

Phenolic and Tyrosyl Ring Deiodination of Iodothyronines in Rat Brain Homogenates

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Research Article

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In cerebral cortical homogenates from normal rats, products of T_4 degradation were as follows (percent added $T_4 \pm$ SEM in nine experiments): T_3 , 1.9 \pm 0.5%; 3,3',5'-triiodothyronine (rT_3), 34.0 \pm 2.4%; 3,3'-diiodothyronine (3,3'- T_2), 5.8 \pm 1.6%; 3'-iodothyronine (3'- T_1), \leq 2.5%; and excess I^- , 4.7 \pm 1.2%. In the same experiments, products of T_3 degradation were 3,3'- T_2 , 63.3 \pm 5.5%, and 3'- T_1 , 12.6 \pm 1.4%. Cerebral cortical homogenates from hyperthyroid rats and normals were similar in [...]

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ABSTRACT Conversion of thyroxine (T_4) to 3,5,3'-triiodothyronine (T_3) in rat brain has recently been shown in *in vivo* studies. This process contributes a substantial fraction of endogenous nuclear T_3 in the rat cerebral cortex and cerebellum. Production of T_4 metabolites besides T_3 in the brain has also been suggested. To determine the nature of these reactions, we studied metabolism of 0.2–1.0 nM [125 I] T_4 and 0.1–0.3 nM [131 I] T_3 in whole homogenates and subcellular fractions of rat cerebral cortex and cerebellum. Dithiothreitol (DTT) was required for detectable metabolic reactions: 100 mM DTT was routinely used. Ethanol extracts of incubation mixtures were analyzed by paper chromatography in *t*-amyl alcohol:hexane:ammonia and in 1-butanol:acetic acid. Rates of production of iodothyronines from T_4 and T_3 were greater at pH 7.5 than at 6.4 or 8.6 and greater at 37°C than at 22° or 4°C. Lowering the pH, reducing the protein or DTT concentrations, and preheating homogenates to 100°C all increased excess I^- production but reduced iodothyronine production.

In cerebral cortical homogenates from normal rats, products of T_4 degradation were as follows (percent added $T_4 \pm$ SEM in nine experiments): T_3 , $1.9 \pm 0.5\%$; 3,3',5'-triiodothyronine (rT_3), $34.0 \pm 2.4\%$; 3,3'-diiodothyronine (3,3'- T_2), $5.8 \pm 1.6\%$; 3'-iodothyronine (3'- T_1), $\leq 2.5\%$; and excess I^- , $4.7 \pm 1.2\%$. In the same experiments, products of T_3 degradation were 3,3'- T_2 , $63.3 \pm 5.5\%$, and 3'- T_1 , $12.6 \pm 1.4\%$. Cerebral cortical homogenates from hyperthyroid rats and normals were similar in regard to T_4 to T_3 deiodination. In contrast, in cerebral cortical homogenates from hypothyroid rats, phenolic ring deiodination rates were increased and tyrosyl ring

deiodination rates were decreased compared with normals.

T_4 to T_3 conversion rates in cerebellar homogenates were greater than rates in cerebral cortical homogenates from the same normal rats and less than rates in cerebellar homogenates from hypothyroid rats. T_4 and T_3 tyrosyl ring deiodination rates were greatly diminished in cerebellar homogenates compared with cerebral cortical homogenates in normal and hypothyroid rats. High-speed (1,000–160,000 g) pellets from cerebral cortical homogenates were enriched in phenolic and tyrosyl ring deiodinating activities relative to cytosol. Fractional conversion of T_4 to T_3 was inhibited by T_4 , iopanoic acid, and rT_3 , but not by T_3 . Tyrosyl ring deiodination reactions were inhibited by T_3 , T_4 , and iopanoic acid, but not by rT_3 .

These studies demonstrate separate phenolic and tyrosyl ring iodothyronine deiodinase enzymes in rat brain. The brain phenolic ring deiodinase serves *in vivo* as a T_4 5'-deiodinase and closely resembles anterior pituitary T_4 5'-deiodinase in physiological and biochemical characteristics. The physiological significance of the tyrosyl ring iodothyronine deiodinase enzyme is unclear; it shares several properties with rat hepatic T_4 5-deiodinase.

INTRODUCTION

In clinical medicine, the brain is clearly responsive to excessive and deficient supplies of thyroid hormones. Congenital hypothyroidism is accompanied by morphological abnormalities of the cerebral cortex and cerebellum and by corresponding derangements of central nervous system function in man and other species (1–5) (see [1–4] for recent reviews). Both hyperthyroidism and hypothyroidism cause abnormalities of cognitive function in adult patients (6, 7). The molecular events necessary for the expression of thyroid hormone effects on the brain remain poorly understood. Schwartz and Oppenheimer (8, 9) identified nuclear

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3,5,3'-triiodothyronine (T_3)¹ binding sites in rat brain cells with physical properties similar to those of nuclear T_3 receptors in other rat tissues. They found differences in the maximal nuclear T_3 binding capacity in different areas of the brain (9), and they and others have described changes in cortical and cerebellar nuclear T_3 binding with age in newborn rats (10, 11). Dratman et al. (12) found localization of [¹²⁵I] T_3 within synaptosomal vesicles from whole rat cerebral hemispheres after in vivo injection of [¹²⁵I] T_3 . Dozin-Van Roye and DeNayer (13) demonstrated saturable binding of T_3 to brain cytosol proteins in vitro. The physiological significance of these observations is not yet known.

There is evidence for metabolism of thyroid hormones in brain tissue in vivo. Dratman and Crutchfield (14) injected [¹²⁵I]-thyroxine ([¹²⁵I] T_4) to thyroidectomized rats given T_4 replacement. In brain homogenates they found T_3 , which was most abundant in the synaptosome fraction, and small quantities of two other compounds, one identified as 3,3',5'-triiodothyronine (rT_3) and the other as a common metabolite of T_4 and T_3 . Vigouroux et al. (15) identified [¹²⁵I] T_3 in extracts of rat brain after in vivo administration of [¹²⁵I] T_4 . Obregon et al. (16) administered [¹³¹I] T_4 and [¹²⁵I] T_3 simultaneously, and found that brain tissue had a higher [¹³¹I] T_3 : [¹²⁵I] T_3 ratio than liver, kidney, heart, and skeletal muscle, suggesting local production in brain. Crantz and Larsen (17) injected normal rats with [¹²⁵I] T_4 and [¹³¹I] T_3 in vivo and isolated cell nuclei from the cerebral cortex and cerebellum. They found that a substantial fraction of nuclear T_3 in these regions was produced locally and that in vivo iopanoic acid treatment abolished the appearance of locally produced nuclear T_3 .

In vitro studies of metabolism of thyroid hormones in brain tissue have also been reported. Tata et al. (18) found degradation of [¹³¹I] T_4 and [¹³¹I] T_3 in brain homogenates of chickens and dogs. They tentatively identified tetraiodothyroacetic acid and triiodothyroacetic acid, or the analogous propionic acid derivatives, as minor products. They also found that small amounts of degraded T_4 sometimes appeared as T_3 , but iodide constituted the great bulk of the identifiable T_4 degradation products in their studies. We (19, 20) and Chopra (21) previously tested brain tissue for T_4 5'-deiodinase activity but found little or no change in radioimmunoassayable T_3 in ethanolic extracts of the homogenates. In these experiments, it is possible that significant T_4 to T_3 conversion occurred in brain homogenates at rates lower than those found in homog-

enates of other tissues (19–21), but was difficult to detect against the background of T_3 contaminating the large quantities of T_4 used.

To establish the biochemical characteristics of T_4 5'-deiodination in brain, we have reexamined T_4 metabolism in vitro, using a sensitive radiometric technique for identification and quantitation of reaction products. We investigated the effects of hypothyroidism and hyperthyroidism, since both of these conditions stimulate T_4 5'-deiodinase activity in some tissues (20, 22, 23). Initial experiments disclosed several degradation products of T_4 besides T_3 in brain homogenates. We have attempted to identify those substances and characterize the factors that influence their production.

METHODS

Animals and reagents. Adult male Sprague-Dawley rats (Zivic Miller Laboratories, Allison Park, Pa.) were used in all experiments. Hypothyroid rats were supplied thyroidectomized with parathyroid implants. Hypothyroidism was judged by a weight gain of <100 g in 60 d after surgery; intact rats of the same age gain 210–300 g in 56 d (data from supplier). Rats meeting this criterion have had uniformly low serum T_4 and high serum thyrotropin concentration in past studies (24), although serum hormone levels were not routinely measured in the present studies. In one experiment, normal rats were made hyperthyroid by injection of T_4 , 10 μ g/100 g body wt s.c. daily for 5 d and sacrificed 24 h after the last injection. In this experiment, the mean (\pm SD) serum levels for the normal and hypothyroid rats (both vehicle injected) and the hyperthyroid rats were as follows: T_4 , 4.8 ± 0.4 , 1.3 ± 1.0 , and 13.3 ± 1.9 μ g/dl; thyrotropin, 190 ± 30 , 864 ± 365 , and 30 ± 10 μ U/ml.

Levorotatory iodothyronines were used exclusively. [¹²⁵I] T_4 was purchased from New England Nuclear, Boston, Mass., or synthesized in our laboratory by the method of Weeke and Orskov (25). [¹³¹I] T_3 and [¹³¹I] rT_3 were synthesized in our laboratory by the same method. Tracers contained <10% I⁻. Non-radioactive iodothyronines were obtained from Henning GmbH, Berlin, and the Sigma Chemical Co., St. Louis, Mo. Iopanoic acid (Telepaque[®], 3-[3-amino-2,4,6-triiodophenyl]2-ethylpropionic acid) was generously donated by Mr. A. E. Soria, Sterling-Winthrop Research Institute, Rensselaer, N. Y. Other reagents and solvents were purchased from Fisher Scientific Co., Pittsburgh, Pa. and Sigma Chemical Co.

Preparation of homogenates. Rats were decapitated under ether anesthesia and the brains removed. Pieces of cerebral cortex, free of white matter, and the whole cerebellums were dissected with a fine scissors, weighed, and homogenized as described (20) in 9 vol ice-cold 0.05 M Tris, pH 7.6–0.25 M sucrose–100 mM dithiothreitol (DTT). The pH of the homogenates was 7.5 at 37°C. Unless noted, tissues from three rats were pooled to make the homogenates. In experiments testing the effects of thiol concentrations, tissues were homogenized in 8 ml/g Tris-sucrose without DTT; then 1 ml/g tissue of various DTT or GSH solutions was added to homogenate aliquots. In fractionation studies, homogenates were centrifuged at 1,000 g, and the resulting supernate was centrifuged for 45 min at 38,000 rpm in a Beckman model L centrifuge (Beckman Instruments, Fullerton, Calif.) with an SW 50 1 rotor. The low- and high-speed pellets were resuspended to the original volume in Tris-sucrose-100 mM DTT as described (20). The high-speed pellet was centrifuged again at 38,000

¹ Abbreviations used in this paper: BAA, 1-butanol:acetic acid:water::78:5:17; DTT, dithiothreitol; TAA, t-amyl alcohol:hexane::2 N ammonia::5:1:6 (upper phase); T_4 , thyroxine; T_3 , 3,5,3'-triiodothyronine; rT_3 , 3,3',5'-triiodothyronine; 3,3'- T_2 , 3,3'-diiodothyronine; 3',5'- T_2 , 3',5'-diiodothyronine; 3'- T_1 , 3'-iodothyronine.

rpm for 45 min and finally suspended to the original volume in Tris-sucrose-100 mM DTT.

Incubation procedure. Incubations were carried out under N_2 at 37°C as detailed previously (20). 90 μ l of homogenate or Tris-sucrose-DTT solution was mixed with 10 μ l of substrate solution to start the reactions. 10 μ l of the substrate solution contained ~40,000 cpm [125 I]T₄ (1,100–2,500 μ Ci/ μ g) and 6,000–20,000 cpm [131 I]T₃ (~3000 μ Ci/ μ g), equal to 0.2–1.0 nM T₄ and 0.1–0.3 nM T₃ (these and all other concentrations are given as concentrations in the incubation mixture at the start of the incubations). T₄ and T₃ concentrations in these ranges were far below saturating concentrations, so that fractional degradation rates were not concentration dependent and data from different experiments could be compared. Other compounds were added to tracer solutions before the tracers were added to homogenates. Incubations were stopped by addition of 200 μ l ethanol plus 50 μ l 0.04 N NaOH containing 50 μ g T₄, 50 μ g T₃, and 285 μ g methimazole. The tubes were then centrifuged, yielding a pellet ~25 μ l volume. 89–97% of both tracer T₄ and tracer T₃ were recovered into the ethanolic supernate.

Analytical methods. 75–125 μ l of the ethanolic extracts was mixed with carrier T₄, T₃, and NaI, and subjected to descending paper chromatography using the t-amyl alcohol:hexane:2 N ammonia:5:1:6 (TAA) system of Bellarbarba et al. (26). Carriers were identified by spraying with color reagents.² In some experiments, various peaks of radioactivity were eluted from unsprayed chromatograms with methanol:ammonia (99:1). These were concentrated under a stream of nitrogen, mixed with carrier compounds, and chromatographed again either in the TAA system or in a descending paper system of 1-butanol:acetic acid:water (78:5:17) (BAA) (27).

In experiments designed to identify reaction products, chromatograms were cut into 1-cm segments. In subsequent experiments, TAA chromatograms were cut into 10 segments. The I⁻, T₄, and T₃ carrier spots were in segments 2, 5, and 9, respectively. Segments 1–9 were ~2.5 cm in length, while segment 10 was between T₃ and the front. Changes in 125 I and 131 I in segments 6–8 were attributed to 3,3'-diiodothyronine (3,3'-T₂), changes in 125 I in segments 3 and 4 were attributed to rT₃ and changes in 131 I in segments 4 and 5 were attributed to 3'-iodothyronine (3'-T₁) (see Results).

Phenolic ring monodeiodination of [125 I]T₄ or [125 I]rT₃ will produce equimolar amounts of 125 I⁻ and [125 I]T₃, or [125 I]3,3'-T₂, respectively. This is true whether one or two 125 I atoms are present on the phenolic ring of T₄ or rT₃. [125 I]3,3'-T₂ and [125 I]3'-T₁, produced from newly generated [125 I]T₃, will also be matched by equal amounts of 125 I⁻ produced along with the intermediate [125 I]T₃. 125 I⁻ appearing in excess of [125 I]T₃ plus [125 I]3,3'-T₂ is designated excess I⁻. Excess I⁻ includes I⁻ corresponding to [125 I]3'-T₁ production (since that compound was not routinely separable from [125 I]T₄ or [125 I]rT₃), I⁻ produced by phenolic ring deiodination of [3,3']T₂ and 3'-T₁, I⁻ produced by the process observed in the boiled homogenates (see Results) and 3',5'-diiodothyronine (3',5'-T₂), which cochromatographs with iodine (28).

² An Appendix has been deposited with the National Auxiliary Publication Service (NAPS) as NAPS document 03696. This information may be ordered from ASIS/NAPS, Microfiche Publications, P. O. Box 3513, Grand Central Station, New York 10017. Remit in advance, in U. S. funds, \$3.00 for microfiche copy or for photocopy, \$5.00 up to 20 pages plus 25¢ for additional pages. Outside the U. S. and Canada add postage of \$3.00 for photocopy and \$1.00 for microfiche. Checks should be made payable to Microfiche Publications.

Paper electrophoresis employed a Durrum-type cell (Beckman Instruments, Inc.) in 0.3 M glycine-0.15 M acetate buffer, pH 8.6. Protein was measured in triplicate by the method of Lowry et al. (29). Since DTT strongly interferes in the protein assay, homogenate aliquots and bovine serum albumin standards were precipitated with 5 ml 7% perchloric acid, pelleted, washed with the same amount of perchloric acid, pelleted again, and dissolved in 0.1 N NaOH.

Calculations. Each experiment included extracts of Tris-sucrose-100 mM DTT made immediately after addition of tracer (zero time) and incubations with Tris-sucrose-100 mM DTT for 2 h. The percent radioactivity in each segment of the chromatograms from the 2 h incubations with Tris-sucrose-100 mM DTT was subtracted from the percent radioactivity in the corresponding segments from incubations containing brain tissue. This figure for each chromatogram segment was divided by the percent radioactivity in the zero-time [125 I]T₄ or [131 I]T₃ spots, as appropriate, to yield the fraction of added T₄ or T₃ degraded or the fractions of added T₄ to T₃ converted by the tissue to the various reaction products. Measured quantities of 5'-deiodination products of T₄ were doubled to correct for simultaneous 125 I⁻ production. Statistical comparisons were made using the *t* test for paired or unpaired values as appropriate, and by two-way analysis of variance, using the statistical package of Hewlett-Packard 9815A programmable calculator (Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Characterization of reaction products. The distributions of radioactivity on chromatograms of zero-time extracts of cerebral cortical homogenates and of Tris-sucrose-100 mM DTT were indistinguishable from the distribution of radioactivity of chromatograms of original tracer solutions. In these chromatograms, peaks of 125 I radioactivity were found only in the I⁻ and T₄ carrier spots, and peaks of 131 I radioactivity were found only in the I⁻ and T₃ carrier spots. There was no evidence of formation, during extraction or thereafter, of T₄ or T₃ esters or of complexes of T₄ to T₃ with ethanol-soluble lipids. After 2–h incubations with Tris-sucrose, with Tris-sucrose-100 mM DTT in the absence of tissue, or with whole cerebral cortical homogenates from normal or hypothyroid rats without DTT, the percent of total 125 I and 131 I radioactivity in the iodothyronine spots was 1–5% lower than that at zero time. Approximately half of this change was accounted for by an increase in radioactivity in the iodide spot, but the rest of the loss of starting material was not matched by the appearance of radioactivity in any other discrete area of the chromatograms. In particular, in these incubations, <1% of added [125 I]T₄ radioactivity appeared in the T₃ spot after 2 h.

Incubation of homogenates of cerebral cortical tissue from normal rats with [125 I]T₄ and [131 I]T₃ in the presence of 100 mM DTT produced five reaction products (Fig. 1): 125 I⁻; [125 I]T₃; a T₄ metabolite migrating in the TAA system between I⁻ and T₄ (compound A); a T₃ metabolite migrating in the TAA system slightly slower than T₄ (compound B); and a common metabolite of T₄ and T₃ migrating in the TAA system midway between T₄ and

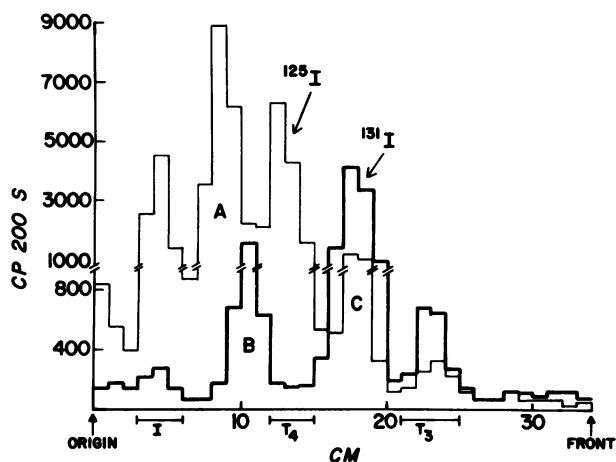


FIGURE 1 Representative TAA chromatogram of ethanolic extract of cerebral cortical homogenate from normal rats incubated for 2 h at 37°C with 0.9 nM [^{125}I]T₄, 0.1 nM [^{131}I]T₃, and 100 mM DTT. The thin line indicates ^{125}I radioactivity and the bold line indicates ^{131}I radioactivity in each 1-cm chromatogram segment. There is no correction in this figure for radioactivity in the starting material. Lines under the abscissa indicate positions of the stained carrier I⁻, T₄, and T₃ spots on this chromatogram.

T₃ (compound C). I⁻ was always separated by at least 1.5 cm from the origin in the TAA system, and appearance of statistically significant quantities of radioactivity at the origin was not observed in the chromatograms. Percent recovery of total ^{125}I and ^{131}I radioactivity in the incubation mixtures into the liquid phase did not change over time: in a representative experiment, recovery of ^{125}I was 95 ± 2 and $97 \pm 3\%$, and recovery of ^{131}I was 95 ± 3 and $96 \pm 1\%$ at 0 and 2 h, respectively. This observation excludes the formation in the incubation mixtures of substantial amounts of ethanol-insoluble, proteinaceous, iodinated "origin material."

Compound A was eluted from several chromatograms, pooled, and rechromatographed in the BAA and TAA system. In both systems, compound A radioactivity cochromatographed with rT₃.² Compound B was eluted and rechromatographed with T₄ standards and with 3'-T₁ standards. Compound B was found to cochromatograph in both systems with 3'-T₁. In the BAA system, compound B was distinct from T₄: R_f of 3'-T₁ and compound B = 0.73, R_f of T₄ = 0.83. Compound C was eluted and rechromatographed in both systems with 3,3'-T₂ standards and with tetraiodothyroacetic acid standards. In both systems, all of the ^{125}I and ^{131}I radioactivity coincided with the 3,3'-T₂ standard. In the BAA system, tetraiodothyroacetic acid, R_f = 0.92, was completely distinct from 3,3'-T₂, R_f = 0.77, and there was no radioactivity in the tetraiodothyroacetic acid spot above background. In some experiments, the formation of 3',5'-T₂ seemed possible. This compound is not

separable from I⁻ in the TAA system (28) and not separable from T₄ in the BAA system (27); however, apparent I⁻ production in individual samples could be compared in both systems. When this was carried out for incubation samples suspected of containing 3',5'-T₂, there was no significant difference by which the presence of 3',5'-T₂ could be inferred. There was never any radioactivity of TAA chromatograms compatible with the formation of triiodothyroacetic acid from T₄ or T₃ (27).

Effects of varying incubation pH, temperature, and time. When the pH of the incubations was adjusted to 6.4 or 8.6, the T₄ to T₃ and T₃ to 3,3'-T₂ conversion rates were <50% of the rates at pH 7.5. Likewise, there was a progressive decrease in the rates of these reactions when the temperature was lowered to 22° and 4°C. Heating the homogenates or high-speed pellets in a boiling water bath for 30 min abolished detectable production of any iodothyronines from T₄ or T₃. When whole cerebral cortical homogenates from normal and from hypothyroid rats were sampled every 30 min for 120 min, plots of log (percent added T₄ or T₃ remaining) vs. time were linear. Thus, the fractional degradation rates of T₄ and T₃ were constant during this period. In the incubations with [^{131}I]T₃, using normal tissue, substantial quantities of [^{131}I]3,3'-T₂ appeared at 30 min, before any detectable [^{131}I]3'-T₁, and the ratio of [^{131}I]3'-T₁ to [^{131}I]3,3'-T₂ increased progressively thereafter. In the incubations with [^{125}I]T₄, using hypothyroid tissue, substantial quantities of [^{125}I]T₃ and [^{125}I]rT₃ appeared at 30 min, when there was barely detectable [^{125}I]3,3'-T₂. In the latter incubations, the ratios of [^{125}I]3,3'-T₂ to both [^{125}I]triiodothyronines increased progressively over the subsequent 90 min.

Excess I⁻ production. Under normal incubation conditions, 0–6% of the [^{125}I]T₄ and 0–2% of the [^{131}I]T₃ was converted to excess I⁻ (e.g., see Table I and Fig. 2). The following alterations in incubation conditions all increased excess I⁻ production up to 40% of the added T₄ and 10% of the added T₃: pH 6.5, decreased protein concentration (particularly in the high-speed pellet), decreased DTT concentration, and preheating to 100°C for 30 min. All of these conditions caused greatly decreased net formation of iodothyronines, and formation of I⁻ from T₃ was never rapid enough to explain more than a small part of the excess I⁻ from T₄. When I⁻ production was analyzed by BAA and TAA chromatography and by paper electrophoresis in incubation mixtures preheated to 100°C, results from all three systems were comparable. In one dilution experiment and in one experiment with 100°C preheating, parallel incubations with [^{125}I]T₄ plus [^{131}I]T₃, and [^{125}I]T₄ plus [^{131}I]rT₃ resulted in ~10% of the T₄ converted to excess I⁻, while simultaneous I⁻ production via T₃ and rT₃ intermediates, taken together, could account for <1% of the added T₄.

Products of T₄ and T₃ degradation in homogenates of cerebral cortex from hypothyroid, euthyroid, and hy-

TABLE I

Products of T₄ and T₃ Metabolism in Homogenates of Cerebral Cortex and Cerebellum of Normal and Hypothyroid Rats

Deiodination products	Cerebral cortex		Cerebellum		P, cortex vs. cerebellum	
	Normal (9 experiments)	Hypothyroid (6 experiments)	Normal (6 experiments)	Hypothyroid (3 experiments)	Normal	Hypothyroid
	mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM		
T ₄ degraded (%)	46.1±3.5	61.7±3.5*	19.5±4.0	40.1±4.3*	<0.001	NS
T ₄ degradation products (% added T ₄)						
T ₃	1.9±0.5	32.0±4.8‡	14.3±2.7	39.6±4.7§	<0.001	NS
3,3'-T ₂	4.4±1.2	15.1±3.0*	1.9±0.6	0.2±0.2	<0.01	<0.001
rT ₃	34.0±2.4	10.8±1.8‡	1.8±0.4	0.5±1.4	<0.001	<0.001
Excess I ⁻	4.7±1.2	3.5±0.8	1.1±0.3	2.9±2.3	<0.01	NS
T ₃ degraded (%)	73.3±4.2	37.7±5.7‡	6.2±2.1	2.8±0.4	<0.001	<0.001
T ₃ degradation products (% added T ₃)						
3,3'-T ₂	63.3±3.5	32.9±4.1‡	5.0±0.9	0.7±1.0 [¶]	<0.001	<0.002
3'-T ₁	12.6±1.4	3.3±0.5‡	0.8±0.4	0.3±0.2	<0.001	NS
I ⁻	0.1±0.1	0.8±0.3	0.9±0.4	0.1±0.1	NS	NS
3'-T ₁ /3,3'-T ₂	0.21±0.03	0.10±0.01 [¶]				
Protein concentration (mg/ml)	12.5±1.9	10.7±1.4	13.1±0.6	8.0±1.6*	NS	NS

In each experiment, pooled homogenates from cerebral cortex or cerebellum of three rats were incubated in triplicate for 2 h at 37°C with 0.2–1.0 nM [¹²⁵I]T₄, 0.1–0.3 nM [¹³¹I]T₃, and 100 mM DTT. Values are means of individual experimental means. Comparisons between normal and hypothyroid tissue were made by the unpaired *t* test. Comparisons between cerebellum and cortex were made by two-way analysis of variance since, in each experiment with cerebellar tissue, values for cerebral cortical tissue from the same rats were available.

* *P* < 0.02 vs. normal tissue from same region.

‡ *P* < 0.001.

§ *P* < 0.005.

¶ *P* < 0.05.

perthyroid rats. In early experiments, using homogenates of cerebral cortex from normal rats, net production of small quantities of [¹²⁵I]T₃ from [¹²⁵I]T₄ was observed. Because we were interested most in T₃ production, we sought a system in which it was more readily studied. Alterations in thyroid status were potentially useful, in view of their effects of pituitary and hepatic T₄ 5'-deiodination (20, 23, 24). Because differences in endogenous T₄ could alter the extent of T₄ degradation in homogenates, incubations were carried out in the presence and absence of 100 nM [¹²⁷I]T₄, a concentration much higher than any conceivably due to endogenous T₄.

Results obtained using cerebral cortical homogenates from normal, hyperthyroid, and hypothyroid rats are shown in Fig. 2. The protein concentrations of the incubation mixtures (mean±SD) were 10.3±0.8, 10.5±0.2, and 9.7±0.3 mg/ml in the euthyroid, hyperthyroid, and hypothyroid groups, respectively. In homogenates of cerebral cortex from normal and hyperthyroid rats (Fig. 2, center and right) the major [¹²⁵I]T₄ degradation product was [¹²⁵I]rT₃. There were also small quantities of [¹²⁵I]T₃, [¹²⁵I]3,3'-T₂, and excess ¹²⁵I⁻. In incubation

mixtures from normal and hyperthyroid rats the estimated upper limit for production of both 3',5'-T₂ and 3'-T₁ was 2.5% of the added T₄ in 2 h. In the incubations of normal and hyperthyroid cerebral cortex, the only significant effect of increasing the T₄ concentration from 0.2 to 100 nM was to decrease the total degradation of T₄ in the normal group from 33.2±7.3 to 27.1±6.8% (*P* < 0.05 by paired *t* test).

In homogenates of cerebral cortex from hypothyroid rats (Fig. 2, left), substantial quantities of [¹²⁵I]T₃ and smaller quantities of [¹²⁵I]3,3'-T₂ and [¹²⁵I]rT₃ were produced from [¹²⁵I]T₄. When the T₄ concentration was increased from 0.2 to 100 nM, the fractional degradation of T₄ decreased from 48.8±6.3 to 28.8±6.4% (*P* < 0.001), largely due to a decrease in T₃ production from 30.0 to 15.3% of the added T₄ (*P* < 0.05). Percent conversion of T₄ to the other products was not significantly different at the two T₄ concentrations. The greater fractional T₄ to T₃ conversion in the hypothyroid preparation with 100 nM T₄ than in the other homogenates with only tracer T₄ shows that T₃ production in cerebral cortical homogenates from normal and hyperthyroid rats was not obscured by endogenous T₄.

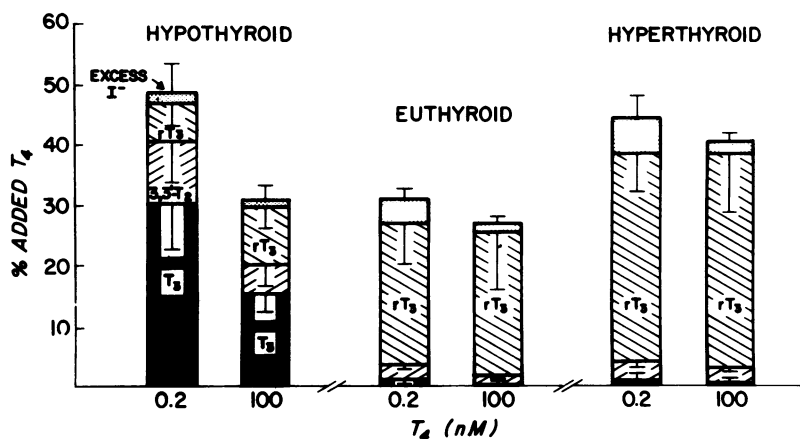


FIGURE 2 Effect of thyroid status on deiodination of [¹²⁵I]T₄ in cerebral cortical homogenates. In this experiment tissue from each rat was homogenized and incubated separately. Three intact rats received 10 μg T₄/100 g body wt. s.c. daily × 5 to render them hyperthyroid, while three intact and three thyroidectomized rats received the T₄ vehicle in the same schedule. All the rats were sacrificed at the same time and the incubations (one tube per rat) were performed simultaneously. Incubations were performed at 37°C for 2 h with 0.2 nM [¹²⁵I]T₄, with and without 100 nM [¹²⁷I]T₄, 0.1 nM [¹³¹I]T₃, and 100 mM DTT. Vertical lines show 1 SD. ■, T₃; ▨, 3,3'-T₂; ▩, rT₃; and ▩, excess I⁻.

The rates of T₃ degradation, 3,3'-T₂ production, and 3'-T₁ production were least in the homogenates from hypothyroid rats, greater in homogenates from euthyroid rats, and greatest in homogenates from hyperthyroid rats. The rates in the hyperthyroid group were two- to fourfold greater than those in the hypothyroid group. There was no effect on [¹³¹I]T₃ metabolism of increasing the T₄ concentration from 0.2 to 100 nM.

Inasmuch as there was clearly no enhancement of T₃ production in cerebral cortical homogenates of hyperthyroid rats, either normal or hypothyroid rats were used in subsequent experiments. The combined results of several experiments are shown in Table I. At tracer T₄ and T₃ concentrations, mean net production of T₃ from T₄ in cerebral cortical homogenates from normal rats was 1.9 ± 0.5% of added T₄, a low but significant rate. In cerebral cortical homogenates from hypothyroid rats, mean net T₃ production was 32%, much greater than in normals. Conversely, cerebral cortical homogenates from hypothyroid rats exhibited significantly less tyrosyl ring deiodination of T₄ and T₃ and, as judged by the difference in the [¹³¹I]3'-T₁/[¹³¹I]3,3'-T₂ ratios, less tyrosyl ring deiodination of 3,3'-T₂ than the cerebral cortical homogenates from the euthyroid rats.

To clarify the sources of [¹²⁵I]3,3'-T₂, we performed parallel incubations, using the same homogenates, with [¹²⁵I]T₄ plus [¹³¹I]T₃, and with [¹²⁵I]T₄ plus [¹³¹I]rT₃.² In cerebral cortical homogenates from both normal and hypothyroid rats, fractional conversion of T₃ to 3,3'-T₂ was greater than fractional conversion of rT₃ to 3,3'-T₂. Conversion of rT₃ to 3,3'-T₂ was greater in the ho-

mogenates from hypothyroid rats than from normals. Given the differences in availability of [¹²⁵I]T₃ and [¹²⁵I]-rT₃ during incubations using cortex from normal and hypothyroid rats (Table I), it could be concluded that T₃ served as the intermediate for much (in normals) or most (in hypothyroid rats) of the 3,3'-T₂ produced from T₄ in cerebral cortical homogenates. It was also clear, however, that rT₃ was the intermediate for some 3,3'-T₂ produced from T₄. Addition of 1 or 2 μM [¹²⁷I]T₃ inhibited [¹³¹I]T₃ degradation to <10% and increased the simultaneous net production of [¹²⁵I]T₃ from [¹²⁵I]T₄, without significantly changing the sum of net [¹²⁵I]T₃ plus net [¹²⁵I]3,3'-T₂ production.

In view of the foregoing results using [¹³¹I]rT₃ and high concentrations of [¹²⁷I]T₃, it appeared that total T₃ production rates in cerebral cortical homogenates from hypothyroid rats could be approximated well as the sum of net T₃ and net 3,3'-T₂ production. In two experiments using 100 nM initial T₄ concentration (one shown in Fig. 2), total T₃ production rates were 8.1 and 17.5 fmol T₃/min per mg homogenate protein. In these experiments, incubations with 1,000 nM T₄ were included: fractional T₄ to T₃ conversion was very low, and changed between 100 and 1,000 nM T₄ approximately in inverse proportion to the concentration. Therefore, 100 nM T₄ was probably greater than the apparent K_m for T₄ in this reaction, and the measured T₃ production rates at 100 nM T₄ approximated maximum reaction rates.

Iodothyronine deiodinating activities in cerebellar homogenates. The mean T₄ to T₃ conversion rate in the cerebellar homogenates from normal rats was con-

siderably greater than that in cerebral cortical homogenates from the normal rats (Table I). The mean T_4 to T_3 conversion rate in cerebellar homogenates from hypothyroid rats was the same as that in cerebral cortical homogenates from the hypothyroid rats, and much greater than in the cerebellar homogenates from the normal rats. In dramatic contrast, there was little or no T_4 to rT_3 conversion in cerebellar homogenates from normal or hypothyroid rats. T_3 to $3,3'$ - T_2 conversion was present in cerebellar homogenates from normal rats but was much slower than in cerebral cortical homogenates. T_3 to $3,3'$ - T_2 conversion was not detectable in cerebellar homogenates from hypothyroid rats.

Thiol dependence of deiodination reactions. Whole homogenates of cerebral cortex from normal and hypothyroid rats were incubated with tracer T_4 and T_3 in the presence of 0–100 mM DTT. There was no detectable degradation of T_4 or T_3 in the absence of DTT. In the hypothyroid preparations (Fig. 3), there was a progressive increase in percent conversion of $[^{125}I]T_4$ to $[^{125}I]T_3$ as the DTT concentration was increased up to 10 mM and no significant further change at 50 or 100 mM DTT. In the same hypothyroid preparations, there was a progressive increase in conversion of $[^{131}I]T_3$ to $[^{131}I]3,3'$ - T_2 up to 100 mM DTT and an increase in net accumulation of $[^{125}I]3,3'$ - T_2 . The low $3,3'$ - T_2 production rates in this experiment did not allow precise definition of the DTT dose-response relationship. In the homogenates of cerebral cortex from normal rats (Fig. 4), the rate of T_4 to rT_3 conversion and the rate of T_3 to $3,3'$ - T_2 conversion increased progressively as the DTT concentration was varied from 0 to

50 mM, with no significant differences between 50 and 100 mM DTT. The production of $3'$ - T_1 from T_3 had a similar relationship to the DTT concentration. In normal rats, there was no significant T_4 to T_3 conversion in cerebellar homogenates, and low rates of T_3 to $3,3'$ - T_2 conversion in cerebral cortical homogenates when 100 mM GSH was used instead of DTT. 100 mM GSH was slightly less effective than 1 mM DTT in stimulating T_3 to $3,3'$ - T_2 conversion.

Subcellular distribution of deiodinating activities. In normal, hypothyroid, and hyperthyroid rats, the extent of conversion of T_4 to T_3 , T_4 to rT_3 , and T_3 to $3,3'$ - T_2 was the same, or only slightly less, in the resuspended low- and high-speed pellets as in the whole homogenates. In the hypothyroid rats, the T_4 5'-deiodination rate per milligram protein was less in cytosol than in other fractions. In all three groups, the rate of T_4 5-deiodination per milligram protein was less in the high-speed supernate than in the high-speed pellet; this was probably true for 5-deiodination of T_3 also, after accounting for the decrease in reaction rate as the substrate became largely depleted. Since the amount of the various products were similar in the washed high-speed pellet and in the whole homogenate in all three groups, differences in cytosol binding of T_4 clearly did not prevent the detection of T_3 production in the whole homogenates of cerebral cortex from normal and hyperthyroid rats.

The effects of dilution of the high-speed pellet was studied.² In the high-speed pellet from hypothyroid rats, the rate of conversion of T_4 to T_3 had a reasonably linear, direct relationship to the amount of protein in the incubation tubes between 1.5 and 11.5 mg protein/

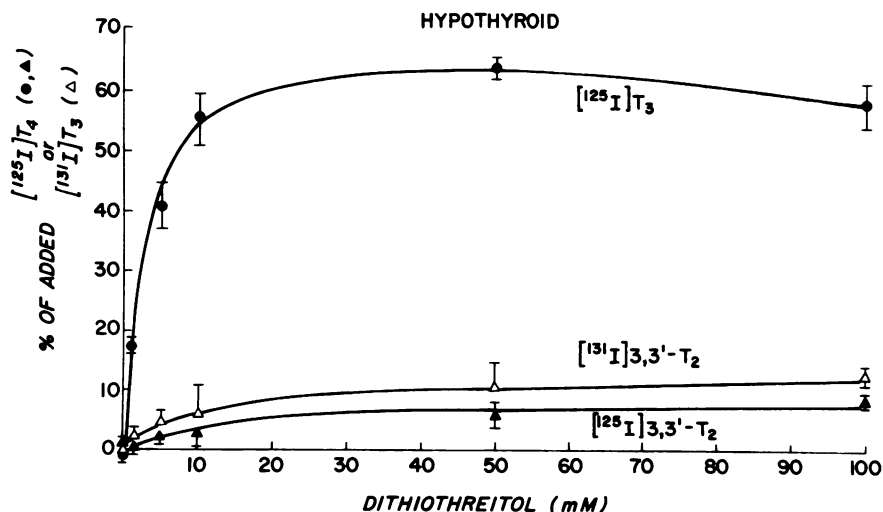


FIGURE 3 Dependence on DTT concentration of T_4 and T_3 deiodination in pooled cerebral cortical homogenates from three hypothyroid rats. Points represent mean \pm SD of triplicate incubations with 0.4 nM $[^{125}I]T_4$ and 0.2 nM $[^{131}I]T_3$ at 37°C for 2 h. Production of $[^{125}I]rT_3$, $[^{131}I]3'$ - T_1 , and excess $^{125}I^-$ were at most 4% of added $[^{125}I]T_4$ or $[^{131}I]T_3$ at all DTT concentrations.

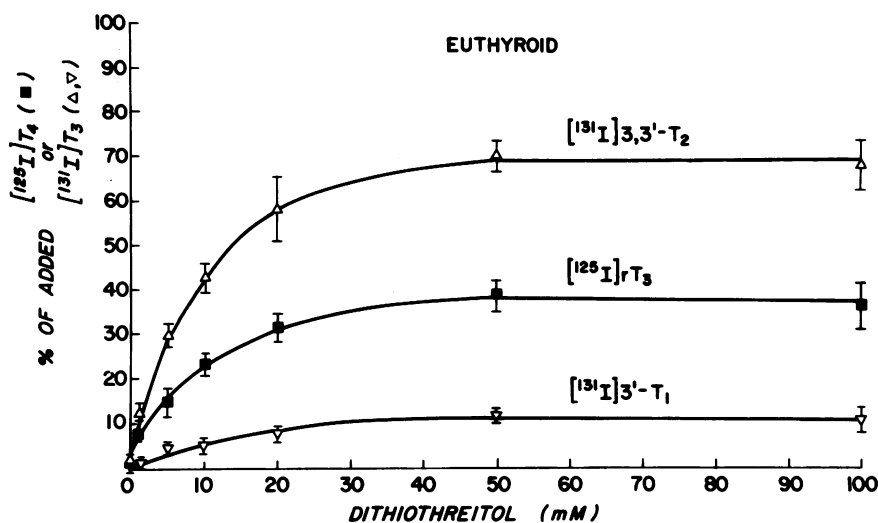


FIGURE 4 Dependence on DTT concentration of T_4 and T_3 deiodination in pooled cerebral cortical homogenates from three euthyroid rats. Points represent mean \pm SD of triplicate incubations with 0.5 nM $[^{125}\text{I}]T_4$ and 0.3 nM $[^{131}\text{I}]T_3$ at 37°C for 2 h. Production of $[^{125}\text{I}]T_3$ was $<2\%$ and production of excess $^{125}\text{I}^-$ was $<4\%$ of added $[^{125}\text{I}]T_4$ at all DTT concentrations.

ml. When the high-speed pellet from normal rats was diluted from 8.8 to 1.1 mg/ml, the percent conversion of T_4 to rT_3 decreased progressively, but the relationship was not linear above 4.4 mg protein/ml.

Inhibition of phenolic and tyrosyl ring deiodination in cerebral cortical tissue by iodothyronines and iopanoic acid. Results are shown in Tables II and III. Both rT_3 and iopanoic acid (Table II) caused dose-dependent inhibition of T_4 to T_3 conversion. Increasing the T_4 concentration from 1 to 1,000 nM also caused a progressive reduction in fractional T_4 to T_3 conversion (Table II). The extent of inhibition of fractional T_4

to T_3 conversion by the agents used in these two experiments may be compared, since total T_4 degradation in the incubations with T_4 up to 1 nM were quite similar ($47.6 \pm 3.2\%$ in experiment 1 and $45.0 \pm 2.0\%$ in experiment 2). The effect of adding 1,000 nM T_3 to the high-speed pellet from hypothyroid rats was the same as in the whole homogenate experiments, i.e., no inhibition of total T_3 production. Thus, for the T_4 to T_3 conversion reaction in the high-speed pellet fraction, the order of inhibitory potency was $T_4 > \text{iopanoic acid} > rT_3 \gg T_3$.

T_3 at 1,000 nM greatly reduced fractional conver-

TABLE II
Inhibition of T_4 5'-Deiodination by Cerebral Cortical Tissue from Hypothyroid Rats *in vitro*

	Inhibitor	$[^{125}\text{I}]T_3$	$[^{131}\text{I}]3,3'-T_2$	$[^{125}\text{I}]T_3$
		production	production	+ $[^{131}\text{I}]3,3'-T_2$
nM		% added $T_4 \pm \text{SD}$		
Experiment 1	None	21.4 \pm 1.6	8.0 \pm 2.0	29.4 \pm 2.6
	rT_3 100	13.2 \pm 1.4	5.0 \pm 1.4	18.2 \pm 2.0
	rT_3 1,000	4.8 \pm 0.6	1.0 \pm 0.9	5.8 \pm 1.1
	Iopanoic acid 300	8.4 \pm 0.4	1.6 \pm 1.2	10.0 \pm 1.3
	Iopanoic acid 1,000	2.6 \pm 1.0	1.2 \pm 1.6	3.5 \pm 1.9
Experiment 2	None	25.5 \pm 2.0	5.2 \pm 1.4	30.7 \pm 2.4
	T_4 10	16.8 \pm 1.7	3.8 \pm 0.3	20.6 \pm 1.7
	T_4 100	9.9 \pm 4.3	3.0 \pm 2.0	12.9 \pm 4.7
	T_4 1,000	0.0 \pm 0.6	0.9 \pm 0.9	0.9 \pm 1.1

Incubations were carried out in triplicate at 37°C for 2 h in the presence of 0.4–1.0 nM $[^{125}\text{I}]T_4$, 0.2 nM $[^{131}\text{I}]T_3$, and 100 nM DTT. In each experiment, cerebral cortical tissue from three hypothyroid rats was pooled and the high-speed pellet fraction was used in the incubations.

TABLE III
Inhibition of T_4 and T_3 5-Deiodination in the Cerebral Cortical
High-speed Pellet Fraction

	Inhibitor	$[^{125}I]T_4$	$[^{125}I]T_3$
		converted to $[^{125}I]rT_3$	converted to $[^{125}I]3,3'-T_2$
	nM	% added $T_4 \pm SD$	% added $T_3 \pm SD$
Experiment 1 (hypothyroid)	None	10.4 ± 1.6	15.3 ± 1.4
	T_4 1,000	4.4 ± 2.9	0.9 ± 2.0
	T_3 1,000	6.2 ± 4.5	-0.4 ± 1.6
Experiment 2 (normal)	None	27.6 ± 3.2	67.8 ± 2.6
	T_4 100	18.5 ± 3.2	37.9 ± 4.2
	T_4 1,000	7.9 ± 4.7	10.3 ± 1.7
	Iopanoic acid 1,000	6.8 ± 2.7	17.0 ± 4.9
	Iopanoic acid 50,000	0.7 ± 2.8	1.7 ± 4.8
Experiment 3 (hypothyroid)	None	10.2 ± 2.4	28.9 ± 2.6
	rT_3 100	10.4 ± 2.0	26.7 ± 2.4
	rT_3 1,000	11.5 ± 2.0	24.1 ± 1.9
	Iopanoic acid 300	2.6 ± 3.0	12.6 ± 2.0
	Iopanoic acid 1,000	0.0 ± 1.8	3.4 ± 1.3

Tissue preparation and reaction conditions were as described in TABLE II.

sion of $[^{131}I]T_3$ to $[^{131}I]3,3'-T_2$ in the high-speed pellet from hypothyroid rats (Table III). The effect of T_3 on T_4 to rT_3 conversion in experiment 1 was indeterminate. T_4 was an effective inhibitor of fractional conversion of $[^{125}I]T_4$ to $[^{125}I]rT_3$ and of $[^{131}I]T_3$ to $[^{131}I]T_2$ (Table III). 1 μM T_4 was approximately as potent as 1 μM T_3 in inhibiting fractional conversion of $[^{131}I]T_3$ to $[^{131}I]3,3'-T_2$. Iopanoic acid inhibited T_4 to rT_3 conversion and T_3 to $3,3'-T_2$ conversion (Table III). The potency of iopanoic acid in the inhibition of 5-deiodination of T_4 and T_3 was approximately equal to that of T_4 in the high-speed pellet from normal rats (Table III). rT_3 at 100- and 1,000-nM concentrations did not inhibit T_4 to rT_3 conversion or T_3 to $3,3'-T_2$ conversion (Table III). From these data the order of inhibitory potency of these compounds for the 5-deiodination reaction in the cerebral cortical high-speed pellet was $T_3 \approx T_4 \approx$ iopanoic acid $\gg rT_3$.

DISCUSSION

The studies we report here demonstrate complex patterns of deiodinative iodothyronine metabolism in rat brain homogenates. It seems clear that rat brain possesses separate phenolic and tyrosyl ring deiodinases. For both of these enzymes, activity is heat labile and thiol dependent. The rates of the two types of reactions in cerebral cortical homogenates vary in opposite directions in hypothyroidism. The activities of these enzymes also vary independently in different regions of the brain. The relationship of our results to those of Tata et al. (18) is obscure, probably due to substantial

methodological differences. It is not clear whether the compounds they identified as triac and tetrac, or the corresponding propionic acid analogues, could have been $3,3'-T_2$ and rT_3 . Certainly, no alterations in the alanine side chain of T_4 or T_3 were observed in the present studies.

The formation of excess I^- from T_4 , particularly in homogenates preheated to 100°C, raises the possibility that we have misinterpreted nonspecific processes (30, 31) as being enzymatic reactions. This seems most unlikely for several reasons. First, the processes that form identifiable iodothyronine products of T_4 and T_3 degradation always behaved as expected for enzymatic reactions. They showed dependence on pH, temperature, and protein concentration, regional specificity within the brain, and dependence on the thyroid status of the animals. Second, we did not observe rapid deiodination of T_3 and rT_3 , the known degradation products of T_4 , in preboiled homogenates. This result excluded sequential deiodination through those intermediates. Third, all of the manipulations that increased excess I^- formation greatly reduced the net formation of all identifiable iodothyronine products. Although the nature of excess I^- formation is unknown, it appeared to be largely unrelated to formation of iodothyronine products of T_4 degradation and may well be non-enzymatic.

The significance of T_4 5'-deiodinase activity in the brain, with respect to the responses of the brain to circulating thyroid hormones, is not yet known. Crantz and Larsen (17) have shown that T_3 is produced from T_4 within the cerebral cortex and the cerebellum of

normal rats in vivo. They have recently reported (32) that >70% of the endogenous nuclear T_3 in these brain regions is produced locally. This means that local T_3 production, compared with T_3 transport from the blood, is at least as important in determining occupancy of nuclear T_3 binding sites in the cerebral cortex and cerebellum as in the anterior pituitary gland (33, 34). Local T_4 to T_3 conversion provides a much lower fraction of nuclear T_3 in other tissues (33–35). Inhibition by iopanoic acid of T_4 5'-deiodination within the anterior pituitary gland prevents physiological doses of T_4 from suppressing TSH release in hypothyroid rats in vivo (35). Iopanoic acid inhibits local T_4 to T_3 conversion in vivo in the rat cerebral cortex and cerebellum (17), just as it does in pituitary (20) and brain homogenates. Hypothyroidism increases local T_4 to T_3 conversion in anterior pituitary fragments (22) and probably in the anterior pituitary in vivo (36, 37). Parallel increases in in vivo T_4 5'-deiodinase activity are readily measured in anterior pituitary and brain homogenates in hypothyroidism. These similarities lead us to anticipate that brain T_4 5'-deiodinase, the properties of which we are beginning to define in vitro, will prove to be necessary for the expression of some of the effects of blood T_4 on the brain.

The estimated maximal T_3 production rates in cerebral cortical homogenates from hypothyroid rats, 8.1–17.5 fmol T_3 /min per mg protein, are higher than the T_4 to T_3 conversion rates we previously measured in cerebral cortical homogenates from normal rats, ~1 fmol T_3 /min per mg protein. In both normal and hypothyroid rats, maximum T_4 to T_3 conversion rates in anterior pituitary homogenates are 40–80 times greater than in cerebral cortical homogenates (20). We have speculated that the increased anterior pituitary T_4 5'-deiodinase activity in hypothyroidism could be caused by increased numbers of thyrotrophs (20). This explanation is obviously inapplicable to brain tissue. It seems more likely, therefore, that metabolic factors are in operation in the brain and the pituitary, e.g., an increased synthesis rate or a decreased turnover rate of the enzyme molecule.

T_4 5'-deiodinase activity is also present in rat liver and kidney (19, 21, 38–42). The 5'-deiodinase enzymes in liver and kidney are very similar to one another and may well be the same (19, 42). Rat liver and kidney T_4 5'-deiodinases share several properties with rat brain and pituitary T_4 5'-deiodinases: inhibition by iopanoic acid and rT_3 , and lack of apparent interaction with T_3 (19, 20, 42, 43). In these tissues, T_4 5'-deiodinase is particulate, and cytosol has very little activity (24, 38–44). In at least one important respect, brain and pituitary T_4 5'-deiodinase differ from the liver and kidney enzymes: in hypothyroidism, reaction rates in brain and pituitary homogenates are supernormal (20, 22), while reaction rates in liver and kidney homog-

enates are subnormal (23, 45–48). Further purification will be required to establish the identity or difference between the T_4 5'-deiodinase enzymes from these different organs.

Enzymatic tyrosyl ring deiodination of T_4 and T_3 has been identified in vitro in rat liver and kidney homogenates and in monkey hepatocarcinoma cells (49–55). Hepatic T_4 to rT_3 conversion is thiol dependent and heat labile (50, 52), and activity is found in particulate fractions as well as in cytosol (50, 54). These characteristics are similar to those that we find for brain iodothyronine 5-deiodinase. The finding of such substantial iodothyronine 5-deiodinase activity in brain tissue was unexpected, and its significance, if any, is unknown. Our data suggest that rT_3 and the other T_4 metabolite (very likely 3,3'- T_2) found by Dratman and Crutchfield (14) in rat brain tissue after ^{125}I - T_4 injection may well be produced within brain tissue in vivo. It is possible that the iodothyronine 5-deiodinase participates in regulation of thyroid hormone effects by providing a mechanism for intracellular T_3 disposal, inasmuch as T_4 and T_3 tyrosyl ring deiodination products produced endogenously have not yet been shown to exert hormonal effects.

From our studies, methodological recommendations for future in vitro studies of production of T_4 , rT_3 , and other iodothyronines in brain tissue can be made. Thiol concentration needs to be carefully controlled. Degradation of reaction products needs to be evaluated along with production, because, at least for T_3 , net increases in T_3 can seriously underestimate total T_3 production. It is desirable to adjust reaction conditions to minimize excess I^- production or, at any rate, to take account of the process because it affects substrate concentrations during incubations. Certainly, production of I^- from T_4 can not be equated with T_3 production.

It is becoming increasingly clear that quantitatively and qualitatively distinct patterns of thyroid metabolism occur within various tissues. Even within the brain, the cerebral cortex and the cerebellum exhibit this diversity according to our studies. Clearer understanding of thyroid hormone action will follow from study of intracellular thyroid hormone metabolism. Accumulating evidence suggests that the circulating pool of T_4 is a reservoir which is drawn upon to provide substrate for closely regulated, local T_3 production. This mechanism may allow tissues to provide themselves with T_3 according to their particular requirements for thyroid hormone effects.

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