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NUTRITIONAL AND HORMONAL REGULATION OF THYROID HORMONE DEIODINASES

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ABSTRACT

Selenocysteine has been identified in the active center of types 1 and 3 iodothyronine deiodinases, two important enzymes regulating the formation and degradation of the active thyroid hormone, 3,5,3′-triiodothyronine (T3). Selenium is thus required for such complex processes as normal growth, brain development, and metamorphosis, all of which are thyroid hormone dependent. Structural and functional analyses of the type 1 deiodinase mRNA allowed identification of the selenocysteine insertion sequence (SECIS) element, a stem-loop structure in the 3′ untranslated region of the mRNA. SECIS elements with conserved sequence and structural features are also present in the 3′ untranslated regions of the mRNAs encoding selenoprotein P and the glu-

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tathione peroxidase family of selenoproteins. These elements are necessary and sufficient for directing selenocysteine incorporation into the deiodinases and the other mammalian selenoproteins.

INTRODUCTION

A nutritional requirement for the essential trace element selenium was established many years ago, but the precise reasons for this requirement are still under investigation. The discovery that type 1 deiodinase is a selenoenzyme led to the identification of the essential role for this element in thyroid hormone action but has also had more far-reaching implications concerning the mechanism of selenocysteine incorporation into eukaryotic selenoproteins in general. The recent discovery that type 3 deiodinase is a selenoenzyme and the characterization of the specific properties of this enzyme have shown that selenoenzymes are not inherently sensitive to heavy metals. Studies on the effects of selenium deficiency provide additional support for the tissue-specific nature of preservation of this important nutrient.

A great deal of information about thyroid hormone deiodination has been published over the years. Hence, in this review we refer to the earlier material only briefly and focus on more recent studies in the field. For earlier reviews, we direct the reader elsewhere (15, 20, 75, 88, 141). Previous publications have used a variety of abbreviations for type 1 deiodinase, some of which are inaccurate because they do not take into account the ability of this enzyme to catalyze both inner- and outer-ring deiodination. In this review, we introduce brief nomenclature for the three deiodinases, D1, D2, and D3, that does not rely on specific properties of the enzymes for accuracy.

TYPE 1 DEIODINASE

Substrates

OUTER-RING DEIODINATION Type 1 iodothyronine deiodinase (D1) can catalyze both outer- and inner-ring deiodination, but the preferred reaction is removal of a phenolic, or outer-ring, iodine from either T4 or reverse T3. Outer-ring deiodination of T4 is thought to be more important physiologically, as this reaction produces the bioactive hormone T3, but D1 exhibits a preference for reverse T3 at least an order of magnitude greater than its preference for T4. Furthermore, outer-ring deiodination of rT3 may also be of biological significance in the elimination of this compound from the circulation. Early studies of type 1 deiodination utilized rat and human liver microsomes to study these reactions and reported K_m values of 2–4 μM for T4 and 0.06–0.35 μM for rT3 (143, 145). The properties of the transiently expressed cloned rat and

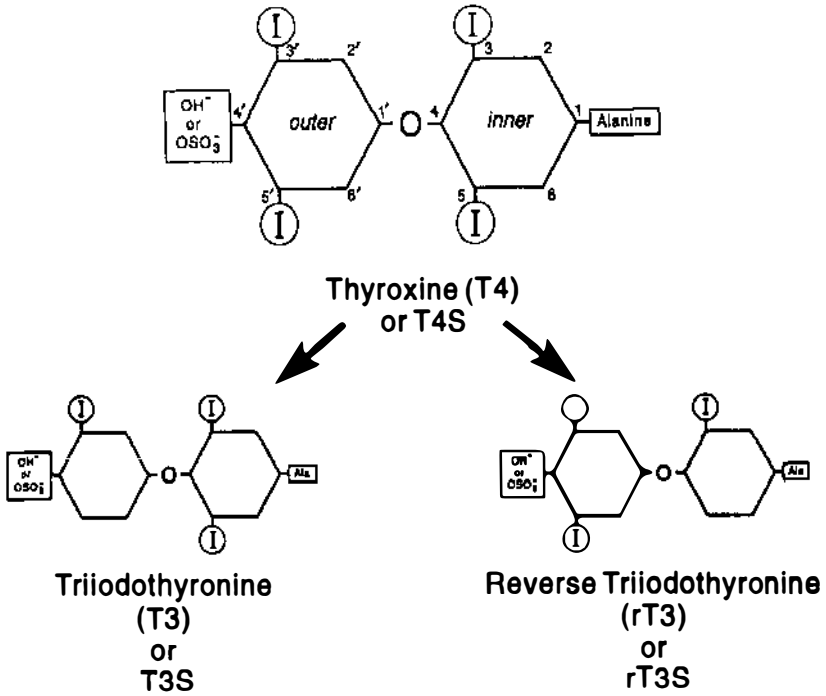

Figure 1 Structures of T4, T3, and reverse T3. Sulfation at the 4′-hydroxyl position produces the sulfate conjugates of T4, T3, and reverse T3. (Reprinted with permission from Reference 20.)

human enzymes with respect to rT3 are quite similar to those of the enzymes in liver microsomes. Measurement of T4 deiodination with the transiently expressed enzymes proved more difficult, presumably owing to the lower V_{max} values for this substrate. However, K_i values for T4 as a competitive inhibitor of rT3 deiodination showed close agreement with the reported results for microsomes (13, 90).

In liver, sulfation of T4, T3, rT3, and T2 occurs at the phenolic 4′-hydroxyl group and has been reported to be a major route of iodothyronine metabolism in neonatal sheep, a model system for neonatal humans (Figure 1) (106). Sulfation of T4 prevents outer-ring deiodination and accelerates inner-ring deiodination. However, 3, 3′-T2S is deiodinated more rapidly at the outer ring than the corresponding unconjugated iodothyronine (141). T4 and T3 glucuronides are excreted by bile, and deiodination of T4 glucuronide by D1 has been demonstrated in vitro (53).

INNER-RING DEIODINATION It was reported a number of years ago that tissues with high levels of 6-n-propylthiouracil (PTU)–sensitive outer-ring deio-

dinase activity also exhibit inner-ring activity, leading to speculation that both activities were catalyzed by a single molecule. This hypothesis is supported by the fact that both activities colocalize to microsomes, coenrich during purification, and exhibit similar inhibitor sensitivities (see 99 for additional references). Transiently expressed rat D1 does catalyze inner-ring deiodination of T3 and T3S, showing that both inner- and outer-ring activities are encoded by a single mRNA (99). The V_{max}/K_m ratios for T3S were approximately 100-fold higher than for T3, as is characteristic of D1, but not of type 3 deiodinase (D3). Like that of T4, the degradation of T3 by successive inner- and outer-ring deiodination is enhanced by its sulfation at the 4′-hydroxyl position (141).

Enzyme Structure and Function

TOPOLOGY Early subcellular fractionation studies using rat liver or kidney have shown a membrane localization of D1 (40, 85). By combining partial purification with assays of marker enzymes, D1 was localized to different membranes in each tissue. In liver, D1 copurifies with markers of endoplasmic reticulum, such as glucose-6-phosphatase, whereas in kidney it copurifies with Na^+K^+-ATPase (82, 84). Renal D1 is specifically expressed in the basolateral membrane of proximal convoluted tubule cells. In situ hybridization studies have shown that D1, like the ATPase, is relatively restricted to the S3 segment of the proximal convoluted tubule (80). These cells have high levels of reduced glutathione (GSH), a potential cofactor for the deiodination reaction. LLC-PK1 cells, a proximal renal tubule cell line, also express high levels of D1, and trypsinization studies have shown that the catalytic portion of the molecule faces the interior of the cell (82).

When reticulocyte lysates containing rat D1 mRNA are complemented with pancreatic microsomes, the ^{35}S-labeled D1 is incorporated into the microsomes, with its N-terminus in the microsomal lumen (133). The transmembrane segment of the protein is found between amino acids 13 and 35. These results are consistent with predictions based on hydrophobicity plots indicating that the only portion of the molecule sufficiently hydrophobic to qualify as a transmembrane sequence is found in this region. Thus, D1 in the liver remains in the endoplasmic reticulum, with its catalytic portion oriented toward the cytoplasm. In the kidney, further processing of the enzyme occurs, with the final result that the amino terminus is found on the external cell surface. Previous physiochemical studies have indicated that D1 may be a homodimer because sedimentation profiles suggest a complex of ~55 kDa, about twice the molecular weight predicted from the sequence (39, 86). Whether the enzyme can function in a soluble form or requires a membrane component for activity remains to be demonstrated.

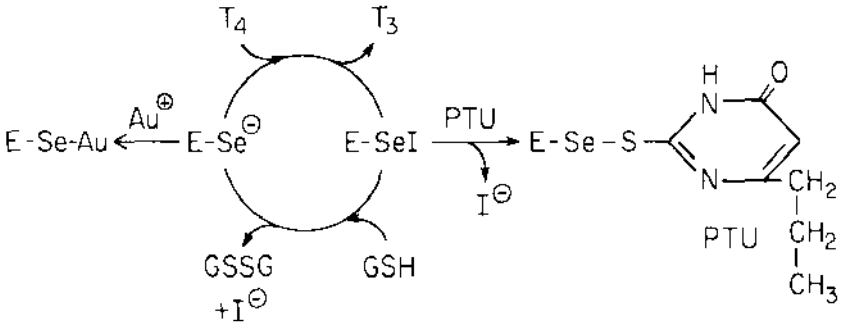

Figure 2 Proposed mechanism for type I iodothyronine deiodination and inhibition by PTU and gold, respectively.

ROLE OF SELENOCYSTEINE Prior to isolation of a D1 cDNA, biochemical evidence suggested the presence of a sulfhydryl active site, and proposed mechanisms of deiodination indicated a sulfenyl-iodide intermediate (88). Upon identification of selenocysteine in the active site of the molecule, these earlier mechanisms had to be reconsidered. Using the D1 cDNA in a mammalian transient expression system, investigators found that site-directed mutagenesis allowed for direct examination of the role of selenocysteine in the enzyme function. Substitution of leucine for the active-site selenocysteine resulted in complete loss of enzyme activity (9), providing the first evidence for the critical role of selenocysteine in catalysis. Thus, the revised reaction mechanism suggests a selenolyl-iodide intermediate (Figure 2). Because cysteine and selenocysteine have similar structures, we examined the effects of this substitution on enzyme activity. The cysteine mutant exhibited a 10-fold higher K_m for rT3 and 100- and 300-fold higher K_i values for gold and PTU, respectively, than did the wild-type enzyme (13), suggesting that the presence of selenocysteine confers specific biochemical properties on D1. Recent studies suggest that the lower turnover number of the mutant enzyme, and not the presence of selenocysteine per se, may be responsible for these differences (see below).

Because UGA is recognized as both a stop and a selenocysteine codon and is thus translated inefficiently, the presence of the selenocysteine codon results in a large decrease in deiodinase protein levels relative to the cysteine mutant both in transiently transfected cells (16) and in reticulocyte lysate in vitro translation reactions (9). This decrease varied from ~20-fold in COS cells to ~400-fold in JEG cells. However, the k_{cat} of the selenoenzyme produced by transient transfection is 300-fold higher than that of the cysteine mutant (16), thus compensating for the low level of expression. This finding may explain

the evolutionary preservation of selenocysteine despite the complex selenoprotein synthesis pathway (see below).

ESSENTIAL HISTIDINES Mol et al utilized the histidine-specific reagents, rose bengal and diethylpyrocarbonate, to demonstrate the presence of at least one essential histidine in rat D1 (98). Cloning of the rat enzyme revealed the presence of four histidine codons, which we examined individually to determine their role in deiodinase function (6). Deletion of the fourth histidine had no effect on catalytic activity. Using site-directed mutagenesis, we mutated each of the remaining three histidines and evaluated the kinetic parameters of the mutant enzymes. Mutagenesis of histidine 174 to glutamine or asparagine produced 20- and 100-fold increases in K_m for rT3, respectively, whereas mutagenesis of histidine 158 to glutamine, asparagine, or phenylalanine resulted in complete loss of deiodinating activity. Conversion of histidine 185 to asparagine had no effect on K_m. Thus, histidines 158 and 174 appear to be critical for enzyme function. Subsequent cloning of human, dog, and mouse D1 revealed that of the four histidines in the rat enzyme, only histidines 158 and 174 are conserved in all four species (90, 134; PR Larsen & MJ Berry, unpublished results).

AMINO ACIDS INVOLVED IN SUBSTRATE BINDING As mentioned above, D1 shows a preference for reverse T3 as a substrate for 5′ deiodination, and T3 sulfate is the favored substrate for inner-ring deiodination (Figure 1). For human and rat D1, the K_m for reverse T3 deiodination is 10- to 20-fold lower than that for T4 (13, 90). An exception is dog D1, for which the apparent K_m for reverse T3 is considerably higher than it is for rat or human D1. Reverse T3 also inhibits the N-bromoacetyl T3 labeling of dog D1 significantly less effectively than it does that of the rat or human enzyme (115). Accordingly, we cloned dog D1 cDNA and examined the kinetic properties of transiently expressed dog D1. We found that the apparent K_m for reverse T3 was in fact 9 μM, approximately 30-fold higher than that for human or rat D1 (134). However, the K_i for T4 inhibition of reverse T3 5′ deiodination differed only slightly from that for deiodination by human D1 (18 vs 6.2 mM; see Table 1).

We compared the deduced dog D1 sequence to those of the rat and human proteins in an attempt to identify specific amino acid differences that could explain the much higher K_m for reverse T3 of dog D1. These three sequences are illustrated in Figure 3. Although dog D1 is highly homologous to human and rat D1, the amino acids nearest the amino-terminal end of dog D1 are less conserved. The most obvious difference between dog D1 and human and rat D1 is the absence in dog D1 of a 5–amino acid peptide, TGMTR, found at positions 47–52 of the human and rat proteins. To identify which portions of the protein were responsible for the difference in K_m for rT3, Toyoda et al

Table 1 Kinetic parameters for deiodination catalyzed by the transiently expressed wild-type or mutant dog and human D1[a]

Value[b]	Dog	Human	Dm 7	Hm 6
K_a (rT3) (mM)	9.0	0.35	0.45	9.0
K_i (T4.rT3) (mM)	18.0	6.2	5.4	28.0
K_b (DTT) (mM)	42.0	5.0	ND[c]	ND
K_i (PTU.rT3) (mM)	1.6	0.17	0.32	1.4
K_i (PTU.DTT) (mM)	0.63	0.014	ND	ND
K_i (GTG.rT3) (nM)	16.0	4.7	6.5	18.0

[a] Reprinted with permission from Reference 134.

[b] All values are for inhibition of 5'-monodeiodination of rT3 under conditions in which the enzyme is rate-limiting. All reported values are the mean of closely agreeing values from at least two experiments. All reactions contained 10 mM DTT.

[c] ND, not done.


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          1                                                  50
  Dog     MGLprpvLWL rRLwvlLqVA vqVavGKVfl kLFPaRVKqh IvAMngK---
Human     ...pqpg... k..wvl.e.. vh.vv...ll i...d...rn .l..ge.tgm
  Rat     ...sqlw... k..vif.q.. le.at...lm t...e...qn .l..gq.tgm

          51                                                 100
  Dog     --NPhFsyDN WaPTlySmQY FWFVLKVqWQ RLEDrtEpGG LAPNCpVVRL
Human     tr..h.sh.. .i..ff.t.. .......r.. ....tt.l.. .....p....
  Rat     tr..r.ap.. .v..ff.i.. .......r.. ....ra.y.. .....t....

          101                                                150
  Dog     SGQrCNiWdF mQGnRPLVLN FGSCT*PSFl fKFDQFKRLi eDFcStADFL
Human     ...r..i.e. m..n..... .........m f........i e..s.i....
  Rat     ...k..v.d. i..s..... .........l l........v d..a.t....

          151                                                200
  Dog     iIYIEEAHAs DGWAFKNNvn IRtHqtLQDR LqAArLLLdR aPpCPVVVDT
Human     v........s ........md ..n.qn.... .q..h...a. s.q.......
  Rat     i........t ........vd ..q.rs.... .r..h...a. s.q.......

          201                                                250
  Dog     MrNQSSQfYA ALPERLfvlQ EGRIlYKGKp GPWNYhPEEV RAVLEKLhs
Human     .q.....l.. ......yii. ....l....s .....n.... .......hs
  Rat     .q.....l.. ......yvi. ....c....p .....n.... .......cip

          251
  Rat     pghmpqf
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Figure 3 Alignment of the predicted amino acid sequences of dog, human, and rat D1. For the amino acid sequences of the human and rat enzymes, only those residues that are not conserved in all species are shown. Dashes indicate amino acids conserved in both the human and rat deiodinases but not present in the dog enzyme. Selenocysteine at position 126 is represented by an asterisk. (Reprinted with permission from Reference 134.)

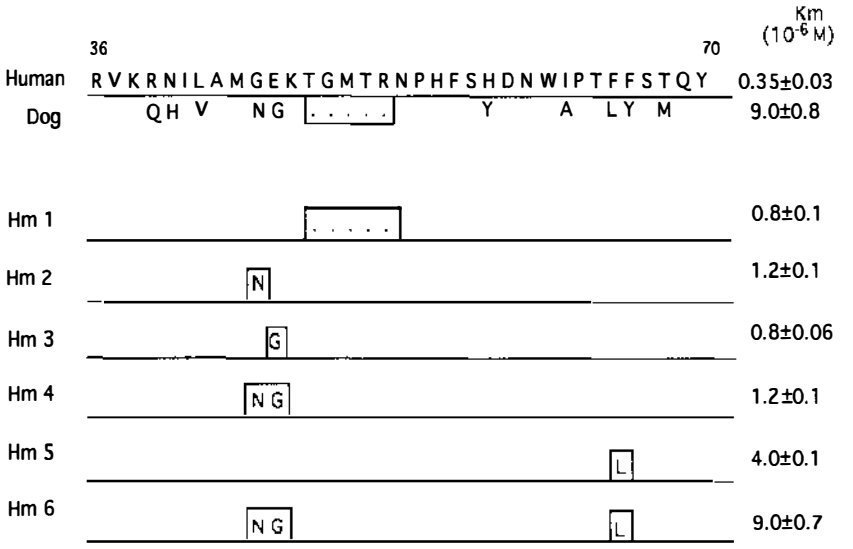

Figure 4 Effect of mutations in amino acid residues 37–70 of the human enzyme on its kinetics. Mutations were made in wild-type human D1 and are designated Hm 1–6. For the amino acid sequences of mutant human proteins, only the amino acids that were deleted or substituted are shown in the boxes. The K_m values are the mean ±SE of the results obtained from at least three separate transfections. Differences between the human and dog amino acid sequences in this region are shown for reference. (Reprinted with permission from Reference 143.)

(134) constructed a series of chimeric dog and human or rat proteins (134). Using these constructs, we localized the portion of the molecule responsible for the difference in reverse T3 deiodination kinetics to a segment of dog D1 located between positions 37 and 65 (37 and 70 in human or rat D1). Analysis of the kinetics of reverse T3 deiodination by transiently expressed mutated dog proteins showed that unique residues at positions 45, 46, 61, and 62 of dog D1 were associated with the higher K_m(rT3) of this enzyme.

To determine the relative role of each of these differences, the human mutants shown in Figure 4 were evaluated. Surprisingly, deletion of the 5 amino acids (TGMTR) not present in the dog from the human D1 protein raised the apparent K_m for reverse T3 only twofold. However, a single mutation in the human enzyme, i.e. a change from phenylalanine 65 to leucine (the amino acid present in the dog enzyme), resulted in a 10-fold increase in the K_m for reverse T3. An additional twofold increase occurred when residues 45 and 46 were changed from glycine and glutamic acid to asparagine and glycine, respectively. These changes in the human protein also reduced the capacity of reverse T3 to inhibit the binding of N-bromoacetyl T3 to the protein. The concentration for half-maximal inhibition by reverse T3 increased from 2 to

18 nM for the human mutant 6 protein. Interestingly, the concentration for half-maximal inhibition by T4 increased only threefold. In these respects, the human mutant 6 protein was now identical to wild-type dog D1, indicating that these amino acid differences were responsible for the kinetic differences in the two enzymes with respect to reverse T3. The more modest differences with respect to T4 kinetics suggested that the binding of this substrate was not greatly affected by these changes. We speculated that this phenomenon could be explained by the fact that the absence of a second iodine on the inner aromatic ring of reverse T3 allowed close approximation of this substrate to phenylalanine 65, where it could develop π interactions with the phenolic ring of phenylalanine. A bulky iodine substitution in this location would prevent close approximation to this site. Therefore, the substitution of leucine for phenylalanine would not likely have an effect on T4 binding.

DEIODINASE INHIBITORS AND COSUBSTRATES The enzymatic reaction for T4 to T3 conversion is illustrated in Figure 2. This schematic is based on numerous studies from many laboratories over the past two decades (27, 28, 85, 142, 143). The modifications of the schema are the substitution of selenium for sulfur in the active site, as discussed above, and the addition of gold as a competitive inhibitor of the iodothyronine substrate (9, 13, 16). The complete reaction, including regeneration of the active enzyme, requires two substrates, iodothyronine and a cellular thiol cofactor, in a ping-pong reaction mechanism. In vitro, it is customary to use dithiothreitol (DTT) as the second substrate so that the enzyme is regenerated. Investigators are still uncertain as to the identity of the physiologic second substrate, although reduced GSH is a leading candidate. For example, the S3 segment of the proximal convoluted renal tubule where rat renal D1 is expressed contains an efficient system for the generation of GSH from glutamate and cysteine, independent of NADPH (80). The reduction in D1 activity during glucose deprivation, which occurs in the liver but not in the kidney (65), likely results from a decrease in NADPH generated by the hexose monophosphate shunt pathway, with a consequent increase in the ratio of GSSG to GSH in the hepatic cytosol.

Several classes of inhibitors for the D1 enzyme have been identified. One class is comprised of substances containing iodophenolic groups such as iopanoic or iopodipic acids, agents formerly used in oral cholecystography (38). These agents compete directly with substrate for the deiodinase. Other competitive inhibitors are the plant flavonoid compounds, the best studied of which is EMD 21388 (73). These compounds are also competitive inhibitors of T4 binding to the circulating binding protein transthyretin (TTR), suggesting that the conformation of the T4-binding site in this serum transport protein is similar to that of D1 (75).

After rat D1 was cloned and the critical role of selenocysteine in the active

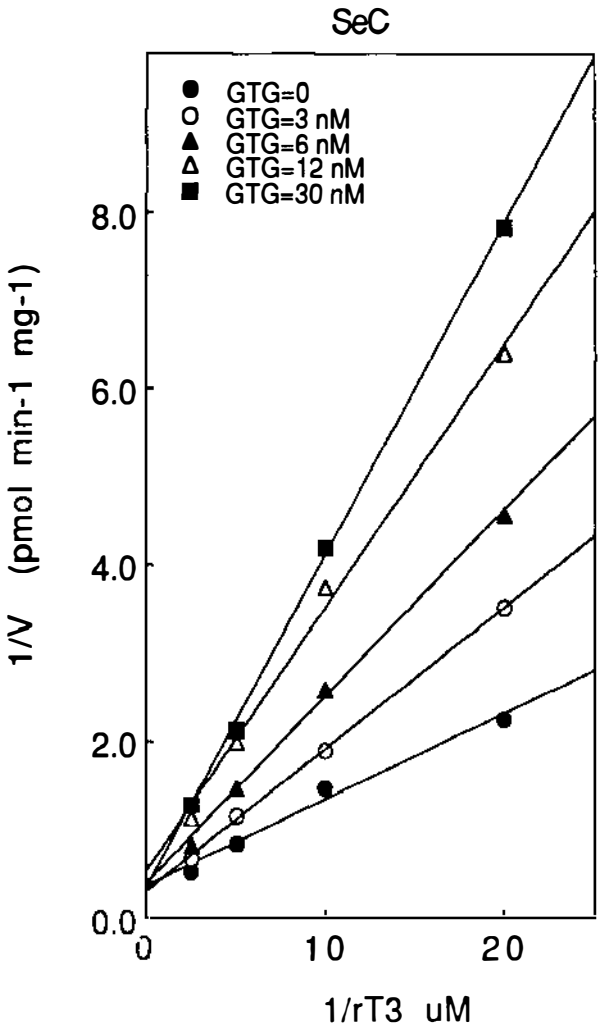

Figure 5 Kinetics of inhibition of rT3 deiodination by GTG. Double-reciprocal plot of deiodination rate vs rT3 concentration at varying GTG concentrations. (Reprinted with permission from Reference 13.)

site demonstrated, we speculated that heavy metals such as gold would be potent inhibitors of deiodination (9, 13). This turned out to be the case (see Figure 5) for gold thioglucose (GTG), which had an apparent K_i of approximately 5 nM for reverse T3 5′-deiodination. Gold competes with the iodothyronine substrate in accordance with the hypothesis that it forms a complex with the negatively charged selenolyl protein (Figure 2). Parenteral GTG

administration to rats causes a marked inhibition of D1 (14). However, in humans given gold compounds for rheumatoid arthritis, we and others (101) have not identified any alterations in the ratio of T3 to T4 in the circulation (SJ Mandel, GA Brent, M Weinblatt, PR Larsen, manuscript submitted). This result suggests that gold is not present in high quantities in hepatic parenchyma, an observation consistent with previous pathological studies (50).

The thiourea compound, PTU, inhibits T4 to T3 conversion in the intact rat and interferes with the effect of T4, but not of T3, in producing thyromimetic effects in thyroidectomized animals (42, 104). Inhibition of T4 action by PTU occurs via interference with the second-half reaction of the enzyme. Thus, deiodination and the formation of a selenolyl-iodide complex must occur for PTU to be effective (147). Although this reaction and the competitive inhibition of PTU with respect to DTT are readily demonstrated in vitro, there is still some disagreement as to the relevance of this effect in vivo. Some argue that the quantity of enzyme present in the tissues is sufficient to render regeneration of D1 by thiol cosubstrates unnecessary for normal T4 to T3 conversion (75). In fact, in athyreotic, levothyroxine-treated humans, administration of 1 g PTU per day causes only a 20–25% decrease in circulating T3 (109, 137). However, in the thyroidectomized, T4-treated rat, approximately two thirds of T4 to T3 conversion can be blocked by PTU coadministration, suggesting that regeneration of enzyme is necessary for the production of T3 from T4 in rats (104).

In the hyperthyroid human, the inhibition of T3 production by PTU is readily demonstrated (1). This is seen when the acute effects of PTU on the ratio of serum T3 to T4 are compared with those of methimazole (Tapazole®). Although both PTU and methimazole block thyroid peroxidase activity, methimazole does not block D1 activity. Thus, the decrease in serum T3 concentrations during PTU treatment is significantly more rapid than it is during treatment with methimazole. Several potential reasons can be given for the greater sensitivity of the hyperthyroid individual to PTU. First, presumably high levels of the D1 enzyme are present in liver, kidney, and thyroid stimulated by the hyperthyroid state (58, 132). Although D1 activity has not been evaluated in human liver and kidney during hyperthyroidism, a marked increase in D1 content in these tissues has been observed in hyperthyroid rats (61, 64, 65, 129). Second, in addition to inducing D1, the elevated serum T4 and T3 concentrations would downregulate type 2 deiodinase (D2) activity (see below) and consequently minimize whatever contribution this PTU-insensitive pathway might make to peripheral T3 production (83, 87, 128). Third, D1 activity is increased in thyroid tissue from patients with Graves' disease (58, 132, 135) owing to stimulation both by the hyperthyroid state and by thyroid-stimulating immunoglobulin (19, 37, 59, 136). Thus, the acute effect of PTU in decreasing circulating T3 might result from inhibition of T3 pro-

duction from thyroidal D1 acting on either intracellular or extracellular (circulating) T4.

In both humans and rats, PTU causes an increase in circulating reverse T3 (49), indicating that D1 is rate limiting for the clearance of reverse T3. In contrast, PTU administration to dogs does not increase reverse T3 but does decrease the ratio of serum T3 to T4 (78). This result can be explained by the relatively high K_m of the dog enzyme for reverse T3 (discussed above) as well as its lower sensitivity to PTU (134).

As mentioned above, the apparent K_i of PTU for uncompetitive inhibition of reverse T3 deiodination by dog D1 is approximately 10-fold higher than that for the human enzyme (Table 1). This value is correlated with a fourfold lower k_{cat} for reverse T3 deiodination by dog D1 (134). Because an identical increase in the apparent K_i for PTU is found in human D1 that has been mutated to resemble dog D1 (mutant 6 in Figure 4), it seems likely that the lower turnover number, rather than a difference in enzyme conformation, is responsible for the reduced sensitivity to PTU. This finding has implications for the interpretation of the studies of PTU susceptibility of other deiodination reactions, such as those catalyzed by D2 and D3, with respect to the issue of whether or not these are selenoenzymes (see below). The cysteine mutant D1 also exhibits reduced sensitivity to PTU and has a lower turnover number (16). Although an enzyme-S-S-PTU complex between a cysteine sulfhydryl and PTU would be more easily reduced than would an enzyme-Se-S-PTU adduct, this observation may not explain the relative resistance to PTU of the cysteine mutant D1. Because the D3 enzyme from *Xenopus* is also a selenoenzyme but is relatively PTU insensitive (29,131), one must conclude that not all selenodeiodinases are inherently PTU sensitive.

Requirements for Eukaryotic Selenoprotein Synthesis

STEPS IN EUKARYOTIC SELENOPROTEIN SYNTHESIS Much of our information about the mechanism of selenoprotein synthesis comes from genetic analyses in *Escherichia coli* and studies of the bacterial selenoprotein, formate dehydrogenase F (fdhF). Characterization of mutants defective in selenoprotein synthesis identified four complementation groups, selA–D (81). The *selC* gene encodes a tRNA species, $tRNA^{[Ser]Sec}$, which is charged with serine by seryl-tRNA synthetase. The selA gene product, selenocysteine synthase, then catalyzes conversion of serine to selenocysteine via an amino-acrylyl intermediate (18). This enzyme is a homodecamer of ~600 kDa and can bind and catalyze conversion of five seryl-tRNA molecules per enzyme molecule. The selD gene product, selenophosphate synthetase, utilizes selenide and ATP to generate selenophosphate, thus providing a reactive selenium donor to the tRNA (140). The final "player" is the selB gene product, a protein with significant homology

to the bacterial elongation factor EF-Tu, which specifically binds selenocysteyl-tRNA and has a low affinity for all other tRNAs, including the seryl-tRNA precursor. This discrimination on the part of selB contributes to the specificity for incorporation of selenocysteine, and not serine, at the appropriate UGA codons. Studies of fdhF mRNA have shown that translation of UGA as selenocysteine requires a stem loop immediately adjacent to the UGA codon in the mRNA for this protein. In an elegant series of RNA-protein mobility-shift assays, Baron et al showed that, in addition to binding selenocysteyl-tRNA, selB binds the stem loop in the fdhF mRNA, bringing the tRNA into proximity with the UGA codon in the ribosome complex (3). These studies have laid the groundwork for studies of eukaryotic selenoprotein synthesis.

To date, several components of eukaryotic selenoprotein synthesis have been identified. The $tRNA^{[Ser]Sec}$ gene has been cloned in higher eukaryotic animals (79) and protists, and the tRNA has been isolated from plant and fungal species (52). Partial purification and characterization of the murine homologue of bacterial selA have also been reported (97). This enzyme, like *E. coli* selenocysteine synthetase, is a large (~500 kDa), presumably multisubunit, pyridoxal phosphate–containing enzyme. Human selenophosphate synthetase, the homologue of the bacterial selD gene product, was recently cloned in our laboratory (SC Low, JW Harney, MJ Berry, manuscript in preparation).

Although the mammalian homologue of selB has not been reported, two papers may provide insight into the existence of such a protein. The first describes autoantibodies with specificity to a selenocysteyl-tRNA-protein complex in patients with autoimmune chronic active hepatitis (47). The ~48-kDa protein in the complex has not been identified, but evidence indicates that it is not seryl-tRNA synthetase, and the authors suggest that it may be eukaryotic selB. A more recent report describes an ~50-kDa protein that protects [^{75}Se]Secys-tRNA from alkaline hydrolysis (148). The similar sizes of the two proteins suggests that they may be identical.

FUNCTIONAL DEFINITION OF THE SECIS ELEMENT Initial deletion mapping analyses of rat D1 cDNA demonstrated that sequences in the 3′ untranslated region (3′ut) of the mRNA were essential for expression of D1 from wild-type cDNA but not from the cysteine mutant cDNA (7), indicating that these sequences were required for translation of UGA as selenocysteine but not for cysteine incorporation. These sequences were localized to an ~250-nucleotide region, which was predicted by computer analysis to form a stable stem-loop structure. We showed that the sequence was critical but that the spacing relative to the coding region was not. Following identification of this sequence in D1, we examined the 3′ut of glutathione peroxidase (GPX) for a similar structure, as this was the only other eukaryotic selenoprotein that had been cloned at the time. A similar structure was in fact present in the GPX 3′ut, which, when

substituted for the D1 3′ut, functioned to direct selenocysteine incorporation at the D1 UGA codon. We designated these structures SECIS (selenocysteine insertion sequence) elements (7). Based on these findings, it was predicted--and in fact demonstrated (118)--that introduction of a UGA codon and a SECIS element would confer selenocysteine incorporation into heterologous mammalian sequences.

Subsequently, isolation of the cDNA for selenoprotein P (Sel P), which contains 10 TGA codons, was reported (54); amino acid composition indicated at least 8 moles of selenium per mole of protein (108). Computer analysis of the 3′ut of Sel P predicted two stable stem loops (55), and chimeric constructs with the D1 coding region confirmed that these stem loops functioned as SECIS elements (8). In fact, the two Sel P SECIS elements in tandem produced an efficiency of readthrough fourfold higher than that of the D1 element. Comparison of the predicted secondary structures and alignment of the deiodinase, GPX, and the two Sel P SECIS elements showed that bases in the loops and unpaired bulges were conserved in all four, as was the spacing between the loops and the bulges. Using site-directed mutagenesis, conserved bases were shown to be critical to function (8). Mutagenesis of bases on one side of the stem in the rat D1 stem loop resulted in loss of function, whereas complementary mutations to the other side of the stem, which restored base pairing, restored function. Thus, both primary sequence and secondary structural features contribute to SECIS element function.

Because Sel P mRNA has 10 UGA codons but only 2 SECIS elements, we examined the ability of a single SECIS element to direct translation of more than one UGA codon. In these experiments, cysteine codons in wild-type deiodinase were mutated to UGA codons, and translation through the wild-type and new UGA codons was assessed. The SECIS element from rat D1 was sufficient to direct translation through two UGA codons, but this process was more efficient when the two Sel P SECIS elements were substituted. Interestingly, the relative activities of the different SECIS elements correlate with the hierarchy of selenoprotein synthesis seen upon selenium repletion (see below) and thus may contribute to this effect at the level of translation. We also examined the effects of position and spacing of the D1 SECIS element on its function. In wild-type D1, the spacing from the UGA to the middle of the stem loop is ~1.2 kb. Decreasing the spacing to ~0.6 kb increased the efficiency of readthrough by 20–40% (7), whereas increasing it to ~2.7 kb had no effect (8). Placement of the SECIS element upstream of the coding region resulted in very low but detectable readthrough.

As discussed above, translation of UGA as selenocysteine in wild-type deiodinase is inefficient compared with translation of the cysteine mutant. To determine which components of the translation machinery might limit this process, we investigated the effects of cotransfecting the *Xenopus* $tRNA^{[Ser]Sec}$

gene on D1 activity, UGA codon readthrough, and ^{75}Se incorporation. In the absence of added $tRNA^{[Ser]Sec}$, approximately one third of the initiated D1 terminated at the UGA codon. Cotransfection of the tRNA resulted in highly efficient readthrough, with only ~7% termination. Deiodinase activity increased approximately twofold with cotransfected $tRNA^{[Ser]Sec}$ (11), and ^{75}Se incorporation into the protein increased approximately threefold (SC Low, JW Harney, MJ Berry, manuscript in preparation).

COMPARATIVE EFFECTS OF SELENIUM DEFICIENCY ON D1 AND OTHER SELENOPROTEINS Early insight into the hierarchy of tissue distribution of selenium was gained from in vivo ^{75}Se labeling studies in rats maintained on a low-selenium diet for three generations (5). Selenium retention was highest in brain, pituitary, thyroid, adrenals, ovaries, and testes. Tissue selenium content following 8 months of depletion was also highest in these tissues. Liver, plasma, muscle, and heart exhibited poor retention. In all tissues, retention of selenium was lower in GPX than in other selenoproteins. The authors proposed a tissue- and protein-specific hierarchy whereby endocrine tissues have priority for selenium over other tissues, and proteins other than GPX have priority over GPX. In rat thyroid, D1 activity is highly resistant to selenium depletion. Whereas short-term selenium deficiency caused a precipitous drop in liver D1 levels, thyroid D1 levels were slightly elevated following either 5 weeks (24) or 3 generations of selenium depletion (93).

It was reported several years ago that selenium regulated GPX mRNA levels (110, 149). Subsequent studies using transiently transfected GPX promoter constructs in MCF-7 human breast cancer cells in culture (100) as well as measurements of endogenous GPX mRNA in human HL-60 cells (22) showed that levels of GPX mRNA were two- to fourfold higher in selenium-replete than in selenium-deficient cultures, but no difference in GPX nuclear run on rates was observed in either system. Thus, the decreases in GPX mRNA levels observed in selenium deficiency appear to result from posttranscriptional regulatory processes.

Other studies have examined the regulatory effects of this trace element on other selenoproteins, focusing on effects at both the mRNA and the protein levels. The porcine kidney epithelial cell line, LLC-PK1, expresses endogenous D1 and GPX activities, both of which are subject to regulation by selenium. Selenium depletion for 4 days decreased the level of both enzymes, and steady-state levels of both mRNAs dropped to ~5–15% of the levels in selenium-replete cultures (51). Northern blot analysis and kinetic experiments in the presence of actinomycin D suggest that selenium regulates D1 at both the transcriptional and posttranscriptional levels (74), but results of nuclear run-on assays have not been reported. Maintenance of rats on a selenium-deficient diet resulted in a greater and more rapid decrease in GPX mRNA than in D1

or selenoprotein P mRNAs (56). These findings and those discussed above support the notion of a tissue- and selenoprotein-specific hierarchy, with D1 and selenoprotein P mRNAs and proteins persisting after loss of GPX.

Hormonal Regulation of Thyroid Hormone Deiodinases

THYROID HORMONE As mentioned above, early studies indicated that the bioactivity of D1 was increased in both liver and kidney from hyperthyroid rats and was low when rats were made hypothyroid (61, 64, 65, 68, 122, 128, 129). Further studies of this phenomenon by several groups showed that these changes in activity resulted from altered levels of the enzyme in the particulate fractions of these two organs and were not explained by alterations of the endogenous cofactor. Subsequent studies revealed that changes in D1 activity in these tissues are paralleled by alterations in D1 mRNA levels (9, 12). T3 is a positive regulator of D1 mRNA not only in liver and kidney, but also in thyroid cells (136). Toyoda et al (136) had previously demonstrated that thyrotropin (TSH) or Graves' immunoglobulin increases D1 activity and had shown that this regulation occurs at the mRNA level. In FRTL-5 cells, pretreatment with cycloheximide does not block the twofold, T3-induced increase in D1 mRNA (136). Subsequent studies have illustrated similar findings in primary cultures of rat hepatocytes (95) and in several pituitary tumor cell lines (88a). In intact animals, the T3-induced increase in hepatic D1 mRNA is on the order of 10- to 50-fold; however, increases in activity are generally much less, on the order of 3- to 4-fold (102). The response of the D1 enzyme to T3 in rat liver is not altered by fasting, nor does fasting reduce hepatic D1 mRNA levels or activity in hyperthyroid rats (102). Thus, T3 can overcome the 50% decrease in hepatic D1 mRNA observed during starvation.

OTHER HORMONAL INFLUENCES In primary cultures of rat hepatocytes, the effect of T3 in increasing D1 mRNA was enhanced by dexamethasone (95). Part, although perhaps not all, of this effect may be nonspecific in that dexamethasone also significantly increased the levels of albumin mRNA. Interestingly, although cycloheximide pretreatment did not affect the response of D1 mRNA to T3, it did block dexamethasone enhancement of both albumin and D1 mRNA.

In humans, high doses of dexamethasone (2 mg every 6h) transiently decrease the ratio of T3 to T4 in the circulation (34). Similar studies in rats show glucocorticoid-induced decreases in D1 activity in liver and kidney (62). However, T3 production from T4 by perfused livers from dexamethasone-treated rats is not altered (62). Thus, one may conclude that the effects of dexamethasone on D1 expression in liver cells are modest and may be indirect, perhaps through enhancement of the effect of endogenous T3.

Fasting and Systemic Illness and T4 to T3 Conversion

Fasting or acute illness in humans leads to a rapid and sustained decrease in serum T3 concentrations and in the ratio of T3 to T4 (31, 130). Because sick or fasted patients remain clinically euthyroid, this decrease in circulating T3 relative to T4 has been referred to as the euthyroid sick syndrome or as nonthyroidal illness. Many studies have evaluated the effect of fasting in rats. Again, decreases in T3 have been found; however, these are paralleled by decreases in serum T4 (71). The 50% decrease in D1 activity in liver of fasted rats can be attributed to reduced T4 and T3 secretion and decreased cofactor (64, 67). In fact, fasting in rats does not alter total-body T4 to T3 conversion (71), even though it does so in humans. Fasting in larger rats with greater fat stores or in those previously given high-fat diets does not alter thyroid function, nor does it lead to a decrease in the ratio of T3 to T4 in the circulation (48). These results suggest that the consequences of fasting in lean animals are due to severe stress, which decreases TSH and, subsequently, thyroidal secretion (71).

This issue was revisited recently using solution hybridization techniques to measure D1 mRNA combined with measurements of deiodinase activity during fasting in both rat liver and kidney (102). Fasting causes an approximately 50% decrease in D1 mRNA within 48 h, which is followed by a modest decrease in hepatic D1 activity. No alteration in either D1 activity or D1 mRNA was observed in renal tissue in the same animals. Administration of T3 to fasted rats resulted in an increase in D1 mRNA similar to that observed in nonfasting rats. These studies, combined with the results of supplementing rats with sufficient exogenous hormone to correct the reduced T3 consequent to the decrease in serum TSH (67), indicate that the effect of fasting on rat hepatic D1 mRNA likely results from reduced thyroid function due to hypothalamic-pituitary hypothyroidism. Again, this finding indicates that the rat is not a reliable model for the changes that occur in the fasted or starved human.

Untreated diabetic patients also exhibit a marked decrease in fractional T4 to T3 conversion that is partially reversed by insulin administration (105). Streptozotocin-induced insulin deficiency in rats decreases serum T3 and reduces the level of D1 activity in both liver and kidney (41, 45, 60, 117). Although somatostatin infusion also decreases D1 activity in intact rats, this effect is independent of alterations in insulin secretion since supplementation with insulin and glucose did not reverse the effects of somatostatin (46). Diabetic rats have decreased serum T4 and T3, an effect that is reversed by treatment with insulin (102). D1 mRNA is reduced approximately 50% in both liver and kidney. This decrease is also reversed by insulin administration, but ~1 week of treatment is required for complete normalization.

These data indicate that although fasting or diabetes in rats will decrease

hepatic D1 activity via effects at a pretranslational level, these effects are likely to be secondary to the modest hypothyroidism induced by this change and are not the cause of the decreased T3 observed under these circumstances. The effect of altered nutritional status on hepatic T3 production in humans thus may occur by other mechanisms, e.g. by alterations in a carbohydrate-dependent thiol cofactor in the cytosol of those tissues in which D1 converts T4 to T3 or by a decrease in tissue T4 uptake. The extent to which hepatic D1 per se contributes to the T3 produced peripherally in humans remains to be defined, since kinetic analyses indicate that extrathyroidal T3 production derives from slowly equilibrating tissue pools rather than from the more rapidly equilibrating T4 pools, such as those in the liver (36).

Studies in a human thyroid carcinoma cell line (FTC-133) show that retinoic acid or 9-*cis*-retinoic acid can induce increases in D1 activity in FTC-133 cells (116). These increases were paralleled by increases in D1 mRNA. In these cells, T3 or T4 did not affect either basal or retinoic acid–induced D1 activity. Whether such cells express T3 receptor is unknown. However, if they do not, it may explain the difference between these results and those previously described in FRTL-5 cells, intact rat thyroid glands, or thyroid tissue from patients with Graves' disease.

As indicated above, both T3 and TSH increase D1 mRNA and protein levels in FRTL-5 cells (19, 136). The effect of TSH can be reproduced by incubation with forskolin or dibutyryl cAMP (19). This observation suggests that the increased cAMP induced by activation of the TSH receptor enables TSH to stimulate D1 mRNA. This effect is additive to that of T3, and the combination of T3 and dibutyryl cAMP increases D1 mRNA four- to fivefold.

These studies indicate that thyroid status is the predominant regulator of D1 activity in rat liver and kidney. Modest changes are associated with various nutritional stresses, such as fasting or diabetes, but these can be attributed to the secondary hypothyroidism induced by these manipulations, at least in the rat. In the thyroid cell, however, D1 responds to T3 and cAMP. Although data have not yet been reported, these effects are likely regulated at the level of transcription, and thyroid hormone and cAMP response elements presumably will be found in the promoters of the genes encoding the D1 mRNA. The regulation of D1 activity by these various hormonal influences is schematically illustrated in Figure 6.

Effects of, and Basis for, Genetic Deficiency of D1

Certain inbred strains of mice exhibit marked differences in D1 activity, segregating into high- or low-activity groups (10, 114). This grouping has allowed the investigation of the regulation of D1 activity in the mouse as well as studies of the physiological and metabolic consequences of congenital reduction of D1 activity. The prototypes of normal and decreased hepatic D1

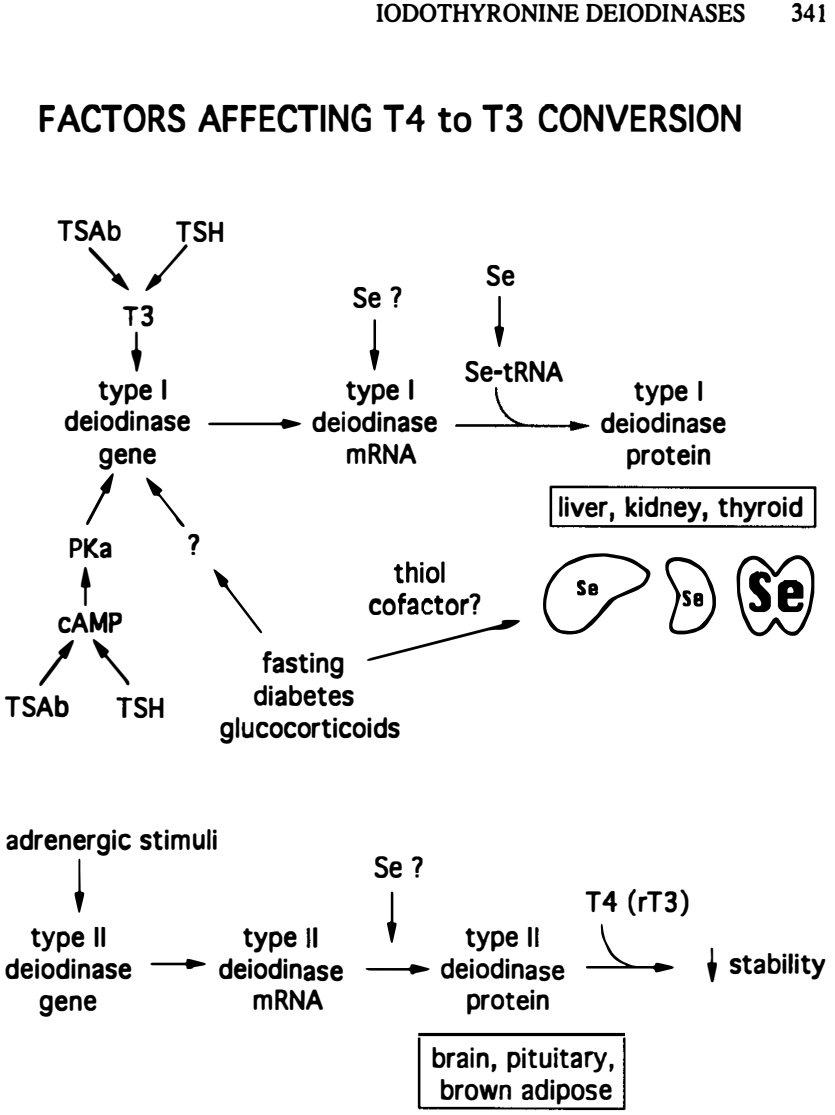

Figure 6 Factors affecting T4 to T3 conversion. Hormonal and nutritional regulation of D1 and D2 mRNA and protein expression. (Reprinted with permission from Reference 15.)

activity are the C57/Bl6 (C57) strain, which exhibits normal activity, and the C3H/HeJ (C3H) strain, which exhibits an approximately 10-fold lower D1 activity in liver and a 5-fold lower D1 in kidney (10). The differences in D1 activity in these two strains are paralleled by alterations in mRNA levels, indicating that they likely result from pretranslational effects. The genes encoding D1 in both strains have been cloned and were found to contain four exons (89). S1 nuclease and RACE analyses have shown that the transcriptional

start sites for both the C57 and C3H mRNAs are identical and that the cDNA sequences exhibit >98% identity. A restriction fragment length variant (RFLV), which segregates with high or low activity in inbred strains, results from the presence of 150 base pairs of repetitive sequence in the second intron of the C3H gene. In addition, slight differences are evident in promoter sequences, and transient expression studies of promoter activity have shown that the C57 promoter and 5′ flanking region are 2–3 times more potent than the C3H promoter. Experiments are currently under way to determine which of the minor differences in promoter sequence might explain this discrepancy. Studies of the intronic sequence differences during in vitro expression suggest that they do not account for the lower D1 mRNA of the C3H mouse. Thus, these differences are not the cause of the altered expression but rather serve as a marker for the D1 gene exhibiting low expression.

Surprisingly, despite the 8- to 24-fold lower activity of D1 in liver and the 4- to 5-fold lower activity in kidney of the C3H mice, serum total and free T3 and TSH did not differ significantly in the two strains (10). However, both serum reverse T3 and serum free T4 were significantly elevated in the D1-deficient strain (10, 114). The D1-deficient strain had an elevated free T4 but similar TSH responsiveness to TSH-releasing hormone (TRH) owing to an approximately 50% reduction in D2 in the pituitary and brain of the C3H strain. This decrease in D2 activity is to be expected given the well-recognized effect of T4 in depressing D2 activities in these tissues (see below). Thus, these mice appeared to undergo a compensatory reequilibration such that the feedback regulation of TSH secretion provided by serum T4 was altered to reduce the local production of T3 in the hypothalamic-pituitary axis. The fact that D2 is encoded by a different gene can be inferred from the observation that D2 activity in the brown adipose tissue (BAT) of cold-exposed C57 mice did not differ from that of C3H mice (10). This finding suggests that the sympathetic activation of BAT D2 synthesis due to cold exposure could override the suppressive effects of the elevated serum free T4. Because D1 activity is important not only for T3 production but also for T3 degradation via its inner-ring deiodination, the preservation of normal T3 levels in the D1-deficient strains likely results from a combination of the elevated serum free T4 and a prolonged half-life of T3 in the D1-deficient strains.

Analysis of one patient, the offspring of a consanguinous union, has shown a hormonal profile quite similar to that described in both experimental selenium deficiency and in D1-deficient mice (72). In this patient, serum total and free T4 were elevated despite a normal free T3. TSH was normal, as was the TSH response to TRH infusion. Studies are under way to characterize the D1 gene in this individual, but as yet no alterations in gene structure have been identified. In another family, six affected members showed an elevation in total and free T4 despite high normal total T3 and TSH levels (91). Although such a

pattern might also be seen in generalized thyroid hormone resistance, the elevation in serum T4 was greater than that in serum T3, suggesting D1 deficiency. The nature of the defect in this family has not been further characterized.

Taken together, these studies illustrate the remarkably modest effect of a severe deficiency of D1 on thyroid hormone physiology. The fact that a chronic decrease in hepatic D1 activity of more than 10-fold does not cause hypothyroidism suggests that a congenital decrease in the efficiency of T4 to T3 conversion can be balanced by a combination of increased T4 secretion and decreased T3 clearance. Presumably the acute reduction in T3 in response to physiological and pathophysiological perturbations of D1 activity in humans occurs too rapidly to allow such compensatory processes.

TYPE 2 DEIODINASE

Type 2 deiodinase (D2) is a low K_m iodothyronine deiodinase with exclusive 5′ deiodinase activity (144, 146). The enzyme does not catalyze inner-ring deiodination of the iodothyronines. Extensive studies of the physiology and regulation of D2 activity have been conducted (77), but more precise evaluation of this enzyme has been impeded by the lack of a cDNA. The low D2 activities in various tissues in which this enzyme is expressed, e.g. brain, pituitary, BAT, placenta, and skin, have frustrated attempts to isolate it. Injection of mRNA from BAT has resulted in only minimal levels of detectable activity in *Xenopus* oocytes (12). Therefore, the cloning strategy used so successfully for isolation of D1 is not useful for D2.

The principal physiological role of D2 is the local (intracellular) production of T3 (76, 120, 127, 128, 138, 139). Locally produced T3 accounts for ~80% of the nuclear T3 in the cerebral cortex (32) and for ~50% in the pituitary and BAT (119, 124). In BAT, however, activation of the sympathetic nervous system (e.g. cold exposure) leads to marked increases in D2 activity through the combined effects of α1 and β adrenergic stimulation (96, 107, 123, 125, 126). The T3 produced thereby causes a complete saturation of the T3 receptors, with a consequent optimization of uncoupling protein synthesis and thermogenic response of the cold-stressed rodent (17). Similar phenomena presumably occur in the human newborn at the time of delivery, when similar increases in D2 occur (57). The various physiological and pharmacological studies of D2 have been reviewed extensively (121).

For the purposes of the present discussion, the most critical issue is whether D2 is also a selenoenzyme. As discussed above, sensitivities to PTU or to gold have been suggested as criteria for the presence or absence of selenium in the active center. However, this proposal is not valid for reasons given above. D2 may have a relatively low turnover number because its V_{max} is approximately

100-fold lower for T4 and reverse T3, its preferred substrates, than it is for D1. Because the D3 enzyme shares a similarly low K_m and V_{max} and is also relatively insensitive to PTU and to gold (21, 29, 92, 131), these criteria for the presence of selenocysteine can no longer be considered absolute.

However, other studies argue against the presence of selenium in D2. Although the reduced level of D2 in brain of selenium-deficient rats was initially thought to suggest that D2 was a selenoprotein, this reduction can be attributed to a decrease in D2 resulting from an elevation in serum T4 that occurs in selenium-deficient animals (4, 25, 26). This decrease is similar to the decrease in D2 activity in D1-deficient inbred mouse strains. It has also not been possible to label D2 with ^{75}Se (111). Still unexplained are the lack of cold-induced increases in D2 in BAT of selenium-deficient rats (2) and the 70% decrease in D2 in brain in selenium-deficient vs selenium-sufficient pregnant animals (23). The latter has been attributed to an increase in serum reverse T3 in the pregnant selenium-deficient rat, but whether such modest levels of reverse T3 could produce this phenomenon remains to be seen (63, 103). As is discussed below, D3 activity in selenium-deficient animals is also relatively resistant to selenium depletion, despite compelling evidence recently presented that this enzyme does contain selenocysteine (131). Thus, a final determination of whether D2 contains an active-site selenocysteine must await characterization of its mRNA.

TYPE 3 DEIODINASE

Although D1 is capable of inner-ring deiodination, as discussed above, the predominant enzyme catalyzing this reaction is D3. In the rat, D3 activity levels are highest in adult brain, skin, and placenta and in fetal liver, muscle, brain, and the central nervous system. This enzyme has been proposed to protect fetal tissues from high levels of T4 and T3 during development by converting them to the inactive iodothyronines rT3 and T2, respectively (20, 66). Considerable effort has been made to characterize the function of D3 in amphibians, in which it plays a critical role in development. The enzyme is present in *Rana catesbeiana* tadpoles from premetamorphosis through the onset of metamorphic climax (44), at which time it declines to nearly undetectable levels. High levels of exogenous thyroid hormone cause uncoordinated development and eventually death in young tadpoles (43). D3 activity in the adult frog ranges from zero to levels seen in premetamorphic tadpoles (44).

The cDNA for *Xenopus laevis* D3 was recently cloned using a polymerase chain reaction(PCR)–based strategy for isolating T3-responsive genes (131). The sequence of the cDNA revealed the presence of an in-frame TGA codon encoding selenocysteine, as in the D1 cDNA. A SECIS element is present in the 3′ut of D3 mRNA, and its deletion results in loss of expression. The

presence of selenocysteine was somewhat unexpected for a number of reasons. First, as mentioned above, the enzyme was shown some time ago to be relatively insensitive to PTU (29) and, more recently, to gold (112). These results were confirmed with the cloned *Xenopus* D3. Second, studies of the effects of selenium deficiency on enzyme activity yielded conflicting results. In one report, maintenance of rats on a selenium-deficient diet for 4 months produced a small but statistically significant decrease in cerebrocortical D3 (94). Another study found no effect on rat placental D3 following 7 weeks of selenium deficiency (23). In light of the earlier studies showing high retention of selenium in brain and endocrine tissues even after long-term maintenance on selenium-deficient diets, these discrepancies may arise from differences in tissue retention and in length of time of depletion.

D3 catalyzes inner-ring deiodination of T4, T3, and T2 but not of the corresponding sulfated iodothyronines (113). D3 in rat cerebrocortical microsomes exhibits a K_m for T4 of 37 nM and for T3 of 6 nM (69). The latter value is very close to that obtained for the cloned *Xenopus* D3 enzyme, i.e. 2 nM for T3 (131). Early studies revealed that D3 required high concentrations of thiol cofactor. This requirement was confirmed in studies of the cloned enzyme. Parallels between D3 activity and thyroid status have been demonstrated in mammals and amphibians. In rats, D3 activity is increased in hyperthyroidism (30) and decreased throughout the central nervous system in hypothyroidism (70). In *Xenopus* tadpoles, D3 is markedly and rapidly stimulated by T3 before metamorphic climax (44). In contrast to D1, D3 appears not to be affected by fasting (35). The enzyme was recently shown to be acutely regulated by growth hormone in chicken liver (33); activity decreases within 2 h of injection.

Despite the considerable progress that has been made in the field of thyroid hormone metabolism in recent years, much work remains to be done. Elucidation of the structure of D2 and investigation of the complex interplay of the three deiodinases during development are the most pressing of the tasks ahead.

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