Metabolism of Thyroid Hormone

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Clinical summary

In healthy humans the thyroid gland produces predominantly the prohormone T4 together with a small amount of the bioactive hormone T3. Most T3 is produced by enzymatic outer ring deiodination (ORD) of T4 in peripheral tissues. Alternative, inner ring deiodination (IRD) of T4 yields the metabolite rT3, the thyroidal secretion of which is negligible. Normally, about one-third of T4 is converted to T3 and about one-third to rT3. The remainder of T4 is metabolized by different pathways, in particular glucuronidation and sulfation. T3 is further metabolized largely by IRD and rT3 largely by ORD, yielding in both cases the metabolite 3,3'T2. Thus, ORD is regarded as an activating pathway and IRD as an inactivating pathway.

Three enzymes catalyzing these deiodinations have been identified, called type 1 (D1), type 2 (D2) and type 3 (D3) iodothyronine deiodinases. All three deiodinases have been cloned and characterized in a variety of species. Together, they form a family of homologous selenoproteins which consist of »250-280 amino acids, including an essential selenocysteine residue in the active center. It is remarkable, therefore, that production and metabolism of thyroid hormone are dependent on two trace elements, namely iodine and selenium.

D1 is expressed mainly in the liver, the kidneys and the thyroid. In particular the hepatic enzyme is thought to contribute importantly to peripheral T3 production and to be the main site for the clearance of plasma rT3. These processes are mediated by the ORD activity of D1. However, D1 also has IRD activity, especially towards sulfated T4 and T3. Therefore, in addition to the bioactivation of T4 to T3, D1 also catalyzes the degradation of thyroid hormone. An important property distinguishing D1 from the other deiodinases is its sensitivity to inhibition by the anti-thyroid drug propylthiouracil (PTU). The important role of D1 in the peripheral production of plasma T3 has been demonstrated by the marked decrease in plasma T3 levels in T4-substituted athyreotic subjects treated with PTU.

D2 has been studied extensively in the central nervous system, the pituitary and brown adipose tissue of experimental animals. D2 has only ORD activity and its expression shows adaptive changes in response to alterations in thyroid state, which serves to maintain tissue T3 levels in the face of varying plasma T4 and T3 levels. These findings have led to the general opinion that D2 is important for the generation of local T3 in these tissues but does not contribute much to the production of plasma T3. However, there is strong evidence that a significant part of plasma T3 may be generated by an extra-hepatic, PTU-insensitive mechanism, in particular in subjects with lowered plasma T4 levels. It remains to be

established to what extent expression of D2 in human skeletal muscle contributes to this process. D2 has also been localized in the human thyroid gland.

D3 mediates the degradation of thyroid hormone since it has only IRD activity. The brain is the predominant D3-expressing tissue in adult animals, and may thus be the main site for the clearance of plasma T3 and for the production of plasma rT3. However, high D3 activities have been demonstrated in the placenta and the pregnant uterus as well as in different fetal tissues. The high D3 activities at these sites appear to prevent exposure of fetal tissues to high T3 levels, allowing the growth of these tissues. T3 is only required at the differentiation stage of tissue development.

Whereas intitial studies focused on the role of the deiodinases in maintaining normal serum T3 concentrations, the paradigm has evolved that these enzymes can locally modify TH bioactivity independent of serum TH concentrations. An example is the critical role of D2 and D3 in cochlear development, since Dio2-/- as well as Dio3-/- mice have severe hearing loss. These enzymes prevent too little or too much hormonal stimulation at inappropriate stages in development. At immature stages, D3 limits stimulation by T3. Postnatally, a double switch occurs with a decline in D3 and an increase D2, resulting in a local T3 surge which is independent of serum T3 levels and triggers the onset of auditory function.

Clinically, the importance of the deiodinases in the regulation of thyroid hormone bioactivity is apparent when their activity is affected by patho-physiological conditions. Examples of such conditions are iodine insufficiency, thyroidal and non-thyroidal illness and malnutrition.

Expression of D1 and D3 is under positive control and that of D2 is under negative control of thyroid hormone. Therefore, the relative contribution of D1 and D2 to peripheral T3 production varies with thyroid state, with D1 prevailing in the hyperthyroid and D2 in the hypothyroid state. The proportions of T3 being produced via D1 or D2 in euthyroid subjects remains to be established.

In iodine deficiency, D1-mediated peripheral T3 production decreases but this is in part compensated by an increased thyroidal T3 production, which is mediated by an increased TSH secretion as well as by increased efficiency of D2-mediated T3 production. Simultaneously, neuronal D3 expression decreases thereby prolonging the local half-life of T3.

In non-thyroidal illness (NTI) plasma T3 is often decreased and plasma rT3 increased; plasma FT4 is still in the normal range depending on the severity of disease. The changes in plasma T3 and rT3 are explained by a diminished conversion of T4 to T3 and of rT3 to 3,3-T2 by D1 in the liver. Although this may be caused to some extent by decreased D1 expression or cofactor levels, a diminished activity of transporter(s) mediating hepatic uptake of T4 and rT3 appears to be another important mechanism. This also holds for the generation of the low T3 syndrome in malnutrition.

In addition to a decreased peripheral T3 production, the low T3 syndrome of NTI may also be caused by stimulated thyroid hormone degradation due to induction of D3 in different tissues. Pathological expression of D3 may be so high that this results in a state of consumptive hypothyroidism with low serum (F)T4 and T3 and very high rT3 levels. This has been shown in different patients with hemangiomas which express very high D3 activities.

Finally, peripheral production of T3 can be inhibited by a variety of drugs, including PTU, dexamethasone, propranolol, and iodinated compounds such as the radiographic agents iopanoic acid and ipodate and the anti-arrhythmic drug amiodarone. PTU is a specific uncompetitive inhibitor of D1,

while iopanoic acid and ipodate are competitive inhibitors not only of D1 but also of D2. In addition, the radiographic agents inhibit hepatic uptake of thyroid hormone. Amiodarone and its metabolite desethylamidarone may also interfere with peripheral thyroid hormone levels by inhibition of deiodinase activities and tissue thyroid hormone transport. Little is known about the mechanisms by which propranolol and dexamethasone inhibit peripheral T3 production. Combinations of these drugs (e.g. PTU, ipodate, dexamethasone and/or propranolol) may be used to acutely decrease plasma T3 levels in patients with severe hyperthyroidism.

Thyroid hormone metabolism in humans

In healthy human subjects with an adequate iodine intake, the thyroid gland produces predominantly the prohormone T4 and a small amount of the bioactive thyroid hormone T3. Roughly 80% of T3 is produced by outer ring deiodination (ORD) of T4 in peripheral tissues . The relative contribution of T3 secretion increases in iodine deficiency and other conditions where the thyroid gland is stimulated by TSH or TSH receptor antibodies, since this is associated with increased de novo T3 synthesis and thyroidal expression of both D1 and D2, and thus increased intra-thyroidal T4 to T3 conversion (see below). Nevertheless, there is good agreement that about 1/3 of T4 daily produced (~130 nmol) in normal humans is converted to T3, which corresponds to about 40 nmol and thus 80% of the estimated total daily T3 production of 50 nmol. For recent comprehensive reviews of thyroid hormone metabolism and the role of the iodothyronine deiodinases therein, the reader is referred to (1-5).

That most plasma T3 is derived from peripheral conversion of T4 is supported by the fact that normal plasma T3 levels are obtained in athyreotic patients treated with sufficient T4 to achieve high-normal plasma (F)T4 levels. Administration of T4 to hypothyroid rats to achieve normal plasma T4 levels results in subnormal plasma T3 levels not only because of the lack of T3 secretion but also because of a decreased T3 production by D1 in peripheral tissues, since this enzyme is under positive control of T3 itself (6). Other studies in hypothyroid rats suggest that optimal restoration of serum and tissue thyroid hormone levels is achieved by the combined administration of specific amounts of T4 and T3 (7).

Also initial studies in humans suggested that replacement with a combination of T4 and T3 is better than replacement with T4 alone (8). However, this has not been confirmed in a large number of subsequent studies (reviewed in (9, 10)). A common drawback of these trials testing the possible beneficial effects of adding T3 to the T4 replacement therapy is that regular T3 tablets were used. Due to its short half-life, this results in substantial fluctuations of serum T3 levels. It remains to be investigated if administration of T3 in a slow-release formula which better mimics the continuous thyroidal T3 secretion (11) may improve the outcome of combined T4 and T3 replacement. Furthermore, psychological well-being and preference for L-T4 + L-T3 combination therapy may be influenced by polymorphisms in thyroid hormone pathway genes, specifically in thyroid hormone transporters and deiodinases (12-14).

Besides ORD to T3, T4 is converted by inner ring deiodination (IRD) to the metabolite rT3 (Fig. 1), which accounts for about 40% of T4 turnover, while thyroidal secretion of rT3 is negligible. T3 and rT3 undergo further deiodination, predominantly to the common metabolite 3,3′-diiodothyronine (3,3′T2), which is generated by IRD of T3 and by ORD of rT3 (1-5). Thus, ORD is an activating pathway by which the prohormone T4 is converted to active T3, whereas IRD is an inactivating pathway by which T4 and T3 are converted to the metabolites rT3 and 3,3′T2, respectively.

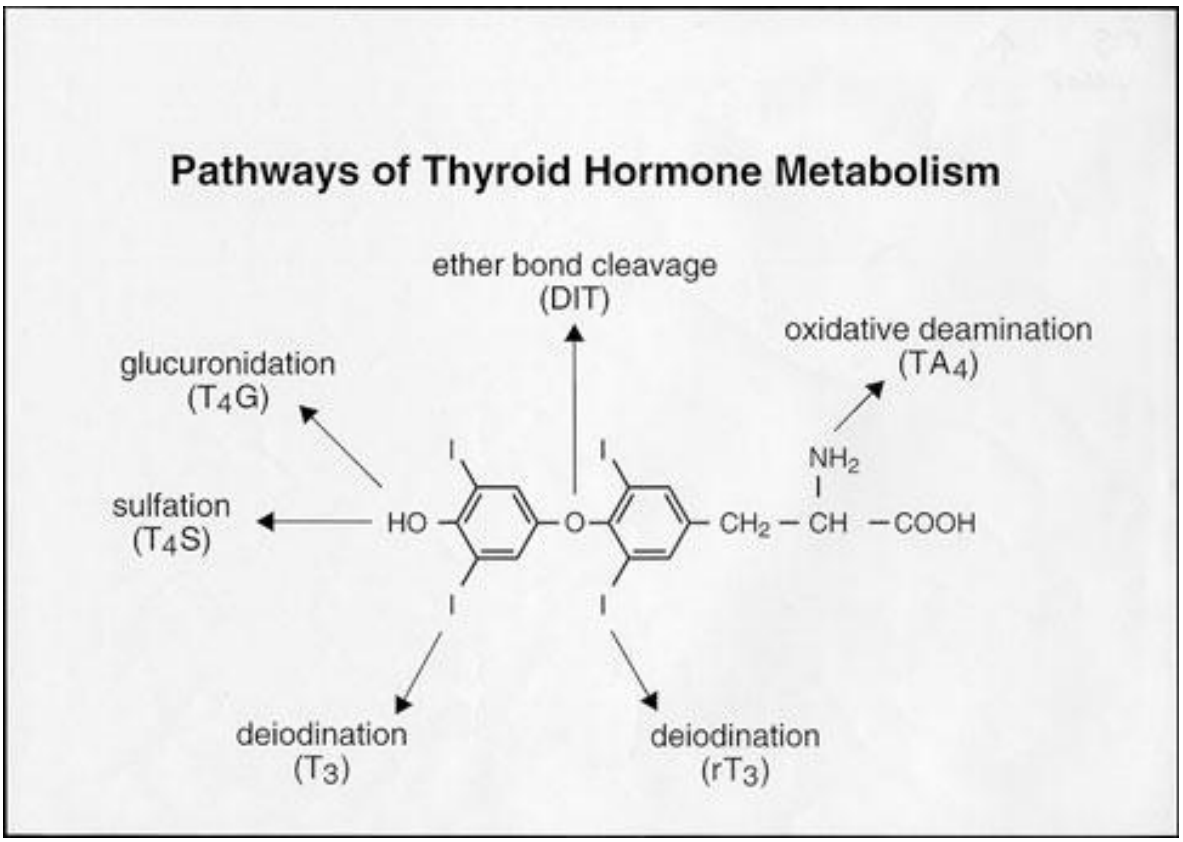

Figure 1. Pathways of Thyroid Hormone Metabolism

In addition to deiodination, iodothyronines are metabolized by conjugation of the phenolic hydroxyl group with sulfate or glucuronic acid (Fig. 1) (15, 16).Sulfation and glucuronidation are so-called phase II detoxification reactions, the general purpose of which is to increase the water-solubility of the substrates and, thus, to facilitate their biliary and/or urinary clearance. However, iodothyronine sulfate levels are normally very low in plasma, bile and urine, because these conjugates are rapidly degraded by D1, suggesting that sulfate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone (17, 18). Plasma levels and, if investigated, biliary excretion of iodothyronine sulfates are increased by inhibition of D1 activity with PTU or iopanoic acid (IOP), and during fetal development, NTI and fasting (16, 18). Under these conditions, T3 sulfate (T3S) may function as a reservoir of inactive hormone from which active T3 may be recovered by action of tissue sulfatases and bacterial sulfatases in the intestine (15-17).

In contrast to the sulfates, iodothyronine glucuronides are rapidly excreted in the bile. However, this is not an irreversible pathway of hormone disposal. After hydrolysis of the glucuronides by bacterial ß-glucuronidases in the intestines, part of the liberated iodothyronines is reabsorbed, resulting in an enterohepatic cycle of iodothyronines (15, 16). Nevertheless, about 20% of daily T4 production appears in the feces, probably through biliary excretion of glucuronide conjugates.

Thyronamines (TAMs) are a novel class of iodothyronine-like endogenous signaling compounds (19). Their structure differs from T4 and T3 only with regard to the absence of the carboxylate group of the alanine side chain. THs and TAMs are designated Tx and TxAM, respectively, with “x” indicating the number of iodine atoms per molecule, thus following the same rules for nomenclature (see (20) for an excellent review). So far, only 3-iodothyronamine (3-T1AM) and thyronamine (T0AM) have been detected in vivo using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (19, 21). 3T1AM and T0AM have been shown to exert acute and dramatic effects on heart rate, body temperature and physical

activity, inducing a torpor-like state (19), but also more subtle effects on neurocognitive function (22). The physiological receptor(s) of TAMs has not been identified unambiguously, but despite their structural similarities, iodothyronines and TAMs appear to signal via different receptors. Initial studies suggested that the TAMs mediated their effects via the G-protein coupled trace amine receptor, TAR1 (19). However, the impressive hypothermic response to 3-T1AM administration is maintained in TAAR-1 knockout (23). Whether other members of the TAAR family or other plasma membrane receptors mediate the TAM response remains to be studied.

Studies in athyreotic patients provide evidence for extrathyroidal formation of 3-T1AM (24), but the pathways of TAM biosynthesis are still unknown. However, it has been shown that iodothyronamines are deiodinated by the different deiodinases (25), which may suggest a role in biosynthesis.
Interesting effects of other natural thyroid hormone derivatives have been described as well. (26)Triac has significant thyromimetic activity and its affinity for the T3 receptor TRα1 is equal to that of T3 and for the TRβ receptor it is even higher than that of T3 (26). As a consequence, administration of Triac has successfully been used to suppress TSH secretion in patients with pituitary and general resistance to thyroid hormone due to mutations in TRβ (27). Interestingly, it was recently shown that the marine invertebrate Amphioxus expresses a TH receptor which is activated by Triac but not by T3 (28), as well as a non-selenoprotein that deiodinates Triac but not T3 (29). This may suggest that Triac is the primordial TH (29). A different natural TH derivative, 3,5-diiodo-L-thyronine (T2), has been shown to prevent adiposity and body weight gain when administered to rats receiving a high-fat diet (HFD) without the unfavorable side effects that are usually caused by T3 (30, 31).

However, the exact biological functions of these iodothyronine, iodothyronamine and iodothyroacetic acid metabolites remain to be established in future studies.

Cleavage of the ether bond connecting the inner and outer ring of iodothyronines represents a relatively minor pathway of thyroid hormone disposal (16) and will not be discussed here. In the following sections especially the biochemical aspects of the deiodination and conjugation pathways will be reviewed.

DEIODINATION

Three iodothyronine deiodinases have been identified, with distinct tissue distributions, catalytic specificities, physiological functions, and regulations (Fig. 2) (1-5). Whereas initial studies focused on the role of the deiodinases in maintaining normal serum T3 concentrations, the paradigm has evolved and it has now clearly been shown in different developmental and clinical conditions that these enzymes can locally modify TH bioactivity independent of serum TH concentrations. This is especially the case for D2 and D3 (see below).

Type	D1	D2	D3
	T4 T3 rT3 T2	T4 T3 rT3 T2	T4 T3 rT3 T2
Tissues, *e.g.*	liver, kidney, thyroid	brain, pituitary skeletal muscle, heart (?)	brain, placenta fetal tissues
Susbtrates	rT3 » T4 T3	T4 > rT3	T3 > T4
Km values	0.1-10 M	1 nM	10 nM
Function	plasma T3 production	local T3 production	T3 degradation
Inhibitors (IC_{50}, M)			
PTU	5	>1000	>1000
IAc	2	1000	1000
GTG	0.05	1	5
Hypothyroidism	decrease	increase	decrease
Hyperthyroidism	increase	decrease	increase

Fig. 2 Characteristics of the three types iodothyronine deiodinases

Fig. 2 Characteristics of the three types iodothyronine deiodinases

D1, D2 and D3 have been cloned in different species, including mammals, frog, chicken and fish. The deduced amino acid sequences of human D1, D2 and D3 are presented in Fig. 3. The deiodinases appear to be homologous proteins, consisting of 249-278 amino acids. A particular lipophilic sequence is present in the N terminal domain of all three deiodinases, which probably represents a membrane-spanning region.

The most remarkable feature of all three iodothyronine deiodinase is that they are selenoproteins, i.e. they contain a selenocysteine (Sec) residue in the center of the amino acid sequence . In all selenoproteins, Sec is encoded by a UGA triplet which is an opal stop codon because it usually signals termination of translation. However, if the 3′ untranslated region (3′UTR) of the mRNA contains a particular stem loop structure, termed selenocysteine-insertion sequence (SECIS) element, the UGA codon specifies the insertion of Sec . Interestingly, it was recently shown that the marine invertebrate Amphioxus expresses a non-selenoprotein that deiodinates Triac but not T3 .

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hD1       MGLPQP GLWLKRLWVL LEVAVHVVVG KVLLILFPDR VKRNILAMGE KTGMTRNP..
hD2   MGILSVDLLI TLQILPVFFS NCLFLALYDS VILLKHVVLL LSRSKSTRGE WRRMLTSEGL
hD3     MLHSLLLH SLRLCAQTAS CLVLFPRFLG TAFMLWLLDF LCIRKHFLGR RRRGKPEPEV

hD1   .......... ......HFSH DNWIPTFFST QYFWFVLKVR WQRLEDTTEL GGLAPNCPVV
hD2   R......... ......CVWK SFLLDAYKQV KLGEDAPNSS VVHVSSTEGG DNSGNGTQEK
hD3   ELNSEGEEVP PDDPPICVSD DNRLCTLASL KAVWHGQKLD FFKQAHE... GGPAPNSEVV

hD1   RLSG.QRCNI WEFMQGNRPL VLNFGSCTUP SFMFKFDQFK RLIEDFSSIA DFLVIYIEEA
hD2   IAEG.ATCHL LDFASPERPL VVNFGSATUP PFTSQLPAFR KLVEEFSSVA DFLLVYIDEA
hD3   LPDGFQSQHI LDYAQGNRPL VLNFGSCTUP PFMARMSAFQ RLVTKYQRDV DFLIIYIEEA

hD1   HASDGWAFKN N....MDIRN HQNLQDRLQA AHLLLARS.. .PQCPVVVDT MQNQSSQLYA
hD2   HPSDGWAIPG DSSLSFEVKK HQNQEDRCAA AQQLLERFSL PPQCRVVADR MDNNANIAYG
hD3   HPSDGWVTTD SP...YIIPQ HRSLEDRVSA ARVLQQGA.. .PGCALVLDT MANSSSSAYG

hD1   ALPERLYIIQ EGRILYKGKS GPWNYNPEEV RAVLEKLHS                    249
hD2   VAFERVCIVQ RQKIAYLGGK GPFSYNLQEV RHWLEKNFSK RUKKTRLAG         273
hD3   AYFERLYVIQ SGTIMYQGGR GPDGYQVSEL RTWLERYDEQ LHGARPRRV         278
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Fig. 3 Alignment of the amino acid sequences of human D1, D2, and D3
U=selenocysteine (Sec)

Type I iodothyronine deiodinase (D1)

Biochemistry

D1 is expressed predominantly by liver parenchymal cells, kidney proximal tubular cells, and thyroid follicular cells. Most evidence points to the localization of D1 in the plasma membrane but older data have suggested that in liver the enzyme may reside in the endoplasmic reticulum (35). D1 catalyzes the ORD and/or IRD of a variety of iodothyronine derivatives, although it is most effective in catalyzing the ORD of rT3, while the IRD of both T4 and T3 is strongly facilitated by sulfation of these iodothyronines (17). Therefore, although D1 is thought to be a major source of circulating T3, the enzyme shows particularly high activity towards TR-inactive metabolites such as rT3 and the different sulfo-conjugates. This suggests that D1 plays an important role in the recovery of iodide from inactive compounds for reutilization in thyroidal hormone synthesis (36). In the presence of dithiothreitol (DTT) as the cofactor, D1 displays high Km and Vmax values.

Studies of the topography of rat D1 have suggested that the major part of the protein is exposed on the cytoplasmic surface of the membrane (37). Older studies using detergent extracts of rat liver and kidney membranes have suggested that the native enzyme largely exists as a homodimer. This has been confirmed in a number of recent studies utilizing cells transfected with different D1 constructs (2, 38-40). These studies have also demonstrated that amino acids 148-163 constitute the dimerization domain of the D1 protein (DFLVIYIEEAHASDGW in human D1).

The D1 gene is located on human chromosome 1p32-33. It consists of four exons, with exon 1 coding for the 5'UTR and amino acids 1-112, exon 2 for amino acids 113-160, exon 3 for amino acids 161-227, and

exon 4 for amino acids 228-249 and the 3'UTR, including the SECIS element. Before D1 was identified as a selenoprotein, this was suspected from findings that D1 activities are strongly reduced in liver and kidney, but not in thyroid, of rats fed on a selenium (Se)-deficient diet (41). This is associated with a small decrease in serum T3 and a marked increase in serum T4. The Sec residue in D1 is essential for deiodinase activity since replacement of Sec by Cys results in a 100-fold decrease in catalytic activity, while substitution of Sec by Leu produces an enzymatically inactive protein (42). In addition, D1 is extremely sensitive to inactivation by iodoacetate due to carboxymethylation of a highly reactive residue, probably Sec, in the enzyme active center which is prevented in the presence of substrate (2, 15). Moreover, D1 activity is inhibited by very low concentrations (□10-8 M) of goldthioglucose (GTG), which is known to form very stable complexes with Sec residues, and this inhibition is also competitive with substrate (43). Therefore, Sec is probably the catalytic center of D1.

Two other observations have provided important clues about the possible catalytic mechanism of D1. Firstly, D1 shows ping-pong type reaction kinetics in catalyzing the deiodination of iodothyronines by DTT (2, 15), suggesting that reaction of iodothyronine substrate with D1 produces an enzyme intermediate, from which native enzyme is regenerated by reaction with thiol cofactor (DTT). Secondly, D1 is potently inhibited by PTU, and this inhibition is uncompetitive with substrate and competitive with cofactor, suggesting that PTU and cofactor react with the same enzyme intermediate. Thiouracil derivatives are particularly reactive towards protein sulfenyl iodide (SI) groups, and presumably even more reactive towards selenenyl iodide (SeI) groups, suggesting that such an intermediate is generated in the catalytic cycle of D1. Therefore, the selenolate (Se-) group of the native enzyme is thought to act as an acceptor of the iodonium (I+) ion which is substituted in the substrate by a proton, and the SeI intermediate thus generated is reduced back to native enzyme by thiols such as DTT or converted into a dead-end complex by PTU (Fig. 4).

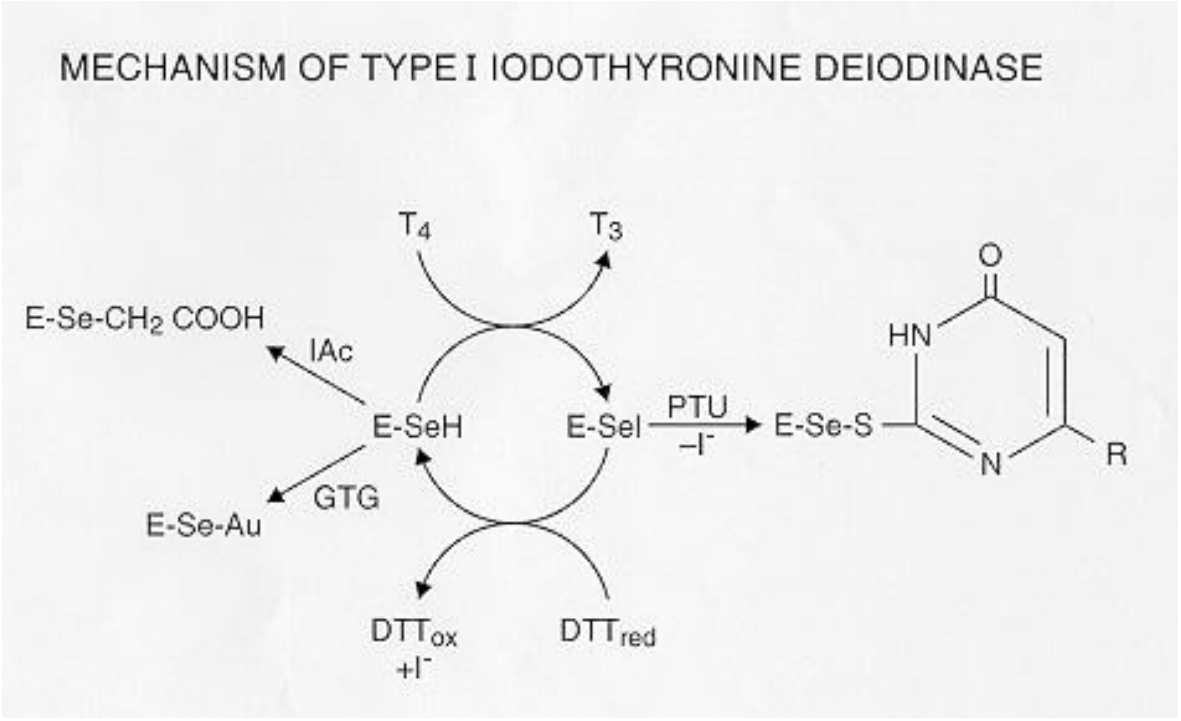

Figure 4. Putative catalytic mechanism of D1 and inhibition by PTU, IAc and GTG.

Unlike the mammalian enzyme, D1 from tilapia was found to be insensitive to PTU inhibition (44). Like all

other characterized deiodinases, tilapia D1 also contains a Sec residue in the corresponding position (44). However, two positions downstream from this Sec residue, tilapia D1 features a Pro residue, which is also the case in other fish species and in frog D1. In contrast, all known PTU-sensitive D1 enzymes have a Ser residue at this position. Remarkably, a Pro residue is also present at this position in all known D2 and D3 sequences, which are also PTU-insensitive. Substitution of Pro by Ser in tilapia D1 did not restore PTU sensitivity (44). However, substitution of Pro by Ser in frog D1 (4) as well as in human D2 and D3 not only made these enzymes susceptible to inhibition by PTU but also changed the kinetic mechanism of these enzymes (45). Therefore, in addition to the Sec residue the amino acid two positions downstream plays an important role in the catalytic mechanism of the deiodinases. The lack of PTU inhibition of the tilapia D1Pro>Ser mutant suggests that additional elements of the protein are important for effect of PTU.

Pathophysiology

D1 activity in liver and kidney is stimulated in hyperthyroidism and decreased in hypothyroidism, representing the regulation of D1 activity by T3 at the transcriptional level (46). T3 response elements (TREs) have been identified in the upstream region of the D1 gene (47, 48). Studies in TR knockout mice have indicated that D1 expression in liver is primarily controlled by the TR□ isoform (49). This agrees well with the colocalization of TR□ and D1 in the pericentral zone of rat liver (50). In thyroid, D1 expression is stimulated by T3, TSH and TSH receptor antibodies, where the effects of the latter are mediated by cAMP (51, 52).

There is controversy about the contribution of D1 to peripheral T3 production. Different animal models have been studied which may provide a clue about this function of D1. Firstly, rats have been raised on a severely selenium-deficient diet, resulting in a dramatic reduction in liver and kidney D1 activity (53). These rats showed a significant decrease in serum T3 and increase in serum T4, compatible with an important role of D1 in peripheral T4 to T3 conversion. Other studies in rats have demonstrated that D2 and D3 activities in other tissues such as brain are much less affected directly by selenium deficiency (54).It should be noted that in mice lacking the plasma Se carrier selenoprotein P (SePP), thyroid hormone metabolism is preferentially maintained indicating that selenoenzymes have a priority in the organism with respect to selenium supply (55).

Findings in mice do not support an important function of liver and kidney D1 in peripheral T3 production as suggested by selenium deficiency in rats. C3H mice show a strongly reduced hepatic and renal D1 expression compared with other mouse strains (56-58). Yet, their serum T3 levels are comparable, although the C3H mice show some increase in serum T4 suggesting that an increased T4 production may compensate for the decreased T4 to T3 conversion. Serum rT3 levels are mildly elevated as well in C3H mice. In another mouse model, hepatic synthesis of selenoproteins, including D1, is disabled by inactivation of the Sec-specific tRNA (59). This does not result in any change in circulating thyroid hormone concentrations. Finally, D1 knockout (D1KO) mice have been generated which do not express D1 in any tissue, including thyroid and kidneys (36). These D1KO mice also show normal serum T3 and TSH levels, but like the C3H mice they have elevated serum T4 and rT3 levels as well.

However, we should be careful to draw conclusions about the contribution of D1 to serum T3 homeostasis based on these knock-out mouse models, since even mice without any ORD (D1KO/D2KO mice) are able to maintain normal levels of serum T3 (60), pointing towards a major role played by the thyroid gland as well. Although these data in D1KO/D2KO mice suggest that D1 and D2 may not be essential for the maintenance of the serum T3 level, both enzymes do serve important roles in thyroid hormone

homeostasis. Fecal excretion of endogenous iodothyronines was greatly increased in D1KO mice, pointing towards an important role in iodide conservation by serving as a scavenger enzyme in peripheral tissues and the thyroid (36). Similarly, despite normal serum T3 levels in D2KO mice, brain T3 levels as well as the expression of certain T3 responsive genes in the brain was reduced.

Many studies have addressed the question about the contribution of a diminished expression of hepatic D1 to the decrease in serum T3 in rats exposed to fasting or NTI. The results of these studies are confounded by the fact that D1 not only produces T3 from T4 but its expression is also stimulated by T3. In fact, D1 expression is a very sensitive indicator of the thyroid state of the liver (61).

So far, no patients with mutations in D1 have yet been identified. However, several candidate gene association studies have reported on significant associations of single nucleotide polymorphisms (SNPs) in D1 with reciprocal changes in serum T3 versus T4 and rT3 levels (62-65). Recently, a large genome wide association meta-analysis was conducted for serum FT4 levels, and a single nucleotide polymorphism in DIO1 was one of the five genome wide significant hits (Porcu E, Medici, M, et al, PLOS Genetics, accepted for publication), strongly suggesting an important role for D1 in peripheral thyroid hormone metabolism in humans as well.

Type II iodothyronine deiodinase (D2)

Biochemistry

D2 is expressed primarily in the brain, the anterior pituitary gland and (rodent) brown adipose tissue (BAT) (1-5). D2 activity has also been shown in human thyroid (66-68) and skeletal muscle (69), while D2 mRNA is also expressed in human heart (70). Localization of D2 mRNA in rat brain by in situ hybridization has indicated that the enzyme is expressed in astrocytes, in particular in tanycytes lining the third ventricles (71). D2 activity is induced in cultured astrocytes by a variety of factors (72-74). Like the other deiodinases, D2 also forms functional homodimers (38, 39, 75). Regarding cellular localization, D2 is largely present in the endoplasmic reticulum (35).

D2 has only ORD activity, exhibiting low Km and Vmax values, and a slight preference for T4 over rT3 as the substrate. In contrast to D1, it does not catalyze the deiodination of sulfated iodothyronines. The amount of T3 in D2-expressing tissues is derived to a large extent from local conversion of T4 by this enzyme and to a minor extent from plasma T3. In general, D2 activity is increased in hypothyroidism and decreased in hyperthyroidism. Part of this negative control is explained by substrate-induced inactivation of the enzyme by T4 and rT3 (1-5). Reaction of these substrates with D2 induces the ubiquitination of the enzyme, which facilitates its degradation in the proteasomes. However, active D2 may also be recovered by de-ubiquitination of the modified enzyme. Thus, ubiquitination/de-ubiquitination is an important, dynamic mechanism in the regulation of D2 activity. For a more detailed discussion of this pathway, the reader is referred to excellent studies and reviews published in this area (38, 76-80). Furthermore, Dio2 is a cAMP-responsive gene and as a consequence the adrenergic/cAMP signaling pathway mediates the transcriptional control of D2 (81).In addition, D2 expression is also importantly regulated by ER stress reducing D2 activity by inhibition of de novo synthesis of the D2 protein (82). Finally, presumably receptor-mediated inhibition of D2 activity by T3 has been demonstrated in pituitary tumor cells (83), and D2 mRNA levels in brain, pituitary and BAT are up-regulated in hypothyroid rats and down-regulated in hyperthyroid animals (84, 85).

The central Sec residue plays an important role in the catalysis and turnover of D2. Replacement of this

Sec with Cys results in a 1000-fold increase in the Km value of the substrates T4 and rT3, and a 10-fold decrease in turnover number (86, 87). Substitution of Sec by Ala completely inactivates the enzyme. Also the mechanism of substrate-induced D2 degradation is strongly or completely impaired by replacement of Sec by Cys or Ala, respectively (88), suggesting that modification of this Sec residue during catalysis may be an essential step in the inactivation of the enzyme. Interestingly, mammalian and avian D2 also have a second Sec residue near the C-terminus which, however, is not important for catalytic activity (89).

The D2 gene is located on human chromosome 14q24.2-q24.3. It consists of 2 exons of 0.7 kb and 6.6 kb, seperated by a 7.4 kb intron (2). The SECIS element in the 3'UTR is separated by ~5 kb from the UGA triplet coding for the catalytic Sec residue, resulting in a poor translation efficiency of the D2 mRNA (90). This is even further hampered by the presence of multiple short open reading frames in the 5'UTR of human D2 mRNA (90).

Pathophysiology

D2 is expressed in human thyroid but not in rat thyroid. Both D2 mRNA and D2 activity in human thyroid are greatly stimulated by TSH and TSH receptor antibodies circulating in patients with Graves' disease (66, 67). The expression of D2 in human thyroid has been associated with functional TTF-1 binding sites in the 5' flanking region of the human D2 gene which are lacking in the 5' flanking region of the rat D2 gene (91). The stimulatory effects of TSH and TSH receptor antibodies on D2 expression in human thyroid are mediated by cAMP, which has been associated with the presence of a cAMP response element (CRE) in the 5' flanking region of the D2 gene (81, 92). Interestingly, follicular thyroid carcinoma may express high levels of D2, and in case of a large (metastatic) tumor mass this may results in strongly elevated serum T3 levels (93-95).

Recently, a D2 knockout (D2KO) mouse has been (96)generated, showing modest phenotypic changes (96). The homozygous D2KO mice have increased serum T4 and increased TSH levels, but normal levels of T3. The combination of increased serum TSH and T4 is in agreement with an important role of D2 in the negative feedback of T4 at the hypothalamus and pituitary level. However, the normal serum T3 suggests that D2 is not essential for maintaining normal serum T3 levels. However, as mentioned above, even D1KO/D2KO mice are able to maintain normal levels of serum T3 (60), pointing towards a major role for the thyroid gland in serum T3 production as well. In skeletal muscle, D2 levels are higher in slow-twitch than fast-twitch mouse skeletal muscle and are increased in hypothyroidism (97). The contribution of muscle D2 to serum T3 production in humans remains to be determined (98).

In contrast to the marked decrease in hepatic and renal (but not thyroidal) D1 activities, the unexpectedly small effects of Se deficiency on tissue D2 and D3 activities in rats, despite that they all appear to be Sec-containing proteins, may be explained by findings that the selenium state of different tissues varies greatly in Se-deficient animals (99). In addition, the efficiency of the SECIS element to complex with protein factors, such as SBP2, necessary for the read-through of the UGA codon may vary between different seleno¬proteins. This could result in the preferred incorporation of Sec into some seleno-proteins, e.g. deiodinases, over others, e.g. glutathione peroxidase (33).

Despite normal serum T3 levels in D2KO mice, brain T3 levels as well as the expression of certain T3 responsive genes in the brain is reduced, again pointing towards the crucial role of D2 in maintaining local T3 concentrations (96). Several other studies point towards a crucial role for D2 (and D3, see below) in regulating local T3 concentrations, and as a consequence it is know well accepted that these deiodinases can regulate thyroid hormone action at the cellular level during development and tissue

stress relatively independent of serum T4 and T3 concentrations (3).

One of the clearest examples of the role of D2 in development is its role in the inner ear. A sharp increase in D2 activity occurs in mouse cochlea at postnatal days 6-8, which is required for normal cochlear development (100). As a consequence, D2KO mice are deaf underlining the importance of D2 in producing local T3 in the cochlea during a critical period of its development (101). Another example of the important role of D2 in development is the observation that D2KO mice have an impaired embryonic BAT development, and as a consequence a permanent thermogenic defect (102, 103). D2KO mice exhibit an impaired thermogenesis in BAT, leading to hypothermia during cold exposure and a greater susceptibility to diet-induced obesity at thermoneutrality (104).

D2 is also essential for maintaining normal local concentrations of T3 in different physiological and pathophysiological situations. In addition to its important role in pituitary and hypothalamic feedback (96, 105), it also plays an essential role for normal myogenesis (106) and in the optimization of bone strength and mineralization (107). Adult D2KO mice have a 50% reduction in bone formation and a generalized increase in skeletal mineralization resulting from a local deficiency of T3 in osteoblasts. (101)
D2 is also required for the regeneration of skeletal muscle after injury (106), since regeneration after injury is markedly delayed in D2KO mice. The increase in muscle D2 is mediated via FoxO3, thereby locally increasing intracellular T3 concentrations. Muscle D2 expression during critical illness is differentially regulated, probably related to differences in the inflammatory response and type of pathology (108). In humans, skeletal muscle D2 mRNA expression is modulated by fasting and insulin, but not by hypothyroidism (109). Also in lung tissue, D2 activity increases upon injury. In a mouse model of ventilator-induced lung injury (VILI), lung D2 activity increased (110). D2KO mice had a greater susceptibility to VILI than WT mice, demonstrated by poorer alveoli integrity and quantified by lung chemokine and cytokine induction. Interestingly, treatment of D2KO mice with T3 reversed part of the lung chemokine and cytokine profiles.

No patients have been identified with mutations in D2. However, a recent study has reported on patients with homozygous or compound heterozygous mutations in the SECIS-binding protein SBP2, which is crucial for the synthesis of selenoproteins (111). Fibroblasts from these patients were shown to express markedly lower D2 activity than cells from normal subjects. These patients have abnormal serum thyroid hormone levels: high (F)T4 and rT3, low T3, and somewhat elevated TSH levels. This resembles the changes in thyroid parameters in D2KO mice, although in patients with SBP2 mutations also the expression of functional D1 and D3 is probably affected. In contrast to the D2KO mice, these patients do not have a hearing problem, but they show delayed growth (101, 103, 111, 112).

Whether polymorphisms in D2 are associated with significant changes in thyroid hormone levels or with insulin resistance is controversial (62, 113-116), but a recent large genome wide association meta-analysis for serum FT4 levels (Porcu E, Medici, M, et al, PLOS Genetics, accepted for publication) or diabetes did not result in genome wide significant hits for the Dio2 locus. However, genetic variation in D2 has been identified as a risk factor for osteoarthritis (117).

Type III iodothyronine deiodinase (D3)

Biochemistry

D3 activity has been detected in a variety of tissues, i.e. brain, skin, liver, intestine, placenta, and the pregnant rat uterus (1-5, 118-120). D3 expression is usually much higher in fetal than in adult tissues. D3

activity is also highly expressed in certain tumors, including hepatocarcinomas, hemangiomas and basal cell carcinomas (121-124). Because of its expression in fetal tissues and tumors, D3 has been named an oncofetal protein. The enzyme appears to be located in the plasma membrane in the form of a homodimer (38, 125, 126). D3 has only IRD activity, catalyzing the inactivation of T4 and T3 with intermediate Km and Vmax values (Fig. 2).

The expression of D3 in placenta, pregnant uterus, embryonic and fetal tissues may protect developing organs against undue exposure to active thyroid hormone. Also in adult subjects, D3 appears to be an important site for clearance of plasma T3 and production of plasma rT3. In brain and skin, but not in placenta, D3 activity is increased in hyperthyroidism and decreased in hypothyroidism, which in brain is associated with parallel changes in D3 mRNA levels (127).

The D3 gene is located on human chromosome 14q32 and consists of a single exon. In all species, D3 is a selenoprotein homologous with the amino acid sequences of D1 and D3, including the essential Sec residue positioned in a strongly conserved region (Fig. 2). It has been shown that D3 expression is predominantly regulated by TRα1 (128), and studies in TRα1-/- mice have demonstrated a reduced clearance rate of TH due to an impaired regulation of D3 (129).

The presence of Sec in a strongly conserved region of the proteins strongly suggests the same mechanism of deiodination for the different deiodinases. This seems to be contradicted by the widely different susceptibilities of D1 versus D2 and D3 to the different mechanism-based inhibitors PTU, IAc and GTG (Fig. 2). It also seems to be in conflict with previous findings that, in contrast to the ping-pong kinetics of D1, the other two enzymes appear to follow sequential-type kinetics, suggesting the formation of a ternary enzyme-substrate-cofactor complex during catalysis. The differences in enzyme kinetics and PTU inhibition between the deiodinases are determined by the presence of Ser (D1) or Pro (D2,D3) two positions downstream of Sec, which may somehow influence the reactivity of the catalytic Sec residue (see above).

Pathophysiology

D3 plays a very important role in the regulation of local and systemic thyroid hormone bioactivity (1, 123). It has been shown that region-specific expression of D3 in fetal and adult human brain is negatively associated with local tissue T3 levels (130, 131). High expression of D3 in vascular tumors may result in subclinical or even severe hypothyroidism in patients with such tumors, which condition has been termed consumptive hypothyroidism (122, 123, 132). Induction of D3 expression has also been demonstrated in liver and skeletal muscle biopsies from patients who died after severe illness, and D3 activities were correleated to both local and serum rT3 concentrations in these severely sick patients (133-135). Therefore, tissue and circulating iodothyronine levels are regulated not only by changes in the T3-producing deiodinases D1 and D2 but also importantly by reciprocal changes in the T3-degrading deiodinase D3.

D3 knockout (D3KO) mice have been generated, showing remarkable neonatal mortality and growth retardation (136-138). In addition, they show largely abnormal thyroid hormone levels, dependent on the age of the animals. Compared with wild-type mice, serum T4 is very low in D3KO mice at all ages, T3 is higher in neonatal mice but much lower in older D3KO mice, while TSH varies between very low in younger to low in older knockout mice. This picture represents a state of central hypothyroidism, suggesting that the setpoint of the hypothalamus-pituitary-thyroid axis is strongly affected by inactivation of D3, which could be due to overexposure of tissues (e.g. the developing hypothalamus) to T3. This is

reminiscent of the reports of congenital central hypothyroidism in newborns from mothers who were hyperthyroid during pregnancy (139).

Heterozygous D3KO mice show either almost normal or strongly decreased D3 expression, depending on whether the defective allele is inherited from the mother or the father, respectively, indicating paternal imprinting of the DIO3 gene (136). The DIO3 gene is located in an imprinted region on human chromosome 14 or mouse chromosome 12 which is about 1 Mb in size and comprises the paternally expressed genes DLK1 (delta-like 1) and DIO3, and a large number of in particular maternally expressed non-coding genes. Remarkably, one such non-coding gene (DIO3OS) is located on the opposite strand from DIO3 and also partially overlaps with DIO3. The exact role of DIO3OS in the regulation of DIO3 expression, however, is unclear. Both Dlk1 and Dio3 expression are elevated in cultured brown pre-adipocytes and down-regulated during differentiation, suggesting that imprinting might control the dosage of these genes to regulate thermogenesis (140). Interestingly, transgenic animals with partial loss of imprinting of this locus show significant lethality in the third postnatal week, associated with developmental delay and failure to maintain UCP1 expression in BAT (141). This defect is the combined result of prolonged elevated expression of Dlk1, leading to a failure in BAT differentiation and subsequent reduced expression of β-adrenergic receptors, and hypothyroidism due to dysregulation of D3.

The important role of hepatic D3 in the regulation of circulating thyroid hormone during development has been investigated in detail in the embryonic chicken (142, 143). These studies have demonstrated that during the last (third) week of incubation there is a gradual increase in plasma T4 levels paralleled by a steady increase in hepatic D1 activity although hepatic D1 mRNA levels do not change much. D3 mRNA and D3 activity show a parallel increase to maximum levels at day 17 of embryonic development, followed by a steep decrease in both parameters in particular immediately before hatching. This is associated with an equally steep increase in plasma T3, strongly suggesting that the latter is importantly and negatively regulated by hepatic D3 activity (142, 143).

A study of the ontogeny of hepatic D1 and D3 during human development has indicated similar profiles of deiodinase expression, with substantial and relatively constant D1 activities from mid-gestation onwards, and high D3 activities at mid-gestation declining to very low levels around term (144). Since in rat liver D1 is not expressed until the last days of gestation, while hepatic D3 expression is low at all stages of rat development (118), these results indicate that the embryonic chicken is a better model than the fetal rat for the regulation of hepatic deiodinases during human development. Injection of the chicken embryo with growth hormone or glucocorticoids induces an acute down-regulation of hepatic D3 mRNA levels and D3 activities, suggesting that the D3 mRNA in the embryonic chicken has a very short half-life, and that transcription of the D3 gene is acutely blocked by these treatments (142). If D3 expression in the fetal human liver is also rapidly down-regulated by GH and glucocorticoids remains to be determined. It is likely that the high D3 activities expressed in the fetal liver, in addition to the high D3 activities in the placenta (145, 146) and perhaps the uterus (119), plays an important role in the regulation of fetal circulating T3 levels and protect the fetus against early T3 exposure.

In recent years, several studies have addressed the role of D3 in regulating local T3 concentrations. It is now well accepted that D3 plays a crucial role in regulating thyroid hormone action at the cellular level during development, relatively independent of serum T4 and T3 concentrations. During development, D3 is expressed in the immature cochlea before D2 (147). Like D2KO mice, D3KO mice display auditory deficits as well. However, in contrast to the retarded cochlear development in D2KO mice, D3KO mice

display an accelerated cochlear differentiation due to premature stimulation of TR□. The additional deletion of TR□ converts the accelerated cochlear phenotype in D3KO mice to one of delayed differentiation (147), indicating a protective role for D3 in hearing development. This clearly illustrates how different tissues can auto-regulate their developmental response to thyroid hormone through both D2 and D2. D3 also plays a crucial role in cerebellar development, since D3KO mice display abnormally accelerated cerebellar differentiation and locomotor behavioral defects, suggesting that D3 protects cerebellar tissues from inappropriate, premature stimulation by thyroid hormone (148). This cerebellar phenotype results specifically from inappropriate stimulation of the TR□1 receptor isoform, since the additional of TR□1 reversed the cerebellar phenotype. Similarly, D3 protects cones to unlimited T3 exposure in the immature mouse retina. As a consequence, approximately 80% of cones are lost through neonatal cell death in D3KO mice (149). Furthermore, protection against untimed T3 exposure by D3 in pancreatic β-cells during development is essential for normal islet function and glucose homeostasis (150). As a consequence, D3KO mice have impaired insulin secretion in response to glucose stimulation. In contrast to most tissues, D3 expression remains throughout adulthood in human and mouse β-cells. However, whether dysregulation of Dio3 might play a role in different states of impaired insulin secretion remains to be explored in future studies (150).

In addition to its crucial role during development, D3 activity is also important in regulating thyroid hormone action at the cellular level in different pathophysiological conditions. Induction of D3 expression has been documented in the hypertrophic or failing heart resulting from pressure overload or myocardial infarction (151-153). Hypoxia-inducible factor 1□ (HIF-1□) induces local thyroid hormone inactivation by inducing D3 during hypoxia (152), suggesting a mechanism of down-regulating metabolism during ischemia. In neuronal hypoxia, translocation of D3 to the nucleus is mediated by Hsp-40, thereby facilitating local inactivation of thyroid hormone and reducing ischemia-induced hypoxic brain damage (154). Heterozygous D3KO mice constitute a model of cardiac D3 inactivation in an otherwise systemically euthyroid animal (155). These mice have normal hearts but later develop restrictive cardiomyopathy due to cardiac-specific increase in thyroid hormone signaling. In addition, heterozygous D3KO mice are more vulnerable to isoproterenol, further worsening the restrictive cardiomyopathy and leading to congestive heart failure and increased mortality (155). D3 activity is also induced in liver and muscle of critically ill patients (133-135). See (156) for an excellent overview of the changes in local thyroid hormone metabolism during illness and inflammation. Interestingly, in a mouse model of turpentine-induced tissue inflammation, high D3 expression in invading granulocytes has also been reported (157, 158).

Several recent studies have demonstrated that local regulation of thyroid hormone action also plays a crucial role in repair mechanisms, for example D3 in liver (159) and brain (160), and D2 in muscle (106). Furthermore, D2 and D3 activities are regulated by a variety of growth factors and morphogens, which are important mediators of tissue injury repair (161). After a large hepatectomy, ‘stem-like’ cells switch from a quiescent state to a proliferative state. During these processes, many fetal genes are reactivated (162). Among them, D3 activity was increased 10-fold and D3 mRNA expression was increased 3-fold 20 h after partial hepatectomy in rats. No significant effects on D1 and D2 activities or mRNA expression were found after partial hepatectomy in mice (159). This leads to the concept that a coordinated regulation of thyroid hormone action is essential in the control of the tight balance between proliferation and differentiation in the regeneration processes. Induction of D3 expression in the early phases of regeneration may therefore very well correlate with a requirement of increased cellular proliferation in these circumstances (3, 161).

The balance between proliferation and differentiation is disturbed in cancer, and D3 is turned on in

several malignant cell lines and human cancers (3, 163). D3 activity in these cancers can be very high and may even lead to so-called consumptive hypothyroidism (123, 132). In basal cell carcinomas, as well as in primary proliferating keratinocytes, Sonic hedgehog (Shh) increases the expression of D3, acting via a conserved Gli2 binding site on the human Dio3 promoter (121). This suggests that Shh may induce local down-regulation of thyroid hormone activity. Interestingly, knockdown of D3 caused a 5-fold reduction in the growth of basal cell carcinoma xenografts in nude mice (121), suggesting that D3 up-regulation provides an advantage for proliferating tumor cells. Interestingly, a recent study in papillary thyroid carcinoma demonstrated an association between increased levels of D3 activity and advanced disease (164). However, since only a few tumors over-express D3, D3 expression seems not be a necessary step in tumorigenesis.

Sulfation

Iodothyronine sulfotransferases

Sulfotransferases represent a family of enzymes with a monomer molecular weight of≈34 kDa, located in the cytoplasmic fraction of different tissues, in particular liver, kidney, intestine and brain (165). They catalyze the transfer of sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to usually a hydroxyl group of the substrate (165). On the basis of substrate specificity and amino acid sequence homology, mainly two sulfotransferase families have been recognized in human tissues, i.e. the phenol sulfo¬trans¬ferases (SULT1 family), including estrogen sulfotransferase, and the hydroxysteroid sulfotransferases (SULT2 family) (165). Different phenol sulfotransferases have been identified with significant activity towards iodothyronines. These include human SULT1A1, 1A2, 1A3, 1B1 and 1C2 (Table 1) (166-174). These studies have indicated a large substrate preference of the recombinant enzymes as well as the native enzymes in human liver and kidney for 3,3′T2, the sulfation of which is catalyzed orders of magnitude faster than that of T3 or rT3, while sulfation of T4 is hardly detectable (168).

Surprisingly, it has also been demonstrated that human estrogen sulfo¬transferase (SULT1E1) is an important isoenzyme for sulfation of thyroid hormone. Although human SULT1E1 shows much higher affinities for estrogens (Km ≈μM) than for iodothyronines (Km □μM), it is about as efficient as other isoenzymes in sulfating 3,3′T2 and T3, and much more efficient in sulfating rT3 and T4 (169). Human tissues known to express SULT1E1 include liver, uterus, and mammary gland (175). In particular the enzyme expressed in the endometrium may be a significant source for the high levels of iodothyronine sulfates in human fetal plasma (see below). Recently, different human SULTs have also been shown to catalyze the sulfation of iodothyronamines (Table 1) (172).

Deiodination of iodothyronine sulfates

Although D1 is capable of converting T4 with similar efficiency by ORD to T3 and by IRD to rT3, this is changed dramatically after sulfate conjugation, i.e. IRD of T4S by rat D1 is accelerated □200-fold, whereas ORD of T4S becomes undetectable (Fig. 5) (17). IRD of T3 by rat and human D1 is also markedly stimulated (□40-fold) by sulfation (Fig. 5)(17). As mentioned before, rT3 is by far the preferred D1 substrate; its ORD is not influenced by sulfation, suggesting that the catalytic efficiency of D1 is already optimal with nonsulfated rT3 (17). While sulfation inhibits ORD of T4 and is without effect on ORD of rT3, it markedly stimulates ORD of 3,3'-T2 (Fig. 5). Thus, sulfation facilitates the IRD of T4 and T3, while it either inhibits (T4), does not affect (rT3) or markedly stimulates (3,3'T2) the ORD of other

substrates (17).

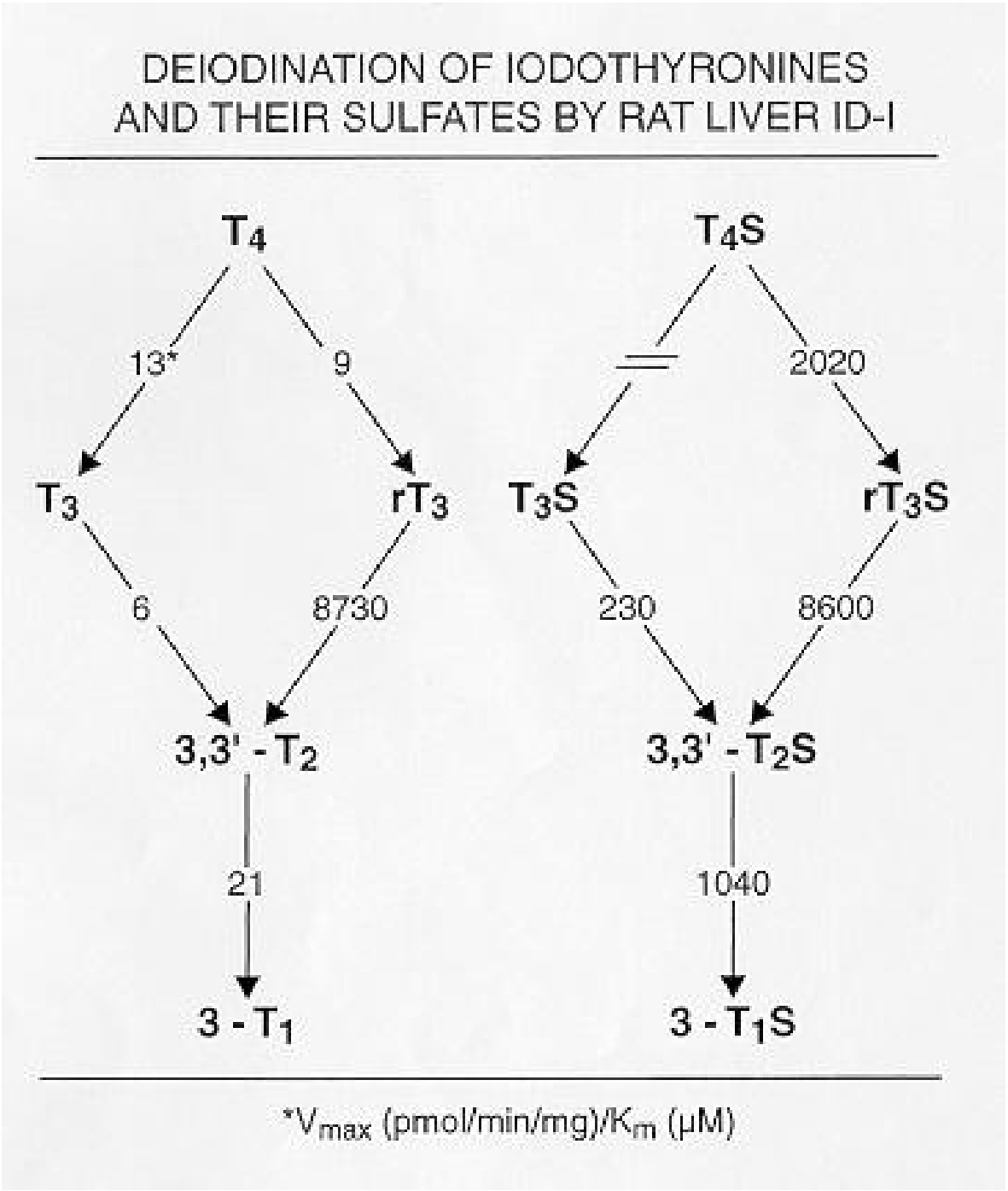

Figure 5. Efficiency of deiodination of iodothyronines and their sulfates by rat liver D1.
*) Vmax (pmol/min/mg protein)/Km (μM) ratio.

The mechanism by which sulfation stimulates especially the IRD of different substrates remains unclear. In some cases sulfation primarily effects an increase in Vmax, while in others there is a predominant decrease in apparent Km value. The facilitated deiodination of sulfated iodothyronines by rat liver D1 may be due to interaction of the negatively charged sulfate group with protonated residues in the active center of this basic protein. The effect of sulfation on deiodination of iodothyronines is both conjugation type and deiodinase type-specific since D1 does not catalyze the deiodination of T3 glucuronide, while D2 and D3 do not accept T4S and/or T3S as substrates.

Importance of thyroid hormone sulfation

Serum concentrations of T4S, T3S, rT3S and 3,3'T2S are low in normal human subjects but they are high in fetal and cord blood, in patients with NTI, and in patients treated with the D1 inhibitor (16, 176). The serum T3S/T3 ratio is also increased in hypothyroid patients (177, 178). High iodothyronine sulfate levels have also been detected in human fetal and neonatal serum and amniotic fluid (16). The high serum iodothyronine sulfate levels during NTI, hypothyroidism and fetal development have been ascribed to a low peripheral D1 activity in these conditions (17, 18). These results are in accordance with studies in rats, showing marked increases in the serum concentration and biliary excretion of iodothyronine sulfates when hepatic and renal D1 activities are decreased by D1 inhibitors or selenium deficiency (17). These changes are not caused by an increased sulfation of iodothyronines but by a decreased clearance of the sulfated iodothyronines by D1.

Thus, sulfation is a primary step leading to the irreversible degradation of T4 and T3 by D1. However, if D1 activity is low, inactivation of thyroid hormone by sulfation is reversible due to expression of sulfatases in different tissues and by intestinal bacteria (179). It has been speculated that especially in the fetus T3S has an important function as a reservoir from which active T3 may be released in a tissue-specific and time-dependent manner(17, 180).

Wu and coworkers have demonstrated the presence of a 3,3'T2S cross-reacting substance, termed compound W, in the serum and urine of pregnant women (16, 181). Interestingly, compound W is derived from the fetus and its concentration in maternal serum may reflect fetal thyroid state (16, 181). The structure of compound W remains to be identified.

Glucuronidation

Like sulfation, glucuronidation is a phase II metabolic reaction that increases the water-solubility of endogenous and exogenous compounds to increase their biliary or urinary excretion. Glucuronidation is catalyzed by UDP-glucuronyltransferases (UGTs) that utilize UDP-glucuronic acid (UDPGA) as cofactor. UGTs are localized in the endoplasmic reticulum of predominantly liver, kidney and intestine. Most UGTs are members of the UGT1A and UGT2B families (182).

Iodothyronines are also metabolized by glucuronidation, although this appears more important in rodents than in humans (183). Especially in rodents, metabolism of thyroid hormone is accelerated through induction of T4-glucuronidating UGTs by different classes of compounds, including barbiturates, fibrates and PCBs (184-186). This may result in a hypothyroid state as the thyroid gland is not capable of compensating for the increased hormone loss. In humans, thyroid function may be affected by induction of T4 glucuronidation by anti-epileptics, but development of overt hypothyroidism is rare (187).

Glucuronidation of T4 and T3 is catalyzed by different members of the UGT1A family (Table 2) (188-191). Usually, this involves the glucuronidation of the hydroxyl group, but human UGT1A3 also catalyzes the glucuronidation of the side-chain carboxyl group, with formation of so-called acyl glucuronides (189). Interestingly, Tetrac and Triac are much more rapidly glucuronidated in human liver than T4 and T3, and this occurs predominantly by acyl glucuronidation (192). Acyl glucuronides are reactive compounds that may form covalent complexes with proteins. It is unknown if this is a significant route for the formation of covalent iodothyronine-protein complexes.

Integrated physiological role of thyroid hormone metabolism

Since most actions of thyroid hormone are initiated by binding of T3 to its nuclear receptors, it is important to consider the role of the processes discussed above in the regulation of nuclear T3. There are two sources of intracellular T3, i.e. T3 derived from plasma T3, and T3 produced locally from T4, and the degree to which they contribute to the occupied receptors varies among the different tissues in different physiological and pathophysiological states (1, 3, 5, 76, 98). The liver and kidneys are typical of most tissues in the body in which most of the T3 specifically bound to the T3 receptor is derived directly from plasma. In cerebral cortex, BAT, and anterior pituitary there is a substantial contribution to nuclear T3 from locally produced T3. Local T3 production may be an autocrine process, where T3 is produced in the same cells where it acts, or a paracrine mechanism, where T3 production and action take place in neighboring cells. The latter appears very important for T3 action in the brain, where neurons are the primary target cells for T3 produced by D2 expressed in nearby astrocytes (193-195).

There seems to be a dual source for plasma T3: a) D1 conversion of T4 in liver and kidneys, and b) D2 conversion of T4 in skeletal muscle. Because of the positive control of D1 and negative control of D2 by thyroid hormone, their relative contributions to plasma T3 production may depend on thyroid state, with D2 being more important in hypothyroidism and D1 in hyperthyroidism. This is supported by findings that the efficacy of PTU in the inhibition of “peripheral” T3 production is greater in hyper- than in euthyroid subjects (2), supporting an increased contribution of D1 to plasma T3 production in hyperthyroid subjects. However, D1 expressed in the thyroid may be also an important source for circulating T3 in hyperthyroid patients (196).

D3 plays an additional important role in maintaining intracellular T3 concentrations in these tissues by catalyzing the degradation of T3 in case of excess or by diverting the metabolism of T4 to rT3. Indeed, the adaptations of deiodinase activities in response to changes in thyroid state are thought to serve the purpose of keeping intracellular T3 in the brain constant. Thus, when T4 supply is decreased in hypothyroidism, both D1 and D3 activities are down-regulated, so that relatively more T4 is available for conversion to T3 by D2 in the brain, the activity of which is up-regulated. Opposite changes occur in hyperthyroidism. These adaptations are not only important for the optimal function of the brain in adult life, they are also essential for the development of the brain which is critically dependent on thyroid hormone. Although the adaptations in deiodinase activities during hypo- or hyper¬thyroidism go a long way in securing T3 availability in the brain, in severe iodine deficiency they may not fully compensate for the extreme decrease in T4 supply. This may result in severe impairment of neurological development in the child even when plasma T3 levels in the mother are sufficient to maintain a euthyroid state.

The critical role of deiodination in regulating local thyroid hormone action is clearly illustrated by the developing cochlea, where D3 is expressed before the onset of D2 activity (101, 147), preventing too much or too little hormonal stimulation at inappropriate stages in development. At immature stages, D3 limits stimulation by T3. Postnatally, a double switch occurs with a decline in D3 and an increase D2, resulting in a local T3 surge which is independent of serum T3 levels and triggers the onset of auditory function. A similar double switch, preventing premature T3 stimulation, occurs in the developing cerebellum (148), and D3 expression has also been shown to be crucial for normal retinal (149) and pancreatic □-cell development (150). Similarly, local thyroid hormone activation by D2 has been shown to be essential formal BAT development (102) and myogenesis (106) as well.

Deiodinases are not only essential in controlling local thyroid hormone action during development, but also for normal function of adult tissues such as hypothalamus, pituitary, bone, and brown adipose tissue (96, 102, 107). Finally, deiodination is also important in regulating thyroid hormone bioactivity in different

pathophysiological conditions, such as hypoxia, myocardial infarction, neuronal ischemia, critical illness, tissue injury, regeneration, and cancer (106, 121, 134, 152-154, 156, 157, 159). D2KO mice are more vulnerable to ventilator induced lung injury (110), whereas heterozygous D3KO mice are more vulnerable to a chemically induced worsening of restrictive cardiomyopathy, leading to congestive heart failure and increased mortality (155). The high expression of D3 in regenerating liver tissue and certain tumors or the crucial role of D2 in muscle regeneration (3, 106, 123, 159, 197) suggest that coordinated regulation of thyroid hormone action is essential in the control of the tight balance between proliferation and differentiation in the regeneration processes, and that high expression of D3 may also be an advantage in proliferating tumor cells (98, 101, 147).

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