Effect of Propylthiouracil on the *in Vivo* Deiodination of Thyroxine Labeled with I^{131} in Different Positions

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ABSTRACT. It has been found, using thyroidectomized, thyroxine-maintained rats, that the administration of propylthiouracil depresses the urinary excretions of iodide I^{131} when labeled L-thyroxine is injected previously. This decrease was found whether the hormone was labeled with I^{131} on the atoms of the phenolic ring, on those of the inner ring, or had been biosynthetically labeled. It is concluded that this thiouracil partially inhibits *in vivo* deiodination of thyroxine on both the phenolic and the inner ring.

IT HAS BEEN shown in intact rats (1–3) and in thyroidectomized rats, maintained with thyroxine or with triiodothyronine (4), that the administration of several thiouracil derivatives results in a depression of the urinary excretion of iodide I^{131} after the injection of thyroid hormones labeled with I^{131} on the phenolic ring. These results have been interpreted as indicating that the extrathyroidal deiodination of thyroid hormones is depressed by these compounds. Braverman and Ingbar (5) have recently shown that the deiodination of thyroxine *in vitro* using rat kidney slices is inhibited partially by the injection of PTU[1] or the addition of TU *in vitro*. Flock and Bollman (6) have suggested, on the basis of chromatographic analyses of bile samples taken from rats on chronic thiouracil treatment and given one dose of thyroxine labeled on the phenolic ring, that deiodination of the iodine atoms of the inner ring is actually increased with respect to that of nontreated rats, though deiodination on the phenolic ring is depressed. These authors had found (6) that the percentage of biliary radioactivity encountered as 3,3′,5′-triiodothyronine glucuronide after injection of labeled thyroxine increased in chronically thiouracil-treated rats, as compared to controls. Since this metabolite of thyroxine is noncalorigenic, Flock and Bollman (6) also indicated the possibility that its increased concentration in the thiouracil-treated animals may account in part for the marked reduction in calorigenic activity of the thyroid hormone administered to animals receiving thiouracils (7).

We have investigated these suggestions directly by studying the effect of the injection of PTU in thyroidectomized, thyroxine-maintained rats previously administered thyroxine labeled with I^{131} in different positions of the molecule. The present paper shows that the administration of this thiouracil depresses the urinary excretion of I^{131} whether the hormone was labeled in the 3′ and 5′ positions, randomly on all four iodine atoms, or in the 3 and 5 positions.

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[1] The following abbreviations are used: 6-n-propyl-2-thiouracil (PTU); 2-thiouracil (TU); L-thyroxine (L-T_4); diiodotyrosine (DIT); diiodo-p-hydroxylphenylpyruvic acid (DIHPPA).

TABLE 1. Effect of 10 mg of PTU/rat on the distribution of I^{131} in thyroidectomized rats, maintained with 3 μg of L-T_4/rat/day and injected once with either 3 μg of L-T_4 labeled biosynthetically with I^{131} (Group I), 3 μg of L-T_4 labeled with I^{131} on the phenolic ring (Group II), or with 6 μg of L-T_4 labeled with I^{131} on the iodine atoms of the inner ring (Group III). In each group, there were five control and five PTU-treated rats.

Group	I^{131} Label on	% of dose of I^{131}-labeled L-thyroxine: mean ± standard deviation								
		Urine			Feces + Intestines			Plasma (1 ml)		
		Controls	PTU-treated	P	Controls	PTU-treated	P	Controls	PTU-treated	P
I	3, 5, 3', 5'	18.8 ± 2.9	8.4 ± 1.5	<0.001	41.7 ± 1.3	50.1 ± 6.6	n.s.*	0.97 ± 0.12	1.21 ± 0.17	0.05–0.02
II	3', 5'	17.3 ± 2.8	7.3 ± 1.5	<0.001	35.7 ± 5.3	44.9 ± 8.9	n.s.	0.98 ± 0.11	1.02 ± 0.15	n.s.
III	3, 5	19.0 ± 0.6	15.5 ± 1.6	<0.001	34.9 ± 5.6	33.2 ± 5.7	n.s.	0.79 ± 0.15	0.72 ± 0.07	n.s.

* Not significant: P higher than 0.05.

Materials and Methods

Adult male Wistar rats were fed a low-iodine, medium-residue modified Remington diet, thyroidectomized and injected daily thereafter with 3 μg of L-T_4/rat, as described previously (4). Two weeks after thyroidectomy the animals were injected intraperitoneally with the labeled thyroxine preparation. Half the experimental group then received 10 mg of PTU/rat by intraperitoneal injection soon after administration of the labeled hormone. The urine and feces were collected individually during a 22-hr period, at the end of which the animals were sacrificed by bleeding under ether narcosis. The distribution of I^{131}-labeled compounds throughout the body was then determined as described previously (8, 9). The data appearing in the present paper are expressed as percentages of the injected dose of labeled thyroxine.

L-*Thyroxine, labeled on the 3' and 5' iodine atoms,* was obtained from Abbott Laboratories, North Chicago, in a 50% propyleneglycol-water solution. It was diluted with stable L-T_4 so as to obtain a final solution containing about 0.6 μc and 3 μg of L-T_4 in a 0.5 ml injection volume of saline containing 10% rat plasma.

L-*Thyroxine, labeled on the 3 and 5 iodine atoms,* was synthesized from I^{131}-labeled diiodotyrosine and stable 3,5-diiodo-4-hydroxyphenylpyruvic acid according to the non-enzymatic procedure described by Yip and Klebanoff (10), with only minor alterations in the concentrations used. Labeled DIT was obtained from Abbott Laboratories, North Chicago, and DIHPPA was supplied by Dr. Rosalind Pitt-Rivers, National Institute for Medical Research, London.

The labeled thyroxine obtained by this procedure was then purified from the other labeled components by chromatography of the mixture on 40-cm wide Whatman No. 3 MM filter paper sheets and n-butanol-ethanol-2N ammonia (5:1:2) as solvent. Location of the thyroxine band was determined by autoradiography and it was eluted with methanol-2N ammonia (3:1). The eluate was evaporated to dryness and rechromatographed on a narrower sheet in the same solvent system. The band containing the labeled thyroxine was again located by autoradiography and eluted into a few drops of methanol-2N ammonia. Samples of the latter eluate were checked for radiochemical purity using n-butanol-ethanol-2N ammonia and n-butanol-acetic acid-water (75:10:15) as solvent systems, together with stable thyroxine, triiodothyronine, mono- and diiodotyrosine and iodide as markers. The proportion of I^{131} in different chemical fractions was assessed by automatic scanning of the chromatograms and planimetry of the resulting areas and by staining and counting the spots in a well-type scintillation counter.

We found it was very important to keep the samples protected from light during the whole purification procedure. In order to minimize deiodination of the labeled thyroxine obtained on paper (11, 12), the application of the reaction mixture to paper, its chromatography, drying of the chromatographic papers, elution of the thyroxine band

and evaporation to dryness of the eluate were carried out in the dark or in blackened containers. For the same reason a small amount of stable thyroxine (about 40 μg) was added for chromatography. Addition of PTU to the chromatograms, which effectively contributes to the protection of thyroxine from deiodination on paper (12), could not be used in the present case, since this compound has practically the same R_f as thyroxine in the n-butanol-ethanol-2N ammonia system and would later be eluted and injected into animals together with the hormone, that is, into both control and PTU-treated groups.

The labeled L-thyroxine thus obtained contained 93% of the I^{131} as thyroxine and the rest as iodide. The concentration of stable T_4 was calculated from the total I^{127} concentration, determined by the modification of the Zak chloric acid method, as described by Benotti and Benotti (13). The labeled thyroxine was then diluted with saline containing 10% rat plasma, and the 0.5 ml injection volume contained about 0.3 μc and 6 μg of L-T_4.

L-*Thyroxine, labeled at random on the 3,5,3' and 5' positions,* was obtained by biosynthesis. Ten rats, previously fed a low-iodine diet for about 2 weeks, were each injected with 60 μc of I^{131}. The animals were sacrificed 24 hr later and the thyroids were excised, homogenized in Tris-HCl buffer, pH 8.6, and hydrolyzed in the manner described by Mayberry and Astwood (14). Only trace amounts of PTU were, however, added to the homogenates for the reasons indicated above. The entire hydrolysate was then applied to two 40-cm wide Whatman No. 3 MM sheets and chromatographed in n-butanol-ethanol-2N ammonia. The band corresponding to the labeled thyroxine was localized by radioautography, eluted with methanol-ammonia and evaporated almost to dryness. The radiochemical purity of the preparation was checked as already indicated.

This biosynthetically labeled L-thyroxine contained more than 92% of the I^{131} as this hormone, the rest of the radioactivity appearing as iodide. Its stable iodine content was determined as described by Morreale de Escobar and Gutierrez Ríos (15), stable L-thyroxine was then added, and the preparation was diluted with saline containing 10% rat plasma. The 0.5-ml injection volume thus obtained contained about 0.2 μc and 3 μg of L-T_4.

Results and Discussion

Typical results obtained with these labeled thyroxine preparations are shown in Table 1. The depression of the urinary I^{131} excretion caused by the administration of PTU to rats previously injected with thyroxine labeled in the 3' and 5' positions and with the biosynthetically labeled hormone show that the over-all deiodination of the hormone is decreased. The results obtained with the biosynthetically labeled hormone indicate that, if deiodination of the atoms on the inner ring were increased, as suggested by Flock and Bollman (6), this effect would not be very intense as compared to the inhibition of deiodination of iodine on the phenolic ring. The results obtained with L-thyroxine labeled on the 3 and 5 positions clarify this point, since they show that deiodination of these iodine atoms is actually somewhat inhibited, not enhanced, after administration of PTU.

The present results do not support, therefore, the suggestion put forward by Flock and Bollman (6) that the enhanced deiodination of iodine atoms on the inner ring of thyroxine, leading to an increased formation of noncalorigenic 3,3',5'-triiodothyronine, might account for the depressed calorigenic effectiveness of thyroxine in thiouracil-treated animals (7). On the contrary, the results reported here support the conclusion drawn from previous work, namely, that the effect of PTU on metabolic actions of the thyroid hormones, such as calorigenic effectiveness (7) and suppression of TSH secretion (16), might be more closely related to the inhibition of the intracellular degradation of the hormone *via* deiodinating mechanism(s) caused by this drug.

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