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Research Article

Triiodothyronine sulfate (T3S) is rapidly deiodinated by the propylthiouracil (PTU)-sensitive type I deiodinase. Here we examined the effects of PTU on plasma T3S levels in rats after intravenous administration of radiolabeled T3 or T3S. Sephadex LH-20 chromatography and high-performance liquid chromatography were used to quantify conjugated and nonconjugated iodothyronines, and iodide was measured as the TCA-soluble radioactivity. In control rats, radioiodide was the main metabolite of both T3 and T3S. Plasma T3S was cleared more rapidly than plasma T3 despite increased binding to plasma proteins. PTU reduced plasma iodide levels by 66 and 78% after T3 and T3S, respectively, and decreased plasma clearance of T3S by 81%. However, PTU had no effect on plasma T3 clearance but increased plasma T3S from injected T3 4.2 times. Biliary excretion of injected T3S was less than 20% in normal rats, in contrast to 70% within 4 h in PTU-treated rats. In conclusion, T3S is an important intermediate in the in vivo metabolism of T3 in rats and accumulates in plasma if type I deiodination is inhibited.

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Accumulation of Plasma Triiodothyronine Sulfate in Rats Treated with Propylthiouracil

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Abstract

Triiodothyronine sulfate (T_3S) is rapidly deiodinated by the propylthiouracil (PTU)-sensitive type I deiodinase. Here we examined the effects of PTU on plasma T_3S levels in rats after intravenous administration of radiolabeled T_3 or T_3S. Sephadex LH-20 chromatography and high-performance liquid chromatography were used to quantify conjugated and nonconjugated iodothyronines, and iodide was measured as the TCA-soluble radioactivity. In control rats, radioiodide was the main metabolite of both T_3 and T_3S. Plasma T_3S was cleared more rapidly than plasma T_3 despite increased binding to plasma proteins. PTU reduced plasma iodide levels by 66 and 78% after T_3 and T_3S, respectively, and decreased plasma clearance of T_3S by 81%. However, PTU had no effect on plasma T_3 clearance but increased plasma T_3S from injected T_3 4.2 times. Biliary excretion of injected T_3S was < 20% in normal rats, in contrast to 70% within 4 h in PTU-treated rats. In conclusion, T_3S is an important intermediate in the in vivo metabolism of T_3 in rats and accumulates in plasma if type I deiodination is inhibited.

Introduction

Stepwise monodeiodination plays a central role in the metabolism of thyroid hormone in peripheral organs such as liver and kidney (1, 2). After the bioactivation of thyroxine (T_4)[1] to 3,3',5-triiodothyronine (T_3) by outer ring deiodination, T_3 is further converted to biologically inactive iodothyronines by successive deiodination in inner and outer rings (3). In humans, metabolic pathways other than deiodination seem equally important for the inactivation and elimination of T_3 (1). Conjugates of T_3 with glucuronic acid or sulfate have been detected by Bollman and Flock (4) in bile and urine of rats and dogs.

In vitro, enzyme kinetic studies with liver type I deiodinase have shown that, in contrast to T_3 itself, T_3 sulfate (T_3S) is rapidly deiodinated in the inner ring (5). The 3,3'-diiodothyronine sulfate (T_2S) produced is also a much better substrate for outer ring deiodination than nonconjugated T_2 (5, 6). Under normal conditions primary cultures of rat hepatocytes metabolize added [^{125}I]T_3 to iodide and T_3 glucuronide (T_3G). Addition of the type I deiodinase inhibitor 6-propyl-2-thiouracil (PTU) results in accumulation of the T_3S intermediate without affecting T_3 clearance (6). Iodide production is also decresed in sulfate-deplete cells or by inhibitors of phenol sulfotransferase, but under these conditions T_3 clearance is diminished as well (6). It was subsequently shown that PTU treatment resulted in a fivefold increase in biliary T_3S after administration of [^{125}I]T_3 to rats without affecting excretion of T_3G[2] (7). Therefore, sulfation and subsequent deiodination is an important metabolic pathway for T_3 in rat liver. To further investigate the physiological relevance of this pathway we analyzed plasma T_3S in rats with impaired type I deiodinase activity.

Methods

Materials. [^{125}I]T_3 was synthesized by radioiodination of 3,5-diiodothyronine (Henning GmbH, Berlin, FRG) with carrier-free Na^{125}I (Amersham Corp., Amersham, UK) using the chloramine T method. The sulfate conjugate of [^{125}I]T_3 was prepared with chlorosulfonic acid in dimethylformamide and purified on Sephadex LH-20 (8). PTU was purchased from Sigma Chemical Co., St Louis, MO. All other chemicals were of analytical grade.

Experimental procedures. Male Wistar rats, 230–350 g body weight (BW), were anesthetized by injection of 5 mg i.p. pentobarbital sodium per 100 g BW. Additional injections of 2–5 mg pentobarbital were administered during the experiment if necessary. Body temperature was maintained by placing the animals under an infrared lamp. A 100-mM PTU solution in 0.1 M NaOH was diluted five times in phosphate-buffered saline. Rats were injected with 220 μl i.v. (0.75 mg PTU) per 100 g BW of this mixture. Controls were studied in parallel and received the same volume of vehicle. 30 min later ~ 10 μCi [^{125}I]T_3 or [^{125}I]T_3S in 500 μl 0.01 M NaOH in saline was injected intravenously ($t = 0$). Blood samples (0.75 ml) were taken from the tail vein at 0.5, 1, and 2 h, and after cervical dislocation at 4 h the animals were bled by heart puncture.

Biliary excretion of intravenously injected [^{125}I]T_3S was studied as follows. The biliary duct of pentobarbital-anesthetized rats (200 g BW) was cannulated[2] and the animals were injected with PTU or vehicle followed by 10 μCi [^{125}I]T_3S as described above. Blood samples (0.3 ml) were obtained in heparinized vials after 0.5, 1, 2, and 4 h, and bile was collected in 10–20 min periods.

In a parallel experiment PTU was administered 2 h before the animals were anesthetized whereupon the bile duct was cannulated and [^{125}I]T_3 injected, i.e., 2.5 h after PTU. Bile and plasma were collected until 4 h after T_3 injection.

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1. *Abbreviations used in this paper:* BW, body weight; G, glucuronide; HPLC, high-performance liquid chromatography; PTU, 6-propyl-2-thiouracil; S, sulfate; SPE, solid-phase extraction; T_2, 3,3'-diiodothyronine; T_3, 3,3',5-triiodothyronine; T_4, thyroxine.

2. De Herder, W. W., F. Bonthuis, M. Rutgers, M. H. Otten, M. P. Hazenberg, and T. J. Visser. Effects of inhibition of type I iodothyronine deiodinase and phenol sulfotransferase on the biliary clearance of triiodothyronine in rats. Submitted for publication.

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Analysis of samples. Serum and bile were kept at −20°C until further analysis. Plasma $^{125}I^-$ was measured as trichloroacetic acid (TCA)–soluble radioactivity. For this purpose, 50–100 μl serum was mixed with 10% (wt/vol) ice-cold TCA to a final volume of 500 μl. After 10 min at 0°C, mixtures were centrifuged and radioactivity was determined in the supernatant. Radioiodide in bile was estimated similarly by addition of 100 μl pooled human serum to 10–25 μl bile followed by 400 μl 10% TCA. > 95% of T_3 or T_3S added to plasma or bile was precipitated by TCA while on average 97% of added $^{125}I^-$ remained in solution.

For analysis of other plasma metabolites, mixtures were prepared consisting of 250 μl serum, 500 μl 0.2 M HCl, and 250 μl ethanol. These were applied to small Sephadex LH-20 columns (bed vol, 1.3 ml) equilibrated in 0.1 M HCl. After rinsing the columns with 0.1 M HCl, conjugated and nonconjugated iodothyronines were eluted successively with 20% ethanol in water and 0.1 M ammonia in ethanol. Fractions of 1 ml were collected and counted for radioactivity. Recovery of T_3S and T_3 added to rat plasma amounted to 91 and 94%, respectively. For further identification of the isolated products by high-performance liquid chromatography (HPLC), peak fractions of corresponding time points were combined within each experimental group. The conjugate pool was lyophylized and the iodothyronine pool was evaporated under a stream of nitrogen at 50°C.

Iodothyronines and their conjugates were isolated from the 4-h plasma samples by solid-phase extraction (SPE) for subsequent analysis by HPLC. In short, 500 μl serum was mixed with an equal volume 0.25 M NaOH and applied to a C_{18}-SPE column (500 mg, J. T. Baker Chemical Co., Phillipsburg, NJ). Columns were washed successively with 2 × 1 ml of each 0.1 M NaOH, H_2O, 0.1 M HCl, and H_2O before elution of both conjugates and iodothyronines with 1 ml methanol. The recovery of T_3 and T_3S added to rat plasma was 90 and 96%, respectively.

HPLC analysis. Reversed-phase HPLC was done on a 10 × 0.3–cm Chromspher C_{18} analytical column in combination with a 10 × 2.1–mm guard column (Chrompack International BV, Middelburg, Netherlands). Elution was performed with a 20-min gradient of 18–40% acetonitrile in 0.02 M ammonium acetate (pH 4). Solvent flow was 0.8 ml/min and fractions of 0.5 min were collected. A nonlinear gradient (No. 7) as programmed by the automated gradient controller (model 680, Waters Associates, Milford, MA) was used. The residues of the plasma extracts were dissolved in mobile phase and the gradient was started at the time of injection. Retention times of possible products were determined using synthetic and biosynthetic reference compounds (9). HPLC analysis of biliary products was performed on 5–25-μl aliquots of bile diluted with 4 vol of mobile phase. Analysis, especially of glucuronide conjugates in bile, was more accurate if a 25-min gradient of 16–40% acetonitrile was used.

Results

Identification of metabolites. All metabolites of interest were well separated using reversed-phase HPLC, and a typical chromatogram is shown in Fig. 1. Neither serum residues nor small amounts of bile in the HPLC samples affected the elution profile seen with pure tracers.

Analysis of plasma T_3 and T_3S metabolites. Fig. 2 shows the distribution of the major radioactive compounds in plasma 2 h after administration of the tracers as determined by LH-20 chromatography. Further analysis of the obtained conjugate and iodothyronine fractions by reversed-phase HPLC is illustrated in Fig. 3. HPLC of the LH-20 fractions of plasma obtained after 0.5, 1, and 4 h indicated similar compositions. On average, 78% of the radioactivity in the conjugate fraction coeluted with T_3S on HPLC, while 84% of the iodothyronine fraction eluted as T_3. If plasma was spiked with labeled T_3 or T_3S, in both cases 89% of the radioactivity in the respective

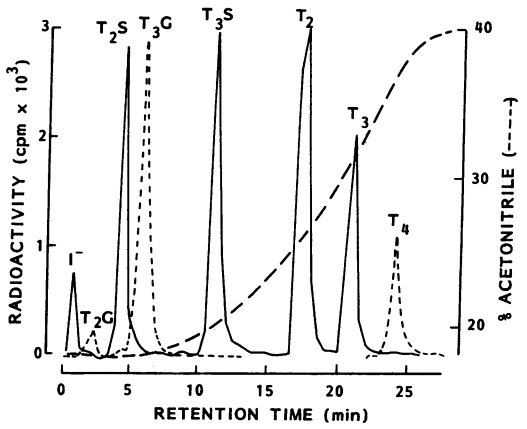

Figure 1. Separation by reversed-phase HPLC of a mixture of ^{125}I-labeled sulfates (*S*), glucuronides (*G*), and nonconjugated iodothyronines. The C_{18} column was eluted at 0.8 ml/min using a 20-min gradient of 18–40% acetonitrile in 0.02 M ammonium acetate (pH 4). (*Dashed curve*) Actual composition of the mobile phase.

LH-20 fractions eluted as T_3 or T_3S on HPLC. Therefore, plasma radioactivity other than iodide as fractionated on LH-20 consisted predominantly of T_3S or T_3. Nonconjugated T_2 was never observed, but T_2S was detected in T_3-injected rats where it comprised on average 10% of the radioactivity in the

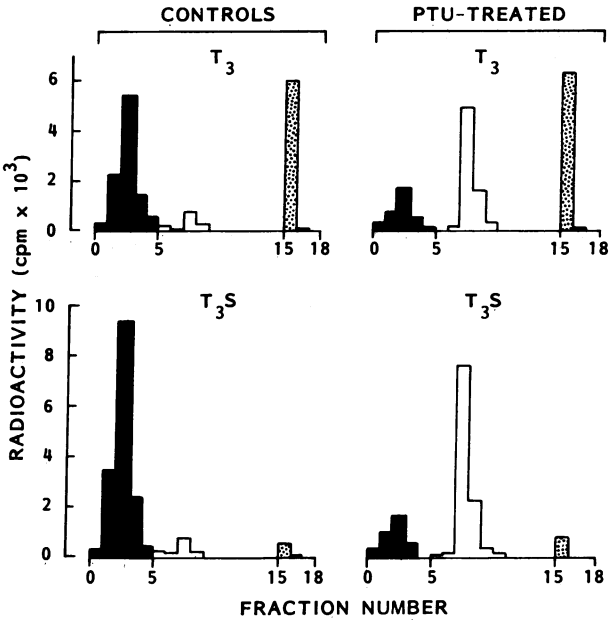

Figure 2. Sephadex LH-20 chromatography of serum from rats injected with T_3 or T_3S. Rats received saline (*left*) or PTU (*right*) 30 min prior to 10 μCi [^{125}I]T_3 (*upper*) or [^{125}I]T_3S (*lower*) by intravenous injections. Serum (0.25 ml) was acidified and applied to Sephadex LH-20 columns as described in Methods. Free iodide, conjugated and nonconjugated iodothyronines were successively eluted with 0.1 M HCl (fractions 1–5), 20% ethanol in water (fractions 6–14), and 0.1 M ammonia in ethanol (fractions 16–18) with a recovery of 95% for I^-, 91% for T_3S, and 94% for T_3. Mean values for each experimental group are given (T_3, n = 5; T_3S, n = 4). Total radioactivity in the samples amounted to 0.39 (T_3), 0.34 (T_3 + PTU), 0.42 (T_3S), and 0.31 (T_3S + PTU) % dose/ml plasma.

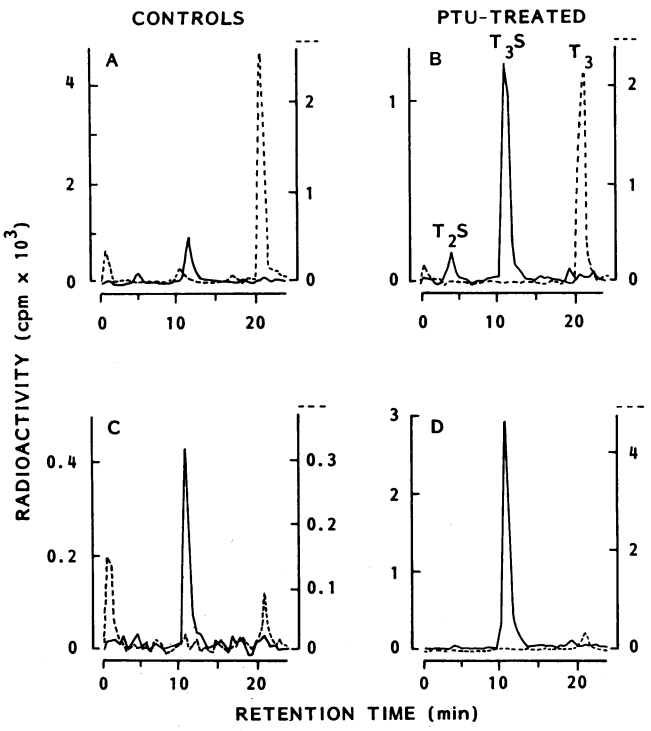

Figure 3. HPLC analysis of serum T_3 and T_3S fractions. Serum was obtained from control and PTU-treated rats 2 h after injection of [^{125}I]T_3 (*A* and *B*) or [^{125}I]T_3S (*C* and *D*) and chromatographed on Sephadex LH-20 (Fig. 2). Corresponding conjugate (*solid line*) or iodothyronine (*dashed line*) fractions were pooled for each experimental group and further analyzed by HPLC (Fig. 1). Chromatograms represent ~ 49, 80, 25, and 78% of total plasma radioactivity due to removal of mainly $^{125}I^-$ by LH-20 from samples *A*, *B*, *C*, and *D*, respectively. Note the smaller scale in *C*, where the first peak represents only 2% of plasma iodide.

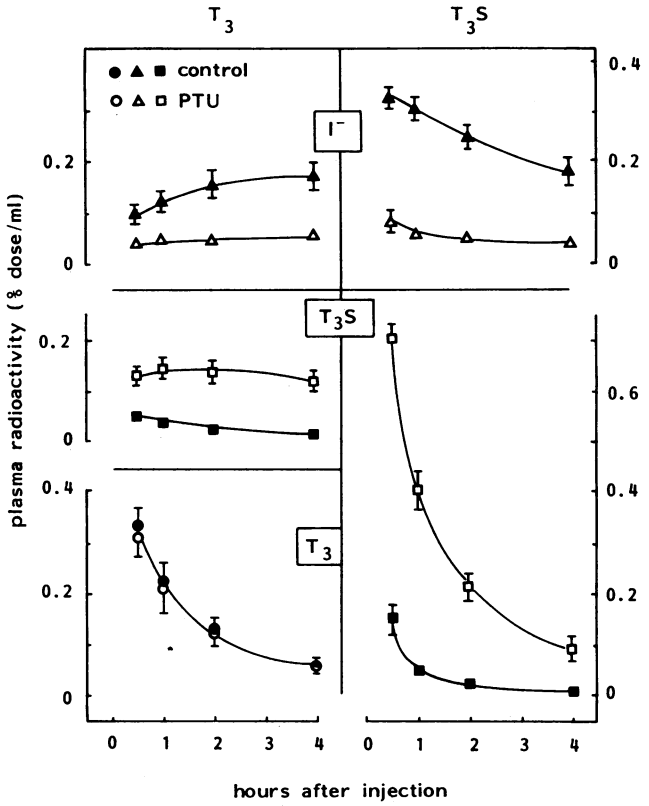

Figure 4. Effect of PTU on plasma T_3 and T_3S metabolites. Serum was analyzed from rats injected with [^{125}I]T_3 (*left*) or [^{125}I]T_3S (*right*) after pretreatment with saline (*solid symbols*) or PTU (*open symbols*). Plasma iodide (*triangles*) was estimated as the TCA-soluble radioactivity, whereas T_3S (*squares*) and T_3 (*circles*) were quantified by Sephadex LH-20 fractionation (Fig. 2). In [^{125}I]T_3-injected rats roughly 10% of the radioactivity in the "T_3S fraction" consisted of T_2S. Radioactivity of compounds is expressed as mean percent dose per milliliter plasma (±SD unless smaller than symbol) and plotted as a function of time after tracer injection. Total amount of plasma radioactivity decreased over the 4-h period from 0.52±0.07 to 0.33±0.05 after T_3 (n = 5), from 0.50±0.08 to 0.26±0.04 after T_3 + PTU (n = 5), from 0.68±0.04 to 0.29±0.04 after T_3S (n = 4), and from 0.82±0.03 to 0.17±0.03 after T_3S + PTU (n = 4). Except for plasma disappearance of T_3, differences between controls and PTU rats were highly significant ($P < 0.001$) as estimated by Student's unpaired t test.

conjugate fraction. After T_3S injection, plasma T_3 was negligible in control rats and represented < 4% of plasma radioactivity in PTU-treated rats. Neither T_4 nor the glucuronides of T_3 and T_2 were observed in any of the samples. HPLC of solid-phase extracts of 4-h plasma samples were in close agreement with analysis of the LH-20 fractions.

> 95% of radioiodide added to plasma eluted in the first five fractions of the LH-20 chromatography. However, coelution of some protein-bound radioactivity or unknown metabolites could not be excluded. Therefore, accurate measurements of free iodide in plasma was performed by TCA precipitation. The radioactivity in the HCl fractions after LH-20 correlated well with the amount of TCA-soluble radioactivity as shown by linear regression analysis. For the means of these parameters in the different experimental groups the following function was derived: y (HCl) = 1.3 x (TCA) + 0.1 (r = 0.997, n = 16) with x and y expressed as percentage of plasma radioactivity. Apparently, the LH-20 method overestimated plasma iodide levels and was not used for calculation of the results.

Effect of PTU on the metabolism of T_3 and T_3S. Results of the measurement of plasma T_3 and T_3S by Sephadex LH-20 and of iodide by TCA precipitation are summarized in Fig. 4. Radioiodide was the main plasma metabolite of both T_3 and T_3S in control rats. PTU did not affect plasma T_3 clearance but decreased T_3S clearance by 81%, as estimated from the area under the plasma T_3S concentration curve. In control rats clearance of plasma T_3S was faster than that of T_3, but the reverse was true after PTU treatment. The administration of PTU diminished plasma iodide levels by 60–71% after T_3 injection and by 74–80% after T_3S. PTU increased plasma T_3S 2.7 times at 0.5 h to 7.5 times at 4 h after T_3 injection, with an average of 4.2 times. This resulted in T_3S being the major radioactive compound in plasma from 2 h after T_3 injection onwards. Similar results were obtained in rats with bile cannules, where T_3 injection was delayed until 2.5 h as opposed to 0.5 h after PTU administration.

Biliary clearance of T_3S (Fig. 5). In control rats < 20% of radioactivity injected as T_3S was excreted in the bile, occurring predominantly during the first 30 min. In PTU-treated rats biliary excretion was greatly increased up to 70% of the dose after 4 h. HPLC analysis demonstrated that T_3S was the only

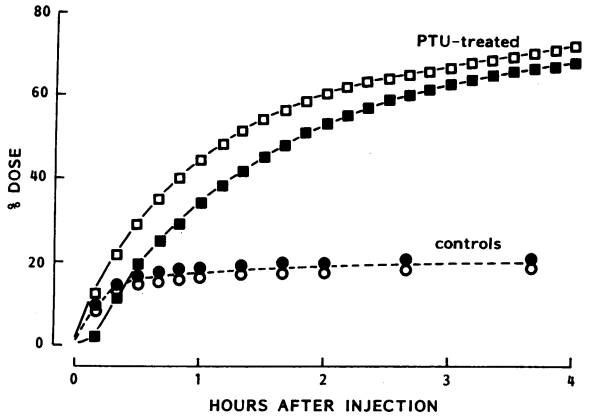

Figure 5. Biliary clearance of T_3S. Rats with bile cannules under pentobarbital anesthesia received intravenous injections of PTU or saline 30 min before 10 μCi [^{125}I]T_3S. Cumulative excretion was estimated by summation of radioactivity in successive 10-min bile aliquots and expressed as percent dose. Different symbols represent individual rats. Total bile volumes collected over 4 h were 3.5 (*open circle*), 4.4 (*solid circle*), 3.7 (*open square*), and 4.2 (*solid square*) ml. Bile flow was low in one of the PTU-treated rats (*solid square*) during the first 10 min.

significant compound in bile. Iodide was excreted only in bile of control rats and amounted to 2.5% of the administered dose. T_2S was observed in bile of PTU-treated rats but accounted for < 3% of the biliary radioactivity. In these cannulated rats distribution of plasma radioactivity was the same as in intact rats and pretreatment with PTU resulted in a 57–84% decrease in plasma iodide over the 4-h period.

Plasma free T_3 and T_3S fractions. The non-protein-bound fractions of T_3 and T_3S in rat plasma were determined in duplicate by equilibrium dialysis. The free fraction was 0.35±0.03 (mean±SD, n = 6) for T_3 and 0.20±0.03 (n = 4) for T_3S, and both were unaffected by 1 mM PTU.

Discussion

The role of conjugation in the metabolism of thyroid hormone, especially in humans, has received little attention in the literature. Extensive conjugation of thyroid hormone has been demonstrated in experimental animals. Bollman and Flock (4) have identified glucuronides as the main excretory products of various iodothyronines in the bile of normal rats. However, the sum of T_2S and T_3S excreted in bile equalled or exceeded that of T_3G after administration of T_3 to dogs (10, 11). Studying the biliary clearance of T_4 in rats, Flock and Bollman (12) observed that thiouracil treatment increased the excretion of an acid-hydrolyzable T_4 conjugate which perhaps represented T_4 sulfate (8). A similar effect was also observed with butyl 4-hydroxy-3,5-diiodobenzoate, which is also an inhibitor of type I deiodinase activity. Treatment of rats with this compound in addition to labeled T_3 or T_4 led to a far greater increase in biliary sulfates compared with glucuronides (13).

Roche et al. (14) reported on the presence of radioactive T_3S in bile and plasma of thyroidectomized rats after injection with labeled T_3. We previously observed an increase in biliary T_3S from exogenous T_3 in rats treated with PTU[2] (7). We have now shown that the same treatment results in a marked accumulation of plasma T_3S. In retrospect, it is possible to explain the findings of Roche et al. (14) as it has become evident that hypothyroidism in rats is associated with an impaired hepatic deiodinase activity (15). Although these investigators did not study euthyroid rats, their results agree with ours, indicating that T_3S accumulates if subsequent deiodination is inhibited.

In control rats, injected T_3S is metabolized more rapidly than T_3 although it binds with higher affinity to plasma proteins, and only radioiodide was detected as a metabolite. Similar rapid deiodinative clearance of T_3S in humans has been reported recently (16). Clearance of T_3S is strongly inhibited by PTU, indicating that it is largely metabolized by type I deiodination. In contrast, T_3 disposition is not affected by PTU as also observed by others (17), illustrating that direct inner ring deiodination of T_3 by the liver type I deiodinase is a negligible metabolic pathway. The slower metabolic clearance rate of T_3S compared with T_3 in PTU-treated rats has also been observed in thyroidectomized rats (18) and entirely explains the increase in plasma T_3S from exogenous T_3. The plasma T_3S levels thus obtained underscore the importance of sulfation as metabolic pathway of T_3.

In PTU-treated rats plasma T_3S is cleared predominantly by biliary excretion. It is possible, therefore, that also after injection of T_3 most T_3S in bile is derived from plasma T_3S. Although T_3 is sulfated and glucuronidated in rat hepatocytes (6), it is not excluded that part of plasma T_3S originates by sulfation of T_3 in other tissues as was observed in hepatectomized dogs (4, 10).

Plasma T_2S was observed in both normal and PTU-treated rats after injection of T_3 but not after T_3S. Therefore, it is probably derived from sulfation of T_2 that is produced by PTU-insensitive (type III) inner ring deiodination of T_3 (2). This would also explain the marked biliary T_2S excretion after T_3 injection to PTU-treated rats[2] (7) in contrast to the negligible amounts of T_2S in bile of T_3S-injected rats (this study).

In conclusion, the present study of plasma T_3S formation in T_3-injected rats extends previous observations in bile, indicating that sulfation is an important pathway of T_3 metabolism in rats. However, unless subsequent type I deiodination of the conjugate is prevented, little T_3S is observed in both body fluids. The finding of significant amounts of T_3S in rat plasma opens the perspectives of studying the importance of T_3 sulfation in humans. The recent development of a radioimmunoassay for T_3S in our laboratory should facilitate such investigations (19). The findings that in human liver T_3 is a substrate for phenol sulfotransferase (20) and that T_3S is rapidly deiodinated (21) suggest that successive sulfation and deiodination of T_3 indeed occurs in humans.

Acknowledgments

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References

1. Engler, D., and A. G. Burger. 1984. The deiodination of the iodothyronines and their derivatives in man. *Endocr. Rev.* 5:151–184.

2. Leonard, J. L., and T. J. Visser. 1986. Biochemistry of deiodina-

tion. *In* Thyroid Hormone Metabolism. G. Hennemann, editor. Marcel Dekker, New York. 189–229.

3. Visser, T. J., D. Fekkes, M. H. Otten, J. A. Mol, R. Docter, and G. Hennemann. 1984. Deiodination and conjugation of thyroid hormone in rat liver. *In* Hormones and Cell Regulation. Vol. 8. J. E. Dumont and J. Nunez, editors. Elsevier/North-Holland, Amsterdam. 179–191.

4. Bollman, J. L., and E. V. Flock. 1965. The role of the liver in the metabolism of I^{131}-thyroid hormones and analogues. *In* The Biliary System. W. Taylor, editor. Blackwell, Oxford. 345–365.

5. Visser, T. J., J. A. Mol, and M. H. Otten. 1983. Rapid deiodination of triiodothyronine sulfate by rat liver microsomal fraction. *Endocrinology.* 112:1547–1549.

6. Otten, M. H., J. A. Mol, and T. J. Visser. 1983. Sulfation preceeding deiodination of iodothyronines in rat hepatocytes. *Science (Wash. DC).* 221:81–83.

7. De Herder, W. W., F. Bonthuis, M. P. Hazenberg, and T. J. Visser. 1986. Biliary excretion and reabsorption of T_3 and its metabolites. *Ann. Endocrinol.* 47:23. (Abstr.)

8. Mol, J. A., and T. J. Visser. 1985. Synthesis and some properties of sulfate esters and sulfamates of iodothyronines. *Endocrinology.* 117:1–7.

9. De Herder, W. W., M. P. Hazenberg, A. M. Pennock-Schröder, G. Hennemann, and T. J. Visser. 1986. Hydrolysis of iodothyronine glucuronides by obligately anaerobic bacteria isolated from human faecal flora. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 35:249–253.

10. Flock, E. V., J. L. Bollman, and J. H. Grindlay. 1960. Conjugates of triiodothyronine and its metabolites. *Endocrinology.* 67:419–429.

11. Flock, E. V., J. L. Bollman, and J. H. Grindlay. 1960. 3,3′-Diiodothyronine, a metabolite of 3,5,3′-triiodothyronine. *Proc. Mayo Clin.* 35:75–81.

12. Flock, E. V., and J. L. Bollman. 1962. The effect of thiouracil on the metabolism of L-thyroxine. *Biochem. J.* 84:621–626.

13. Flock, E. V., J. L. Bollman, C. A. Owen, Jr., and P. E. Zollman. 1965. Conjugation of thyroid hormones and analogs by the Gunn rat. *Endocrinology.* 77:303–314.

14. Roche, J., R. Michel, J. Closon, and O. Michel. 1959. Sur la sulfoconjugaison hépatique de la 3,5,3′-triiodothyronine et la présence d'un ester sulfurique de cette hormone dans la bile et la plasma. *Biochim. Biophys. Acta.* 33:461–468.

15. Kaplan, M. M. 1986. Regulatory influences on iodothyronine deiodination in animal tissues. *In* Thyroid Hormone Metabolism. G. Hennemann, editor. Marcel Dekker, New York. 231–254.

16. Nimalasuriya, A., L. Y. Mizuno, C. A. Spencer, A. Marcus, and J. T. Nicoloff. 1984. Program of the 60th Annual Meeting of the American Thyroid Association, New York, NY. 57. (Abstr.)

17. Silva, J. E., M. B. Gordon, F. R. Crantz, J. L. Leonard, and P. R. Larsen. 1984. Qualitative and quantitative differences in the pathways of extrathyroidal triiodothyronine generation between euthyroid and hypothyroid rats. *J. Clin. Invest.* 73:898–907.

18. Roche, J., R. Michel, J. Closon, and O. Michel. 1960. Sur le métabolisme du sulfoconjugué de la 3,5,3′-triiodothyronine chez le rat. *Biochim. Biophys. Acta.* 38:325–332.

19. Eelkman Rooda, S. J., E. Kaptein, M. A. C. Van Loon, and T. J. Visser. 1986. Sulfate conjugation of T_3 by rat hepatocytes as analysed by a T_3 sulfate radioimmunoassay. *Ann. Endocrinol.* 47:24. (Abstr.)

20. Young, W. F., C. A. Gorman, and R. M. Weinshilboum. 1986. Triiodothyronine (T_3): a substrate for two forms of human phenol sulfotransferase (PST). Program of the 61st Annual Meeting of the American Thyroid Association, Phoenix, AZ. 45. (Abstr.)

21. Visser, T. J., and E. P. Krenning. 1983. Deiodination of iodothyronines in human liver. Program of the 59th Annual Meeting of the American Thyroid Association, New Orleans, LA. 31. (Abstr.)