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Research Article

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Tyrosyl ring deiodination rates of T_4 and T_3 were more than three times as great in cerebral homogenates from 1-d-old rats as in adult cerebral homogenates. In cerebellar homogenates from 1-d-old rats, tyrosyl ring deiodination rates were much greater than the rates in adult cerebellar homogenates, but less than those in 1-d cerebral homogenates. In 1-d hypothalamic homogenates, tyrosyl ring deiodination rates were the highest of all the tissues tested, whereas rates in adult hypothalamic homogenates were similar to those in adult cerebral homogenates.

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ABSTRACT To explore the control of thyroid hormone metabolism in brain during maturation, we have measured iodothyronine deiodination in homogenates of rat cerebrum, cerebellum, and hypothalamus from 1 d postnatally through adulthood. Homogenates were incubated with ^{125}I-L-thyroxine (T_4) + [^{131}I]3,5,3′-L-triiodothyronine (T_3) + 100 mM dithiothreitol. Nonradioactive T_4, T_3, and 3,3′,5′-triiodothyronine (rT_3) were included, as appropriate. The net production rate of [^{125}I]T_3 from T_4 in 1-d cerebral homogenates was similar to the rate in adult cerebral homogenates (9.9 ±2.5[SEM]% vs. 8.9±1.2% T_4 to T_3 conversion in 2 h). Production of T_3 was not detectable in 1-d cerebellar and hypothalamic homogenates. The net T_3 production rate in adult cerebellar homogenates was twice as great as, and that in adult hypothalamic homogenates similar to, the rate in cerebral homogenates.

Tyrosyl ring deiodination rates of T_4 and T_3 were more than three times as great in cerebral homogenates from 1-d-old rats as in adult cerebral homogenates. In cerebellar homogenates from 1-d-old rats, tyrosyl ring deiodination rates were much greater than the rates in adult cerebellar homogenates, but less than those in 1-d cerebral homogenates. In 1-d hypothalamic homogenates, tyrosyl ring deiodination rates were the highest of all the tissues tested, whereas rates in adult hypothalamic homogenates were similar to those in adult cerebral homogenates.

During maturation, T_4 5′-deiodination rates increased after 7 d and exceeded adult rates between 14 and 35 d in cerebral and cerebellar homogenates, and at 28 and 35 d in hypothalamic homogenates. In cerebral homogenates, the peak in reaction rate at 28 d reflected an increase in the maximum enzyme activity (V_{max}) of the reaction. T_4 and T_3 tyrosyl ring deiodination rates decreased progressively with age down to adult rates, which were attained at 14 d for cerebrum and cerebellum and at 28 d for hypothalamus.

These studies demonstrate quantitative differences in T_4 5′-deiodinase activities in cerebrum, cerebellum, and hypothalamus at all ages, with the overall maturational pattern differing from the developmental patterns of both the pituitary and hepatic T_4 5′-deiodinases. Iodothyronine tyrosyl ring deiodinase activities also vary quantitatively among these same brain regions and exhibit a pattern and a time-course of maturation different from that of the T_4 5′-deiodinase. These enzymes could have important roles in the regulation of intracellular T_3 concentrations and, hence, on the expression of thyroid hormone effects.

INTRODUCTION

Phenolic ring, or 5′-deiodination of L-thyroxine (T_4),[1] which occurs in rat brain in vivo (1–3), supplies much of the endogenous 3,5,3′-L-triiodothyronine (T_3) in the cerebral cortex and cerebellum, including that T_3 which occupies the nuclear T_3 binding sites (2, 3). After intravenous injection of ^{125}I-T_4, [^{125}I]T_3 is also found in various extranuclear brain fractions, especially the synaptosomal fraction (4), and more ^{125}I-T_3 is found in extracts of whole brain tissue and brain nerve cell bodies in 10-d-old rats than in 30-d-old rats (5). Also after intravenous ^{125}I-T_4 injection, [^{125}I]3,3′,5′-L-triiodothyronine (rT_3) and a compound that may be [^{125}I]3,3′-L-diiodothyronine (3,3′-T_2) have been found in rat brain extracts (4). The in vivo occurrence of tyrosyl ring deiodination of T_4 and T_3 is thus suggested. We have

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[1] *Abbreviations used in this paper*: DTT, dithiothreitol; 3,3′-T_2,3,3′-L-diiodothyronine; 3′-T_1, 3′-L-iodothyronine; T_4, L-thyroxine; T_3, 3,5,3′-L-triiodothyronine; rT_3, 3,3′,5′-L-triiodothyronine; V_{max}, maximum enzyme activity.

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demonstrated in vitro T_4 5′-deiodinase activity in homogenates of rat cerebral cortex and cerebellum and found in vitro activity of a separate tyrosyl ring deiodinase that converts T_4 to rT_3 and converts T_3 sequentially to 3,3′-T_2 and 3′-L-iodothyronine (3′-T_1) (6). Both enzymes are largely particulate and require thiol reducing agents for detectable in vitro activity (6).

Thyroid hormone metabolism in fetal and neonatal rat tissues differs from that in adult tissues. T_4 5′-deiodination rates in liver homogenates from neonates are much lower than in adult liver homogenates, but the differences are abolished by addition of the thiol reducing agent, dithiothreitol (DTT) (7, 8). In contrast, in vitro T_4 5′-deiodination is more rapid in neonatal pituitary tissue than in adult pituitary tissue (8, 9). If orderly changes in patterns of thyroid hormone metabolism were also to occur in the brain, then the argument for the biological significance of changes in in vitro rates of iodothyronine deiodination reactions in brain tissue in altered physiological states would be strengthened. Further, since T_3 is both produced and degraded by brain homogenates, knowledge of relative reaction rates at different ages could facilitate the interpretation of previous (5) and future in vivo studies of brain tissue T_3 concentrations. We have therefore investigated changes in deiodinative metabolism of thyroid hormone in vitro occurring during maturation. Cerebrum, cerebellum, and hypothalamus were examined separately, since our previous studies showed differences between adult cerebral cortex and cerebellum in rates of T_4 5′-deiodination and of T_4 and T_3 5-deiodination (6). Determinations of the kinetic parameters for T_4 5′-deiodination in cerebral homogenates were also made by modifying the incubation conditions to minimize concomitant reactions that interfere with measurements of T_3 production.

METHODS

Nonradioactive iodothyronines were obtained from the Sigma Chemical Co., St. Louis, Mo. and Henning GMBH (Berlin, West Germany). High specific activity tracers were prepared in our laboratory (6). Timed-pregnant Sprague-Dawley rats and adult male Sprague-Dawley rats were supplied by Zivic-Miller Laboratories, Allison Park, Pa. Male and female pups were used before age 21 d and only males were used at older ages. Ages of rats weighing ≥200 g were estimated from their weights according to growth curves from the supplier. Three to four pups (ages ≤43 d) and three to four adult rats (ages >60 d) were used simultaneously, in anticipation of possible inter-experiment variation. In some experiments two groups of pups and one group of adults were used. Animals were anesthetized lightly with ether and decapitated. Brains were removed to iced 0.05 M Tris, pH 7.6–0.25 M sucrose (Tris-sucrose) and subsequent operations were performed at 0–4°C. For each group, tissue from the rats was pooled. Brains were divided into cerebellum, hypothalamus, and cerebral hemispheres (with brainstem removed). Whole cerebral hemispheres were used since there was no clear demarcation of the cerebral cortex in 1- and 7-d-old brains. In some experiments, adult hypothalami were dissected according to Glowinski and Iversen (10), yielding 90–110 mg tissue per hypothalamus. In other experiments, the cephalad cut was made at the floor of the third ventricle, yielding 30–40 mg tissue per hypothalamus. There was no difference in reaction rates occurring in homogenates of the larger or smaller hypothalamic pieces. Hypothalamic weights in the 1-d-old animals were 9–13 mg and increased to adult values by 28 d.

Tissue was homogenized in 9 vol Tris-sucrose containing 100 mM DTT. Details of the tissue preparation and incubation procedures have been reported (6). In some experiments, a high-speed pellet (material sedimenting between 1,000 and 160,000 *g*) was prepared as described (6), and resuspended to the original homogenate volume. 90 μl of the homogenates were incubated in triplicate at 37°C under nitrogen for 2 h with 10 μl substrate solution, giving concentrations in the incubation mixtures of ≈0.2 nM ^{125}I-T_4 (40,000 cpm) + ≈0.1 nM [^{131}I]T_3 (20,000 cpm) and either 1 μM nonradioactive T_3 or 1 μM nonradioactive rT_3. Endogenous T_4 and T_3 contribute ~0.15 nM each to the concentrations in the incubation mixtures (11). Variations in tracer T_4 and T_3 concentrations between 0.1 nM and 1 nM do not affect fractional degradation rates. Hypothalamic homogenates from 1-d-old rats were incubated only with 1 μM T_3 because of the small amount of tissue available. Other homogenates were incubated with 1 μM T_3 and, separately, with 1 μM rT_3. T_3 inhibits 5-deiodination, but not 5′-deiodination, of T_4 and inhibits degradation of newly formed T_3 (6). rT_3 inhibits T_4 5′-deiodination but not T_4 or T_3 5-deiodination (6). The data from incubations with 1 μM rT_3 thus show maximal T_4 5-deiodination rates, not inhibited by T_3.

Incubations were terminated by the addition of 200 μl ethanol and 50 μl of 0.04 N NaOH containing nonradioactive L-T_3 + L-T_4 + methimazole (6). The ethanolic extracts were analyzed by descending paper chromatography in t-amyl alcohol:hexane:ammonia, 5:1:6. Further details of the analytical methods and calculations have been published (6). Identifiable products of T_4 degradation were T_3, rT_3, 3,3′-T_2, and I^-. Identifiable products of T_3 degradation were 3,3′-T_2, 3′-L-iodothyronine (3′-T_1), and I^-. Results are expressed as percent ^{125}I-T_4 or [^{131}I]T_3 converted to the various products. In all experiments reported here, excess I^- production (6) amounted to ≤5% of ^{125}I-T_4 and <2% of [^{131}I]T_3. Experimental protocols always included 0 time and 2-h control incubations with no tissue in the buffer. Reaction rates in the presence of tissue were corrected for nonenzymatic reactions observed in the buffer controls. Boiled tissue controls were not appropriate owing to the occurrence of nonenzymatic deiodination of T_4 without T_3 or rT_3 production (6). In the buffer control incubations, <5% of T_4 was degraded, <5% of T_3 was degraded, and T_3 production from T_4 was <1.5%. Protein was measured by the method of Lowry et al. (12), after DTT was removed by perchloric acid precipitation of the protein (6).

In the statistical analyses, each mean of triplicate incubations within an experiment was treated as a single value. When data were not normally distributed or variances were not homogeneous, nonparametric statistics were used. To compare reaction rates at different ages, overall significance was tested by Kruskal-Wallis one-way analysis of variance (13). If the χ^2 was significant, comparisons of rates at different ages were made by the Mann-Whitney test (13), if three or more values were present. All the values for rats 60 d or older were grouped together, inasmuch as no measurement varied with age within this group. Also, inter-experiment variation of adult results was found not to differ from intra-experiment adult results when tissues from individual rats were prepared separately. Inter-experiment variation, therefore, seems largely to reflect biological variation. Values for 4-d-old and 7-d-old rats

and for 41–48-d-old rats were grouped together to minimize the data sets with $n < 3$. This grouping does not materially affect the conclusions.

RESULTS

T_4 5′-deiodination in homogenates of cerebrum, cerebellum, and hypothalamus from 1-d-old and adult rats (Table I). The net production rate of [^{125}I]T_3 in 1-d cerebral homogenates was similar to the rate in adult cerebral homogenates. Degradation of [^{131}I]T_3 and removal of ^{125}I-T_4 by conversion to rT_3 were more rapid in the cerebral homogenates from neonates than in those from adults (Table I, lines 2 and 5). Net production of [^{125}I]3,3′-T_2 was 2.50±0.5 (SE)% of added T_4 in 1-d cerebral homogenates and not detectable in adult cerebral homogenates. These findings suggested that the capacity for T_4 to T_3 conversion might be greater in neonatal cerebral tissue than in adult cerebral tissue.

In 1-d rats, net production of T_3 was not detectable in cerebellar and hypothalamic homogenates. Net production of [^{125}I]3,3′-T_2 from ^{125}I-T_4 was also very low or undetectable (<1% conversion) in incubations of cerebellar and hypothalamic homogenates from 1-d-old rats; therefore, the possibility can be excluded that substantial quantities of T_3 were produced and rapidly degraded. Even considering the differences between the age groups in homogenate protein content (Table I), and in rates of T_4 depletion via 5-deiodination (see below), the capacities of neonatal cerebellar and hypothalamic homogenates to catalyze T_4 5′-deiodination were clearly much lower than those of the corresponding adult homogenates. In homogenates from adult rats, the mean T_4 5′-deiodination rate was highest in cerebellar tissue (Table I), $P < 0.001$ vs. cerebrum and hypothalamus.

Tyrosyl ring deiodination of T_4 and T_3 in cerebrum, cerebellum, and hypothalamus from 1-d-old and adult rats (Table I). The mean rates of tyrosyl ring deiodination of T_4 and T_3 were more than three times as great in cerebral homogenates from 1-d-old rats than in adult cerebral homogenates in incubations with 1 μM T_3. In incubations using 1 μM rT_3 instead, 61±6% of T_4 was converted to rT_3 in the 1-d homogenates vs. 33±3% in the adult homogenates ($P < 0.001$). In cerebellar homogenates from 1-d-old rats (Table I), tyrosyl ring deiodination rates of T_4 and T_3 were about half the rates in 1-d cerebral homogenates, but were much greater than the rates in adult cerebellar homogenates, the latter having very little activity. In cerebellar incubations with 1 μM rT_3, similar findings were noted: T_4 to rT_3 conversion was 41.4±3% in the 1-d homogenates and 1.1±0.3% in adult homogenates ($P < 0.001$). In hypothalamic homogenates from 1-d-old rats (Table I), tyrosyl ring deiodination rates of T_4 and T_3 were the highest of all the tissues tested, whereas rates in the adult hypothalamic homogenates were similar to the rates in adult cerebral homogenates.

To test the effects of differences in protein content on tyrosyl ring deiodination rates, tyrosyl ring deiodination of 1 μM T_3 was measured in adult tissue homogenates diluted with 1 vol Tris-sucrose-100 mM DTT. In the three brain regions, rates were reduced in the diluted homogenates to 42–76% of the rates in

TABLE I

Iodothyronine Deiodination in Cerebral, Cerebellar, and Hypothalamic Homogenates from 1-d-old and Adult Rats

Measurement	Cerebrum		Cerebellum		Hypothalamus	
	1 d	Adult	1 d	Adult	1 d	Adult
T_4 5′-deiodination (percent T_4 converted to T_3)	9.9±2.5	8.9±1.2	0.4±0.4*	17.6±1.6	0.2±0.2*	7.6±1.0
T_4 5-deiodination (percent T_4 converted to rT_3)	20.8±1.7*	5.6±2.3	10.4±1.7*	0.3±0.5	46.2±7.5*	7.2±1.2
T_3 tyrosyl deiodination (percent added T_3)						
3,3′-T_2	27.6±3.2*	9.6±1.3	13.3±2.9*	0.3±0.2	34.3±1.0*	8.2±1.4
3′-T_1	6.8±2.1*	1.0±0.2	2.8±0.5*	0.0±0.1	17.7±3.3*	1.1±0.3
3,3′-T_2 + 3′-T_1	34.4±3.8*	10.6±1.4	16.1±2.7*	0.3±0.3	52.0±3.8*	9.3±1.6
Protein content, *mg/ml*	5.3±0.5	6.8±0.5	5.9±1.7‡	10.0±0.5	5.6±1.0‡	8.6±0.5

Results are mean±SEM of individual experimental means for 60–100-d-old adult rats (12 experiments) and 1-d-old neonatal rats (three experiments). Incubations were performed at 37°C for 2 h with 0.2 nM ^{125}I-T_4 + 0.1 nM [^{131}I]T_3 + 1 μM [^{127}I]T_3 + 100 mM DTT. Values for neonatal and adult tissues were compared using the *t* test with *n* = number of experiments. No correction was made for degradation of newly formed [^{125}I]T_3 or ^{125}I-rT_3.

* $P < 0.001$.

‡ $P < 0.01$ vs. adult tissue from the same region.

corresponding undiluted homogenates. Correction for the differences in homogenate protein content would therefore magnify the differences between neonates and adults in tyrosyl ring deiodination rates seen in Table I, but would not greatly alter the comparison between the different regions.

Changes in T_4 5′-deiodination rates during maturation. T_4 5′-deiodination rates (Figs. 1A, 2A, and 3A) increased after 7 d, and exceeded adult rates between 14 and 35 d in cerebral and cerebellar homogenates and at 28 and 35 d in hypothalamic homogenates. The mean rates at the peak ages were two to three times the mean adult rates. T_3 production rates in the period 14–48 d could be compared directly to adult rates since there were no significant differences in T_4 depletion and T_3 degradation (see below) or homogenate protein content.

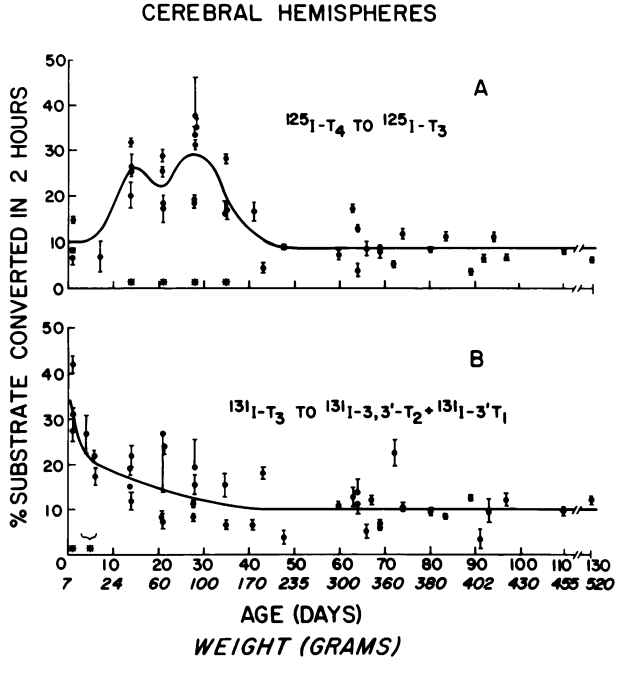

FIGURE 1 T_4 5′-deiodination and T_3 tyrosyl ring deiodination in homogenates of rat cerebral hemispheres. Each point is the mean±SD of triplicate incubations using pooled tissues from three to four rats. Incubations were carried out at 37°C under N_2 for 2 h in the presence of ≈0.2 nM ^{125}I-T_4 + ≈0.1 nM [^{131}I]T_3 + 1 μM [^{127}I]T_3 + 100 mM DTT. Asterisks indicate ages at which rates are significantly different ($P < 0.05$ or less) from the rates in the adult rats (60 d and older) by the Mann-Whitney test. Values for weights, in italics below the abcissa, are average weights of male rats at the indicated ages. The curves are drawn through the mean rates for ages with multiple points. (A) Net conversion of ^{125}I-T_4 to [^{125}I]-T_3 in 2 h. No correction was made for [^{125}I]T_3 degradation. (B) Conversion of [^{131}I]T_3 to [^{131}I]3,3′-T_2 + [^{131}I]3′-T_1. For technical reasons, only T_3 deiodination could be measured in the 4 d incubations. More detailed analysis of the 1-d and adult incubations is given in Table I.

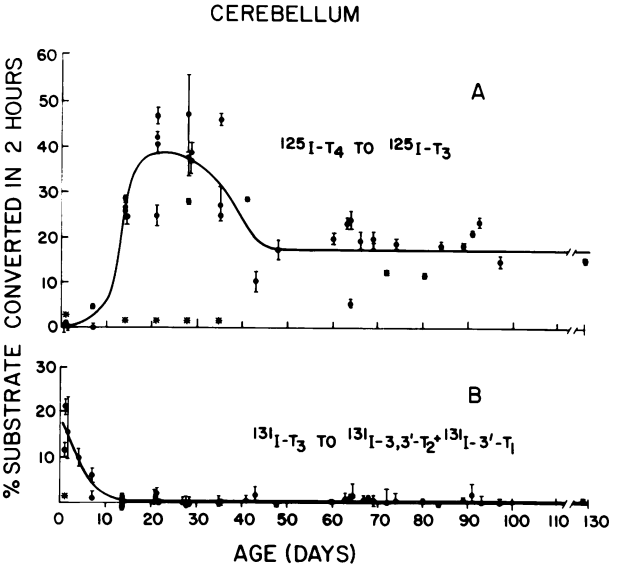

FIGURE 2 T_4 5′-deiodination and T_3 tyrosyl ring deiodination in homogenates of rat cerebellum. Experimental details are given in the legend to Fig. 1.

Because of the remaining uncertainty about the relative T_4 5′-deiodinase activities in the 1-d and adult cerebral homogenates, and because the peak rates seen between 14 and 35 d could result from changes in either the Michaelis constant (K_m) for T_4 or maximum enzyme activity (V_{max}), or both, we performed T_4 dose-response experiments. Cerebral homogenates from 1-, 28-d-old and adult rats were incubated for 2 h with 2 μM T_3 (instead of 1 μM) and with 0.2, 2, 5, 10, and 20 nM T_4. There was a progressive decrease in all the

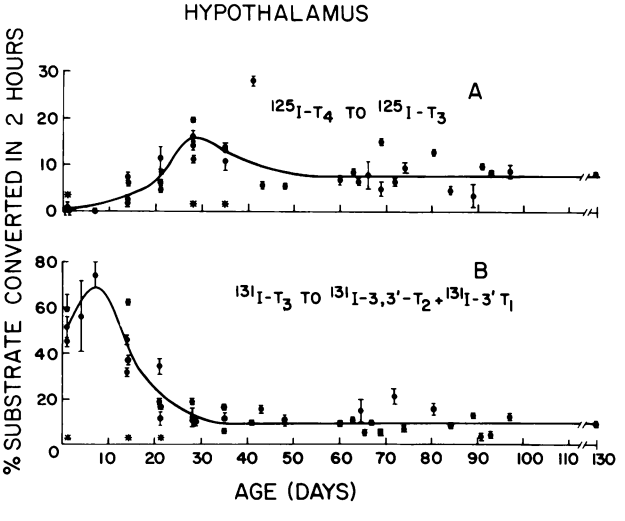

FIGURE 3 T_4 5′-deiodination and T_3 tyrosyl ring deiodination in homogenates of rat hypothalamus. Experimental details are given in the legend to Fig. 1.

homogenates in percent conversion of ^{125}I-T_4 to [^{125}I]-T_3 as the T_4 concentration was increased, but when rates were translated from percent conversion to femtomoles T_3 produced per minute per milligram protein, the molar reaction rates were found to increase with the T_4 concentration. In these incubations the extent of [^{131}I]T_3 degradation was reduced to 6–15%, the extent of T_4 degradation (largely to rT_3) was reduced to 16–25% (compare Table I), and the protein concentrations were similar in the homogenates from the pups and the adults. The higher T_3 concentration thus facilitated comparisons of $T_4$5′-deiodination rates. Eadie-Hofstee plots of (reaction rate ÷ average T_4 concentration) vs. molar reaction rate (14) were linear. The kinetic parameters calculated from the plots are given in Table II. The apparent K_m for T_4 was 34% lower in the 1-d-old rats than in adults, whereas it was 45% higher in the 28-d-old rats than in adults. The V_{max} was not significantly different in the 1-d-old and adult rats, but the V_{max} in the 28 d rats was considerably higher than in other groups, 2.6 times as high as that of the adult rats.

Changes in T_3 tyrosyl ring deiodination rates during maturation. Total T_3 tyrosyl ring deiodination rates (Figs. 1B, 2B, and 3B) declined progressively with age, attaining adult values at 14 d for cerebrum and cerebellum and at 28 d for hypothalamus. In parallel incubations, using <1 nM T_3, fractional T_3 tyrosyl ring deiodination rates were too rapid (>75%) in cerebral and hypothalamic homogenates at all ages to allow differences to be detected. However, in cerebellar homogenates incubated with <1 nM T_3, mean fractional T_3 tyrosyl ring deiodination fell from 70% at 1 d to 6% at 14 d and remained at that level thereafter. These data show that adult cerebellum has real, but low level, tyrosyl ring deiodinase activity which becomes saturated at 1 μM T_3.

The conversion of ^{125}I-T_4 to rT_3 in the presence of 1 μM T_3 or 1 μM rT_3 showed the same pattern of decrease with age as did total tyrosyl ring deiodination of T_3. That is, for each brain region, T_4 tyrosyl ring deiodination rates declined progressively from the high neonatal values (Table I) and reached adult levels at the same age as total T_3 tyrosyl ring deiodination.

TABLE II
Kinetic Parameters of T_4 5′-deiodinase in Cerebral Homogenates

Age	Apparent K_m for T_4	V_{max}
	nM	*fmol T_3 per min per mg protein*
1 d	9.5 (9.2, 9.9)	2.3 (2.0, 2.9)
28 d	21.0 (17.4, 26.4)	3.9 (3.8, 4.1)
Adult	14.3 (12.9, 16.1)	1.5 (1.0, 2.4)

Values in parentheses are 95% confidence limits.

DISCUSSION

The present studies extend the information available about in vitro thyroid hormone metabolism in brain. From a methodological standpoint, routine inclusion of 1–2 μM L-T_3 in the incubations greatly facilitated quantitation of T_4 5′-deiodination, allowing initial estimates of kinetic parameters for T_4 5′-deiodinase in normal rat brain. In heterogenous homogenates such as those employed here, these parameters must be interpreted cautiously, because nonenzymatic binding of substrate can influence them greatly. They do have some value in comparing physically similar systems. With that in mind, the apparent K_m for T_4 of T_4 5′-deiodinase in adult rat cerebral homogenates, 14 nM, was found to be very close to the apparent K_m for T_4 of rat anterior pituitary homogenate T_4 5′-deiodinase, 8.8 nM (15). Both of these values are 35–1,000-fold less than the K_m for T_4 of rat liver and kidney homogenate T_4 5′-deiodinases (16–23). From this kinetic evidence and from the responses of brain and anterior pituitary T_4 5′-deiodinase activity in hyper- and hypothyroidism (6, 8, 15), the suggestions may be drawn that the brain and anterior pituitary T_4 5′-deiodinases could be the same, or very similar, and that both may well be different from the liver and kidney T_4 5′-deiodinase (17).

The maturational pattern of T_4 5′-deiodinase activity in the brain proved to be complex. T_4 5′-deiodinase activity in homogenates of neonatal tissue was highest in the cerebrum and virtually absent in cerebellum and hypothalamus. In all three regions, the T_4 5′-deiodination rates increased over the first 4 wk of life, attaining peaks above adult values, then declining to adult rates. For the cerebrum, this peak reflected an increase in the total enzyme activity (V_{max}); the modest difference in apparent K_m for T_4 in the 28-d-old vs. adult rats was in a direction that would tend to lower rates in the 28-d-old rats at tracer T_4 concentrations. The quantitative differences between regions at all ages reinforce our previous findings of regional diversity of T_4 5′-deiodinase activity (6).

Other tissues show different developmental patterns. In rat anterior pituitary tissue, T_4 5′-deiodination rate in vitro are elevated in the neonatal period, after 1 d, and decline to adult values by 28-45 d (8, 9). In rat liver homogenates, T_4 5′-deiodination rates are much lower in fetal and neonatal tissue than in adult tissue (7, 8) but the differences between neonatal and adult liver are lessened or abolished by DTT supplementation (7, 8). There is some uncertainty about the time course of change of T_4 5′-deiodinase activity in liver: Harris et al. (24) found normalization of rates in liver homogenates at 5 d with (24) or without (7) a peak above adult rates at 7 d, whereas we observed persistently low rates at 9 d and normal adult rates at 21 d (8).

For iodothyronine tyrosyl ring deiodination, the pattern of changes was completely different. Hypothalamic tissue showed the highest rates initially and remained above adult values longest. In all three tissues, there was a progressive decline in iodothyronine tyrosyl ring deiodinase activity until a plateau was reached at the adult values. Tanaka et al. (25) have reported in preliminary form that T_4 and T_3 tyrosyl ring deiodination is more rapid in homogenates of fetal rat brain than in homogenates of adult rat brain. Their observations are in accord with ours. The parallel changes of T_4 tyrosyl ring deiodination and T_3 tyrosyl ring deiodination in these and previous experiments (6) leave little doubt that a single brain tyrosyl ring deiodinase accepts T_4 and T_3 as substrates. In contrast, the present results support our previous conclusion (6) that the brain tyrosyl ring deiodinase is not the same enzyme as the T_4 5′-deodinase: neonatal hypothalamic homogenates have abundant tyrosyl ring deiodinase activity and are devoid of T_4 5′-deiodinase activity (Table I), whereas cerebellar homogenates from hypothyroid adult rats have abundant T_4 5′-deiodinase activity and are devoid of iodothyronine tyrosyl ring deiodinase activity (6). Rat liver and monkey hepatocarcinoma cells contain iodothyronine tyrosyl ring deiodinases with catalytic properties generally similar to the rat brain enzyme (26–28), and adult rat liver has higher activity than neonatal rat brain (26, 27). Nonetheless, complete physical separation of hepatic phenolic and tyrosyl ring deiodinase activities has not been achieved (29). Our findings in brain homogenates lead us to predict that hepatic phenolic and tyrosyl ring deiodinases will prove to be separate molecules.

By analogy with observations concerning T_4 to T_3 conversion in the anterior pituitary, we have reasoned that local T_3 production in the brain is likely to be physiologically significant (6). A recent preliminary report indicates that after ^{125}I-T_4 injection, the fraction of injected radioactivity that appears as T_3 is greater in brain extracts from hypothyroid rats than in extracts from normal rats (30). This finding is in agreement with our data that in vitro T_4 5′-deiodinase activity is much greater in cerebral cortex and cerebellum of hypothyroid adult rats than in normal tissue (6). The coherent patterns of change in activities of both deiodinases in brain tissue during maturation reinforce the idea that these enzymes may have an important role in the expression of thyroid hormone effects. An obvious possibility is that the balance of intracellular T_3 production and T_3 degradation is a major determinant of steady state T_3 tissue concentrations. It is difficult to relate the in vivo data of Vigoroux et al. (5), to the present results, since they extracted whole brains and since in vitro T_3 production and T_3 degradation rates at 10 d are changing rapidly and in opposite directions. The data in Figs. 1–3 suggest that 1–7, 28 and >60 d would be more suitable ages at which to compare brain tissue levels of iodothyronines and in vivo iodothyronine metabolism to obtain further direct evidence of the physiological significance of the iodothyronine metabolic pathways observed in vitro.

These studies, together with previous reports, suggest that there is a complex system of local regulation of thyroid hormone metabolism in target tissues, serving to modulate the expression of thyroid hormone effect. Many other factors that may regulate thyroid hormone secretion and actions on target tissues undergo changes in the first several weeks of life in the rat. These include the number of T_3 nuclear receptors in different brain regions and in the liver, the sensitivity of thyrotropin secretion to thyrotropin-releasing hormone and to circulating iodothyronine concentrations, the plasma activity of the proteases that degrade thyrotropin-releasing hormone, the sensitivity of the thyroid gland to inhibition of secretion by iodide, and the sensitivity of the liver to plasma T_3 concentrations (31–39). Much additional work will be required to integrate these diverse observations into a complete picture of the regulation of thyroid hormone action in the developmental period.

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