Evidence for Two Tissue-specific Pathways for In Vivo Thyroxine 5′-Deiodination in the Rat

J. ENRIQUE SILVA, JACK L. LEONARD, FRANK R. CRANTZ, and P. REED LARSEN,
Howard Hughes Medical Institute Laboratory and Thyroid Diagnostic Center, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Propylthiouracil (PTU) is a well known inhibitor of thyroxine (T_4) to triiodothyronine (T_3) conversion as evidenced by its effect in several in vitro systems and by the decrease in serum T_3 caused by this drug in either rats or man receiving T_4 replacement. However, the failure of PTU to decrease the intrapituitary T_3 concentration and to completely blunt the serum T_3 concentration in T_4-replaced athyreotic rats suggest that there may be a PTU-insensitive pathway of T_4 to T_3 conversion in some tissues. To address this question, we have studied the in vivo effect of PTU treatment on the generation of $[^{125}I]T_3$ from $[^{125}I]T_4$ in the serum and cerebral cortex (Cx), cerebellum (Cm), liver (L), and anterior pituitary (P) of euthyroid rats. Whereas PTU decreased the concentration of $[^{125}I]T_3$ in the serum, L homogenates, and L nuclei after $[^{125}I]T_4$, it did not affect the concentration of $[^{125}I]T_3$ in homogenates or nuclei of Cx, Cm, or P. Iopanoic acid pretreatment significantly reduced the $[^{125}I]T_3$ concentration in serum, homogenates, and cell nuclei of all these organs. Neither agent affected the metabolism or tissue distribution of simultaneously injected $[^{131}I]T_3$. The presence of PTU in these tissues was evaluated by in vitro assessment of iodothyronine 5′-deiodinating activity using both $[^{125}I]rT_3$ and $[^{125}I]T_4$ as substrates. In agreement with the in vivo findings, generation of $[^{125}I]T_3$ from T_4 in vitro was not affected by PTU in Cx, Cm, P but it was inhibited by 76% in L. However, rT_3 5′-deiodination, known to be sensitive to PTU in these tissues, was inhibited in all four indicating that the PTU given in vivo was present in significant amounts. These results demonstrate that in rat Cx, Cm, and P unlike liver, PTU does not inhibit T_4 to T_3 conversion in vivo despite the presence of the drug in the tissues in amounts that significantly inhibit reverse T_3 5′-deiodination. These results show that in vivo 5′-deiodination of T_4 proceeds via a PTU-insensitive pathway in the central nervous system and pituitary, while this pathway is not quantitatively important in the L. This mechanism accounts for the "locally generated" T_3 in central nervous system and pituitary and could also provide the approximately one-third of extrathyroidally produced T_3 not blocked by PTU administration in athyreotic T_4-replaced rat.

INTRODUCTION

3,5,3′-Triiodothyronine (T_3)[1] appears to be the major active thyroid hormone at the cellular level (1). Studies in man and in rat indicate that more than two-thirds of extrathyroidal triiodothyronine (T_3) is produced via 5′-deiodination of thyroxine (T_4), a reaction which occurs in many tissues (2). In some (liver, kidney, and heart), the bulk of intracellular T_3 exchanges rapidly with plasma (3, 4). However, in the pituitary, the T_3 produced from T_4 does not immediately equilibrate with the plasma T_3 (2). Furthermore, this locally produced T_3 makes a contribution of 50% or more to the intracellular T_3 in this tissue (2, 5). T_4 to T_3 conversion, at rates sufficiently rapid to make a significant contribution to total intracellular T_3, has been recently identified in the rat central nervous system (6–8). Similarly this locally generated T_3 does not readily exchange with serum T_3. Furthermore, whereas hypothyroidism increases the T_4 5′-deiodination in the pituitary (9) and brain (10), this condition decreases the activity of this process in the liver (9). The reason for this difference between various organs is not apparent and could reflect intrinsic differences in the pathways of T_3 generation.

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[1] *Abbreviations used in this paper*: Cm, cerebellum; Cx, cerebral cortex; DTT, dithiothreitol; IOP, iopanoic acid; L, liver; MMI, methimazole; N/P ratio, nuclear to plasma ratio; $N[^{125}I]T_3$, nuclear$[^{125}I]T_3$; P, anterior pituitary; PTU, propylthiouracil; rT_3, reverse T_3; T_3, triiodothyronine; T_4, thyroxine.

A number of studies on the effect of propylthiouracil (PTU) on T_4 to T_3 conversion are compatible with the idea that there may be more than one enzymatic pathway of extrathyroidal T_3 generation. Thus, this agent does not cause >70% inhibition of serum T_3 generation in in vivo studies in thyroidectomized, T_4-replaced rats (11–14) despite the fact that T_4 to T_3 conversion is inhibited over 90% in liver and kidney homogenates from PTU-treated rats (15, 16). On the other hand, in the anterior pituitary, the generation of intracellular T_3 in vivo is not inhibited by PTU (17) nor is T_4 to T_3 conversion affected in vitro by this agent (9, 18). These results could be explained by a PTU-insensitive pathway for T_3 production in anterior pituitary. Alternatively, recent studies in GH-3 cells raise the possibility that PTU may not enter some cells in effective concentrations (19). Recent studies have shown that 5'-deiodination of reverse T_3 (rT_3) in cerebral cortex may proceed by two different mechanisms, one of which is insensitive to PTU and has a lower K_m for rT_3 than the classical PTU-sensitive pathway (20). Nanomolar concentrations of T_4 inhibit only the PTU-sensitive reaction. Taken together, these data raise the possibility that a significant fraction of extrathyroidal T_4 to T_3 conversion may proceed via a process that is PTU insensitive. This pathway may be predominant in some tissues like the central nervous system and pituitary, but because of their small size and the incomplete exchange of intracellular T_3 in these tissues with serum T_3, the contribution of this source of T_3 to the total extrathyroidal T_3 pool may have been underestimated (2). The physiological importance of such a source of T_3 is difficult to approach in vitro, since these studies involve alteration of tissue structure by homogenization, addition of artificial cofactors, nonspecific binding of substrates to tissue proteins, etcetera. In addition, since one of the enzyme pathways may have a lower K_m than the other (20), one should use the appropriate endogenous concentration of T_4 for each tissue to determine what fraction of the substrate is metabolized by each enzymatic pathway. Accordingly, we have chosen to study these questions in vivo by evaluating the effect of PTU on the generation of $[^{125}I]T_3$ from $[^{125}I]T_4$ in cerebral cortex (Cx), cerebellum (Cm), anterior pituitary (P), and liver (L). The results show that virtually all the $[^{125}I]T_3$ found in the nuclei of Cx, Cm, and P is PTU insensitive, whereas serum as well as L $[^{125}I]T_3$ was significantly decreased by PTU pretreatment. PTU was present in significant amounts in all tissues examined.

METHODS

In vivo studies. Euthyroid male Sprague-Dawley rats weighing 175–250 g were obtained from Zivic-Miller, Allison Park, PA. The isotopic methods to study the sources of T_3 in different tissues in vivo have been published (5, 6, 17, 21). $[^{125}I]T_4$ is injected intravenously with or followed by $[^{131}I]T_3$. The latter is used to calculate the contribution of plasma T_3 to intracellular T_3 as well as being recovery standard in the various extraction procedures. Knowing the concentration of $[^{131}I]T_3$ in the plasma and in the tissues, and the $[^{125}I]T_3$ in plasma, the $[^{125}I]T_3$ found in a given tissue that is due to plasma $[^{125}I]T_3$ can be calculated. The tissue $[^{125}I]T_3$ in excess of that derived from plasma is that generated locally in the tissue (T_3 $[T_4]$).

In the present experiments, $[^{125}I]T_4$, ~100 μCi/100 g body wt (4,200 μCi/μg sp act), and $[^{131}I]T_3$, ~10 μCi/100 g body wt (2,800 μCi/μg sp act), were injected intravenously simultaneously 3 or 18 h before killing the rats (7). Tracers were dissolved in 0.1–0.2 ml of 10% normal rat serum in isotonic saline containing 100–150 μg NaI to prevent the recirculation of radioactive iodine. Experiments performed 18 h after injecting $[^{125}I]T_4$ (Table I) were intended to estimate the effect of PTU in steady-state conditions and therefore they required that, for each tissue, both, locally generated nuclear (N) $[^{125}I]T_3$ and $[^{131}I]T_3$ be equilibrated with serum $[^{125}I]T_4$ and $[^{131}I]T_3$, respectively (4–7, 17, 21). At 18 h the N T_3 $[T_4]$ is equilibrated with plasma $[^{125}I]T_4$ in all tissues examined (5, 7). However, the nuclear to plasma (N/P) ratio for $[^{131}I]T_3$ present 18 h after T_3 injection is greater than that at the time of equilibrium (t_m) in these tissues. This is due to the more rapid decrease in plasma than tissue tracer T_3 as we have previously discussed (5). Therefore, the observed N/P ratio for $[^{131}I]T_3$ at 18 h must be corrected to obtain the equilibrium N/P ratio for each tissue. The necessary correction factors have been determined in parallel experiments and were 0.52, 0.59, and 0.46 for Cx, Cm (7), and for L, respectively. The corrected N/P ratios were multiplied by the observed plasma $[^{125}I]T_3$ to compute the tissue $[^{125}I]T_3$ derived from plasma (Table I). These correction factors allow use of the same rats for analyses of different tissues and avoid the physiological perturbations associated with a second injection of $[^{131}I]T_3$. For time intervals within a few hours of the t_m for T_3 for L, Cx and Cm, differences between the observed N/P and that present at the t_m are not experimentally demonstrable (4, 5, 7). Therefore, no corrections are required when both isotopes were given within 3 h of tissue analyses (Tables II and III).

Iopanoic acid (IOP, Telepaque R) was supplied by Mr. A. E. Soria, Winthrop Laboratories Co., New York. This was dissolved in alkalinized isotonic saline and 5 mg/100 g body wt were given intraperitoneally at various intervals before sacrifice. PTU was similarly dissolved and injected intraperitoneally at a dose of 1 mg/100 g body wt at indicated times. Control animals received the same vehicle with identical timing.

The animals were killed by aortic exsanguination under light ether anesthesia and perfused with 30 ml of cold isotonic saline through the inferior vena cava, to minimize the contribution of trapped plasma to the tissue radioactivity (22). Cell nuclei from Cx, Cm, P, and L were prepared as previously described (6, 22). Identification and quantitation of $[^{131}I]T_3$ and $[^{125}I]T_3$, and $[^{125}I]T_4$ bound to nuclei and present in tissue homogenates were also performed as described earlier (17, 22). When $[^{131}I]T_3$ in a given sample was present alone, i.e., in isolated nuclei or in serum from which all non-T_3 ^{131}I was eliminated by affinity chromatography (23), it was used to correct for the losses of $[^{125}I]T_3$ during paper chromatography. Sufficient counts were accumulated to reduce counting error to <5%. Serum $[^{125}I]T_3$ and $[^{131}I]T_3$ were isolated by affinity chromatography followed by paper chromatography (5, 23).

In vitro studies

Tissue preparation. 5'-Deiodinating activity was measured in homogenates of L, Cx, and P and in Cx microsomes. Tissues were homogenized in 5 vol (wt/vol) of a solution containing 0.32 M sucrose, 10 mM HEPES pH 7.0, and either 10 mM (P, Cx) or 1 mM (L) dithiothreitol (DTT), unless otherwise indicated. To prevent any effect of PTU contaminating the tissue on the enzyme during homogenization, we added 5 mM methimazole (MMI) to this buffer (24). Microsomes of Cx were the 10,000–100,000 *g* pellet. MMI concentrations in the final homogenates were reduced by dilution to <0.6 mM. The final wash of the microsomal pellet did not contain MMI.

Deiodination assays. 5'-Deiodination of iodothyronines was measured in a total volume of 100 μl of reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.0) 1 mM EDTA, 1–20 mM DTT, as indicated, and either $[^{125}I]T_4$ or $[5'\text{-}^{125}I]rT_3$. The concentrations of iodothyronines in each assay are specified in the Results. Reactions were started by adding tissue (70–150 μg protein in 40–50 μl) and incubating at 37°C under nitrogen. Incubation times and protein concentrations were designed to keep the fraction of substrate consumed to <20%. Reactions were terminated by addition of 50 μl serum followed by 350 μl ice-cold 10% TCA (rT_3 assay) and by cold 95% ethanol containing 0.2 mg T_4 and T_3/ml and 1 mM MMI (T_4 to T_3 conversion assay) (15, 16). ^{125}I released from rT_3 was quantitated by column chromatography (25). Samples of the rT_3 deiodination products under various assay conditions showed that the $^{125}I/3'\ ^{125}I\text{-}T_2$ ratio was 1.0. $[^{125}I]T_3$ formation was quantitated by paper chromatography (16).

Reagents. $[^{125}I]T_4$ and $[^{131}I]T_3$ were prepared by chloramine-T iodination and purified by paper chromatography (5). $[^{125}I]rT_3$ was obtained from New England Nuclear, Boston, MA. Other reagents were obtained from Sigma Chemicals Co., St. Louis, MO or Fisher Scientific Co., Newburgh, NY.

TABLE I

*Effect of Pretreatment with PTU on Serum and NT3 from Various Tissues 18 h after Injecting $[^{125}I]T_4$ and $[^{131}I]T_3$**

Serum (% dose/ml)			
	$[^{125}I]T_3$ ($\times 10^{-3}$)		$[^{131}I]T3$ ($\times 10^{-2}$)
Control	8.0±1.7		3.3±1.0
PTU	4.1±0.6		2.4±0.3
P	<0.005		NS
Cx [(%/mg DNA) × 10^{-4})]			
	$[^{125}I]T_3$ Total	Local	$[^{131}I]T3$
Control	78±24	64±20	131±43
PTU	59±11	50±13	120±14
P	NS	NS	NS
Cm [(%/mg DNA × 10^{-4})]			
Control	11±1	6.0±2.2	35±6
PTU	10±2	6.0±1.4	37±4
P	NS	NS	NS
Liver [(%/mg DNA × 10^{-4})]			
Control	25±3	7±3	132±22
PTU	13±1	4±2	147±28
P	<0.001	NS	NS

Data represent mean±SD.

* Groups of four euthyroid rats were injected with either PTU 1 mg/100 g body wt or vehicle (controls) 28, 18, and 4.5 h before sacrificing the animals. $[^{125}I]T4$ and $[^{131}I]T_3$ were mixed and injected intravenously 18 h before killing. Local $N[^{125}I]T_3$ was calculated as described in Methods. To compute the equilibrium N/P ratio of T_3, the observed ^{131}I N/P in Cx, Cm, and liver were multiplied by 0.52, 0.59, and 0.46, respectively.

RESULTS

Effect of PTU on in vivo *N $[^{125}I]T_3$ content after the injection of $[^{125}I]T_4$.* Since $[^{125}I]T_3$ generated locally from $[^{125}I]T_4$ (T_3 [T_4]) equilibrates slowly with serum $[^{125}I]T_4$ we first examined the effect of PTU pretreatment on the $N[^{125}I]T_3$ in Cx, Cm, and L 18 h after $[^{125}I]T_4$ injection. At this time the Cx and Cm $NT_3[T_4]$ are equilibrated i.e., the specific activity of $N[^{125}I]T_3$ is maximal and similar to serum $[^{125}I]T_4$ (7). This should provide the most sensitive reflection of steady-state conditions during PTU administration. PTU was given 28, 18, and 4.5 h before killing the animals. The results are shown in Table I. PTU treatment caused a 50% decrease in the concentration of serum $[^{125}I]T_3$ ($P < 0.005$). However, PTU had no effect on $N[^{125}I]T_3$ in Cx and Cm, while in the liver total $N[^{125}I]T_3$ was decreased by 50%. As judged by the concentration of $[^{131}I]T_3$ in serum or the $N[^{131}I]T_3$, the PTU effect cannot be explained on the basis of changes in T_3 metabolism. There was no significant effect of PTU pretreatment on locally produced T_3 in any of the three tissues, though local T_3 in liver nuclei was depressed from 7±3 to 4±2% of the dose per milligram DNA × 10^{-4}. In confirmation of previous studies, ~85 and 60% of the $N[^{125}I]T_3$ in the Cx and Cm, respectively was locally produced, whereas only 30% of the liver $N[^{125}I]T_3$ was locally derived (6, 7). The serum $[^{125}I]T_4$ concentrations also were not different in controls and PTU-treated animals (data not shown). In two parallel experiments, where tracers were not given, there was no significant change in the serum T_4 concentration (radioimmunoassay) with this regimen of PTU administration; in only one of these there was a slight fall in serum T_3 (radioimmunoassay) of 0.17±0.09 ng/ml ($P < 0.01$).

Since IOP is a potent inhibitor of T_4 to T_3 conversion known to affect the pituitary and Cx T_3 [T_4] in vivo, we performed further experiments comparing its effect with that of PTU. Although the equilibration of $N[^{125}I]T_3$ with serum $[^{125}I]T_4$ is slow, both in central nervous system tissue and in pituitary, accumulation of $[^{125}I]T_3$ is significant at 3 h and at this time the effect of IOP is also evident. Accordingly, the following experiments were done 3 h after the injection of $[^{125}I]T_4$. Table II shows a typical experiment comparing the effects of both drugs on serum and $N[^{125}I]T_3$ and $[^{131}I]T_3$. PTU caused a 63% and IOP an 80% fall in serum $[^{125}I]T_3$ while neither drug affected the concentration of $[^{131}I]T_3$. Total $N[^{125}I]T_3$ was not affected by PTU in Cx, Cm, and pituitary but it was reduced by 60% in the liver. In contrast, the $N[^{125}I]T_3$ was markedly reduced in all four tissues in IOP-treated rats. Locally generated T_3 was not affected by PTU in Cx, Cm, and pituitary but liver NT_3 (T_4) was reduced to virtually zero. Neither IOP nor PTU affected the nuclear content of $[^{131}I]T_3$ except in the Cx where it was higher.

To demonstrate that the $N[^{125}I]T_3$ measurements were a reflection of alterations in $[^{125}I]T_4$ to T_3 conversion in the whole tissue, $[^{125}I]T_3/[^{125}I]T_4$ ratios in nuclei and homogenates were measured (Table III). The small amount of tissue precluded such comparisons in the pituitary where only the nuclear data were available. PTU did not affect the T_3/T_4 ratio in Cx, Cm, and pituitary but did cause a significant reduction of this ratio in the liver. In contrast, the ratio was lower in all four tissues in IOP-treated rats. These results are quite consistent with those that would be predicted from the effects of these agents on $N[^{125}I]T_3$ in Table II.

Iodothyronine 5′-deiodinase activity in tissues from animals treated with PTU and IOP. The assays of iodothyronine 5′-deiodinase activity in microsomes from Cx are shown in Fig. 1. PTU pretreatment did not affect T_4 to T_3 conversion while in IOP-treated rats there was a 70% decrease in this reaction rate. The lack of effect of PTU on T_4 to T_3 conversion in the Cx microsomes either in vivo or in vitro could be due to the failure of the drug to penetrate the central nervous system in adequate amounts. Since rT_3 5′-deiodination

TABLE II

Effect of PTU and IOP Pretreatment on Serum and Nuclear T_3 of Various Tissues 3 h after the Injection of $[^{125}I]T_4$ and $[^{131}I]T_3$

Serum (% dose/ml)	$[^{125}I]T_3$ ($\times 10^{-3}$)	P*	$[^{131}I]T_3$ ($\times 10^{-2}$)	P*
Control	4.1±0.6		11.1±2.5	
PTU	1.5±0.6	<0.001	9.5±1.4	NS
IOP	0.8±0.3	<0.001	12.2±3.5	NS

	$[^{125}I]T_3$				^{131}I-T_3	
	Total		Local			
Cx [(% dose/mg DNA) × 10^{-4}]						
Control	24±3		21±3		108±33	
PTU	29±6	NS	26±5	NS	144±40	NS
IOP	8±1	<0.001	7±1	<0.001	190±30	<0.025
Cm [(% dose/mg DNA) × 10^{-4}]						
Control	2.0±0.6		1.1±0.5		22±5	
PTU	1.3±0.4	NS	1.0±0.4	NS	19±4	NS
IOP	0.1±0.0	<0.001	0	<0.001	28±6	NS
Liver [(% dose/DNA) 10^{-4}]						
Control	20±5		5±3		436±99	
PTU	8±1	<0.005	0±0	<0.025	480±62	NS
IOP	5±1	<0.001	1±1	<0.05	498±70	NS
Pituitary [(% dose/pituitary) × 10^{-4}]						
Control	2.2±0.4		0.7±0.1		42±7	
PTU	1.9±0.9	NS	1.2±0.7	<0.001	47±2	NS
IOP	0.5±0.4	<0.001	0	<0.001	56±9	NS

Data represent mean±SD.

* P vs. control. Groups of four euthyroid rats were injected intraperitoneally with either IOP 5 mg/100 g body wt of PTU 1 mg/100 g body wt 24, 16 and 1.5 h prior to tracer injections. The control group was injected with vehicle at the same time. $[^{125}I]T_4$ and $[^{131}I]T_3$ were injected simultaneously intravenously.

TABLE III
Effect of PTU and IOP on the Observed $[^{125}I]T_3/[^{125}I]T_4$ Ratios in Cell Nuclei and Homogenates from Various Tissues 3 h after Injection of $[^{125}I]T_4$*

		Control	PTU	P‡	IOP	P‡
Cx	H	0.48±0.13	0.40±0.07	NS	0.16±0.02	<0.001
	N	5.33±0.95	4.78±1.50	NS	0.85±0.27	<0.001
Cm	H	0.13±0.08	0.11±0.06	NS	≃0	<0.025
	N	7.27±3.35	9.57±8.91	NS	0.13±0.03	<0.001
Pituitary	N	10.3±2.1	6.70±2.79	NS	0.38±0.18	<0.001
Liver	H	0.07±0.01	0.04±0.006	<0.005	0.02±0.005	<0.001
	N	0.58±0.11	0.24±0.06	<0.005	0.15±0.04	<0.001

Data represent mean±SD.

* $[^{125}I]T_3$ cpm/$[^{125}I]T_4$ cpm in paper chromatograms of butanol extracts of the samples. No correction was made for specific activity differences. Experiment performed as in Table II. H, homogenate; N, nuclear pellet.

‡ *P* vs. control.

in the euthyroid Cx microsomes is inhibited by PTU (20), rT_3 deiodination rates can be used as a "bioassay" of tissue PTU concentrations. Fig. 1 shows that the total rT_3 5'-deiodination rate is reduced by more than 50% in the PTU-treated rats. That PTU is an effective inhibitor of rT_3 deiodination in this tissue is evident from the significant decrease in rT_3 deiodinase activity found in microsomes from all three groups in the pres-

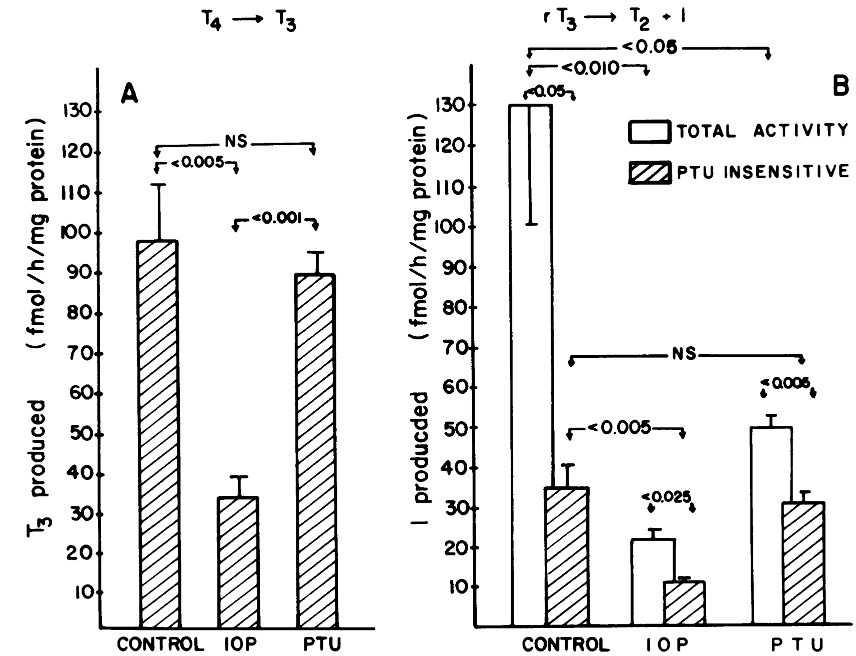

FIGURE 1 Iodothyronine 5'-deiodinase activity in microsomes from rat Cx. Groups of euthyroid rats ($n = 4$) were treated with PTU or IOP (Methods). Homogenization and microsomal preparation was carried out in 0.32 M sucrose, 0.010 M Hepes pH 7, 10 mM DTT, and 5 mM MMI as described. T_4 to T_3 conversion was measured by the production of $[^{125}I]T_3$ from 5 nM $[^{125}I]T_4$ in the presence of 15 mM DTT and 1 μM T_3 (panel A). rT_3 deiodination was measured by the release of ^{125}I from 2 nM $[5'\text{-}^{125}I]rT_3$ in the presence of 15 mM DTT. PTU-insensitive refers to the reaction performed in the presence of 1 mM PTU (panel B).

ence of 1 mM PTU added in vitro (Fig. 1). This procedure reduced the activity in PTU-treated animals to levels identical to those found in control tissue, implying that the difference in total activities between control and in vivo PTU-treated rats was due to the presence of this drug in the tissue. As expected, IOP-treated rats also showed an inhibition of rT_3 5'-deiodinase activity.

Table IV shows the results of experiments where the 5'-deiodinase activity was assayed in crude homogenates of Cx, pituitary, and liver from similarly treated animals. In experiment 1, T_4 to T_3 conversion was assayed at 2 nM T_4 and 20 mM DTT in all tissues. In vivo treatment with PTU did not affect T_4 to T_3 conversion in Cx or pituitary while it decreased the activity by 75% in the liver. In a second experiment the T_4 5'-deiodinase activity was also measured in the presence of PTU (1 mM) added in vitro. In this assay the liver activity was measured at 100 nM T_4 and 1 mM DTT to optimize the conditions for demonstrating an effect of PTU. The results were comparable to those of experiment 1. Thus, while no inhibition was detected in Cx and pituitary, there was ~90% inhibition in the liver. The addition of PTU in vitro did not inhibit the activity in Cx and pituitary homogenates, but caused a 90% inhibition in the livers from control animals. PTU added in vitro did not further inhibit 5'-deiodination of T_4 in livers of PTU-treated rats.

In the first experiment, pretreatment with PTU caused a significant fall in the rate of 5'-deiodination of rT_3 in both Cx (~40%, $P < 0.005$) and pituitary (30%, $P < 0.05$), which contrasts with the lack of effect of this treatment on the T_4 to T_3 conversion assay (Table IV). In the liver the pretreatment with PTU induced a 95% fall in 5'-deiodination of rT_3. Since the conditions of the assay in this experiment might have favored the effect of small amounts of PTU in the liver and partially overcome the effect of PTU in pituitary and Cx, a second experiment was carried out where the concentration of rT_3 and DTT in the liver homogenates were the same as those used for the assay of the pituitary and Cx. In this case the liver was also homogenized in buffer containing 10 mM DTT as were the Cx and the pituitary. The inhibition observed in the liver and Cx of PTU-treated rats was entirely comparable to that of the first experiment. However, the rT_3 5'-deiodination rates observed in the pituitaries in this experiment were not significantly different in the PTU-treated rats. Earlier studies have shown that 1 mM PTU gives maximal inhibition of PTU-sensitive rT_3 5'-deiodination in rat Cx (20). The data from the control rats in Table IV show that this amount of PTU causes only 50–70% inhibition of rT_3 5'-deiodination in Cx and pituitary but 90% or greater inhibition in the liver. Since the in vivo PTU treatment caused at least 60% inhibition of the PTU-sensitive fraction of rT_3 5'-deiodinase, it suggests that roughly comparable quantities of PTU were present in all three tissues.

DISCUSSION

The enzyme converting T_4 to T_3 in the central nervous system is critically important to the thyroid status of this tissue since recent studies in euthyroid rats indicate that locally generated T_3 accounts for >70% of the nuclear T_3 in Cx and 50% of that in Cm (6, 7). In that respect, the tissue resembles the rat anterior pituitary in which 50% of the nuclear T_3 is derived from local T_4 to T_3 conversion whereas <30% of nuclear T_3 in rat liver and kidney is derived from this source (5). The present experiments show further similarities of central nervous system T_4 to T_3 conversion to that in anterior pituitary. Pretreatment of rats with amounts of PTU previously shown to inhibit extrathyroidal T_3 production by 70% in T_4-treated athyreotic rats (13, 14) had no effect on the local production of T_3 from T_4 in the Cx or Cm. Similar results have been observed in rat pituitary and are confirmed in this study (17). Though PTU and IOP both reduced the serum $[^{125}I]T_3$ present 3 h after $[^{125}I]T_4$, the amount of T_3 was differentially affected by the two agents in the various tissues. Whereas IOP decreased the concentration of $[^{125}I]T_3$ in all organs, PTU did so only in the liver. The fact that PTU decreased the T_3/T_4 ratio in the liver to the same extent as the tissue $[^{125}I]T_3$ (Table III) while in the other tissues this ratio did not change, indicates that the differences observed cannot be ascribed to differential effects of PTU on the tissue uptake of $[^{125}I]T_4$. Likewise, the fact that the $[^{131}I]T_3$ concentration was not decreased by either PTU or IOP in tissues or serum indicates that the differences cannot be explained by differential effects of PTU on T_3 metabolism or distribution. Taken together, these findings point to a difference in the sensitivity to PTU of the process responsible for the generation of T_3 in these tissues.

In agreement with in vivo data, T_4 to T_3 conversion was not affected when assayed in vitro in Cx microsomes from animals treated with PTU while it was decreased in about the same proportion as the $[^{125}I]T_3$ content in this tissue was decreased in vivo in rats that received IOP. There are two lines of evidence that indicate that PTU was present in Cx; the first is that when iodothyronine 5'-deiodinase was assayed by $[^{125}I]rT_3$ deiodination there was a significant difference between controls and PTU-treated animals (Fig. 1B). Second, when PTU was added to the reaction mixture, it induced a 70% fall in rT_3 5'-deiodination in the Cx microsomes from controls while it only modestly decreased deiodination in the PTU-treated animals. The

TABLE IV

Effect of PTU Pretreatment on Iodothyronine 5′-Deiodinase Activity in Various Tissues of the Rat

	Experiment 1 (n = 5)					Experiment 2 (n = 6)				
(A.) T_4 to T_3 Conversion	In vivo Rx*		In vitro PTU‡ —	In vitro PTU‡ +	*P*§	In vivo Rx		In vitro PTU —	In vitro PTU +	*P*§
Cx										
2 nM T_4 20 mM DTT	Control		25.9±11.8	–		Control		22.3±16.3	19.3±12.9	NS
(*fmol/h/mg protein*)	PTU		22.1±12.8	–		PTU		24.2±12.1	20.9±11.7	NS
		P	NS				*P*	NS	NS	
Pituitary										
2 nM T_4 20 mM DTT	Control		332±143	–		Control		259±89	311±107	NS
(*fmol/h/mg protein*)	PTU		278±45	–		PTU		392±200	366±102	NS
		P	NS				*P*	NS	NS	
Liver										
2 nM T_4 20 mM DTT	Control		39.0±8.3	–		–		—	—	
(*fmol/h/mg protein*)	PTU		9.3±1.8	–		–		—	—	
		P	<0.01				—			
100 nM T_4 1 mM DTT	—		—	—		Control		3.4±0.3	0.3±0.2	<0.005
(*pmol/h/mg protein*)	—		—	—		PTU		0.4±0.3	0.4±0.3	NS
		–					*P*	<0.001	NS	
(B.) rT3 5′-deiodination										
Cx										
2 nM rT3 20 mM DTT	Control		20.1±3.6	6.1±3.8	<0.01	Control		47.4±15.8	11.8±1.6	<0.001
(*fmol/h/mg protein*)	PTU		11.3±2.4	7.0±2.4	NS	PTU		21.7±6.6	14.5±5.4	<0.001
		P	<0.005	NS			*P*	<0.01	NS	
Pituitary										
2 nM rT3 20 mM DTT	Control		0.15±0.01	0.08±0.001	<0.005	Control		1.0±0.1	0.3±0.1	<0.001
(*pmol/h/mg protein*)	PTU		0.11±0.01	0.09±0.01	<0.025	PTU		0.8±0.2	0.5±0.1	<0.001
		P	<0.05	NS			*P*	NS	<0.05	
Liver										
2 nM rT_3 20 mM DTT	Control		—	—		Control		36±12	4.3±1.6	<0.001
(*pmol/h/mg protein*)	PTU		—	—		PTU		2.6±0.8	0.5±0.6	<0.001
		P	—	—			*P*	<0.001	<0.001	
1 μM rT_3 1 mM DTT	Control		946±182	25±2	<0.001	Control		—	—	
(*pmol/h/mg protein*)	PTU		45±9	26±2	NS	PTU		—	—	
		P	<0.001	NS						

Data represent mean ±SD.

* Rx, treatment; PTU given as in Table 1.

‡ In the absence or the presence of 1 mM PTU added in vitro.

§ No PTU vs. PTU added in vitro by paired t test.

amount of PTU-insensitive activity was unaffected by PTU in vivo, suggesting that PTU was present in the tissue though at less than maximally inhibitory concentrations. Since PTU might have been present only in trapped plasma or in cells other than those possessing 5′-deiodinase activity and possibly artifactually affected deiodinase activity, tissues were homogenized and microsomes isolated in the presence of 5 mM MMI. This drug does not inhibit 5′-iodothyronine deiodinase but has been shown to prevent the effect of PTU, subsequently added, on kidney deiodinase (15). The MMI was present throughout the preparation of the microsomes and removed in the final step before the assay. These observations, therefore, suggest that PTU was already bound to the enzyme in vivo.

Comparable results were observed in tissue homogenates (Table IV) all taken from the same animals. T_4 to T_3 conversion activity in the Cx and pituitary was not affected by PTU-pretreatment while it was reduced by 75% (first experiment) and 90% (second experiment) in the liver. In the first experiment the assay conditions (2 nM T_4, 20 mM DTT) were the same in all three tissues. Accordingly, the differences in PTU-sensitivity among tissues cannot be attributed to differences in the assay conditions. Again the presence of PTU in the Cx and pituitary was evidenced by the inhibition of rT_3 5′-deiodination, although in pituitary this was not consistent. The addition of 1 mM PTU in vitro further inhibited the reaction in all three tissues from control animals. In PTU-treated rats, the additional inhibition caused by in vitro PTU relative to the basal activity (controls measured in the absence of PTU) was only modest indicating the presence of PTU in these tissues at significant, though submaximal, concentrations. Thus the differences between Cx, pituitary, and liver with respect of rT_3 5′-deiodinase activity after in vivo PTU can be attributed to the fact that in liver, a higher fraction of this activity, if not all, is PTU sensitive. In the second experiment, shown in Table IV, the rT_3 deiodination in liver was assayed under the same conditions as were the pituitary and the Cx (2 nM rT_3, 20 mM DTT) to eliminate the possibility that the different conditions used in the liver in experiment 1 could have favored the effect of PTU (more substrate, less cofactor, [15]). The degree of inhibition was comparable to that observed in experiment 1.

Evidence for two pathways for rT_3 5′-deiodination in Cx in vitro has been published recently (20). One mechanism similar to that previously identified in liver (16) and kidney (15), has a K_m of 31 nM, is inhibited by PTU but little if at all by up to 1 μM T_4. A second mechanism has a low K_m for rT_3 (2.7 nM) is insensitive to 1 mM PTU but is inhibited by low concentrations of T_4 (K_i, half maximal inhibitory concentration for $T_4 \sim 2$ nM). The present results indicate that in vivo virtually all the local T_3 production in Cx, Cm, and pituitary occurs by a PTU-insensitive mechanism. However, $[^{125}I]T_3$ content in the liver is quite sensitive to PTU as reflected in the reduced ratio of $[^{125}I]T_3/[^{125}I]T_4$ in this organ. Although this may be due to a rapid equilibration of $[^{125}I]T_3$ formed via a PTU-insensitive pathway with serum $[^{125}I]T_3$, the in vitro findings (75–95% inhibition of T_4 to T_3 conversion by in vivo PTU and similar inhibition induced by in vitro added PTU) suggest that the low hepatic $[^{125}I]T_3$ content after injection of $[^{125}I]T_4$ in PTU-treated rats reflects the minor importance of the PTU-insensitive pathway in the liver.

There are several implications of these findings. Firstly, it is known that maximal doses of PTU can reduce the serum concentration of T_3 in thyroidectomized T_4-maintained euthyroid rats by only 70%. Since there is near maximal inhibition of 5′-deiodinase activity in the liver and kidney under these circumstances, it is reasonable to speculate that the PTU-nonsuppressible serum T_3 in these conditions is generated via a pathway similar to that found in the central nervous system and pituitary. Secondly, in the Cx, Cm, and pituitary, T_3 (T_4) accounts for 50% or more of the tissue T_3, while in the liver and kidney T_3 (T_4) is of much less importance (5, 7). This seems to be more than a coincidence though our present ignorance about the precise nature of the mechanisms involved in T_4 deiodination allows us only to call attention to it. Thirdly, since the PTU administration is more likely to affect those tissues like liver and kidney whose T_3 content depends heavily on serum T_3, while not affecting as markedly the concentration of T_3 in tissues like pituitary and Cx (provided the serum T_4 concentration is not reduced), it should be a useful tool to study the physiological relevance of these two sources of intracellular T_3.

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