Evidence for Two Pathways of Iodothyronine 5'-Deiodination in Rat Pituitary That Differ in Kinetics, Propylthiouracil Sensitivity, and Response to Hypothyroidism

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ABSTRACT We have studied 5'-deiodination of thyroxine (T_4) and 3,3',5'-triiodothyronine (rT_3) in rat pituitary tissue in vitro, with respect to substrate specificity, reaction kinetics, effects of 6-*n*-propyl-2-thiouracil (PTU), and the time course of effects of thyroid hormone depletion and repletion. Removal of one phenolic iodine or both tyrosyl iodines from the T_4 molecule resulted in compounds that were not deiodinated, but alterations in the alanine side chain had little effect.

5'-Deiodination of 2 nM rT_3 by pituitary microsomes from euthyroid rats was inhibited >90% by 1 mM PTU, but was inhibited <10% by 100 nM T_4. The apparent Michaelis constant (K_m) and maximum velocity (V_{max}) for rT_3 at 20 mM dithiothreitol (DTT) were 33 nM and 84 pmol/mg protein per h. This reaction followed ping-pong type reaction kinetics when concentrations of DTT were varied. PTU inhibition was competitive with DTT and uncompetitive with rT_3. In contrast, when pituitary microsomes from hypothyroid rats (21 d postthyroidectomy) were used, deiodination of 2 nM rT_3 was inhibited only 20% by 1 mM PTU and up to 80% by 100 nM T_4. At 20 mM DTT, the apparent K_m and V_{max} in hypothyroid microsomes were 4.7 nM rT_3 and 16 pmol/mg protein per h. T_4 was a competitive inhibitor of PTU-insensitive rT_3 5'-deiodination (K_i = 1.3 nM). T_4 5'-deiodination by hypothyroid microsomes was not affected by PTU, was competitively inhibited by rT_3 (K_i, 1.7 nM), and exhibited sequential type reaction kinetics with DTT as cosubstrate. When T_4 5'-deiodination was measured in euthyroid and hypothyroid microsomes, respectively, the apparent K_m and V_{max} for T_4 at 20 mM DTT, were 0.9 nM and 0.55 pmol/mg protein per h (euthyroid), and 0.8 nM and 6.9 pmol/mg protein per h (hypothyroid).

The T_4 5'-deiodination rate and the PTU-insensitive, but not total, rT_3 5'-deiodination rate (i.e. measured in the presence and the absence of 1 mM PTU, respectively) in pituitary homogenates were significantly elevated 24 h after thyroidectomy. PTU-insensitive activity continued to increase until at ≥30 d after thyroidectomy it was 11 times the PTU-insensitive activity in controls. At the latter time, PTU-sensitive rT_3 5'-deiodinase activity appeared to be decreased. The increase in PTU-insensitive T_4 and rT_3 5'-deiodination observed 48 h after thyroidectomy was prevented by replacement doses of T_4 or T_3. The PTU-insensitive activity of long term hypothyroid pituitaries was decreased by 71% and ≥84% 4 h after injection of 20 and 200 μg T_3, respectively, with no change in PTU-sensitive rT_3 deiodination.

These data show that rat pituitary tissue contains two distinct iodothyronine 5'-deiodinating pathways that differ with respect to substrate specificity, PTU sensitivity, reaction kinetics, and regulation by thyroid hormone. One of these resembles the 5'-deiodinase of liver and kidney, and predominates in euthyroid pituitary tissue in vitro. The other, also found in rat brain, predominates in hypothyroid pituitary tissue,

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is rapidly responsive to changes in thyroid hormone availability, and, as judged by previous, in vivo studies, appears to account for all the T_3 produced locally in the pituitary and, thereby, 50% of the intracellular T_3 in this tissue.

INTRODUCTION

The anterior pituitary gland is an important target tissue for thyroid hormone. Besides the well-documented negative feedback control of thyrotropin (TSH)[1] secretion, thyroid hormone stimulates growth hormone (GH) production by the rat pituitary (1). In GH_3 cells, derived from rat pituitary tumor tissue, thyroid hormone modulates the rate of synthesis of at least six proteins, including GH (2). Ample evidence has been presented that these actions are initiated by the binding of 3,3',5-triiodothyronine (T_3) to a nuclear chromatin-associated receptor (3–6). Yet, on some occasions a better correlation of serum TSH with serum thyroxine (T_4) than with serum T_3 concentrations is observed (7). This may be explained by the finding that in rat pituitary one-half of the intracellular T_3 is derived from local 5'-deiodination of T_4, the other half being taken up from the circulation (8, 9).

Conversion of T_4 to T_3 has been observed in rat pituitary fragments and homogenates (10–15), and in GH_3 cells (16). Like the deiodinase activity of rat liver and kidney, this is a thiol-dependent process (12). Neither in vivo nor in vitro pituitary T_4 to T_3 conversion is affected by 6-propyl-2-thiouracil (PTU) (9, 12, 17). In contrast, PTU is a potent inhibitor of the 5'-deiodination in liver and kidney (18–20). However, iopanoic acid, the cholecytographic dye containing a triiodoaminophenyl group, inhibits T_3 production in all tissues thus far examined (7, 11, 12, 21, 22). With respect to both TSH and GH secretion, T_4 action on the pituitary is blocked by iopanoic acid, or the similar drug ipodic acid, but not by PTU (17, 23, 24). Pituitary T_4 5'-deiodinase activity in homogenates and tissue fragments is increased in hypothyroidism and decreased in hyperthyroidism (11, 12), whereas opposite changes are observed slices, homogenates, and microsomes from liver and kidney, as reviewed recently (7). 3,3',5'-Triiodothyronine (reverse T_3, rT_3) is a more suitable substrate than T_4 for the 5'-deiodinase activities of liver and kidney, judging from the higher ratio of V_{max} to K_m for rT_3 (25, 26). We have recently described two pathways of iodothyronine 5'-deiodination catalyzed by rat cerebral cortical microsomes (27, 28). One pathway shares several properties with hepatic and renal 5'-deiodination, including sensitivity to PTU. The other pathway is not sensitive to PTU, sharing that property with T_4 5'-deiodination in rat pituitary tissue (9, 11, 12, 17). Much less is known about the deiodination of rT_3 in the pituitary.

We have now examined reaction kinetics and compared the effects of PTU and thyroid status on the 5'-deiodination of T_4 and rT_3 in rat pituitary. The results suggest two different pathways of iodothyronine 5'-deiodination in this tissue, with distinct biochemical and physiological characteristics.

METHODS

Animals and reagents. Male Sprague-Dawley rats, weighing 175–200 g, were obtained from Zivic-Miller Laboratories, Allison Park, PA. Thyroidectomies and sham operations were performed by us or the supplier. Pituitaries were harvested after exsanguination of the animals via the abdominal aorta under ether anesthesia. The effectiveness of the thyroidectomy (and thyroid hormone replacement) was confirmed by measurements of serum T_4, T_3, and TSH at the time of death (29).

Levorotatory iodothyronines were used in all studies, unless otherwise specified. [^{125}I]rT_3, specific radioactivity ≃625 Ci/mmol, was obtained from New England Nuclear, Boston, MA, and was purified by paper electrophoresis within 2 h of its use in an assay, to remove $^{125}I^-$. Iopanoic acid was generously provided by the Sterling-Winthrop Research Institute, Rensselaer, NY. [^{125}I]T_4, specific radioactivity ≃1,750 Ci/mmol, was prepared in this laboratory (30) and purified by paper chromatography (21). 3,3',5,5'-Tetraiodothyroacetic acid (tetrac), 3,3',5-triiodothyroacetic acid (triac), 3,3',5,5'-tetraiodothyropropionic acid (tetraprop, 3,3'-diiodothyronine (3,3'-T_2), 3',5'-T_2 and 3'-iodothyronine (3'-T_1) were also iodinated with ^{125}I in our laboratory by the chloramine T method, and purified on Sephadex G-25 in 0.01 N NaOH (30). Tracers as thus synthesized were labeled only on the phenolic ring, at the 3' or 5' positions. Unlabeled T_4, T_3, and analogs were obtained from Henning GmbH, Berlin, West Germany, and rT_3 from Calbiochem Behring, Inc., La Jolla, CA. Other reagents were obtained from Calbiochem-Behring, the Sigma Chemical Co., St. Louis, MO, and Fisher Scientific Co., Pittsburgh, PA.

Tissue preparations. In initial characterization studies, anterior pituitaries were used, and homogenates and subfractions were prepared in 0.25 M sucrose, 0.05 M Tris, pH 7.5, 100 mM dithiothreitol (DTT). Subsequently, in homogenate studies, whole pituitaries were homogenized on ice in 80 vol of 0.32 M sucrose, 10 mM Hepes (pH 7.0), containing 10 mM DTT. In experiments using in vivo treatments, pituitaries from each animal were homogenized separately. For microsomal preparations, whole pituitaries were collected from a group of 14 normal 150–175-g rats, and from a group of 10 hypothyroid rats, weighing 175–200 g at the time of thyroidectomy 21 d previously. Each pool was homogenized in 10 ml 0.32 M sucrose, 10 mM Hepes (pH 7.0), containing 10 mM DTT, and centrifuged for 10 min at 3,500 *g* at 4°C. The pellets were resuspended in 10 ml sucrose-Hepes-DTT buffer and centrifuged again under the same conditions. The

[1] *Abbreviations used in this paper*: DTT, dithiothreitol; GH, growth hormone; PTU, 6-*n*-propyl-2-thiouracil; rT_3, 3,3',5'-triiodothyronine; tetrac, tetraiodothyroacetic acid; tetraprop, tetraiodothyropropionic acid; T_3, triiodothyronine; T_4, thyroxine; TSH, thyrotropin; triac, 3,3',5-triiodothyroacetic acid; 3,3'-T_2, 3,3'-diiodothyronine; V_{max}, maximum velocity.

combined supernatants were centrifuged for 60 min at 100,000 *g* and 4°C. The pellets were rinsed with 0.1 M potassium phosphate, 1 mM EDTA (pH 7.0), containing 2 mM DTT, and homogenized in 7.5 ml of this buffer. 1-ml aliquots of these suspensions were rapidly frozen in a dry ice-acetone bath and stored until use at −20°C. The 3,500–100,000 *g* pellets are referred to as crude microsomal fractions, but contain mitochondria as well. The protein content of the microsomal preparations from the euthyroid and hypothyroid pituitaries amounted to 0.45 and 0.50 mg/ml, respectively. The protein content of the tissue preparations was measured with the dye-binding assay of Bradford (31), using ovalbumin as the standard, reagents from Bio-Rad Laboratories (Richmond, CA), and detailed procedures as specified in the instructions from Bio-Rad.

Deiodination measurements. Tissue 5′-deiodinase activities were measured by the release of radioactive iodide from [^{125}I]T_4 or [^{125}I]rT_3 (assay A) (26), or the production of radioactive T_3 from [^{125}I]T_4 (assay B) (21). For assay A, 50 μl (22.5–25 μg protein) of the homogenates or microsomes were mixed with 50 μl 0.2 M potassium phosphate, pH 7.0, containing (with final concentrations in the reaction mixtures in parentheses) 100,000 cpm labeled iodothyronine of varying specific activites EDTA (1 mM) and DTT and PTU as indicated. The reaction was started by the addition of microsomes and allowed to continue for 45–120 min at 37°C in air. Tissue-free incubations served as controls. Analyses of reaction products were performed as described (21). The reaction was stopped by successive additions of 50 μl 50% human serum containing PTU, and 350 μl of cold 10% trichloroacetic acid. The acid soluble radioiodide was isolated in the unretarded fractions after chromatography of the supernatants on 1.2 ml Dowex 50W-X8 columns (Dow Corning Corp., Midland, MI), equilibrated, and eluted with 10% acetic acid (2 × 1 ml). The coefficient of variation of replicate analyses was <5%.

The reductive conditions of the reaction mixture for assay A that contained both EDTA and DTT were designed to inhibit totally peroxidase-catalyzed deiodination. In fact, no I^- release was observed in the absence of reduced thiols. In addition, no I^- release from rT_3 occurred in the presence of 20 mm DTT using heat-inactivated (100°C for 10 min) pituitary homogenates from hypothyroid or euthyroid rats supporting the enzymatic nature of the 5′-deiodination of rT_3. When pituitary homogenates from hypothyroid and euthyroid rats were incubated with [^{125}I]rT_3 with or without 10^{-3} M PTU, and the products identified by paper chromatography, the I^-/3,3′T_2 ratio was not significantly different from unity (1.07). There was also no effect of PTU on this ratio (1.09) control, (1.05 PTU), and no other products were found. In addition, similar studies using pituitary microsomes from hypothyroid rats and [^{125}I]T_4 as substrate over a broad range of fractional T_4 to T_3 conversion rates showed that the mean ratio of $^{125}I^-$ to [^{125}I]T_3 generated was 0.98, $r^2 = 0.99$. These results confirm that under these reaction conditions, quantitation of $^{125}I^-$ release from [^{125}I]rT_3 or [^{125}I]T_4 is a valid measure of the rate of 5′-deiodination as has been reported previously for both kidney (26) and cerebral cortex (27, 28) microsomes.

For assay B, 90 μl (40–45 μg protein) of homogenate was mixed with 10 μl 0.25 M sucrose, 0.05 M Tris, pH 7.5, containing 40,000 cpm [^{125}I]T_4 (≃0.2 nM), unlabeled T_4 (0 or 10 nM), T_3 (0 or 1 μM) and DTT (100 mM). Incubations were carried out for 60 min at 37°C under nitrogen. In control incubations, homogenate was replaced by buffer. The reaction was stopped by addition of 200 μl ethanol and 50 μl 0.04 N NaOH containing 50 μg T_4, 50 μg T_3, and 285 μg methimazole. After centrifugation and addition of carrier I^-, T_3, and T_4, aliquots of the supernatants were subjected to descending paper chromatography in t-amyl alcohol/hexane/2 N ammonia (5:1:6). The coefficient of variation of replicate determinations was 6.5%. The chromatograms were developed and counted as described (21). Deiodination of [^{125}I]3′-T_1 and 3′,5′-T_2 was measured by paper electrophoresis (21).

Data analysis. Product formation was corrected for nonenzymatic deiodination and contamination of substrates by subtraction of the respective control value, which always amounts to <1% (I^-) or 3% (T_3) of radioactivity added. Recovery of $^{125}I^-$ through the column procedure was better than 97%. A factor of 2 was applied to account for the fact that the specific radioactivity of the products was half that of the substrates, randomly labeled in the equivalent 3′ and 5′ positions. Each experimental point was determined in duplicate (T_3 by paper) or triplicate (I^- by column). In the study of the influence of thyroid status on pituitary deiodinase activities, group means were tested by analysis of variance and, if significant differences were present, individual experimental groups were compared to the controls by Dunnett's multiple *t* test (32). In the study of the kinetics of the 5′-deiodination by pituitary microsomes, conditions were chosen such that <20% of the substrates were consumed during the reaction. Linear regression lines in kinetic analyses were calculated by unweighted least squares analysis. Equations for the components of nonlinear Eadie-Hofstee plots were calculated by the method of Rosenthal (33), with five iterations of the cross corrections.

RESULTS

Initial characterization of the reaction system. Assay B was used for the studies in this section. The only products other than T_4 observed on the chromatograms after incubation of T_4 with pituitary homogenates or pituitary microsomes were T_3 and I^-, and the only products observed after incubations with rT_3 were 3,3′-T_2 and I^-. With both substrates, the amounts of I^- and the deiodinated iodothyronine did not differ significantly. Product formation, i.e. T_3 and I^- from T_4 and 3,3′-T_2 and I^- from rT_3, was linear with microsomal protein concentration to at least 100 μg/ml, and with incubation time to at least 120 min. All subsequent experiments with microsomes were carried out within these confines.

T_4 5′-deiodinase activity was associated predominantly with the 1,000–100,000-*g* pellet of pituitary homogenates from chronically hypothyroid rats. Relative to the homogenate (defined as 1.0), the specific 5′-deiodinase activity of the low-speed pellet was found to be 0.7±0.1 (mean±SE, $n = 3$), that of the 1,000–100,000-*g* pellet ("microsomes") 2.9±0.6, and that of the cytosol 0.3±0.1. A dispersed enzyme preparation was obtained by treatment of the microsomes with 0.5% cholate, followed by removal of excess cholate from the nonpelletable fraction by dialysis. The specific activity of this clear preparation was 3.9±1.0.

The nature and the extent of the reaction of several radioiodine-labeled T_4 analogs with the dispersed enzyme at 37°C, pH 7.6 and 100 mM DTT was investigated by paper chromatography. L-T_4, D-T_4, tetrac, tetraprop, and rT_3 were all actively deiodinated. The only products observed were the 5'-monodeiodinated compounds, i.e. T_3, triac, triprop, or 3,3'-T_2, and equal amounts of I^-. T_3, 3,3'-T_2, 3',5'-T_2, 3'-T_1, and triac were not deiodinated to any discernable extent. The rate of deiodination by the dispersed enzyme of tracer [^{125}I]T_4 (≃0.2 nM) was inhibited >50% by addition of 20 nM D-T_4, tetrac or tetraprop. It required 500 nM iopanoic acid to attain 50% inhibition of T_4 5'-deiodination, and the reaction was not measurably inhibited by 1 μM T_3, 3,3'-T_2, 3,5-T_2 or diiodotyrosine.

Using the dispersed enzyme and 100 mM DTT, the 5'-deiodination of T_4 was found to obey simple saturation kinetics with an apparent K_m value of 4.3±1.0 nM and V_{max} of 48±5 pmol/mg/protein per h (mean±SE, n = 4). However, studied under the same conditions, the kinetics of rT_3 5'-deiodination were more complex, with a nonlinear Eadie-Hofstee plot (34) that was concave upward (not shown). The results were best described by invoking two enzymatic processes with approximate K_m values of 3.1 and 152 nM, and V_{max} values of 2.1 and 26 pmol/mg protein per h, respectively.

Kinetics of 5'-deiodination by pituitary microsomal fractions. To investigate further the possibility of more than one 5'-deiodination reaction in the pituitary, detailed kinetic analysis of this reaction was performed using microsomes from euthyroid and hypothyroid rats as sources of enzyme and rT_3 and T_4 as substrates.

Stimulation of rT_3 5'-deiodination by DTT. 5'-Deiodination of rT_3 by euthyroid microsomes at 20 mM DTT followed simple saturation kinetics, characterized by a mean apparent K_m of 33 nM and V_{max} of 84 pmol/mg protein per h (Table I). Both parameters were, however, greatly influenced by the DTT concentration, as illustrated in Fig. 1A. Double-reciprocal plots of the data showed parallel, downward displacements with increasing fixed concentrations of DTT, suggesting "ping-pong" type reaction kinetics (34). Apparent K_m and V_{max} values increased with the DTT concentration between 10 and 50 mM DTT, but higher concentrations of thiol were inhibitory (see below).

Inhibition of rT_3 deiodination by PTU. The 5'-deiodination of rT_3 (2 nM) by the microsomal fraction of euthyroid pituitaries in the presence of 20 mM DTT was progressively inhibited by the addition of increasing concentrations of PTU. Over 90% inhibition was reached with 1 mM PTU (Fig. 2, left panel). The mode of inhibition by PTU was investigated in incubations of 10–125 nM rT_3 with euthyroid microsomes and 20 mM DTT in the absence or the presence of 1 or 2 μM of the inhibitor. Addition of PTU resulted in parallel, upward shifts of the double-reciprocal plots of rT_3 deiodination (Fig. 1B), demonstrating that PTU is an uncompetitive inhibitor of this reaction.

The interaction of PTU with the cofactor, DTT, was then studied by measuring deiodination at a single rT_3 concentration (25 nM) and varying DTT (10–125 mM) concentrations, with or without addition of 1 or 2 μM PTU. Fig. 1C shows that PTU only affected the slope but not the y-intercept of the double-reciprocal plots,

TABLE I
Kinetic Parameters of T4 and rT3 5'-Deiodination by Rat Pituitary Microsomes

Microsomes	Substrate	PTU added	n	K_m	V_{max}
		mM		*nM*	*pmol/mg protein per h*
Euthyroid	rT3	—	3	33 (30–36)	84 (69–94)
	T4	—	1	0.9	0.55
Hypothyroid (21 d post-thyroidectomy)	rT3	—	3	4.7 (3.7°–6.7)	16 (8°–30)
		1	2	2.9 (1.6°–4.2)	4.7 (3.5°–5.9)
	T4	—	3	0.6 (0.55–0.7)	8.3 (7.2–10.2)
		1	1	0.8	6.9

Incubations were performed in triplicate at pH 7.0 and 37°C, with products quantitated by assay A. The DTT concentration was 20 mM in all experiments except one, in which 15 mM DTT was used (values marked with °). n, number of determinations of the kinetic parameters. For n > 1, results are given as the mean and range of values from different experiments.

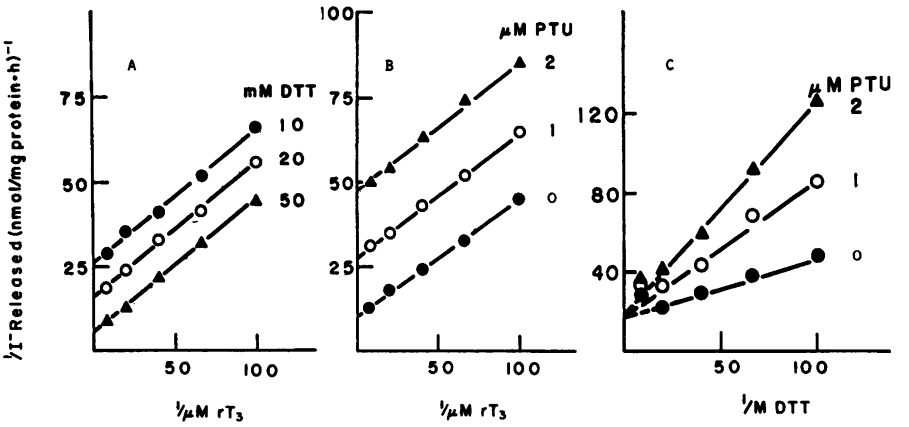

FIGURE 1 Double-reciprocal plots of the rate of rT_3 5'-deiodination by euthyroid rat pituitary microsomes (panel A) as a function of rT_3 concentration at 10, 20, or 50 mM DTT; (panel B) as a function of rT_3 concentration at 20 mM DTT and 0.1 or 2 μM PTU; (panel C) as a function of DTT concentration at 25 nM rT_3 and 0, 1 or 2 μM PTU. Points are means of triplicate determinations. Iodide release was measured by the ion-exchange assay (assay A).

indicating that PTU inhibition is competitive with the cofactor. Fig. 1C also shows the inhibition observed at DTT concentrations > 50 mM. The intercept-replot of Fig. 1B and the slope-replot of Fig. 1C (not shown) yielded approximate K_i values for PTU of 0.6 and 0.7 μM, respectively.

In contrast to the above results with euthyroid pituitary microsomes, 1 mM PTU caused <20% inhibition of rT_3 5'-deiodination in microsomes from 21 d thyroidectomized rats (Fig. 2, left panel). These results indicated that hypothyroidism was associated with an increase in the activity of a 5'-deiodinase mechanism for rT_3, which was PTU-insensitive. This phenomenon was explored further by evaluation of the relative sensitivities of euthyroid and hypothyroid microsomal deiodinations of rT_3 to inhibition by T_4.

Inhibition of rT_3 deiodination by T_4. The 5'-deiodination of rT_3 by pituitary microsomes from rats thyroidectomized 21 d previously was characterized by a mean apparent K_m value of 4.7 nM and V_{max} of 16 pmol/mg protein per h at 20 mM DTT (Table I). Both of these values were much lower than those for euthyroid microsomes. Nonlinear double-reciprocal plots were observed at high (>25 nM) rT_3 concentrations (not shown), again indicating the involvement of more than one enzymatic process. The 5'-deiodination of rT_3 (2 nM) by these microsomes was progressively inhibited by increasing concentrations of T_4, until, with 100 nM T_4, a plateau was reached at 20% of the control activity (Fig. 2, right panel). Much less inhibition by T_4 was observed in microsomes from euthyroid rats, with >90% of the activity remaining in the presence of as high as 100 nM T_4 (Fig. 2, right panel). These data demonstrate a further difference between euthyroid and hypothyroid rats with respect to rT_3 5'-deiodination.

To elucidate the nature of the interaction between rT_3 5'-deiodination and T_4, hypothyroid microsomes were reacted with 1.5–12.5 nM rT_3 and 0–10 nM T_4. Addition of T_4 affected the slope but not the y-intercept in the double-reciprocal plot, demonstrating that inhibition by T_4 was competitive with rT_3 (Fig. 3). However, the increase in the slope was not propor-

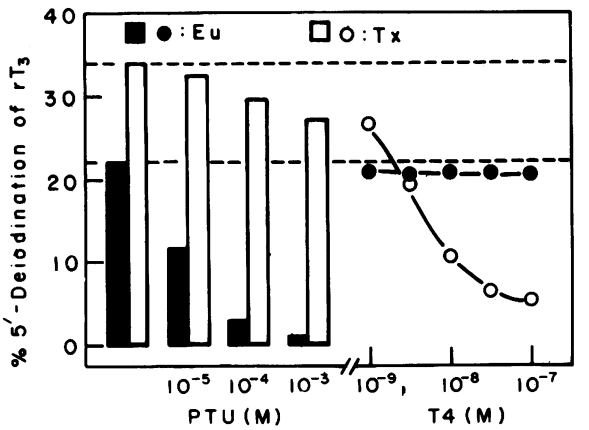

FIGURE 2 Effects of PTU (left panel) and T_4 (right panel) on 5'-deiodination of 2 nM rT_3 during 2-h incubations with 20 mM DTT and either 50 μg euthyroid pituitary microsomes or 55 μg of hypothyroid pituitary microsomes. Results are means of triplicate determinations, using assay A. Solid symbols designate data from euthyroid microsomes, and open symbols designate data from hypothyroid microsomes. The upper broken line shows the control rate (no T_4 or PTU added) for the hypothyroid preparation and the lower broken line shows the control rate for the euthyroid preparation.

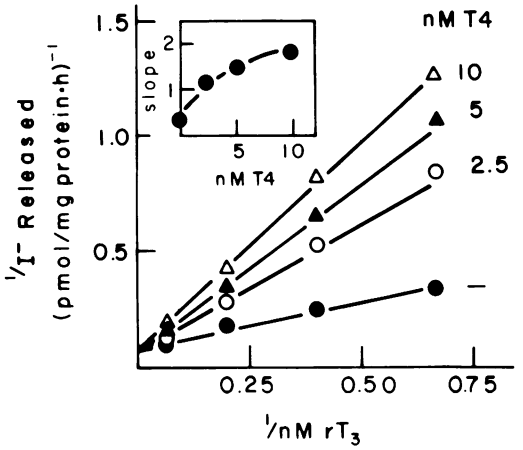

FIGURE 3 Double-reciprocal plots of the rate of rT_3 5′-deiodination by hypothyroid rat pituitary microsomes as a function of rT_3 concentration in the presence of 20 mM DTT and 0, 2.5, 5, or 10 nM T_4. Points are means of triplicate determinations, using assay A. Inset: Replot of slopes of the double reciprocal lines as a function of T_4 concentration.

tionate to the T_4 concentration, and the replot, slope vs. T_4 (Fig. 3, inset), was clearly hyperbolic, concave down. This suggested that only one of two putative pathways for the deiodination of rT_3 was inhibited by T_4. From the data in Fig. 2, this was thought most likely to represent inhibition of only the pathway insensitive to PTU. This hypothesis was tested in the next series of experiments.

The 5′-deiodination of rT_3 by hypothyroid microsomes and 15 mM DTT was determined in incubations containing 1.5–37.5 nM rT_3 and 0 or 1 mM PTU. The presence of PTU resulted in a decrease of both the apparent K_m and the V_{max} for rT_3 deiodination, i.e. 1.6 vs. 3.7 nM and 3.5 vs. 8 pmol/mg protein per h (Table I). The difference between the activities measured in the absence and in the presence of PTU, i.e. PTU-sensitive rT_3 5′-deiodination, also yielded a linear double-reciprocal plot, characterized by values for K_m and V_{max} of 17 nM rT_3 and 6 pmol/mg protein per h, respectively. Similar effects of 1 mM PTU on the parameters of rT_3 5′-deiodination were also evident at 20 mM DTT (Table I).

The effects of increasing concentrations (1.25–5 nM) of T_4 on the PTU-insensitive deiodination of rT_3 (1.5–12.5 nM) by hypothyroid microsomes and 20 mM DTT was investigated in the presence of maximally inhibiting PTU concentrations (1 mM). Again, inhibition by T_4 was found to be competitive with rT_3 but the slope of the double-reciprocal plot was now linearly dependent on the T_4 concentration (data not shown). From this slope replot an apparent K_i value of 1.3 nM for T_4 was estimated.

T_4 5′-deiodination. Deiodination of T_4 by hypothyroid microsomes was much more extensive than that observed with euthyroid microsomes. Measurement of the kinetic parameters in the presence of 20 mM DTT revealed that although apparent K_m values were very similar (0.6–0.9 nM) in microsomes from euthyroid and hypothyroid rats, maximal reaction rates (apparent V_{max} derived from double reciprocal plots) were 16-fold higher in the hypothyroid preparation than in the euthyroid preparation, 8.3 vs. 0.55 pmol/mg protein per h, respectively, (Table I). Note that the higher V_{max} for T_4 5′-deiodination given in the first section of Results was determined at a fivefold higher DTT concentration, using a more enriched enzyme preparation.

The stimulation of T_4 deiodination by DTT was analyzed in incubations containing 0.5–5 nM T_4, 2.5–20 mM DTT, and hypothyroid pituitary microsomes. Double-reciprocal plots of deiodination rate vs. T_4 concentration, each at a fixed level of DTT, intersected in a single point to the left of the vertical and close to the horizontal axis (Fig. 4). A similar set of intersecting lines was obtained by plotting reciprocals of deiodination rate vs. DTT at fixed T_4 concentrations (not shown). In each case the intercept replot as a function of the fixed substrate was linear (e.g. Fig. 4, inset) providing values for the limiting Michaelis constants; K_a = 0.7 nM T_4, extrapolated to infinite DTT; K_b = 9 mM DTT, extrapolated to infinite T_4; and V_1 = 13 pmol/mg protein per h. Addition of 1 mM PTU did not affect T_4 5′-deiodinase activity (Table I), but rT_3 was found to be a competitive inhibitor of T_4 conversion by hypothyroid microsomes in the presence of 20 mM DTT (not shown), with an apparent K_i value for rT_3 of 1.7 nM.

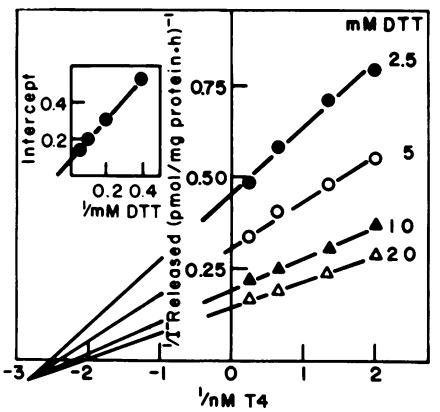

FIGURE 4 Double-reciprocal plots of the rate of T_4 5′-deiodination by hypothyroid rat pituitary microsomes as a function of T_4 concentrations in the presence of 2.5, 5, 10, or 20 mM DTT. Points are means of triplicate determinations, using assay A. Inset: Replot of the vertical intercept of the double reciprocal lines vs. the reciprocal of the DTT concentration.

Acute effects of changes in thyroid status on pituitary 5'-deiodinase activities. The marked qualitative and quantitative differences in 5'-deiodination reactions between euthyroid and hypothyroid pituitary microsomes led to studies of the time dependence of these changes after thyroidectomy. In pituitary homogenates of the sham controls, the mean (±SE) deiodination rate for rT_3 was 534±66 (n = 10) fmol I^-/mg protein per h. No significant alterations were observed until 10 d postthyroidectomy, when the mean rate was 920±121 fmol I^-/mg protein per h (n = 4, $P < 0.05$) (Fig. 5). However, PTU-insensitive rT_3 deiodination increased more rapidly. PTU-insensitive rT_3 5'-deiodinase activity in the euthyroid, sham-operated controls was 84±5 fmol I^-/mg protein per h, representing, on the average, 16% of the total activity. PTU-insensitive activity was significantly elevated 48 h after thyroidectomy, $P < 0.01$ (Fig. 5), and continued to increase gradually to 938±66 fmol I^-/mg protein per h in chronically (≥30 d after thyroidectomy) hypothyroid rats. PTU-insensitive activity in the latter animals was 87% of the enzyme activity measured in the absence of PTU. The difference between total and PTU-insensitive rT_3 5'-deiodination, i.e. PTU-sensitive activity, was 0.45 pmol/mg protein per h in pituitary homogenates from the euthyroid rats, compared to only 0.14 pmol/mg protein per h in homogenates from chronically hypothyroid rats.

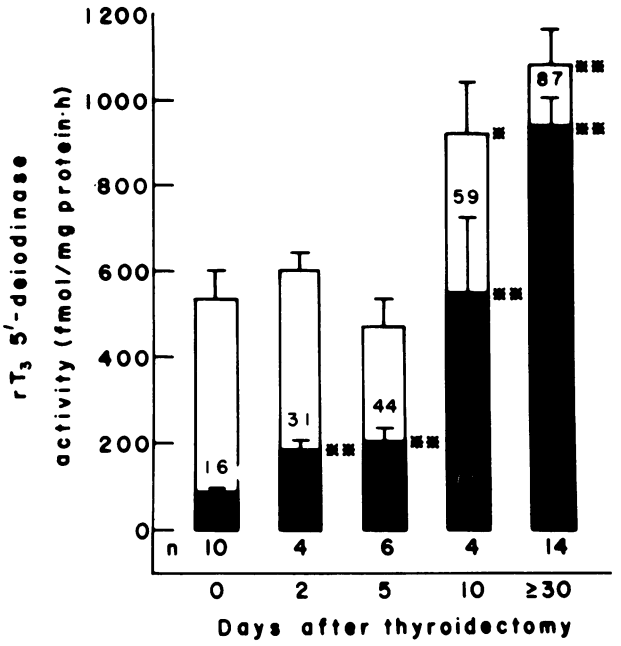

FIGURE 5 The rate of rT_3 5'-deiodination in rat pituitary homogenates as a function of time after thyroidectomy. Control rates (0 days after thyroidectomy are pooled values from sham-operated rats killed 2, 5 and 10 d after surgery, simultaneously with the 2-, 5-, and 10-d thyroidectomized animals. Pituitaries from each animal were homogenized separately. Rates were determined by assay A, performed in triplicate for each pituitary. Reaction mixtures contained 2 nM rT_3, 20 mM DTT, and no PTU (total bars) or 1 mM PTU (filled portions of bars). Numbers within the bars represent the mean rate in the presence of PTU as a percentage of that in the absence of PTU. Values are mean±SEM. $^*P < 0.05$ compared to corresponding (i.e. total or PTU-insensitive) value in euthyroid controls. $^{**}P < 0.01$.

Changes in deiodination rates at still shorter times after thyroidectomy and with T_4 or T_3 repletion were then investigated using both rT_3 and T_4 as substrates (Table II). Production of T_3 from tracer T_4 (~0.2 nM) was 0.22±0.03% of added $T_4/\mu g$ protein per h (mean±SE, n = 18) in the sham-controls. 1 and 2 d after thyroidectomy T_4 to T_3 conversion rates were increased to 183±31% of control (n = 6, $P < 0.05$) and 247±32% of control (n = 12, $P < 0.01$), respectively. Likewise, PTU-insensitive rT_3 5'-deiodination was elevated to 263 and 221% of control 24 and 48 h after thyroidectomy, though the value at 24 h was not significantly higher than in controls ($0.1 > P > 0.05$ by Dunnett's test). The increments in T_4 5'-deiodination and PTU-insensitive rT_3 5'-deiodination at 2 d were prevented by the administration of replacement doses of T_4 (0.8 μg/100 g body wt per d) or T_3 (200 ng/100 g body wt/twice daily) to the thyroidectomized rats. Injection of sham-controls with the same quantity of T_3 did not affect pituitary T_4 5'-deiodinase activity. None of these treatments significantly altered rT_3 5'-deiodination measured in the absence of PTU.

In rats thyroidectomized 12–18 d previously, mean T_3 production from 10 nM T_4 decreased as early as 4 h after injection of 20 and 200 μg T_3/100 g body wt to 29 and 1% of the hypothyroid control value, respectively (Table III). PTU-insensitive rT_3 5'-deiodination by pituitary tissue from thyroidectomized rats was also significantly suppressed to 16% of hypothyroid control values 4 h after 200 μg T_3/100 g body wt (Table III). Total rT_3 5'-deiodination was also significantly decreased 4 h after 200 μg T_3, but the difference between total and PTU-insensitive rT_3 5'-deiodination was not significantly altered by this T_3 dose: 33 fmol I^-/mg protein per h in the hypothyroid controls vs. 40 fmol I^-/mg protein per h in the T_3-treated animals.

DISCUSSION

The findings detailed above establish the presence of two pathways of iodothyronine 5'-deiodination in rat pituitary tissue, similar to the pathways we have described in rat brain (27, 28). Both pathways accept rT_3 as a substrate but 5'-deiodination of T_4, and inhibition of rT_3 5'-deiodination by T_4, are only detectable via a PTU-insensitive pathway.

TABLE II

Acute Effects of In Vivo Thyroid Hormone Depletion and Repletion on Pituitary Iodothyronine 5'-Deiodination

Treatment	T_4 5'-deiodination		rT_3 5'-Deiodination			
			Total		PTU-insensitive	
	n	*% euthyroid*	*n*	*% euthyroid*		*% total*
Euthyroid	18	100±13	10	100±12	100±6	16±1
24 h Tx	6	183±31*	5	146±20	263±56	22±9
48 h Tx	12	247±32‡	4	112±8	221±21‡	31±3‡
48 h Tx + T_4	4	101±28		N.D.		
48 h Tx + T_3	10	138±23	3	117±10	136±29	18±4
Euthyroid + T_3	8	110±15	4	138±21	129±21	15±2

Results are mean±SEM. Euthyroid rats were sham thyroidectomized. T_4 treatment was 800 ng per 100 g body wt s.c. 8 and 32 h after thyroidectomy. T_3 treatment was 200 ng per 100 g body wt s.c. 8, 14, 32, and 40 h after thyroidectomy. Rats were killed 48 h after sham operation or thyroidectomy, except the 24-h Tx group, which were killed 24 h after thyroidectomy. rT_3 5'-deiodination was measured at 2 nM rT_3 and 20 mM DTT, with I^- release quantitated by assay A. To measure PTU-insensitive activity, 1 mM PTU was added to the incubations. T_4 5'-deiodination was measured at 0.2 nM T_4 and 100 mM DDT, with reaction products quantitated by assay B. Tx, thyroidectomized rats, ND, rates were not determined. * $P < 0.05$ compared with euthyroid rats. ‡ $P < 0.01$. There was no significant difference between the 24- and 48-h sham-operated controls; therefore their results were pooled.

The PTU-sensitive pathway in pituitary, like PTU-sensitive iodothyronine 5'-deiodination in the cerebral cortex, is similar to the iodothyronine 5'-deiodinase activity in rat liver and kidney (25, 26). As in kidney, liver, and rat brain, deiodination follows ping-pong type kinetics with DTT as cosubstrate, and inhibition of this pathway by PTU is noncompetitive with respect to rT_3 but competitive with respect to DTT (Fig. 1). In cerebral cortex and kidney, this pathway is also inactivated by extremely low (1 μM) concentrations of iodoacetate, a sulfhydryl active reagent (26, 28). This evidence suggests the participation of a highly reactive sulfhydryl group in the reductive removal of the 5'-iodine, and, further, that the reaction mechanism comprises two half reactions: $E\text{-}SH + rT_3 \rightarrow E\text{-}SI + 3,3'\text{-}T_2$; $E\text{-}SI + DTT_{reduced} \rightarrow E\text{-}SH + DTT_{oxidized} + HI$.

As in the brain (27, 28), this PTU-sensitive pathway

TABLE III

Acute Effects of Supraphysiological Doses of T_3 on Pituitary Iodothyronine 5'-Deiodination

Treatment	T_4 5'-Deiodination		rT_3 5'-Deiodination			
			Total		PTU-insensitive	
	n	*% hypothyroid control*	*n*	*% hypothyroid control*		*% total*
Hypothyroid control	9	100±10	6	100±9	100±12	64±8
Hypothyroid + 20 μg T_3, 4 h	6	29±4*		N.D.		
Hypothyroid + 200 μg T_3, 4 h	11	1±0.3*	5	54±12‡	16±3*	10±2*

Rats were thyroidectomized 18–21 d before study. T_3 or vehicle was injected into the jugular vein under light ether anesthesia 4 h prior to death. Values are mean±SEM. Reaction conditions are as given in Table II. * $P < 0.01$. ND, not determined. ‡ $P < 0.05$ vs. hypothyroid control.

of in vitro rT_3 5′-deiodination prevails in pituitary tissue from euthyroid rats, even at substrate concentrations well below the K_m for rT_3, owing to the high V_{max} compared to that of the PTU-insensitive pathway. The substrate specificity of the PTU-sensitive pathway in pituitary is not entirely clear. Under our experimental conditions, 5′-deiodination of T_4 via this pathway was not detectable, nor was deiodination of 2 nM rT_3 inhibited by 100 nM T_4 (Fig. 2). However, in light of the difference between tissues in rT_3 5′-deiodination rates, these would be expected findings if V_{max}/K_m ratios for PTU-sensitive 5′-deiodination of rT_3, and T_4 were similar in pituitary as in liver and kidney (25, 26).

The PTU-insensitive 5′-deiodinating pathway in the pituitary is similar to that we have recently identified in rat cerebral cortex (27, 28). The apparent K_m for T_4 and rT_3 are similar in both tissues, <5 nM. Each of these iodothyronines is a competitive inhibitor of 5′-deiodination of the other, with similar apparent K_m and K_i: 0.6–0.9 nM and 1.3 nM for T_4, and 2.9 and 1.7 nM for rT_3 in pituitary microsomes. as detailed above. In cerebral cortex, this pathway is not affected by PTU or 100 μM iodoacetate, and the reaction follows sequential type kinetics as the DTT concentration is varied (27, 28), findings similar to those in pituitary microsomes (Fig. 4). This kinetic pattern suggests that both the iodothyronine and the thiol combine with the enzyme before the reaction takes place, and that the iodine removed from the iodothyronine is not transferred to an enzyme sulfur, but rather, perhaps, to the cofactor −SH group: $E + T_4 + DTT_{reduced} \rightarrow E\text{-}T_4\text{-}DTT \rightarrow \rightarrow E + T_3 + DTT_{oxidized} + HI$. Other, more complex, reaction mechanisms are also possible (34).

As in brain tissue (27, 28), the PTU-insensitive pathway of rT_3 5′-deiodination predominates in microsomes from hypothyroid rats when the rT_3 concentration is 2 nM. This is due both to an increase in the activity of the PTU-insensitive pathway and a decrease in the activity of the PTU-sensitive pathway. However, because the V_{max} of the PTU-sensitive pathway is severalfold higher than that of the PTU insensitive pathway (even in hypothyroid tissue), an increase of the rT_3 concentration in the assay to ≃10 nM or higher would make the PTU-insensitive pathway difficult to detect.

The relationship between the two pathways of 5′-deiodination in pituitary and brain is unclear. Possibly, they represent two different enzymes. If so, the similarities in the properties of the PTU-sensitive pathway in pituitary, brain, liver, and kidney suggest the possibility that a single enzyme is present in all of these tissues, with ~40-fold higher concentrations in pituitary than in cerebrocortical microsomes, and 1,000–2,000-fold more in liver and kidney microsomes than in pituitary (25–28). Another possibility is that the two 5′-deiodination pathways correspond to different forms of the same enzyme. This possibility is suggested by the similarity in subcellular distribution of the two activities in brain tissue (35). Purification of the deiodinating activities will be necessary to distinguish between these possibilities.

In vivo administration of PTU has no effect on in vivo intrapituitary conversion of T_4 to T_3 (8), as is true also for cerebral cortex and cerebellum (36). Likewise, PTU has no effect on T_4 5′-deiodination by pituitary tissue in vitro [(12, 17) and present results]. In contrast 5′-deiodination of rT_3 by pituitary tissue in vitro is inhibited by pretreatment of rats in vivo with PTU (36). It therefore appears that the PTU-insensitive 5′-deiodinating pathway is responsible for all of the locally produced T_3 in rat pituitary, which amounts to >50% of the T_3 in that tissue. This is in marked contrast to the inhibitory effect of PTU on T_4 to T_3 conversion in liver and kidney (18–20). The physiological significance of the PTU-sensitive 5′-deiodination pathway in the pituitary or brain, is not yet known. If it is an altered form of the PTU-insensitive enzyme, it could serve as a reservoir of latent enzyme, available for transformation into PTU-insensitive activity, which could then produce T_3.

We previously reported increases in pituitary T_4 5′-deiodinase activities in chronically hypothyroid rats (11, 12), recently confirmed by Maeda and Ingbar (13). They observed an increase in T_4 5′-deiodination by rat hemipituitaries by 24 h (but not 4–6 h) after thyroidectomy, with further increases at 7 and 14 d. We have reported such a rapid increase for cerebral cortex 5′-deiodinase after thyroidectomy (29). Our present results for the time course of the increase of PTU-insensitive rT_3 5′-deiodination and T_4 5′-deiodination in pituitary tissue are similar (Fig. 5 and Table II). In addition, we found that the increase in activity is selective for the PTU-insensitive process: the PTU-sensitive activity showed a gradual decrease after thyroidectomy, like that observed for hepatic iodothyronine 5′-deiodination (13, 29). We have also noted decreases in pituitary T_4 5′-deiodination in thyroid hormone-treated rats (11, 12). Maeda and Ingbar found a decrease of ~50% in T_4 5′-deiodination 4 h after treatment of hypothyroid rats with 1.5 μg/T_3 per 100 g body wt (13). Though assay methods were not identical, comparison of their results with those in Table III suggest that the higher T_3 doses used here caused an even greater reduction in PTU-insensitive 5′-deiodination, and that an effect of T_3 treatment might well be demonstrable after shorter time intervals. If so, the reduction in PTU-insensitive 5′-deiodination may be as rapid a response to T_3 as is inhi-

bition of thyrotropin release, which can be demonstrated by 1 h after T_3 or T_4 (37). Physiological replacement doses of either T_4 or T_3 prevented the increase in PTU-insensitive 5′-deiodination 48 h after thyroidectomy (Table II), suggesting that control of this enzyme activity is not a unique property of the T_4 molecule itself.

In conclusion, we have demonstrated two pathways for the 5′-deiodination of iodothyronines in rat pituitary. The processes differ with respect to substrate specificity, susceptibility to PTU-inactivation and reaction kinetics, probably reflecting distinct enzyme entities with different catalytic mechanisms. Moreover, opposite changes in these two enzyme activities are observed in this tissue in response to thyroid hormone depletion and repletion. It is conceivable that these compensatory changes prevent large fluctuations in intracellular T_3, and thereby GH secretion rates, in the face of variations in plasma thyroid hormone concentrations. The benefits of such a mechanism in the regulation of thyrotroph function, however, is less clear, or even doubtful, inasmuch as it would appear to interfere with the optimal negative feedback control of TSH secretion. The possible association of the PTU-insensitive 5′-deiodinase with specific cell populations in the anterior pituitary and the mechanism of regulation of these enzyme activities by thyroid hormone, are important subjects for future investigations.

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