 and D2) activate thyroid hormone whereas the type 3 deiodinase (D3) inactivates both T4 and T3 [9]. Sequence analysis coupled with hydrophobic cluster analysis (HCA) revealed that the deiodinases share an overall 50% sequence similarity. Most similarity lies within the conserved thioredoxin-fold (TRX) domain composed of $\beta\alpha\beta$ and $\beta\beta\alpha$ motifs [10]. Deiodinases are anchored in cell membranes via a single transmembrane domain with its catalytic globular domain facing the cytosol [11, 12]. This model was further refined by crystallization of mouse D3 globular domain [13]. The cDNA encoding the three deiodinases predict a molecular weight between 29 and 33 kDa [14]. However, in gel filtration studies deiodinases are found in higher molecular weight complexes, between 44 and 200 kDa [15], suggesting that they could be part of multi-protein complexes or be organized in multimeric forms. Indeed, deiodinases are homodimers and dimerization is required for catalytic activity [11, 16–18]. Dimerization involves the trans-membrane and globular domains of the deiodinases, and may be mediated by disulfide bonds [17].

The deiodinase active center is a pocket within the globular domain defined by a $\beta\alpha\beta$ motif and a highly conserved intervening element that is also found in α -L-iduronidase, a lysosomal enzyme. In this pocket is the rare amino acid selenocysteine (Sec), critical for the nucleophilic attack that takes place during the deiodination reaction [10]. Given the topology of all three deiodinases, thyroid hormones need to enter cells in order to be metabolized by deiodinases. Cell entry is mediated by membrane transport proteins [19], with the monocarboxylate 8 (MCT8) likely being the most relevant one. Mutations in the MCT8 gene are associated with neurodevelopmental problems and endocrine dysfunctions [19–21]. T3 binds two nuclear thyroid hormone receptors (TRs), TR α and β , that modify transcription of multiple sets of thyroid hormone-responsive genes, initiating the biological effects of thyroid hormone [22]. Given that TR saturation depends on the concentration of intracellular T3, it is also recognized that D2 and D3 play antagonist roles in thyroid hormone action by inversely affecting intracellular T3 levels. D2 produces T3 and thus increases thyroid hormone signaling whereas D3 inactivates T4 and T3, silencing thyroid hormone signaling. Both pathways are relatively independent of circulating T4 or T3 levels [23].

A corollary of such studies is that thyroid hormone action can be customized on a time- and cell-dependent fashion via the coordinated expression of D2 and D3 [1, 24]. A well-known model that illustrates such interplay is vertebrate development. In most developing tissue D3 activity predominates, preventing rapidly developing cells from being exposed to thyroid hormone. As the embryo matures, D3 expression is minimized and D2 increases, enhancing

thyroid hormone signaling. D1, on the other hand, does not contribute significantly to the local control of thyroid hormone signaling. This is explained by its localization in the plasma membrane, which facilitates rapid exit of D1-generated T3 back to the circulation. Indeed, in rodents D1 is responsible for approximately half of the daily extrathyroidal production of T3, but in humans D1 plays a lesser role in maintaining circulating T3 [25, 26]. In addition, studies with D1 knock out (KO) mice suggest that D1 also plays a scavenger role, preferentially deiodinating sulfated forms of iodothyronines in the process of being eliminated in the bile and urine [27].

2.1 Regulation of the D2 Pathway

Intrinsic biological properties make D2 a key enzyme regulating local thyroid hormone signaling. For example, D2 has a relatively short half-life (approximately 40 min) that is due to ubiquitination and destruction in the proteasomes, a process that is accelerated by interaction with T4, its natural substrate [28]. This provides rapid control of intracellular T3 production and TR occupancy.

D2 and the ubiquitin-proteasome system: Subcellular localization studies indicate that D2 is an ER resident protein by virtue of being retained in the ER [12, 29]. Residency in the ER is critical for D2's ability to contribute to thyroid hormone signaling. Physical proximity with the nucleus allows for D2-generated T3 to gain easy access to the TR-containing nuclear compartment. In tissues where D2 is expressed, at least 50% of the TR-bound nuclear T3 is made intracellularly via the D2 pathway [9]. Notably, ER residency places D2 physically close to an array of proteins that interact and modify the D2 molecule via ubiquitination and targeting to the proteasomal system, explaining its relatively short half-life. In addition, interaction with its natural substrate T4 accelerates disposal of D2 via the proteasomal system [30]. Substrate interaction with the enzyme's catalytic site triggers D2 ubiquitination [28]. Two Lys residues in D2 are involved in this process, K237 and K244. Although a mutation in either one of these residues does not affect D2-ubiquitination, mutation of both prevented D2 ubiquitination and prolonged D2 half-life [18].

Both ubiquitin conjugases UBC6 and or UBC7 interact with D2 and support D2 ubiquitination [31, 32]. In addition, two E3 ligases mediate D2 ubiquitination: the sonic hedgehog (Shh) inducible WD-40 repeat and SOCS box-containing 1 (WSB-1 or SWiP) and the ERAD enzyme TEB4 (the human ortholog of yeast Doa10). WSB-1 is part of the ECSWSB1 multi-protein catalytic complex that contains Elongin B and C, Cullin5, RBX1 and the E2 ligase UBC7 [33]. The D2-WSB-1 interaction requires the 18 amino acid "instability loop" in D2 and the WSB-1 WD-40 propeller-shaped domain. In the developing chicken bone growth plate, perichondrial/periosteal cells express both D2 and WSB-1 [33]. In this setting, Indian hedgehog (Ihh) secreted from chondrocytes leaving the proliferative pool induce expression of WSB-1. This in turn accelerates D2 ubiquitination, decreasing thyroid hormone signaling, which in turn induces PTHrP expression [33]. The other D2 ubiquitin ligase is TEB4, an ER resident protein that is a component of the ER associated degradation (ERAD) machinery. TEB4 was shown to be a D2-interacting protein in human cells, and when co-expressed with D2 it decreases D2 protein levels and enzymatic activity. In contrast, cellular knock down of TEB4 slows down D2 protein/activity turn-over [34].

Like WSB-1, TEB4 requires the instability loop in D2 in order to interact and mediate D2 ubiquitination [34].

Ubiquitin moieties can be removed from targeted proteins by specific enzymes, avoiding proteasomal destruction. These enzymes are known as deubiquitinases or DUBs. Two DUBs, USP33 (or VDU-1) and USP20 (or VDU-2), interact with and regulate D2 [35]. USP33 and USP20 share a high degree (~59%) of sequence homology at the carboxyl and amino termini, being ubiquitously expressed in human and mouse tissues [36, 37]. Co-expression of USP33/20 decreases the levels of ubiquitinated D2 molecules and prolongs D2 protein half-life [35]. USP33 is up regulated in BAT by cold-exposure, which supports the several-fold increase in D2 activity. USP33-mediated deubiquitination of D2 can also take place in the brain, as USP33 and D2 are co-expressed in astrocytes and tanycytes [38].

An example of how D2 ubiquitination plays important physiological roles is the existence of tissue-specific differences in D2 ubiquitination [39]. These differences were implicated in the relatively higher T4/T3 plasma ratio observed in adult L-T4-treated thyroidectomized rats. While treatment with L-T4 decreased whole-body D2-dependent T3 production, D2-mediated conversion of T4 to T3 in the hypothalamus was only minimally affected by L-T4. In vivo studies in mice harboring an astrocyte-specific *Wsb1* deletion as well as in vitro analysis of D2 ubiquitination driven by tissue extracts indicated that D2 ubiquitination in the hypothalamus is relatively less efficient than what it is observed in the rest of the body. Because T4-induced down-regulation of D2 is turned off in the hypothalamus, the TRH-producing neuron is wired to have increased sensitivity to T4. Therefore, tissue-specific differences in D2 ubiquitination are an inherent property of the TRH/TSH feedback mechanism [39].

D2 and ER stress: ER stress is recognized as an important condition that can be caused by accumulation of unfolded/misfolded proteins within the ER. A number of physiological and pathological conditions have been recognized as exhibiting different levels of ER stress [40]. To neutralize the effects of ER stress, cells trigger a conserved ER-to-nucleus signaling cascade that (1) stops global mRNA translation, (2) increases expression of chaperone proteins such as HSP40 and BiP, and (3) accelerates input of misfolded proteins into the ER stress-induced (ERAD) machinery [41–43]. D2 expression is greatly affected by ER stress [44]. Exposure of D2-expressing cells to different ER stressors reduced D2 activity in as little as 1 h, with significant increase in ER stress markers [44]. This down regulation was independent of transcriptional modulation of the D2 gene (*Dio2*), as ER stress did not change *Dio2* mRNA levels. This loss of D2 activity leads to a significant decrease in D2-mediated T3 production, which shows that D2-expressing cells under ER stress are hypothyroid [44].

Molecules regulating the D2 pathway: *Dio2* is a cAMP responsive gene [45–47]. Thus, any signaling pathway or molecule that increases cAMP concentration will stimulate D2 activity. For example, flavonols are a class of small polyphenolic compounds widely found in today's diet, induce D2 expression and intracellular D2-generated T3 via activation of cAMP production [48]. Kempferol treatment increased D2 activity up to 50-fold in various cell models via transcriptional mechanisms, while increasing oxygen consumption

by 30% and the expression of key metabolic genes such as PGC1 α , CPT-1, uncoupling protein 3 (UCP-3) and mitochondrial transcription factor 1 (mtTFA) [48]. Another example is bile acids, which have been linked with activation of the mitogen-activated kinase pathways, FXR α nuclear receptors and the G-coupled protein receptor TGR5 [49, 50]. Diet supplementation with cholic acid protects against diet-induced obesity and reversed the body weight gain in animals kept on a high-fat diet for 120 days via TGR5 activation [50]. Dietary bile acid supplementation also increased oxygen consumption that coincided with an increase in BAT expression of PGC1 α and β , CPT-1, UCP-3 and D2 [50].

Two chemical chaperones, tauroursodeoxycholic acid (TUDCA) and 4-phenyl butyric acid (4-PBA) were shown to increase D2 expression and intracellular T3 production [51]. D2 expression was induced via transcriptional activation of the *Dio2* gene [51]. Similar results were also observed in the setting of in vitro differentiated primary brown adipocytes, leading to acceleration in cellular oxygen consumption, lasting up to 72 h. Strikingly, the TUDCA- or 4-PBA-induced oxygen consumption was reduced or lost in primary brown adipocytes isolated from *Dio2*^{-/-} mice, indicating a D2-dependent effect on oxygen consumption [51].

2.2 Physiological Roles of D2

D2 in developmental settings.—D2 expression is low in most tissues during development, only increasing toward birth and the perinatal period [52]. The availability of D2 knockout mice (D2KO) allowed a much better understanding of D2's role in developing tissues [1, 53, 54]. Examples of developing tissues in which D2 plays well established roles include cochlea [55], bone [33, 56–59], brown adipose tissue (BAT) [60]. Although the subject of much attention, it is less clear that D2 plays a role in muscle development [61, 62]. The liver was recently found to be a site of important developmental roles played by D2 [63]. In mice, at around the first day of life, there is a transient surge in hepatocyte D2 that modifies the expression of ~165 genes involved in broad aspects of hepatocyte function, including lipid metabolism. Hepatocyte-specific D2 inactivation revealed that the surge in D2 expression causes hundreds of differentially methylated local DNA regions (DMR) that map to sites of active/suppressed chromatin, thus qualifying as epigenetic modifications. These DMRs underlie a dramatic metabolic phenotype that involves adult susceptibility to diet-induced steatosis, hypertriglyceridemia, and obesity [63].

Regulation of the hypothalamus-pituitary-thyroid axis: TSH (and TRH) stimulates thyroid activity and hormone production/secretion. Both circulating T4 and T3 play a role in the negative feedback loop that inhibits both TRH and TSH secretion [64]. However, T4 is only minimally active. Thus, D2 plays a central role in this regulatory loop, as it converts T4 to T3 inside thyrotrophs (cells that produce TSH) [2, 3]. Consequently, an increase in circulating T4 is expected to increase thyrotroph T3 concentration and to shut down TSHb gene expression, while a drop in circulating T4 results in an opposite effect [3]. Accordingly, inactivation of the *Dio2* gene in mice leads to central resistance to T4 [65], a phenotype that is also seen in mice treated with the D2 inhibitor amiodarone [66]. D2 also plays a role in TRH regulation [4]. D2-generated T3 in tanycytes, specialized ependymal cells located in the walls of the III ventricle, mediate the negative feedback on TRH neurons via paracrine mechanisms [67]. These two pathways function in a coordinated fashion, as elucidated in

studies of two mouse strains with pituitary- and astrocyte-specific D2 knockdown (pit-D2 KO and astro-D2 KO mice, respectively). Such coordination of T4-to-T3 conversion between thyrotrophs and tanocytes is critical to maintain normal plasma T3 levels [68].

Adaptive thermogenesis and metabolic control: When exposed to cold, mammals minimize heat loss to the environment at the same time that accelerates energy expenditure (and consequently heat production), a process known as adaptative thermogenesis [69]. Release of norepinephrine (NE) in a number of tissues, including the brown adipose tissue (BAT), increases cAMP and up-regulates cAMP responsive genes, such as PGC-1 α and Dio2. Although activation of all three β -adrenergic receptor subtypes increases cAMP production, they do play slightly different roles in metabolic control [70–72]. In the BAT, for example, the several-fold increase in D2 activity is critical for thermogenic function, as it potentiates cAMP production and directly induces uncoupling protein 1 (UCP-1) expression [47, 73, 74]. Concomitant induction of the ubiquitin-specific protease 33 (USP-33 or VDU-1) extends D2 half-life by deubiquitination and elevates further D2 activity [35]. D2KO mice are cold intolerant, developing hypothermia when exposed to cold [75].

D2KO animals develop obesity and severe hepatic steatosis when kept on a thermoneutral environment and fed on a high fat diet [76]. To understand the mechanisms underlying this phenotype, systemically euthyroid fat-specific (FAT), astrocyte-specific (ASTRO), or skeletal-muscle-specific (SKM) D2 knockout (D2KO) mice that were developed. The ASTRO-D2KO mice exhibit lower diurnal RQ and greater contribution of fatty acid oxidation to energy expenditure, but no differences in food intake. In contrast, the FAT-D2KO mouse exhibit greater contribution of carbohydrate oxidation to energy expenditure as illustrated by sustained (24 h) increase in RQ, food intake, tolerance to glucose, and sensitivity to insulin. Furthermore, FAT-D2KO animals that were kept on a high-fat diet gained more body weight and fat, indicating impaired BAT thermogenesis and/or inability to oxidize the fat excess. Acclimatization of FAT-D2KO mice at thermo-neutrality dissipated both features of this phenotype. Notably, muscle D2 does not seem to play a significant metabolic role given that SKM-D2KO animals exhibited no metabolic phenotype [77]. These studies indicate that brain D2 plays a dominant albeit indirect role in fatty acid oxidation via its sympathetic control of BAT activity. In addition, D2-generated T3 in BAT accelerates fatty acid oxidation and protects against diet-induced obesity [77].

Food availability is another factor that plays a role in thyroid hormone signaling via D2-pathway. While the activity of the thyroid gland is stimulated by food intake (via leptin-induced TRH/TSH expression), food availability also stimulates D2-mediated T3 production in mouse skeletal muscle and in a cell model transitioning from 0.1 to 10% FBS [78]. Dio2 inhibition normally occurs via FOXO1 binding to the Dio2 promoter. Transcriptional de-repression occurs through insulin signaling, which takes place via the mTORC2 pathway. Therefore, FOXO1 represses DIO2 during fasting and that de-repression occurs via nutritional activation of the PI3K-mTORC2-Akt pathway [78].

D2 has a role in behavior and mood: Despite reduced T3 content in the neonatal D2KO brain, the D2KO mouse exhibits minimal neurological phenotype, suggesting the existence of potent compensatory mechanisms that minimize functional abnormalities

caused by the absence of the D2 [79]. This includes diminished agility, and an altered global gait pattern (mice walked slower, with shorter strides and with a hindlimb wider base of support than wild-type mice). However, there was also impairment on coordination and prehensile reflex and a reduction of muscle strength [80]. However, D2 inactivation does seem to play a role in behavioral and mood processes in the adult mouse [81]. The ASTRO-D2KO mouse was found to exhibit anxiety-depression-like behavior despite normal serum T3 levels. This was found during a comprehensive battery of tests that included open field and elevated plus maze studies and when tested for depression using the tail-suspension and the forced-swimming tests. Despite normal neurogenesis, microarray gene expression profiling of the ASTRO-D2KO hippocampi identified an enrichment of three gene sets related to inflammation and impoverishment of three gene sets related to mitochondrial function and response to oxidative stress [81]. Increased anxiety and fear memory was also reported in the D2KO mouse [82]. These findings suggest that human defects in Dio2 expression in the brain could, potentially, result in mood and behavioral disorders.

D2 in the heart: The healthy human (but not rodent) myocardium expresses D2 and thus is potentially capable of generating T3 inside the muscle fiber. In fact, inhibition of T4 deiodination to T3 has been proposed as a contributory mechanism to the antiarrhythmic efficacy of amiodarone [83]. To better understand the role played by D2 in the myocardium, a transgenic mouse was created that expresses the human D2 gene in the myocardium under the α -myosin heavy chain (α -MHC) promoter [84]. This mouse has normal thyroid function tests but exhibits a discrete increase in myocardial T3 content and a gene expression profile compatible with increased thyroid hormone signaling, i.e., increased mRNA levels of HCN2 (an ionic channel that is key to the cardiac pacemaker) and decreased mRNA levels of β -MHC [84]. In perfused ex vivo studies, the α -MHC-D2 heart has about a 20% higher heart rate and decreased levels of phosphocreatine and ADP, indicating accelerated metabolic rates. This is supported by in vivo studies in which glucose uptake is increased by about 2.5-fold in the α -MHC-D2 heart [84]. These “thyrotoxic” effects are associated with an increased capacity of the α -MHC-D2 heart to generate cAMP in response to catecholamine stimulation [85]. Cardiac-specific increase in thyroid hormone signaling was confirmed in a second α -MHC-D2 mouse model conditionally expressing human D2 in the myocardium [86]. This model further demonstrated that myocardial D2 expression provides a functional advantage such as increased fractional shortening, velocity of circumferential fiber shortening, peak aortic outflow velocity and aortic velocity acceleration [86]. Thus, by virtue of accumulating in the myocardium and being a noncompetitive D2 inhibitor, amiodarone/DEA can potentially decrease thyroid hormone signaling in the heart.

Skeletal muscle: D2 expression is found in minimal amounts in both humans and murine muscle tissue [87]. However, D2 can be induced several-fold and play a role in muscle regeneration [88] and exercise [77, 89]. D2 expression in skeletal muscle is upregulated by acute treadmill exercise through a β -adrenergic receptor-dependent mechanism. Pharmacological block of D2 or disruption of the Dio2 gene in skeletal muscle fibers impaired acute exercise-induced PGC-1 α expression and mitochondrial citrate synthase activity in chronically exercised mice [77].

2.3 Regulation of the D3 Pathway

D3 is an obligatory inner-ring deiodinase with a half-life of about 12 h [90, 91]. The D3 gene (*Dio3*) is unique amongst the deiodinases in being an imprinted gene [92] that is assembled in a cluster of genes that share a common regulatory element [93]. Typically, D3 is highly expressed in embryonic tissues, in the brain and in the placenta [23, 94]. However, D3 expression can be reactivated in multiple tissues during disease states, contributing to the low circulating levels of T3 observed in these situations [95–97].

D3 traffics through different cell compartments: Like many other proteins anchored in the plasma membrane, D3 is internalized and becomes part of the endosomal vesicles [12]. These predominantly clathrin-coated vesicles are also capable of recycling internalized D3 back to the cell surface. Thus, under normal circumstances newly synthesized D3 transits to the plasma membrane and becomes part of the pool recycling between the plasma membrane and the early endosomes.

D3's intracellular trafficking can be modified in different settings. For example, hypoxia leads to nuclear import of D3 in neurons, without which thyroid hormone signaling and metabolism cannot be reduced. After unilateral hypoxia in the rat brain, D3 protein level is increased predominantly in the nucleus of the neurons in the pyramidal and granular ipsilateral layers, as well as in the hilus of the dentate gyrus of the hippocampal formation. In hippocampal neurons in culture as well as in a human neuroblastoma cell line, a 24 h hypoxia period redirects active D3 from the endoplasmic reticulum to the nucleus via the co-chaperone Hsp40. Preventing nuclear D3 import by Hsp40 knockdown resulted in an almost doubling in the thyroid hormone-dependent glycolytic rate and quadrupling the transcription of thyroid hormone target gene ENPP2. In contrast, Hsp40 overexpression increased nuclear import of D3 and minimized thyroid hormone effects in cell metabolism, possibly functioning to reduce ischemia-induced hypoxic brain damage [98].

2.4 Physiological and Patho-physiological Roles of D3

D3 in developmental settings: Embryonic tissues express high levels of D3. This ensures that thyroid hormone signaling is kept at low levels during development. The timed and tissue-specific reduction of D3 during development coordinated with the slow increase in D2 activity provide tighter control of thyroid hormone action [9]. TGF β and the Hedgehog family of proteins are key molecules that stimulate D3 expression [99, 100]. In fact, the Hedgehog family has been recently identified as a major player in determining thyroid hormone signaling through coordinated effects mediated via D2 and D3. For example, in the chicken developing growth plate, Hedgehog signaling inhibits D2-mediated T3 production by inducing WSB-1, an ubiquitin ligase that inactivates D2 by transiently disrupting its dimeric conformation [18, 33]. At the same time, Hedgehog signaling stimulates *Dio3*, which will inactivate thyroid hormone and further decrease thyroid hormone action. The stimulation of *Dio3* by Gli proteins, which are downstream messengers of the Hedgehog cascade, has recently been characterized in keratinocytes from both normal skin and basal cell carcinomas, the most common human malignancy [100].

Pancreatic β -cells express D3: D3 plays a role in pancreatic islet function and glucose homeostasis [101, 102]. Dio3 expression and D3 protein is highly expressed in embryonic and adult pancreatic islets, predominantly in β -cells in both humans and mice. D3KO animals were found to be glucose intolerant due to in vitro and in vivo impaired glucose-stimulated insulin secretion, without changes in peripheral sensitivity to insulin. D3KO neonatal (postnatal day 0) and adult pancreas exhibited reduced total islet area due to reduced β -cell mass, insulin content, and impaired expression of key β -cells genes. D3 expression in perinatal pancreatic β -cells prevents untimely exposure to thyroid hormone, the absence of which leads to impaired β -cell function and subsequently insulin secretion and glucose homeostasis [102]. Studies in adult heterozygous mice with disruption of the Dio3 gene indicated that Dio3 is preferentially expressed from the maternal allele in pancreatic islets and that the inactivation of this allele is sufficient to disrupt glucose homeostasis by reducing the pancreatic islet area, insulin2 gene expression, and glucose-stimulated insulin secretion [101].

D3 in the hypertrophic heart: Severe illness that is associated with ischemia/hypoxia results in ectopic cardiac expression of D3, which inactivates thyroid hormone and causes localized hypothyroidism [103, 104]. D3 expression has also been observed in animal models of adverse remodeling such as myocardial infarction [105] and chronic pulmonary hypertension with right ventricular hypertrophy and ventricular failure (treatment with monocrotaline) [106, 107]. These studies served as the basis for a clinical trial that enrolled patients undergoing elective open heart surgery to assess thyroid hormone deiodination in the human heart [108]. Myocardial thyroid hormone metabolism was assessed by analyzing the difference in serum thyroid hormone levels between the aortic root (incoming blood) and the coronary sinus (outgoing blood) of patients undergoing cardiac surgery. Immediately before cardiopulmonary bypass, blood flowing through the myocardium of patients with aortic stenosis (with left ventricular hypertrophy) exhibited ~5% reduction in T3 and ~7% increase in rT3 levels, decreasing the serum T3/rT3 ratio by ~10%. In contrast, no myocardial thyroid hormone metabolism was observed in patients with coronary artery disease (no ventricular hypertrophy). These data indicate that there is accelerated thyroid hormone inactivation in the myocardium of patients with aortic stenosis, which is likely the result of D3 expression. Notably, no evidence to suggest thyroid hormone activation in the myocardium was obtained [108].

Drugs and tumors: Hemangiomas are common tumors of infancy that express variable levels of D3. Depending on the size of the tumor, D3 expression can be so intense that inactivates circulating thyroid hormone faster than the thyroid gland can secrete, resulting in what it is known as consumptive hypothyroidism [109]. A similar condition has been observed in patients with metastatic renal cell carcinoma or gastrointestinal stromal tumors (GISTs) receiving treatment with the tyrosine kinase inhibitor sunitinib [110]. Hepatic D3 activity increased markedly in rats undergoing similar treatment with this kinase inhibitor, indicating that D3 induction plays a role in sunitinib induced hypothyroidism [110]. In fact, similar to hemangiomas, GISTs themselves can produce consumptive hypothyroidism caused by marked overexpression of D3 within the tumor [111].

3 Methods to Study Deiodinases

Deiodinases can be detected and quantified studying enzyme kinetics [112]. Deiodinase kinetics are typically studied in broken cell/tissue preparations in the presence of co-factors such as dithiothreitol (DTT) and radioactive iodothyronines as substrates followed by quantitation of the radioactive products per unit time. In many cases deiodinase protein can also be assessed by immunohistochemistry [112]. Expression level can also be determined by measuring mRNA levels by standard reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) techniques [112].

For deiodinase reactions enzyme kinetics are measured based on production of tracer iodide or of a specific reaction product. Background controls with no deiodinase activity must always be included. For D1 kinetics the preferred substrate is rT3 and the reaction buffer is based on PBS containing 1–2 mM EDTA, 0.25–0.3 M sucrose and 10–50 mM DTT, whereas the substrate concentration is 0.1–2 μ M. The amount of tissue sonicate is adjusted to ensure <30% deiodination in about 1 h. The assay for D2 is carried out similarly, except that the preferred substrate is T4 (0.5–2 nM) and 1 mM PTU is included to inhibit potential background D1 activity. The preferred substrate for D3 is T3 (0.5–2 nM), which can be used in a similar setting as for D2 studies.

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