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Kinetic evidence suggesting two mechanisms for iodothyronine 5′-deiodination in rat cerebral cortex

(thyroxine/triiodothyronine/reverse triiodothyronine/propylthiouracil)

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ABSTRACT **Enzymatic 5′-deiodination of 3,3′,5′-triiodothyronine (rT3) and 3,3′,5,5′-tetraiodothyronine (thyroxine, T4) was studied in microsomal preparations of rat cerebral cortex. Evidence was obtained for the existence of two thiol-dependent 5′-deiodinase entities. One of these predominates in tissue from euthyroid and long-term hypothyroid rats, is specific for rT3, follows "ping-pong" kinetics with dithiothreitol as the cosubstrate, and is inhibited by propylthiouracil (PrSUra) and iodoacetate. Inhibition by PrSUra is uncompetitive with rT3 and competitive with dithiothreitol. These properties are shared with the 5′-deiodinase activity of liver and kidney. The activity of a second type of 5′-deiodinase is highest in cerebral cortex from short-term hypothyroid rats, prefers T4 to rT3 as the substrate, is insensitive to PrSUra and iodoacetate, and follows "sequential" reaction kinetics. A similar PrSUra-insensitive 5′-deiodinase activity is also found in pituitary but is not detectable in liver and kidney; it seems, therefore, characteristic of tissues in which local T4 to 3,3′,5-triiodothyronine (T3) conversion supplies a major portion of the total intracellular T3.**

The main secretory product of the thyroid gland, 3,3′,5,5′-tetraiodothyronine (thyroxine, T4) is converted to the biologically more potent 3,3′,5-triiodothyronine (T3) in many tissues of the body by a process termed 5′-deiodination. There appears to be a differentiation between tissues such as the liver and the kidneys, where most of the T3 produced is returned to the circulation, and tissues such as the brain and the pituitary, where the T4 to T3 conversion seems to serve local purposes (1). Another distinction between the deiodinase activities at these loci is the effect of changes in thyroid status. Thus, hypothyroidism is associated with decreased T4 to T3 conversion in rat liver and kidney, whereas increased conversion rates are observed in the pituitary and the brain (2–5). Not only are the changes opposite but also they occur more rapidly in the central organs (6,*).

3,3′,5′-Triiodothyronine (reverse T3, rT3), an inactive intermediary product in the metabolism of T4, is in fact a better substrate for the enzymes that convert T4 to T3 in the liver and the kidney (7–9). The enzymes are located in membrane-derived fractions of these tissues, depend on thiols for activity, and are effectively inhibited by derivatives of 2-thiouracil—e.g., 6-propyl-2-thiouracil (PrSUra) (7–15).

A third difference with T4 5′-deiodinase activities of the liver and the kidney is that conversion of T4 to T3 in the pituitary and the brain is not affected by PrSUra in vivo or in vitro (3, 4, 16, 17). However, brain rT3 5′-deiodination is sensitive to PrSUra (18, †). Recent, preliminary studies (18) have yielded a clue to this apparent discrepancy: part of the rT3 5′-deiodinase activity in the cerebral cortex and the pituitary of the rat is inhibited by low concentrations of T4 (<0.1 μM) but not by PrSUra, whereas the remainder is inhibited by PrSUra but not by these concentrations of T4. This suggested that there are two pathways of 5′-deiodination in brain and pituitary tissue. The purpose of the present study was to test this hypothesis.

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MATERIALS AND METHODS

Preparation of Microsomal Fractions of Cerebral Cortex. Thyroidectomized male Sprague–Dawley rats with parathyroid implants, weighing 175–200 g at the time of thyroidectomy, were obtained from Zivic-Miller, Allison Park, PA. Rats were anesthetized with ether and exsanguinated via the abdominal aorta. Cerebral cortex was dissected free from white matter, and pooled tissue from 10 rats (approximately 5 g wet weight) was homogenized in 5–6 vol (vol/wt) of ice-cold 0.32 M sucrose, 10 mM Hepes (pH 7.0), containing 10 mM dithiothreitol (DTT), using a hand-driven, all-glass homogenizer. The homogenate was centrifuged at 4°C for 15 min at 15,000 × *g*. The pellet was washed once by resuspension in the original volume of the sucrose/Hepes/DTT buffer and centrifugation as above. The supernatants were combined and centrifuged at 4°C for 60 min at 100,000 × *g*. The pellet was rinsed with 0.1 M potassium phosphate/1 mM EDTA (pH 7.0) containing 2 mM DTT and subsequently suspended in 15 ml of this buffer. One-milliliter aliquots of this suspension were rapidly frozen in a dry ice/acetone bath and kept at −20°C until use. In this way microsomal fractions were prepared from groups of rats that had been thyroidectomized 2 months (2-mo Tx), 12 days (12-d Tx), and 21 days (21-d Tx) previously. The protein contents of these preparations were measured by the method of Bradford (19), using human gamma globulin as the standard, and amounted to 7.6, 7.0, and 4.4 mg/ml, respectively.

Measurement of 5′-Deiodinase Activity. [3′,5′-^{125}I]rT3, specific radioactivity 625 Ci/mmol (1 Ci = 3.7 × 10^{10} becquerels), was purchased from New England Nuclear. [3′,5′-^{125}I]T4 was prepared by radioiodination of T3 by the chloramine-T method (20) and purified by descending paper chromatography using *t*-amyl alcohol/hexane/2 M ammonia, 5:1:6 (vol/vol), as the solvent, yielding a specific radioactivity equal to that of the ^{125}I used (New England Nuclear; 1,750 Ci/mmol). Both radiolabeled substrates were over 90% pure, with ^{125}I$^-$ as the

Abbreviations: T4, 3,3′,5,5′-tetraiodothyronine (thyroxine); T3, 3,3′,5-triiodothyronine; rT3, 3,3′,5′-triiodothyronine (reverse T3); 3,3′-T2, 3,3′-diiodothyronine; PrSUra, 6-propyl-2-thiouracil; DTT, dithiothreitol; IAcO, iodoacetate; 2-mo Tx, 12-d Tx, and 21-d Tx, rats thyroidectomized 2 months, 12 days, and 21 days, respectively, before use.

* Maeda, M. & Ingbar, S. H. (1980) *Program of the 62nd Annual Meeting of the Endocrine Society*, Washington, DC, June 18–20, 1980 (abstr. 305).

† Visser, T. J., Leonard, J. L. & Larsen, P. R. (1981) *Program of the 57th Annual Meeting of the American Thyroid Association*, Minneapolis, MN, Sept. 16–19, 1981, p. T-43 (abstr.).

main contaminant. The products were further purified by paper electrophoresis (9), resulting in a decrease of $^{125}I^-$ to less than 0.7%. Unlabeled T4 and rT3 were obtained from Henning (Berlin) and Calbiochem (La Jolla, CA), respectively. All other reagents were of the highest purity commercially available.

The measurement of 5'-deiodinase activity was based on the release of radioiodide from the ^{125}I-labeled substrates (8). Reactions were carried out with 100,000 cpm of [^{125}I]T4 or [^{125}I]rT3 plus unlabeled T4 or rT3 to make up the indicated concentrations of substrates in 200 μl of 100 mM potassium phosphate, 1 mM EDTA (pH 7.0), containing the indicated concentrations of DTT. Reactions were started by the addition of the microsomal suspension and continued for usually 1 hr at 37°C under air, yielding deiodination rates that were 10–20% lower than those measured after incubation under nitrogen. Control incubations were performed by addition of excess (100 times apparent K_m value) unlabeled substrate or by omission of microsomes, yielding identical results. Reactions were terminated by addition of 50 μl of ice-cold 50% normal human serum, containing an excess of rT3- and T4-binding proteins. Protein-bound substrate was precipitated with 350 μl of ice-cold 10% trichloroacetic acid. Radioiodide was separated from remaining substrate by passing 500 μl of the trichloroacetic acid supernatant through a small Dowex 50W-X2 column (bed volume 1.2 ml), equilibrated in 10% (vol/vol) acetic acid. The $^{125}I^-$ was eluted with two 1-ml portions of 10% acetic acid and its radioactivity was measured (8). Over 99% of the substrates remained adsorbed onto the columns under these conditions. The recovery of $^{125}I^-$ through the entire procedure was better than 97%. Paper chromatography (5) of extracts of reaction mixtures demonstrated that I^- release from rT3 equalled 3,3'-diiodothyronine (3,3'-T2) production. There was also a strict correlation between I^- release and T3 production from T4 under widely varying reaction conditions ($r = 0.99$), under which more than 85% of the I^- formed could be accounted for by T3 generation.

Data Analysis. The amount of $^{125}I^-$ recovered from the incubation mixtures was corrected for nonenzymatic deiodination and contamination of the substrates with radioiodide by subtraction of the control value, which usually amounted to less than 1% of radioactivity added. Because the substrates were randomly labeled with ^{125}I in the equivalent 3' and 5' positions of the phenolic ring, the specific radioactivity of the iodide released was half of that of the iodothyronines, which was accounted for in the analysis of the data. Reaction conditions were chosen such that less than 10% of the substrate was consumed by enzymatic deiodination. Each experimental point was determined in triplicate with coefficients of variation of less than 5%. Regression lines of the Lineweaver–Burk plots were calculated by unweighted least-squares analysis, as were the intercept and slope replots of these data.

RESULTS

Enzymatic I^- production from both T4 and rT3 was linear with time of incubation with cortex microsomal fractions and 20 mM DTT at 37°C for at least 2 hr. In all subsequent experiments incubation time varied between 45 and 90 min, but usually was 60 min. Enzymatic deiodination was abolished by preheating the microsomes for 30 min at 56°C. Under our assay conditions, 5'-deiodination of T4 and rT3 showed a linear relation with the amount of microsomal protein in the reaction mixture up to about 1 mg/ml. All subsequent experiments were done with protein at 0.95 mg/ml or less.

In the presence of 20 mM DTT, 5'-deiodination of rT3 by 2-mo Tx microsomes followed simple saturation kinetics, with linear double-reciprocal plots, characterized by a mean apparent K_m of 18.6 nM and a V_{max} of 1.6 pmol/mg of protein per hr (Table 1). However, the magnitudes of these parameters were dependent on the thiol concentration. Product formation from various concentrations (7–50 nM) of rT3 by 2-mo Tx microsomes was investigated at different fixed levels (5–20 mM) of DTT. Fig. 1 shows that increases in the DTT concentration resulted in parallel, downward shifts of the double-reciprocal plots of deiodination rate versus rT3 concentration, indicating "ping-pong" kinetics for this reaction (21). Lineweaver–Burk plots of deiodination rate versus DTT concentration at fixed levels of rT3 gave another set of parallel lines. In both cases the plot of the ordinate intercepts as a function of the reciprocal concentration of the fixed substrate was linear. From these replots the limiting Michaelis constants K_a (33 nM) for rT3 (extrapolated to infinite DTT concentrations) and K_b (16 mM) for DTT (extrapolated to infinite rT3 concentrations), and the value for the maximal limiting forward velocity V_1 (2.8 pmol/mg of protein per hr) were derived.

Table 1. Kinetic parameters of T4 and rT3 5'-deiodination by rat cerebral cortex microsomal preparations and 20 mM DTT, with or without addition of 1 mM PrSUra or pretreatment with 10 μM iodoacetate

Microsomes	Substrate	Inhibitor	K_m, nM	V_{max}, pmol/mg protein per hr
2-mo Tx	rT3	—	18.6 ± 3.7	1.60 ± 0.38
	T4	—	3.1	0.31
12-d Tx	rT3	—	8.7 ± 3.2	0.91 ± 0.28
	rT3	PrSUra	2.8 ± 1.2	0.34 ± 0.14
	T4	—	1.1 ± 0.2	0.64 ± 0.09
21-d Tx	rT3	—	6.3*	1.12*
	rT3	PrSUra/IAcO	4.1*	0.59*

Values with an error measurement are mean ± SD ($n = 4$). IAcO, iodoacetate.
* See Fig. 8.

Enzymatic rT3 5'-deiodination was inhibited by PrSUra. With 2-mo Tx microsomes and 5 mM DTT, Lineweaver–Burk diagrams of deiodination rate as a function of rT3 concentration at 0, 2.5, 5, and 10 μM PrSUra were parallel (Fig. 2), showing that inhibition was uncompetitive with rT3. From the plot of the intercept with the $1/v$ axis against PrSUra concentration an apparent K_i value for PrSUra of 4 μM was calculated.

Because PrSUra addition affected the kinetics of rT3 deiodination in the same way as did reduction of the DTT concentration, the possibility was considered that the cofactor and the inhibitor interact with a common site in the deiodination sequence. This was verified by measuring 5'-deiodination by 2-mo Tx microsomes with a single rT3 concentration (7 nM) and various DTT concentrations (5–20 mM) without or with 2.5–10

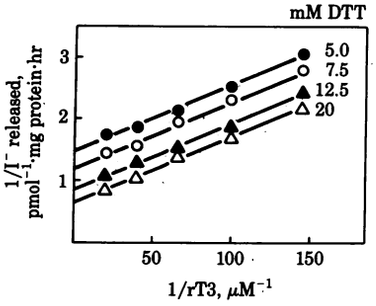

FIG. 1. Double-reciprocal plot of the rate of rT3 5'-deiodination by 2-mo Tx cortex microsomes as a function of rT3 concentration at 5.0 (●), 7.5 (○), 12.5 (▲), or 20 (△) mM DTT.

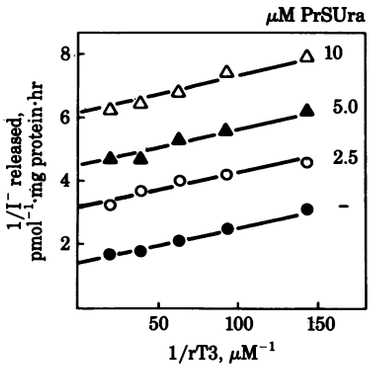

FIG. 2. Double-reciprocal plot of the rate of rT3 5′-deiodination by 2-mo Tx cortex microsomes as a function of rT3 concentration at 5 mM DTT in the absence (●) or presence of 2.5 (○), 5.0 (▲), or 10 (△) μM PrSUra.

μM PrSUra added (Fig. 3). Indeed, addition of PrSUra affected the slope but not the $1/v$ intercept of the double-reciprocal plots, demonstrating that PrSUra inhibition was competitive with DTT. The slope of the lines was a linear function of the PrSUra concentration, from which an apparent K_i value of 6 μM was deduced.

Essentially the same observations were made concerning the reaction kinetics of rT3 5′-deiodination as well as the mode of inhibition by PrSUra when cerebral cortex microsomes from euthyroid animals were used (not shown).

T4, tested at 5–25 nM, was found to be a competitive inhibitor of the 5′-deiodination of rT3 in incubation mixtures containing the substrate at 7–50 nM (Fig. 4). However, the increase in the slope of the lines in the Lineweaver–Burk plots was not proportionate with the concentration of T4. This was substantiated by the plot of these slopes as a function of T4 concentration, which was clearly hyperbolic and concave down (Fig. 4 *Inset*). This suggested different pathways of rT3 deiodination by 2-mo Tx microsomes, only a minor one of which was inhibited by T4.

In a single experiment, the 5′-deiodination of 0.3–30 nM T4 by 2-mo Tx microsomes and 20 mM DTT was measured. From the linear Lineweaver–Burk diagram an apparent K_m value of 3.1 nM and a V_{max} value of 0.31 pmol/mg of protein per hr were estimated. 5′-Deiodination of T4 was not affected by up to 1 mM PrSUra but was completely inhibited by 100 nM rT3.

Enzymatic rT3 5′-deiodination by 12-d Tx microsomes and 20 mM DTT was found to follow simple saturation kinetics as well, with the mean apparent K_m (8.7 nM) and V_{max} (0.9 pmol/mg of protein per hr) values being less than those for 2-mo Tx

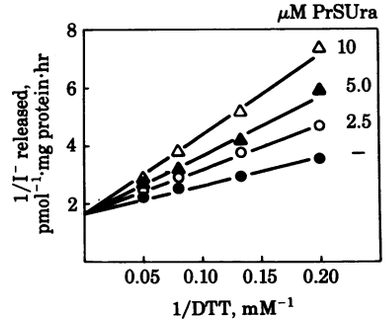

FIG. 3. Double-reciprocal plot of the rate of rT3 5′-deiodination by 2-mo Tx cortex microsomes as a function of DTT concentration at 7 nM rT3 in the absence (●) or presence of 2.5 (○), 5.0 (▲), or 10 (△) μM PrSUra.

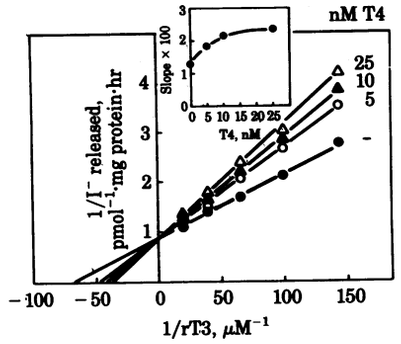

FIG. 4. Double-reciprocal plot of the rate of rT3 5′-deiodination by 2-mo Tx cortex microsomes as a function of rT3 concentration at 20 mM DTT in the absence (●) or presence of 5 (○), 10 (▲), or 25 (△) nM T4. (*Inset*) Plot of slope as a function of T4 concentration.

microsomes (Table 1). 5′-Deiodination of rT3 by 12-d Tx microsomes was only partially inhibited by PrSUra, and substantial activity remained in the presence of PrSUra at concentrations as high as 1 mM. Lineweaver–Burk plots of deiodination rate versus rT3 concentration (1–12.5 nM), as estimated in the presence of 20 mM DTT and 1 mM PrSUra, were linear and were characterized by a mean apparent K_m value of 2.8 nM and a V_{max} value of 0.34 pmol/mg of protein per hr (Table 1). The effect of T4 (1.25–5 nM) on the deiodination of rT3 (1–12.5 nM) by 12-d Tx microsomes was reinvestigated in the presence of 1 mM PrSUra. Again, T4 inhibited the reaction competitively with rT3 (Fig. 5). Now, however, the slope of the double-reciprocal plots showed a linear dependence on the T4 concentration. From the slope plot an apparent K_i value of 1.9 nM was estimated (Fig. 5 *Inset*).

Enzymatic 5′-deiodination of T4 by 12-d Tx microsomes and 20 mM DTT was characterized by simple saturation kinetics with a mean apparent K_m value of 1.1 nM and V_{max} of 0.64 pmol/mg of protein per hr (Table 1). Again, 1 mM PrSUra did not affect this reaction, but rT3 was a competitive inhibitor (Fig. 6). The slope plot was linear with rT3 concentration and yielded an apparent K_i value of 4.5 nM (Fig. 6 *Inset*).

Reaction kinetics of enzymatic T4 5′-deiodination were analyzed in experiments in which the T4 concentration was varied between 1 and 12.5 nM, and DTT between 2.5 and 20 mM. Double-reciprocal plots of the data with T4 in the abscissa gave a set of lines, each with a fixed level of DTT, that converged

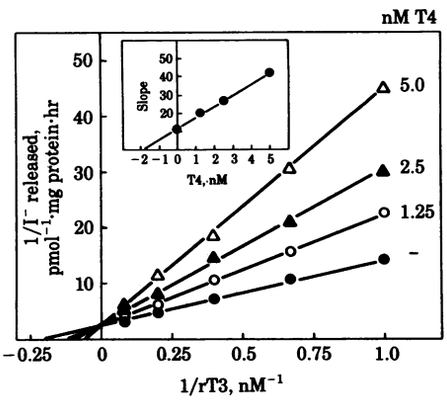

FIG. 5. Double-reciprocal plot of the rate of rT3 5′-deiodination by 12-d Tx cortex microsomes as a function of rT3 concentration at 20 mM DTT and 1 mM PrSUra in the absence (●) or presence of 1.25 (○), 2.5 (▲), or 5.0 (△) nM T4. (*Inset*) Plot of slope as a function of T4 concentration.

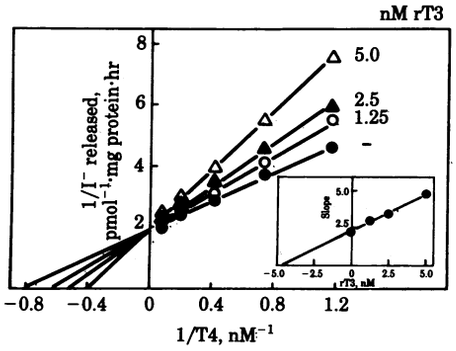

FIG. 6. Double-reciprocal plot of the rate of T4 5'-deiodination by 12-d Tx cortex microsomes as a function of T4 concentration at 20 mM DTT in the absence (●) or presence of 1.25 (○), 2.5 (▲), or 5.0 (△) nM rT3. (*Inset*) Plot of slope as a function of rT3 concentration.

to a single point left of the vertical and somewhat below the horizontal axis (Fig. 7). A similar set of intersecting lines was produced by plotting deiodination rate as a function of DTT concentrations at different fixed levels of T4. From the linear plots of the intercepts as a function of concentration, limiting Michaelis constants K_a for T4 and K_b for DTT as well as V_1 were estimated as 1.4 nM, 28 mM, and 1.4 pmol/mg of protein per hr, respectively. Similar evidence for "sequential" type reaction kinetics (21) of PrSUra-insensitive deiodination was obtained by studying this reaction with various rT3 and DTT concentrations in the presence of 1 mM PrSUra (not shown). Limiting kinetic constants for PrSUra-insensitive rT3 5'-deiodination were 5 nM (K_a), 30 mM (K_b), and 0.8 pmol/mg of protein per hr (V_1), respectively.

5'-Deiodination of rT3 (2 nM) in euthyroid rat cortex was inhibited by 90% after treatment of the microsomes for 15 min at 37°C and pH 7 with 1 μM IAcO or 10 μM iodoacetamide, whereas only 25% inhibition was observed with 21-d Tx microsomes. No further inhibition was observed on increasing the IAcO concentration to 10 μM (not shown). Fig. 8 demonstrates that pretreatment with 10 μM IAcO lowered both apparent K_m (3.6 vs 6.3 nM) and V_{max} (0.57 vs 1.12 pmol/mg of protein per hr) of total rT3 5'-deiodination by 21-d Tx microsomes and 20 mM DTT but had no effect on the kinetic parameters measured in the presence of 1 mM PrSUra (4.4 vs. 4.4 nM and 0.59 vs. 0.61 pmol/mg of protein per hr, respectively).

DISCUSSION

We have previously shown (18) that, assayed at 2 nM rT3, almost all 5'-deiodinase activity of cerebral cortex microsomes from

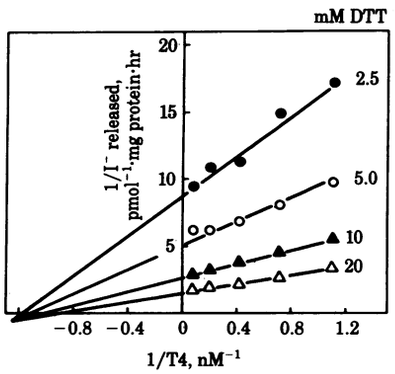

FIG. 7. Double-reciprocal plot of the rate of T4 5'-deiodination by 12-d Tx cortex microsomes as a function of T4 concentration at 2.5 (●), 5.0 (○), 10 (▲), or 20 (△) mM DTT.

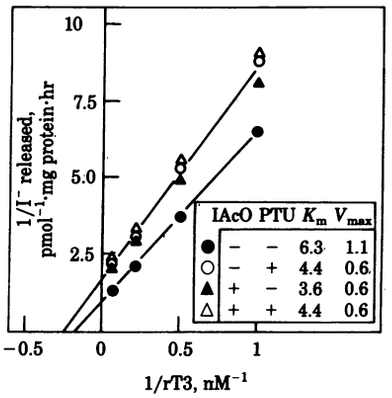

FIG. 8. Effect of pretreatment of 21-d Tx cortex microsomes with IAcO on the kinetics of total and PrSUra-insensitive rT3 5'-deiodination. Microsomes (2.2 mg of protein per ml) were preincubated for 15 min at 37°C with or without 10 μM IAcO in phosphate/EDTA buffer (pH 7) containing 1 mM DTT. 5'-Deiodination of rT3 (1–15 nM) was subsequently measured at 0.55 mg of microsomal protein per ml and 20 mM DTT with or without 1 mM PrSUra (PTU) added.

euthyroid rats, and a major fraction of that of long-term hypothyroid animals, is inhibited by PrSUra. Most of the activity in the cortex from short-term hypothyroid rats, however, is insensitive to PrSUra.

The kinetics of rT3 5'-deiodination by cortex microsomes of euthyroid or long-term hypothyroid rats (2-mo Tx) rats, as illustrated in Figs. 1–3, are reminiscent of previous observations of the 5'-deiodination in liver (9, 14) and kidney (8, 13, 15). Enzymatic 5'-deiodination in all these instances appears to follow ping-pong kinetics with DTT as the cosubstrate. The mode of inhibition by PrSUra—i.e., uncompetitive with rT3 and competitive with DTT—as well as the susceptibility to SH-blocking reagents, also points to the similarity of these 5'-deiodinase activities. A tentative model envisaging PrSUra-sensitive 5'-deiodination as a two-stage reaction has been put forward (8, 13, 14) and is illustrated in Fig. 9. Central in this model is the essential enzyme sulfur alternating (ping-pong) between the reduced (SH) state, subject to carboxymethylation, and the oxidized (SI) form, susceptible to PrSUra inactivation by formation of a mixed disulfide.

Another point of interest is the substrate specificity of these catalytic sites. 5'-Deiodination by liver or kidney microsomes is characterized by apparent K_m values for rT3 that are 1 to 2 orders of magnitude lower than those for T4, and also V_{max} values differ to a similar extent in favor of rT3 deiodination (7, 8). If corresponding differences in kinetic parameters for rT3 and T4 exist with respect to the PrSUra-sensitive activity in cortex, this would explain why T4 5'-deiodination by this process was too slow to be detectable under our assay conditions. In support of this hypothesis, only very high concentrations (>1 μM) of T4 inhibit 5'-deiodination of rT3 by the PrSUra-sensitive pathway (18). In summary then, this activity has all the character-

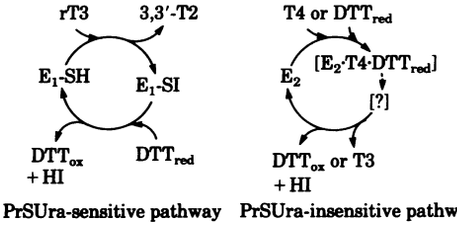

FIG. 9. Tentative pathways of iodothyronine 5'-deiodination in brain.

istics associated with enzymatic 5'-deiodination in the liver and the kidney.

In contrast, T4 is a potent competitive inhibitor of brain rT3 5'-deiodination by a PrSUra-insensitive process. In the absence of PrSUra, T4 only partially inhibited rT3 5'-deiodinase activity, with a plateau being reached with about 25 nM T4 (Fig. 4). Elimination of the PrSUra-sensitive pathway for the 5'-deiodination of rT3 by addition of excess PrSUra (1 mM compared with its K_i value of ≈ 5 μM) resulted in a reduction in both K_m and V_{max} values (Table 1). The residual activity was completely and competitively inhibited by T4 with an apparent K_i value of 1.9 nM (Fig. 5).

5'-Deiodination of T4 by 12-d Tx microsomes was characterized by an apparent K_m value—at 20 mM DTT—of 1.1 nM (Table 1), was unaffected by PrSUra, and was completely and competitively inhibited by rT3 (Fig. 6). The close agreement between the K_m of rT3 for the PrSUra-insensitive deiodinase activity and its K_i in the inhibition of T4 deiodination (2.8 and 4.5 nM, respectively), and the similarity of the Michaelis and inhibitory constants for T4 strongly suggest that these iodothyronines are substrates for a common PrSUra-insensitive deiodinase in brain. T4 appears to be the preferred substrate in this case, with a V_{max}/K_m ratio 5–6 times higher than that for rT3 at limited as well as infinite DTT concentrations.

The sequential reaction kinetics of the PrSUra-insensitive deiodination suggest that both iodothyronine and thiol must combine with the enzyme before reaction can take place (ref. 21; Fig. 9). The lack of effect of IAcO (and PrSUra) suggests that a reactive enzyme sulfur does not participate in this reaction.

In conclusion, our results allow the distinction in rat cerebral cortex of two 5'-deiodinase activities based on differences in substrate specificity, reaction kinetics, and susceptibility to inhibition by PrSUra and SH-blocking reagents. Most likely this implicates distinct enzyme entities with different catalytic mechanisms, although the exact time sequence of their expression after thyroidectomy remains poorly understood.

As to the potential function of the PrSUra-insensitive enzyme, experiments to date show its presence only in tissues such as brain and pituitary, where a major portion of intracellular T3 is derived from local conversion of T4, in contrast to tissues such as liver and kidney, where intracellular T3 is mainly derived from plasma T3 (1). That does not alter the fact that the latter tissues are quantitatively more important sites for the total body production of T3 from T4. The fate of the T3 produced in peripheral tissues is, however, different from that produced in, say, brain in that it appears to be directed mainly to supply T3 to the circulation rather than to local receptor sites.

It is tempting to speculate that the PrSUra-insensitive T4 5'-deiodinase activity is instrumental in securing an optimal occupancy of brain nuclear receptors by T3. Recent studies have demonstrated that over 70% of the specifically bound nuclear T3 in the rat cerebral cortex derives from T4 5'-deiodination within cortical cells via a mechanism that is not inhibited by PrSUra administration (17, 22). The 3- to 5-fold increase in its activity in hypothyroidism (6) suggests that a normal receptor occupancy may be maintained despite a reduction in circulating thyroid hormone levels. Tight control of the intracellular T3 level in the brain is further suggested by the finding of a specific, PrSUra-insensitive 5-deiodinase (ref. 5, unpublished observations). This enzyme degrades T3 by conversion to 3,3'-T2 and is clearly different from the 5'-deiodinase activities described here (which do not act upon T3), and from the PrSUra-sensitive 5-deiodinase activity of rat liver (23, 24). The 5-deiodinase in brain is also regulated by thyroid status but in an opposite direction, as its activity is decreased in hypothyroidism and increased in hyperthyroidism (5, 6).

Many questions remain to be answered. Some of these relate to the physiological cofactor of the deiodinations, to the precise role of thyroid hormone in adult brain, and to the exact location—i.e., cell type and organelle—of the different deiodinases. Nevertheless, the present study makes it untenable to regard iodothyronine 5'-deiodination as a single class of enzymatic reactions, and suggests that enzymes with different catalytic mechanisms may exist within the same tissue.

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