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Brain Cortex Reverse Triiodothyronine (rT_3) and Triiodothyronine Concentrations under Steady State Infusions of Thyroxine and rT_3

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ABSTRACT. T_4 and reverse T_3 (rT_3) can inhibit 5′-deiodinase type II activity in rat brain cortex, pituitary, and brown adipose tissue, raising the possibility that T_4 may act *in vivo* after conversion to rT_3. The aim of this study was to measure in hypothyroid (Tx) rats the content of brain cortex rT_3 during a constant 7-day infusion of either [^{125}I]T_4 alone, corresponding to 12 pmol T_4/day·100 g body weight (BW), or together with 400 pmol T_4/day. [^{125}I]T_4, rT_3, and T_3 were extracted from brain cortex, pituitary, kidney, and liver with a combination of adsorption chromatography on Sephadex G-25, HPLC, and immunoprecipitation. [^{131}I]T_4, T_3, or rT_3 were used as internal standards.

[^{125}I]rT_3 could be detected in brain cortex, liver, and kidney in Tx rats infused with [^{125}I]T_4 (12 pmol T_4/day·100 g BW) and in those infused with 400 pmol T_4/day·100 g BW. The highest rT_3 concentrations were found in brain cortex, where it represented 6% to 10.5% of the local T_4 concentration.

During an infusion of 400 pmol T_4/day·100 g BW, brain cortex T_3 concentration was 6 times higher in the brain cortex than in serum, and even exceeded that of T_4. In Tx rats receiving [^{125}I]T_4 alone the brain cortex to serum T_3 ratio was 3:1, but the total serum T_3 concentration, measured by RIA, was much higher than that due to conversion [0.50 ± (SE) 0.1 pmol/ml *vs.* 0.018 ± 0.002 pmol T_3/ml], indicating thyroidal secretion.

The effect of the blood-brain barrier on rT_3 was measured by infusing [^{125}I]rT_3 over 4 days. After killing, rT_3 was isolated as above. Approximately 3% of serum rT_3 was retrieved from the brain cortex, whereas during the T_4 infusion 40–50% of serum rT_3 was found demonstrating that brain cortex rT_3 is locally produced. (*Endocrinology* **120:** 1590–1596, 1987)

MOST of the intracellular T_3 in brain cortex and pituitary is locally produced by the 5′-monodeiodination of T_4 (1–4). The enzyme involved is called the 5′-deiodinase type II (5′D-II) (5–9). It can also be found in placenta and brown adipose tissue. It is particularly active in tissues of hypothyroid rats (10–12) and can be rapidly inhibited by single injections of T_4, T_3, reverse T_3 (rT_3) (13) and 3′,5′-diiodothyronine (T_2) (14).

In earlier studies, our group established that a continuous infusion of rT_3 exerted its inhibitory effect at a serum concentration of 7 pmol/ml (15). Concentrations of this magnitude are not encountered in pathophysiological conditions. However, under physiological conditions rT_3 is probably produced locally, explaining why our earlier studies underestimated the concentration of rT_3 present in the brain cortex. The work of Obregon *et al.* (16) supports the hypothesis of local production.

The present investigations confirm and extend the studies of Obregon *et al.* (16), and allow speculations on the physiological role of brain rT_3 as an inhibitor of the conversion of T_4 to T_3.

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Materials and Methods

Reagents

The iodothyronines (T_4, T_3, and rT_3) were purchased from Henning Co. (West Berlin, West Germany). Carrier-free Na ^{125}I and Na ^{131}I were obtained from the Institut für Reaktorforschung (Würenlingen, Switzerland). Chloramine-T was used to iodinate rT_3, T_4, and T_3 with ^{125}I or ^{131}I. Low specific activity (SA) of T_4 (54 μCi/μg) was obtained by addition of an adequate amount of unlabeled T_4 (17, 18). Osmotic minipumps (Alzet no. 2001, Alza, London, UK) were used for infusions. HPLC was performed with equipment using a reverse phase Bondapak C18 column (Waters Associates, Milford, MA). TLC was performed on Merck Silicagel 60 F 254 aluminium sheets, with chloroform-methanol-formic acid (16:3:1) used as solvents.

In vivo procedures

Male SIVZ rats, a strain derived from the Wistar rat, were purchased from the breeder (Tierzuchtanstalt, University of Zürich, Zürich, Switzerland) and were rendered hypothyroid (Tx) by thyroidectomy when they reached 150–200 g body weight (BW). The minipumps were implanted on the seventh day after operation, and the animals were killed 1 week later. Two days before and during the experiments, they received 20 mg potassium iodide/liter in their drinking water.

Infusions

Three types of experiments were performed. In Exp I, the minipumps were filled with [^{125}I]T_4 of high SA (2000 μCi/μg), which was infused at a rate of 12 pmol T_4/day·100 g BW (9.3 ng T_4/day). [^{125}I]T_4 was dissolved in 0.02 N NaOH, 0.05 M sodium carbonate, 0.9% NaCl, and 10% Tx rat serum. Four Tx rats received infusions for 7 days.

In Exp II, the minipumps were filled with [^{125}I]T_4 of low SA (54 μCi/μg), which was infused at a rate of 400 pmol/day·100 g BW (311 ng T_4/day). The infusion also lasted 7 days. Eight Tx rats were used.

In Exp III, the Tx rats received [^{125}I]rT_3. The minipumps were filled with [^{125}I]rT_3 with a SA of 900 μCi/μg, which was infused at a rate of 100 pmol rT_3/day·100 g BW (54 ng rT_3/day). Four Tx rats received infusions for 3 days.

Before implantation, the minipumps were left to equilibrate overnight at room temperature in 0.9% NaCl, 1% BSA, and then implanted ip. At the end of the infusion the animals were killed by abdominal aortic exsanguination under light ether anesthesia. To reduce plasma contamination of the tissues, the rats were gently perfused with 25–30 ml ice-cold 0.9 NaCl containing 0.1 mM propylthiouracil (PTU) and 1 mM iopanoic acid via the opening in the aorta. Outflow was obtained by puncturing the inferior vena cava. The heart continued pumping the infused medium until the end of the procedure. The liver, kidneys, cerebral cortex, and anterior pituitary of each animal were rapidly removed, frozen immediately in liquid nitrogen, and kept at −70 C until processed.

In order to measure the degradation of [^{125}I]T_4 and [^{125}I]rT_3 during the 3 or 7 days of infusion, the minipumps were placed for 4 h in 0.9% NaCl, 1% BSA, 10 mM PTU, and 0.5 mM iopanoic acid. Aliquots of this and of the initial solutions were analyzed by chromatography on Sephadex G-25. During the infusions about 10% of the iodine in T_4 was released as I^-, and about 20% of that in rT_3.

Extraction procedures

In vitro degradation of the iodothyronines was always measured by addition of their ^{131}I-labeled tracers. 5′-Monodeiodination of rT_3 is very active in the liver and the kidney and had to be inhibited rapidly (see below). *In vitro* degradation in the brain cortex and pituitary could be inhibited by serum. In these organs the iodothyronines were therefore extracted by immunoprecipitation. This method was also used for extraction of the more stable T_3 in liver and kidney homogenates.

The procedure of rapid denaturation of proteins and extraction by column chromatography is illustrated in Flow Chart 1. Homogenization was performed in a solution consisting of 0.02 N NaOH, 5 mM PTU, 0.5 mM iopanoic acid, 1% Triton X-100, and approximately 10,000 cpm [^{131}I]rT_3 (alkaline solution). A small aliquot (0.5 ml) was frozen for later extraction of T_4 (Chart 1a). Eight milliliters of the homogenate were extracted on a column of Sephadex G-25 (30-ml bed volume in a 60-ml syringe, equilibrated with 0.02 N NaOH, 1% Triton X-100) (Chart 1b). After addition of the homogenate, the columns were rinsed with 30 ml 2 N acetic acid and 40 ml distilled water. The labeled hormones were eluted with 95 ml 0.02 N NaOH. The eluate was acidified with 4 ml acetic acid. This solution was passed through a Dowex 50 WX2 cation exchange column equilibrated with 1.74 N acetic acid (1-ml bed volume in a 2-ml syringe). After washing of the column with 8 ml acetone-H_2O (1:1), the hormones were eluted with 3 ml 7 N NH_4OH-ethanol (7:3) and dried under vacuum. The recovery of [^{131}I]rT_3 was 85%. HPLC was performed according to the method of Van Der Walt and Cahnmann (19), with use of an acetonitrile gradient from 25% to 50% in 20 mM ammonium acetate, pH 4.

Immunoprecipitation (Chart 2) was performed in 25 mM Tris-HCl buffer, pH 8.2, 0.0036% NaCl, 10% human serum (HS), 5 mM PTU, 0.5 mM iopanoic acid (Tris-HCl-HS buffer), and appropriate amounts of [^{131}I] standards of the iodothyronines. The immunoprecipitations were adapted from the

CHART 1. rT_3 extraction from liver and kidney, T_4 extraction from liver and kidney.

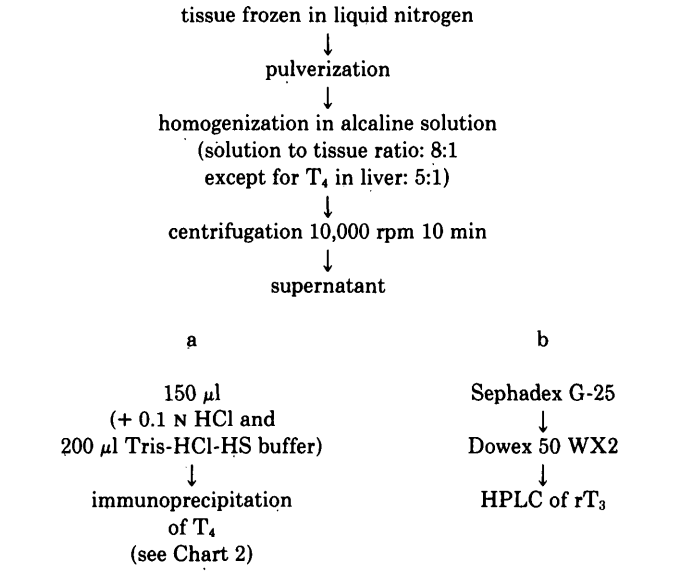
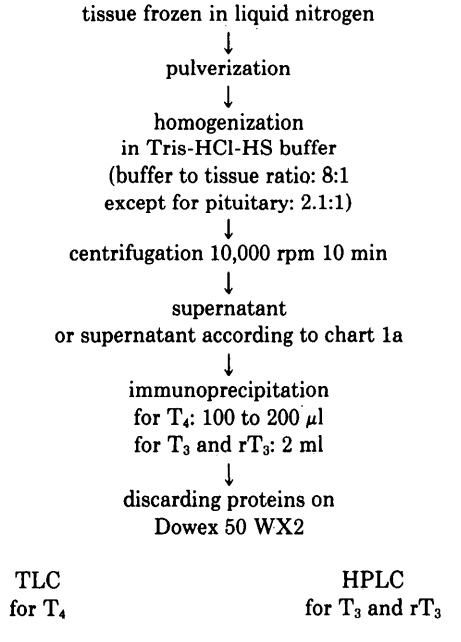


CHART 2. Extraction and immunoprecipitation of T_4, T_3, and rT_3 from brain cortex, pituitary, liver, and kidney.



method of Engler *et al.* (20).

One hundred to 200 μl homogenate were incubated for T_4 immunoprecipitation. For the liver and kidney, the alkaline solution was first neutralized with 0.1 N HCl and 200 μl Tris-HCl-HS buffer (Chart 1a). Serum binding was inhibited with 8-anilinonaphthalene-1-sulfonic acid (14 μg/100 μl Tris-HCl-HS buffer), and T_4 was precipitated with 20 μl rabbit anti-T_4 serum (1:10).

For T_3 and rT_3 1 to 3 times 2 ml homogenate were incubated with 200 μg 8-anilinonaphthalene-1-sulfonic acid. To ensure maximum extraction of T_3 and rT_3, each sample was incubated with both antisera [20 μl rabbit anti-T_3 (1:10) and 60 μl anti-rT_3 serum (1:10)].

The incubations were identical for all types of immunoprecipitations. The samples were kept at 37 C for 10 min and at 4 C overnight. Bound and free hormones were separated by precipitation with a goat antirabbit antiserum (Antibodies Inc., Davies, CA). As the immunoprecipitates could not be injected as such into the HPLC column, the proteins were eliminated on the Dowex column (see above). T_3 and rT_3 were then separated by HPLC and T_4 by TLC. The recoveries of [^{131}I]T_4, [^{131}I]T_3, and [^{131}I]rT_3 were 83 ± 3% (mean ± SE).

Serum T_4, T_3 and rT_3 were also extracted by immunoprecipitation, as adapted from Engler *et al.* (20). In addition, the pellets of immunoprecipitated hormones were further processed as above, with the use of a Dowex 50 WX2 column to eliminate the proteins. The T_4 extracts were then chromatographed by TLC, whereas rT_3 and T_3 extracts were analyzed by HPLC.

Degradation of rT_3 during tissue preparation

In order to evaluate rT_3 degradation during tissue preparation, we injected three groups of Tx rats (thyroidectomized 4 weeks previously) iv via the jugular vein with 10 μCi [^{125}I]rT_3 (Amersham, Buckinghamshire, UK; SA 1200 μCi/μg) dissolved in 0.02 N NaOH and diluted with 10% rat serum in 0.9% NaCl. After 30 min, the rats were killed as previously described. In the first group, the liver was immediately removed and frozen. In the second and third groups, we waited 1 and 3 min, respectively, before removing the liver. Cerebral cortices were removed immediately after the liver, so that the time interval was also 1 and 3 min between the groups. Tissues and serum were processed as above. Dissection time did not affect the rT_3 content of the two tissues (Table 1).

Calculations and statistical analysis

As in the Tx rats, endogenous serum T_4 levels were undetectable and the SA of the infused T_4 was identical with that in serum and tissues. Serum T_4 concentration could therefore be measured by RIA or calculated from its SA. Serum rT_3 and T_3 could also be calculated from the SA of [^{125}I]T_4, taking into account that [^{125}I]T_4 was labeled in the 3′ or 5′-position. Hence, only one out of two T_4 molecules converted to T_3 was radioactive, reducing the SA of [^{125}I]T_3 to half that of [^{125}I]T_4. The SA of [^{125}I]rT_3 was the same as that of T_4. The percent T_3 due to conversion was calculated from the ratio of the serum T_3 concentration due to conversion and the serum T_3 concentration measured by RIA.

Student's *t* test for means and Wilcoxon's test (if $n > 7$) were used to assess the significance of any observed difference. The values are given as means ± SE.

TABLE 1. Tissue to serum [^{125}I]rT_3 ratio (Exp IV)

Time between perfusing of the animal and removal of the organ	Liver	Cerebral cortex
Group 1: 0 min	27.1 ± 6.1%	5.36 ± 0.87%
Group 2: 1 min	27.9 ± 6.7%	5.03 ± 0.37%
Group 3: 3 min	27.3 ± 5.4%	5.87 ± 0.37%

Values are given as mean ± SE.

Results

During infusion of 400 pmol T_4/day·100 g BW, serum T_4 concentration measured by RIA was 40.3 ± 1.7 pmol/ml, which was very similar to the serum T_4 level of 38.6 ± 3.6 pmol/ml (30 ng/ml) calculated from the SA of the infused T_4 (Table 2). Table 2 shows that T_4 concentration was much lower in the brain cortex than in serum, whereas liver and kidney T_4 concentrations were substantial, although below the serum T_4 concentration. Pituitary and serum T_4 concentrations did not differ.

In the same experiment serum T_3 concentration was 0.89 ± 0.17 pmol/ml (0.58 ng/ml) measured by RIA and 0.65 ± 0.07 pmol/ml (0.42 ng/ml; Table 3) calculated on the basis of its SA. A large proportion of the circulating T_3 could therefore be attributed to conversion.

Based on the SA of T_3, the highest concentration of T_3 was found in the pituitary, followed by brain cortex and kidney. T_3 concentration in the liver was markedly lower, but nevertheless 4 times higher than in serum.

TABLE 2. T_4 concentrations per ml serum or g tissue during T_4 infusion

	Serum	Brain cortex	Pituitary	Liver	Kidney
a) 400 pmol T_4/day·100 g BW					
		pmol T_4/ml serum or g tissue			
n	8	8	8	8	8
Mean	38.6	1.91	44.9	30.1	19.1
SE	3.6	0.14	7.3	2.4	0.7
P^a		0.001	NS	NS	0.001
		ng T_4/ml serum or g tissue			
Mean	30.0	1.48	34.9	23.4	14.8
b) 12 pmol T_4/day·100 g BW					
		pmol T_4/ml serum or g tissue			
n	4	4	3	4	4
Mean	2.16	0.041	0.79	0.62	0.48
SE	0.13	0.005	0.09	0.03	0.02
P^a		0.05	0.05	0.05	0.05
		ng T_4/ml serum or g tissue			
Mean	1.68	0.032	0.61	0.48	0.37

NS, Not significant (<0.05).

[a] Significance compared to serum values.

TABLE 3. T_3 concentrations per ml serum or g tissue during T_4 infusion

	Serum	Brain cortex	Pituitary	Liver	Kidney
a) 400 pmol T_4/day·100 g BW					
		pmol T_3/ml serum or g tissue			
n	8	8	8	8	8
Mean	0.650	3.948	5.700	2.712	3.798
SE	0.070	0.283	0.515	0.198	0.223
P^a		0.001	0.001	0.001	0.001
		ng T_3/ml serum or g tissue			
Mean	0.423	2.570	3.711	1.766	2.472
b) 12 pmol T_4/day·100 g BW					
		pmol T_3/ml serum or g tissue			
n	4	4		4	4
Mean	0.018	0.064		0.035	0.063
SE	0.001	0.010		0.004	0.002
P^a		0.050		0.05	0.05
		ng T_3/ml serum or g tissue			
Mean	0.012	0.042		0.023	0.041

[a] Significance compared to serum values.

TABLE 4. rT_3 concentrations per ml serum or g tissue during T_4 infusion

	Serum	Brain cortex	Liver	Kidney
a) 400 pmol T_4/day·100 g BW				
		pmol rT_3/ml serum or g tissue		
n	8	7	7	8
Mean	0.276	0.114	0.216	0.184
SE	0.029	0.013	0.021	0.010
P^a		0.001	NS	0.01
		ng rT_3/ml serum or g tissue		
Mean	0.179	0.074	0.141	0.120
b) 12 pmol T_4/day·100 g BW				
		pmol rT_3/ml serum or g tissue		
n	4	4	4	2
	0.0079	0.0043	0.0300	0.0266
SE	0.0006	0.0007	0.0112	
P^a		0.05	0.05	
		ng rT_3/ml serum or g tissue		
Mean	0.0051	0.0028	0.0195	0.0173

NS, Not significant (>0.05).

[a] Significance compared to serum values.

The highest concentration of rT_3 was found in serum. Brain cortex, kidney, and liver rT_3 concentrations amounted to 41%, 67%, and 78% of serum levels, respectively (Table 4).

In Fig. 1 serum or tissue T_3 and rT_3 concentrations are expressed as percentages of local T_4 concentrations. The figure clearly shows the peculiarity of the brain cortex, with a T_3 concentration exceeding that of T_4. Although rT_3 did not exceed 6% of T_4, the highest ratio of rT_3 to T_4 was also found in brain cortex.

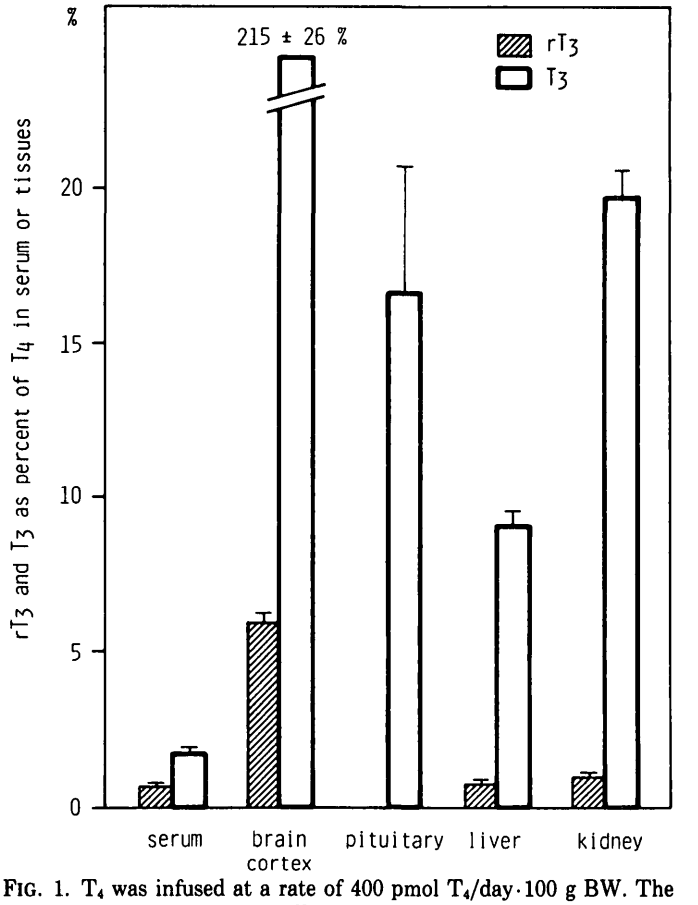


FIG. 1. T_4 was infused at a rate of 400 pmol T_4/day·100 g BW. The resulting concentrations of [^{125}I]rT_3 and [^{125}I]T_3 are represented as percentages of the [^{125}I]T_4 concentration in the same tissue or serum. Clearly marked differences between tissues are seen. For both rT_3 and T_3, the highest percentage is found in the brain cortex.

With infusions of 12 pmol T_4/day·100 g BW (Table 2), serum T_4 levels were similar whether they were measured by RIA (2.3 ± 1.3 pmol/ml or 1.8 ng/ml) or calculated on the basis of the infusion rate (2.16 ± 0.13 pmol/ml or 1.7 ng/ml). The T_4 concentrations in the brain cortex were again small compared to those in the serum and the other tissues that were studied. In these still severely Tx animals T_3 measured by RIA differed markedly from T_3 resulting from conversion. Serum T_3 was 0.50 ± 0.10 pmol/ml (0.33 ng/ml) when measured by RIA, whereas serum T_3 due to conversion [0.018 ± 0.002 pmol/ml or 0.012 ng/ml (Table 3)] only amounted to 3.6% of the circulating T_3 level. Its distribution in serum and tissue was calculated on the basis of its SA and was comparable to the distribution observed during infusion of 400 pmol T_4/day·100 g BW, the highest concentrations being found in brain cortex and renal tissue. The pituitary was not studied. The concentration of T_3 in brain cortex was again higher than the concentration of T_4. During the infusion of 12 pmol T_4/day·100 g BW the highest concentrations of rT_3 were found in liver and kidney (Table 4). Tissue rT_3 to T_4 ratio in brain cortex

was higher than in the other two tissues. Pituitary rT_3 concentration was too low for detection.

Infusion rates in terms of counts per min [^{125}I]T_4 were identical in both experiments. However, Fig. 2 shows that the serum levels of infused [^{125}I]T_4 differ markedly according to whether 400 or 12 pmol T_4/day·100 g BW were infused. The higher [^{125}I]T_4 serum level during an infusion of 12 pmol T_4/day·100 g BW indicated a lower plasma clearance rate than during an infusion of 400 pmol T_4/day·100 g BW (0.47 ± 0.03 ml/h·100 g BW *vs.* 0.91 ± 0.07 ml/h·100 g BW). However, the brain cortex and kidney count per min/[^{125}I]T_4 per g tissue were not affected by the infusion of 400 pmolT_4/day·100 g BW, and during this infusion there was a significant increase in the hepatic [^{125}I]T_4 concentration.

During the [^{125}I]rT_3 infusion its calculated serum level was 0.028 ± 0.03 pmol/ml (0.02 ng/ml), yielding a plasma clearance rate of 78 ± 8 ml/h·100 g BW. [^{125}I]rT_3 was unmeasurable in the pituitary and was also low in brain cortex. In Table 5 results are also expressed as percentages of serum [^{125}I]rT_3 values. They show that [^{125}I]rT_3 in the brain cortex represented only 3.3% of serum [^{125}I] rT_3. In the liver and kidney the values were 71% and 38% of serum rT_3 concentration, the difference between liver and kidney being significant ($P<0.05$).

Discussion

Iodothyronines with two iodine atoms on the phenolic ring (T_4, rT_3, and 3′,5′-T_2) are the most potent inhibitors of the high 5′D-II activity Tx rats. These iodothyronines, and rT_3 in particular, are also capable of inhibiting the activity of this enzyme in cell cultures in the absence of thyroid hormones (22–24).

There is little doubt that T_4 is the major 5′D-II inhibitor *in vivo*. However, the question of whether T_4 acts directly or after conversion to rT_3 remains unresolved. Ideally, a specific inhibitor of the enzyme converting T_4 to rT_3 in brain tissue, placenta and skin, the so called 5-deiodinase type III (25, 26), would provide the answer, but unfortunately no such compound is available. Knowledge of brain cortex rT_3 concentrations could also throw light on the problem. Obregon *et al.* (16) have already addressed this question. Their estimation of brain cortex rT_3 concentration was based on the single injection technique. Our earlier studies with continuous infusions of rT_3 and T_4 established the serum concentrations of rT_3 and T_4 required to inhibit 5′D-II activity to the same extent. The question therefore arose of the brain cortex rT_3 concentrations in the two types of experiments, and the present study was designed to answer it. We studied serum and brain cortex rT_3 concentrations during T_4 infusion and estimated the extent to which the rT_3 blood brain barrier excludes rT_3 during an infusion of the hormone. The results of the T_4 infusion showed that for serum levels of 40 pmol T_4/ml the rT_3 concentration was 0.114 pmol/g in the brain cortex and 0.276 pmol/ml in serum. The concentration of rT_3 in the brain cortex is therefore approximately 40% that of rT_3 in serum. However, during continuous infusions of [^{125}I]rT_3, brain cortex rT_3 concentration was only 3.3% of its serum concentration. We therefore infer that most of the rT_3 found in

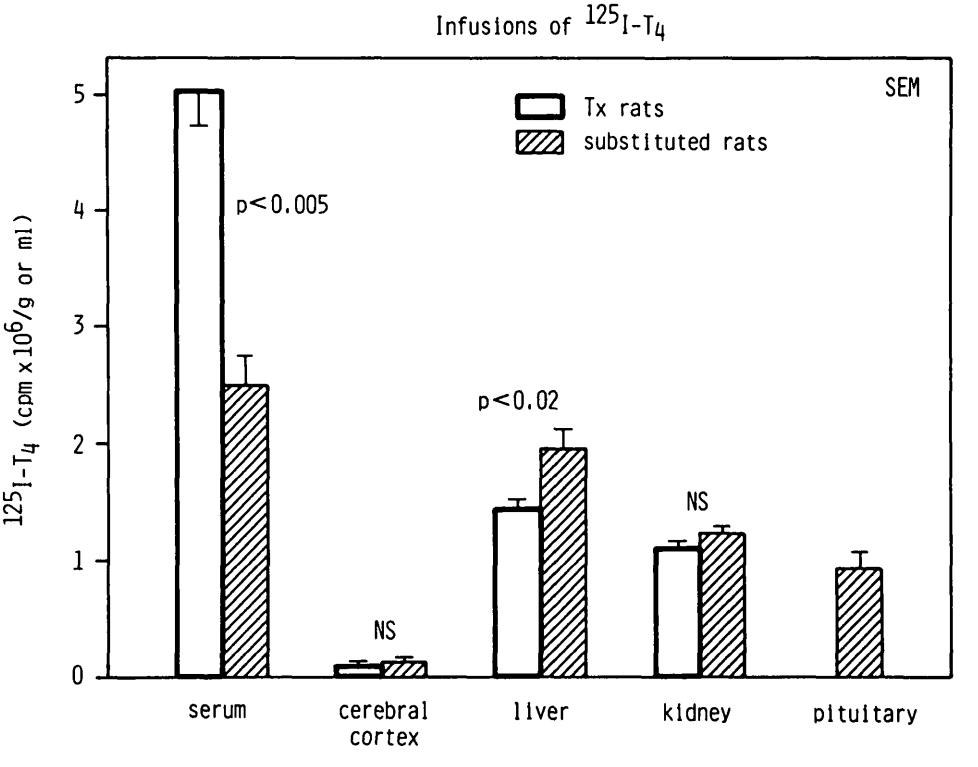


FIG. 2. Tissue or serum [^{125}I]T_4 concentrations (counts per min × 10^6/g or ml) during [^{125}I]T_4 infusion in the absence (□, n=4) or presence of 400 pmol T_4/day·100 g BW (▨, n=8). Results are expressed as mean ± SE.

TABLE 5. Infusion of [^{125}I]rT_3

	Serum	Brain cortex	Liver	Kidney
	cpm rT_3/g tissue or ml serum			
n	4	4	4	4
Mean	25108	805	17970	9480
SE	2555	28	3648	1409
P^a		<0.05	NS	<0.05
	cpm rT_3/g tissue expressed as % cpm in serum			
n		4	4	4
Mean		3.3%	71.0%	37.8%
SE		0.4%	10.5%	5.1%

NS, Not significant (>0.05).

[a] Significance compared to serum values.

the brain cortex results from local conversion of T_4 to rT_3. On the other hand, the quantity of infused rT_3 required to inhibit brain cortex 5'D-II activity to the same extent as 400 pmol T_4/day·100 g BW has been established (15) as 13.5 nmol/day·100 g BW, yielding a serum level of 7 pmol rT_3/ml. On the basis of the present experiments, brain cortex rT_3 corresponding to a serum concentration of 7 pmol/ml can be predicted to be 0.24 pmol/g tissue. This value is not dissimilar to the measured value of 0.114 pmol rT_3/g, and suggests that brain cortex rT_3 concentration contributes to *in vivo* inhibition of 5'D-II by T_4.

No corrections were made for trapped plasma in cerebral cortex which, according to Silva and Matthews (27), is less than 1%. Yet, as indicated above, only small amounts of infused rT_3 (3.3%) could be found in brain cortex homogenates. Our values of brain cortex rT_3 concentration during the infusion of [^{125}I]rT_3 may therefore be slightly overestimated, whereas during the infusion of [^{125}I]T_4 the brain cortex concentration of [^{125}I]rT_3 is much too high to be affected by a contamination by plasma rT_3. The error due to trapped plasma is certainly greater in the highly vascularized liver and kidney.

The tissue T_3 concentrations observed confirm earlier work (1–4). They also show that in the presence of low to moderate serum T_4 brain cortex T_3 concentration is even greater than brain cortex T_4 concentration. This can mainly be attributed to local conversion, although we have clearly demonstrated that the efficiency of brain cortex T_4 to T_3 conversion in hypothyroidism does not increase sufficiently to maintain brain cortex T_3 concentrations in the euthyroid range. Using measurements based on the conversion of unlabeled T_4, brain cortex T_3 concentrations in Tx rats reached only 1.5% of the concentrations in rats infused with 400 pmol T_4/day·100 g BW. In Tx rats serum T_3 values measured by RIA were greater than those measured as having arisen by conversion, indicating thyroidal secretion of T_3. This T_3 diffuses into brain cortex, thus increasing brain tissue concentrations, although it may be limited by the blood-brain barrier.

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