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Comparison of the Effects of Propylthiouracil and Selenium Deficiency on T_3 Production in the Rat*

IRINI E. VERONIKIS†, LEWIS E. BRAVERMAN, SHARON ALEX, SHIH LIEH FANG, BETH NORVELL, AND CHARLES H. EMERSON

Division of Endocrinology and Metabolism, University of Massachusetts Medical School, Worcester, Massachusetts 01655

ABSTRACT

Selenium deficiency and propylthiouracil (PTU) treatment both decrease hepatic type I T_4 5′-deiodinase activity (5′D-I), which is considered to be an important regulator of the serum T_3 derived from peripheral T_4 to T_3 conversion (T_3 neogenesis). The effects of PTU treatment or a selenium-deficient diet on T_4 and T_3 kinetics were compared in thyroid-ablated rats infused with stable T_4 to determine whether PTU treatment is a more potent inhibitor of T_3 neogenesis than selenium deficiency and to compare the degree of inhibition of T_3 production with the degree of inhibition of 5′D-I. PTU treatment and selenium deficiency (Se) did not affect the T_3 MCR (control, 46.0 ± 2.5; PTU, 41.7 ± 2.8; Se^-, 41.1 ± 4.0 ml/h·100 g BW), but did reduce serum T_3 concentrations by 29% and 25%, respectively (control, 58.7 ± 2.6; PTU, 41.5 ± 1.0; Se , 43.9 ± 2.7 ng/dl; $P < 0.01$ for PTU or Se *vs.* control) and the T_3 production rate by 35% and 32%, respectively (control, 26.6 ± 1.0; PTU, 17.3 ± 2.0; Se , 18.0 ± 1.9 ng/h·100 g BW; $P < 0.01$ for PTU or Se *vs.* Control). PTU treatment and selenium deficiency significantly increased serum T_4 concentrations by 36% and 32%, respectively, due to a decrease in T_4 MCR (control, 1.4 ± 0.1; PTU, 1.1 ± 0.1; Se^-, 1.1 ± 0.04 ml/h·100 g BW; $P < 0.05$ for PTU or Se *vs.* control). Assuming that the concentration of T_4 available for T_3 neogenesis is proportional to the serum T_4 concentration, the increase in serum T_4 concentrations caused by PTU treatment or Se would probably have proportionally increased the rate of T_3 neogenesis. Based on these considerations, the apparent decrease in T_3 neogenesis in the PTU-treated animals was 52%. This is less than the 79% and 67% inhibition of 5′D-I noted, respectively, in the liver and kidneys of these rats. Similarly, the apparent decrease in T_3 neogenesis in the Se^- rats was 48%, again less than the 85% and 64% inhibition of 5′D-I in their liver and kidneys, respectively. These studies suggest that PTU and Se^- have similar effects on T_3 neogenesis. The more potent effects of these treatments on liver and kidney 5′D-I activities than on T_3 neogenesis suggest that the activities of these enzymes in these tissues are not the only important determinants of the serum T_3 that is derived from nonthyroidal sources. (*Endocrinology* **137:** 2580–2585, 1996)

PREVIOUS STUDIES have suggested that type I 5′-deiodinase activity (5′D-I) is the major source, in euthyroid rats, of the T_3 in serum that is generated by peripheral deiodination of T_4 to T_3. Experiments in athyreotic rats replaced with physiological doses of T_4 demonstrated that administration of propylthiouracil (PTU), a potent 5′D-I inhibitor, reduced serum T_3 concentrations or the T_3 production rate by as much as 77% (1–3). Surprisingly, in athyreotic selenium deficient (Se) T_4-replaced rats, in which 5′D-I activities in liver and kidney are significantly decreased (4–6) due to the fact that 5′D-I is a selenoprotein (7), the serum T_3 concentration was decreased by only 20% or less (8). It is difficult to explain the finding that serum T_3 concentrations decline to a far lesser extent in thyroidectomized T_4-replaced rats that receive a Se^- diet than in thyroidectomized T_4-replaced rats treated with PTU (1–3, 8). To determine whether there is a discrepancy between the effects of Se^- and PTU treatment and to evaluate the relative contribution of 5′D-I to the serum T_3 pool, we compared the effects of PTU and Se^- on liver and kidney 5′D-I activities and on the production of serum T_3 that arises by peripheral conversion of T_4 to T_3 in rats.

Materials and Methods

These studies were approved by the animal research committee and complied with the institutional assurance certificate of the University of Massachusetts Medical Center. Weanling male Sprague-Dawley rats supplied by Charles River Laboratories (Wilmington, MA) were used. A preliminary study was performed to test the relationship between the dose of PTU administered in the diet and the degree of inhibition of 5′D-I in liver homogenates. Groups of rats (n = 4/group) received from 0–0.1% PTU in their diet. During this period, they were also treated with T_4 (1 μg/100 g BW twice daily) so that they would not be rendered hypothyroid by the PTU treatment. After 11 days of PTU and T_4 treatment, rats were killed, and 5′D-I in liver homogenates was measured as described below for the main kinetic study. The percent inhibition of 5′D-I in liver homogenates was similar in rats receiving either 0.01%, 0.05% or 0.1% PTU in their diet (92.8 ± 0.7% *vs.* 93.6 ± 1.8% *vs.* 95.0 ± 0.6%, respectively). Serum T_4, T_3, and TSH concentrations were also similar in the three groups of PTU-treated rats (serum T_4, 8.5 ± 1.3, 10.2 ± 0.7, and 7.9 ± 1.8 μg/dl, respectively; serum T_3, 44 ± 5, 43 ± 4, and 44 ± 6 ng/dl, respectively; serum TSH, 56 ± 14, 47 ± 7, and 38 ± 12 μU/ml, respectively).

Experimental protocol

Three groups of rats were compared: a control group (C), a PTU-treated group, and a Se^- group. Rats were housed in stainless steel cages and provided with distilled water containing 2% calcium lactate *ad libitum* for the duration of the study. Calcium lactate was provided in the drinking water because the rats were scheduled to undergo thyroidectomy in several days. A Torula yeast-based semisynthetic diet (Teklad

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Address all correspondence and requests for reprints to: Charles H. Emerson, M.D., Division of Endocrinology and Metabolism, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01655.
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† Present address: Division of Endocrinology and Metabolism, Boston University Medical School (Boston, MA).

Premier Laboratory Diets, Madison, WI) was used to provide a Se^- diet. The Se^- diet (TD 86298) contained less than 16 μg/kg selenium. This compares to 200 μg/kg in the Teklad selenium-sufficient diet (TD 91259).

All rats were surgically thyroidectomized under 70% ketamine-30% rompun anesthesia. This anesthesia was employed for all subsequent surgical procedures. Two days later, the Se^- group was switched from standard laboratory chow to the Se^- diet. Four days later, all rats were started on thyroid hormone replacement consisting of daily ip doses of T_4 (1.5 μg/100 g BW). Thirty days after the ip injections of T_4 were started, thyroid hormone replacement was switched from ip injections to continuous T_4 infusion. Miniosmotic pumps (Alza Corp., Palo Alto, CA) were primed by incubation in normal saline at 37 C for 4 h. They were weighed before and after filling to ensure correct loading and implanted sc under anesthesia, and their outflow was directed into the right atrium via a PE60 catheter (Clay Adams, Parsippany, NY). Before implantation, the pumps were loaded and calibrated to deliver T_4 at a rate of 1.5 μg/day·100 g BW and [^{125}I]T_3 at a rate of 6 μCi/day·100 g BW. Three days before implantation of the miniosmotic pumps, the diets of the various groups of rats were changed from the pelleted to the powdered form, and PTU was added in a dose of 0.015% to the diet in the PTU group.

The infusion of stable T_4 and [^{125}I]T_3 was maintained for 7 days. Rats were then anesthetized, and blood, liver, kidney, and osmotic minipumps were harvested. Livers and kidneys were homogenized in 4 vol (wt/vol) 250 mM sucrose, 20 mM HEPES buffer (pH 7.0), 1 mM EDTA, and 1 mM dithiothreitol (DTT) to measure their 5′D-I activities. Serum was separated from blood for measurement of thyroid hormone concentrations, chromatographic analysis of the ^{125}I-labeled constituents, and γ counting. The osmotic minipumps were analyzed for their residual [^{125}I]T_3. All tissue samples and serum were stored at −20 C until assayed.

Serum hormone and tissue enzyme assays

Because the radioactivity of serum samples was high, Ciba Corning ACS 180 (Ciba Corning Diagnostic Corp., Medfield, MA) chemiluminescent immunoassays were used to measure serum T_4 and T_3 concentrations. To confirm the validity of these methods, they were compared with the rat serum T_4 and T_3 RIAs. The results using the two methods were highly correlated (T_4: r = 0.968, $P < 0.0001$, n = 27; T_3: r = 0.989, $P < 0.0001$, n = 10). Liver and kidney 5′D-I activities were determined by the release of ^{125}I from 10 μM rT_3 in the presence of 20 mM DTT at 37 C (9), and results were expressed as units per mg protein. One unit of enzyme activity represents the release of 1 pmol I^-/min. In a complementary study, different substrate and/or DTT concentrations were employed to gauge the apparent inhibition of 5′D-I induced by PTU treatment. This study compared livers from rats receiving no PTU with livers from rats receiving 0.015% PTU in their diet, the same dose of PTU used for the main kinetic study. The enzyme assay conditions tested were 10 μM rT_3 with 20 mM DTT, 1 μM rT_3 with 20 mM DTT, 2 nM rT_3 with 20 mM DTT, 1 μM rT_3 with 1 mM DTT, and 2 nM rT_3 with 1 mM DTT. The percent inhibition of 5′D-I in the livers from PTU-treated rats was less when substrate concentrations of 10 μM rT_3 were used than with lower substrate concentrations. The values for the above concentrations were, respectively, 82.3 ± 1.6, 87.1 ± 1.1, 88.8 ± 0.4, 90.0 ± 0.6, and 91.5 ± 0.3 (n = 8/group; $P < 0.01$ for incubations performed with a rT_3 substrate concentration of 10 μM *vs.* incubations performed at lower substrate concentrations). In the presence of 1 mM PTU added *in vitro* to livers from PTU-treated rats, 5′D-I was inhibited by 95.7 ± 0.4% using enzyme assay conditions of 2 nM rT_3 with 20 mM DTT.

Serum TSH was measured by RIA using materials supplied by the National Pituitary Agency, NIH, after samples were held to allow for decay of ^{125}I radioactivity. All samples were run in duplicate in the same assay and in random order. Protein was measured by the method of Bradford (10).

Analysis of serum and osmotic minipumps for [^{125}I]T_3

Serum samples and aliquots of the [^{125}I]T_3 solution that were loaded into the osmotic minipumps were counted in a Crystal Multi Detector γ-counter (Packard Instrument Co., Downers Grove, IL). To determine the fraction of the contents of the osmotic minipumps that had been infused into each rat, the minipumps were counted before they were implanted sc and again after they were removed from the rats. Counting was performed using a CRC-7 Radioisotope Calibrator (Capintec, Ramsey, NJ). After counting the osmotic minipumps, material from the pumps was removed, and it along with the serum samples and the [^{125}I]T_3 solution used to load the pump were subjected to analysis by HPLC. One half milliliter of these samples was extracted with 1 ml methanol-1% NH_3. After centrifugation at 2600 × *g*, the supernatant was concentrated under N_2-gas to 0.1 ml. The samples were combined with 0.005 ml of a methanol-1% NH_3 solution containing stable iodide carrier and iodothyronine markers (NaI, monoiodotyrosine, diiodotyrosine, 3,5-diiodothyronine, T_3, T_4, and rT_3). They were then subjected to HPLC using a 3.9 × 300-mm Waters C_{18} μBondapak column (Millipore Waters Chromatography, Milford, MA). The column was equilibrated in 65% 0.1% trifluoroacetic acid-35% acetonitrile, and the bound iodothyronines were eluted at a flow rate of 1 ml/min using a linear gradient from 35–41% acetonitrile. Fractions were collected every minute, and the effluent was monitored at 24 nm to detect the specific iodothyronine peaks (11). The radioactivities of the fractions were measured in a Packard γ-counter, and the percentage of T_3 was calculated. This value was used to calculate the percentage of the total radioactivity that was authentic T_3.

T_4 and T_3 kinetics

Noncompartmental kinetics for T_4 and T_3 were calculated using the formula MCR = PR/PC, where MCR was the (plasma) MCR, PR was the production rate, and PC was the plasma concentration (12). The known factors in the formula were the stable T_4 and [^{125}I]T_3 PCs and PRs, as these were obtained by measurement and by the infusion rates, respectively. The MCR of stable T_4 was calculated directly because the other elements of the formula were known. The MCR of [^{125}I]T_3 was similarly calculated, and this value was used as the MCR for stable T_3, thus permitting the PR of stable T_3 to be calculated (12).

Statistics

Statistical significance of difference between the groups was determined by one-way ANOVA using the Student Newman-Keuls multiple comparisons test. All results are presented as the mean ± SE.

Results

T_4 infusion rate and body weight (Table 1)

The body weights of the different groups of rats were similar at the beginning and end of the period during which T_4 was infused by osmotic minipumps. The mean T_4 infusion rates were similar in the three groups and ranged from 172–

TABLE 1. Body weights and L-T_4 infusion rates in control, PTU-treated, and selenium-deficient (Se^-) rats

Group	BW (g)			T_4 (ng/h)	T_4 (μg/100 g · day)
	Preinfusion	Postinfusion	Mean		
Control	276 ± 10	314 ± 9	295 ± 10	172 ± 5	1.4 ± 0.05
PTU	277 ± 12	297 ± 20	287 ± 16	176 ± 9	1.5 ± 0.13
Se^-	294 ± 14	315 ± 15	304 ± 14	191 ± 5	1.5 ± 0.08

Values are the mean ± SE.

191 ng/h. This corresponded to 1.4 ± 0.05 μg T_4/100 g mean BW·day in the C group and 1.5 ± 0.13 and 1.5 ± 0.08 in the PTU and Se groups, respectively. These values were similar to the intended infusion rate of 1.5 μg 100 g BW/day.

Liver and kidney 5′D-I activities in athyreotic T_4-replaced rats

Liver 5′D-I activity was decreased by 79% in PTU-treated rats and by 85% in Se⁻ rats compared to that in the C groups ($P < 0.001$; Fig. 1). Similarly, kidney 5′D-I activity was decreased by 67.2% and 63.5% in PTU-treated and Se rats, respectively ($P < 0.001$; data not shown).

T_4 and T_3 kinetics

The serum T_4 concentrations were modestly higher in the PTU-treated and Se⁻ groups of rats, by 36% and 32%, respectively ($P < 0.05$; Fig. 2). A major reason for this was that the MCR of T_4 was significantly decreased in both the PTU and Se groups, by 25% and 16%, respectively, compared to that in the C rats ($P < 0.05$; Fig. 3). The relationship between the increase in serum T_4 and the decrease in MCR in the PTU-treated and Se⁻ groups was not identical to that in the C group because these treated groups received slightly more T_4 than the C group. Serum T_3 concentrations were modestly decreased by both PTU and selenium deficiency ($P < 0.001$; Fig. 4). Serum T_3 values were 58 ± 2.6 ng/dl in the C rats and decreased to 41.5 ± 1.0 ng/dl in PTU-treated rats and to 43.9 ± 2.7 ng/dl in the Se group. The MCR of T_3 was slightly, but not significantly, lower in the PTU-treated and Se groups compared to that in the C rats (Fig. 5). Although selenium deficiency inhibited liver 5′D-I by 85% and kidney 5′D-I by 64%, the T_3 production rate was decreased by only 32%. Similarly, PTU treatment decreased liver 5′D-I by 79% and kidney 5′D-I by 67%, but decreased T_3 production by only 35% ($P < 0.001$; Fig. 6).

Serum TSH concentrations

Mean serum TSH concentrations were almost identical in the C and Se⁻ rats. PTU-treated rats had significantly higher serum TSH concentrations compared to those in rats in the C or Se⁻ groups ($P < 0.01$; Fig. 7).

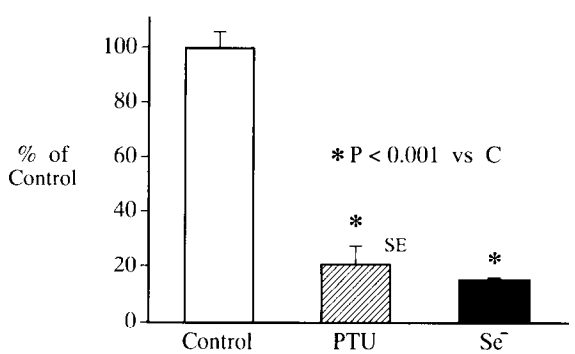

FIG. 1. Liver 5′D-I activity in C, PTU-treated, and Se thyroidectomized T_4-infused rats.

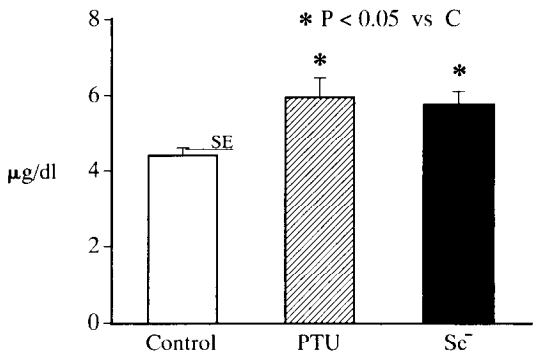

FIG. 2. Serum T_4 concentrations in C, PTU-treated, and Se⁻ thyroidectomized T_4-infused rats.

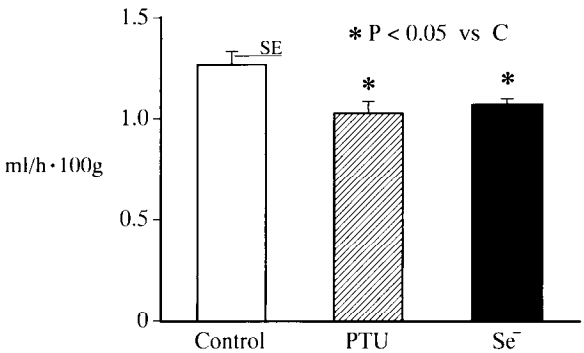

FIG. 3. MCR of T_4 in C, PTU-treated, and Se thyroidectomized T_4-infused rats.

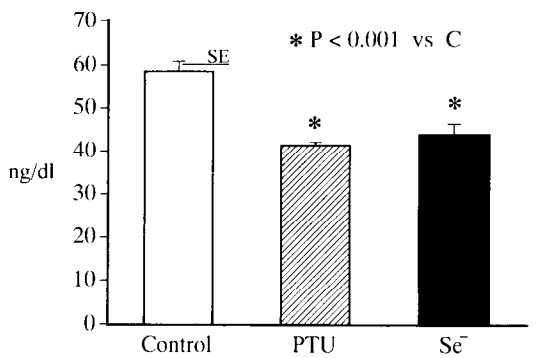

FIG. 4. Serum T_3 concentrations in C, PTU-treated, and Se thyroidectomized T_4-infused rats.

Discussion

5′D-I is an enzyme abundant in the liver and kidney, is inhibited by PTU, and is down-regulated in hypothyroidism and up-regulated in thyrotoxicosis (13). The 5′D-I enzyme has been cloned and is a selenoprotein (7, 14) with a selenocysteine at the enzyme's active site (7). Selenium-depleted liver and kidney have markedly diminished 5′D-I activity (4–6). Surprisingly, plasma T_3 concentrations are decreased only modestly or not at all (4, 6, 15) in Se rats. Additionally, in recent experiments with Se⁻ thyroidectomized T_4-replaced rats, the serum T_3 concentrations decreased by only 20% compared to those in selenium-sufficient rats (8). In contrast to the results in Se⁻ rats, previous experiments in athyreotic rats replaced with physiological doses of T_4 or rats whose endogenous T_4 production was inhibited demon-

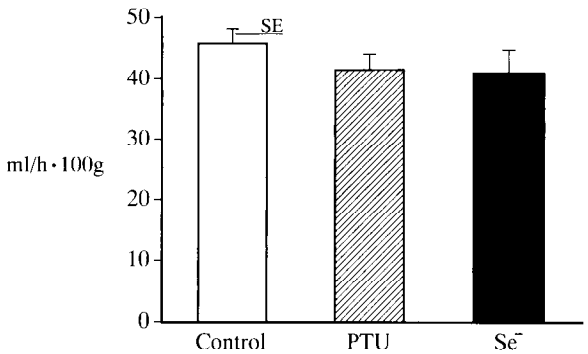

FIG. 5. MCR of T_3 in C, PTU-treated, and Se thyroidectomized T_4-infused rats.

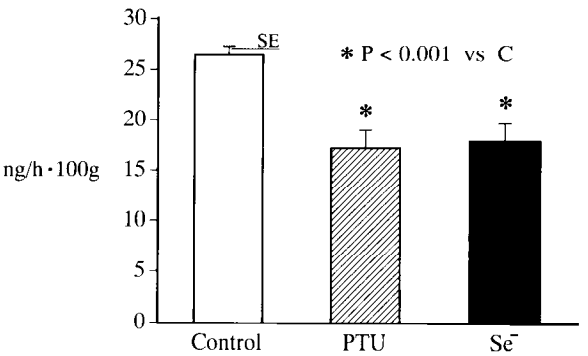

FIG. 6. The production rate of T_3 in C, PTU-treated, and Se^- thyroidectomized T_4-infused rats.

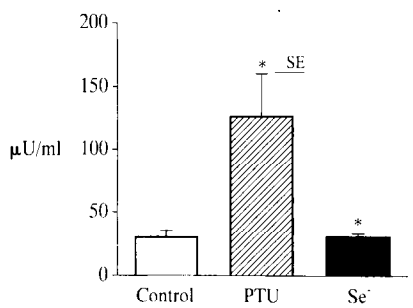

FIG. 7. Serum TSH concentrations in C, PTU-treated, and Se thyroidectomized T_4-infused rats.

strated that PTU administration reduced plasma T_3 concentrations or T_3 production rates by about 50–80% (1–3). Whereas the results in Se^- rats suggested that 5'D-I might have a relatively small role in generating the plasma T_3 that comes from peripheral T_4 to T_3 conversion, the reports using PTU suggested that 5'D-I has a major role in this process (1–3, 16).

The present study directly compared the effects of both selenium deficiency and PTU administration on serum T_4 and T_3 concentrations and kinetics, and hepatic and kidney 5'D-I activities in T_4-replaced, thyroid-ablated rats. Hepatic and kidney 5'D-I activities were equally inhibited by both treatments, and the serum T_3 concentrations and T_3 production rates were similarly decreased by both selenium deficiency and PTU treatment. The data in Se^- rats extends the work of a previous study (8) by demonstrating that in athyreotic T_4-replaced rats, selenium deficiency has similar effects on the production rate of serum T_3 and serum T_3 concentrations. In contrast, the findings in PTU-treated rats leave a different impression from that conveyed by earlier data (1–3) regarding the relative importance of 5'D-I in the generation of serum T_3 arising from peripheral conversion of T_4. Frumess and Larsen (1), Larsen and Frumess (2), and van Doorn *et al.* (3) noted that plasma T_3 concentrations decreased by 47–77% in thyroidectomized T_4-replaced rats treated with PTU compared to those in similar groups of rats that did not receive PTU. In the present study, PTU treatment was associated with a 28% decrease in plasma T_3 concentrations. Similarly, the PTU-induced decline in the T_3 production rate was 77% in the study of van Doorn *et al.* (3) and 35% in the present study.

The difference between the present study and previous reports (1–3) concerning the effect of PTU on serum T_3 levels or T_3 production in athyreotic T_4-replaced rats does not appear to be related to differences in the doses of PTU employed. The dose of PTU administered in the present study was about 3.2 mg/rat·day. This is slightly more than the approximately 2.6 mg/day administered by some (1, 2) and somewhat less than the 5 mg/day administered in a third study (3). Moreover, in our preliminary study, doses of PTU that were lower as well as higher than the dose used in our kinetic studies caused similar degrees of inhibition of 5'D-I activity in liver homogenates. PTU was administered as a single daily dose by the ip route (1, 2) or was administered in the diet in the present study and that of van Doorn *et al.* (3). Oral administration of PTU is probably as efficient as ip administration because PTU is almost completely absorbed when administered by the oral route (16a). Oral doses of PTU are likely to result in more stable plasma PTU concentrations than ip doses because rats tend to feed throughout the lights-off period.

It follows that if similar doses of PTU were employed for the present and previous studies (1–3), it is unlikely that the lesser effect of PTU on serum T_3 concentrations or the T_3 production rate in our study is due to liver 5'D-I activity being less inhibited by PTU treatment. Tissue 5'D-I activities and *in vivo* T_3 production in the various studies cannot be compared, however, because they were both only measured in the present study. In a different study, Silva *et al.* (16) reported data on liver 5'D-I in rats receiving ip PTU treatment, but this was a study of the acute effects of PTU on the nuclear sources of T_3. Furthermore, higher doses of PTU were administered (16) than in earlier studies (1, 2). In earlier studies (1, 2), PTU was administered in a dose of 1 mg/100 g BW·day, ip, whereas Silva *et al.* (16) administered 1 mg/100 g BW PTU, ip, 4.5, 18, and 28 h before the rats were killed. Using this higher PTU dose regimen, the inhibition of liver 5'D-I was 92.7%, a value greater than that reported in the present study. However, lower rT_3 substrate and DTT concentrations were used for the 5'D-I assay (16) than those employed in the present study (1 μM rT_3 *vs.* 10 μM rT_3 and 1 mM DTT *vs.* 20 mM DTT). As the data presented in *Materials and Methods* indicates, these lower rT_3 substrate and DTT concentrations result in a greater degree of *in vitro* 5'D-I inhibition in livers from PTU-treated rats. The percent inhibition of liver 5'D-I in the present study would have been significantly higher, therefore, had the rT_3 substrate concen-

trations and DTT concentrations used by Silva *et al.* (16) been employed in the present study. *In vivo* inhibition of 5′D-I may have been slightly submaximal in our study as well as in the study of Silva *et al.* (16). In the latter study, using enzyme assay conditions of 2 nM rT_3 with 20 mM DTT, the addition of 1 mM PTU *in vitro* increased the inhibition of 5′D-I in the livers of PTU-treated rats from 92.7% to 98.6%. In the present study, using similar substrate and DTT concentrations, the addition of 1 mM PTU *in vitro* resulted in 95.7% inhibition of 5′D-I in livers from PTU-treated rats, about 7% greater inhibition than that noted in the absence of PTU added *in vitro* (see *Materials and Methods*).

It should be noted that some of the earlier studies (1, 2) were more concerned with the effect of PTU on thyroid hormone action in T_4-treated athyreotic rats. Accordingly, these studies did not derive kinetic data, and T_4 was administered in bolus doses rather than by constant infusion as in the present study and that of van Doorn *et al.* (3). The former technique is not as satisfactory as constant infusion for noncompartmental kinetic studies.

One difference between the present study and the previous studies in thyroidectomized T_4-treated rats is that in the present study, there was little or no period after thyroid ablation during which T_4 replacement was withheld. In contrast, in the studies of Frumess and Larsen (1, 2), there was a period of many weeks after thyroidectomy and before starting T_4 replacement during which the rats were almost certainly hypothyroid because their growth plateaued. Similarly, in the van Doorn *et al.* study (3), there was a 2-month period after radiothyroidectomy during which no thyroid hormone was administered. In these rats, serum T_4 and T_3 were undetectable when assessed 6 weeks after thyroidectomy and 2 weeks before T_4 and PTU were administered. The fact that there was a period of hypothyroidism before thyroid hormone replacement in previous studies (1–3), but not in the present report does not provide an obvious explanation for why extrathyroidal T_4 to T_3 conversion was more sensitive to PTU inhibition in previous studies.

PTU treatment and selenium deficiency also cause a decrease in the T_4 MCR and thereby an increase in plasma T_4 concentrations. This should provide increased T_4 substrate concentrations for 5′D-I mediated deiodination and, if this were the only factor, cause serum T_3 concentrations to increase. The increase in T_4 substrate available for 5′D-I activity is probably directly proportional to the increase in serum T_4 concentrations (17, 18). Based on the increase in serum T_4, T_3 production in PTU-treated rats should increase by 36% if 5′-deiodination of T_4 is also not inhibited. In this context, the observed decrease in T_3 production of 35% in PTU-treated rats can be viewed as equivalent to a 52% inhibition of T_3 neogenesis. Although substantial, this is still less than the 79% and 67% inhibitions of 5′D-I noted, respectively, in the liver and kidneys of these rats. Similarly, the apparent decrease in T_3 neogenesis in the Se^- rats was 48%. This is also less than the 85% and 64% inhibitions of 5′D-I noted, respectively, in their heptatic and renal tissues. The discrepancy between the decrease in T_3 neogenesis and the inhibition of 5′D-I in liver and kidney homogenates would be even greater if the actual values for the T_3 production rate were used as the index for T_3 neogenesis or, in the case of PTU-treated rats, if lower rT_3 substrate concentrations were used to assess liver 5′D-I.

Kinetic studies performed by DiStefano and co-workers (19) demonstrated that 35% or less of the total T_3 production rate was generated in the fast pool, postulated to be T_4 to T_3 conversion in the liver and kidney. The bulk of the T_3 production rate (53%) was suggested to be generated by T_4 to T_3 conversion in tissues that constitute the slow pool, *i.e.* extrahepatic and extrarenal tissues such as heart and muscle (19). If this is indeed the case, our results suggest that these slow pool sites are resistant to both Se^- diets and PTU treatment and/or must have a large store of preformed T_3 available to the blood (20). One possibility is that there are tissues that conserve selenium despite the low selenium diets, such as the thyroid (8). Although extremely unlikely, it is possible that there are 5′D-I enzymes that are not selenoproteins. It is also possible that PTU does not reach sufficiently high concentrations to inhibit 5′D-I activity at the slow pool sites.

Other possibilities currently being considered are that the increased T_3 sulfate generation in the liver, reported to occur with both selenium deficiency and PTU administration (6, 21), could result in increased availability of T_3 for absorption across the gut mucosa after removal of the sulfate by bacterial sulfatase activity (22–24), but this is probably not the case (25). Another possibility is that type II deiodinase activity participates in the extrathyroidal production of serum T_3 even in euthyroid rats (25a). Genetic rat models with knockout 5′D-I and other deiodinase gene mutations are probably the most efficacious way to unravel some of the possibilities outlined above.

In an earlier study (1) it was noted that serum TSH concentrations were higher in thyroid-ablated T_4-injected rats that were treated with PTU than in those that did not receive PTU. This was consistent with the fact that in that study, PTU treatment reduced serum T_3 concentrations by 49–63%. We also noted significantly higher serum TSH concentrations in thyroid-ablated T_4-treated rats that received PTU compared to those that did not receive PTU. As noted, however, PTU treatment resulted in a far more modest decrease in serum T_3 than previously observed. Interestingly, unlike the PTU-treated group, serum TSH concentrations were not increased in the Se^- group despite the fact that the effects of PTU treatment and selenium deficiency on serum T_3 and T_4 concentrations in the thyroid-ablated T_4-infused rats were similar. This raises the possibility that the intrapituitary deiodination of T_4 to T_3 by the PTU-resistant 5′D-II isoenzyme and 5′D-I is different in PTU-treated and Se^- rats. Selenium deficiency is associated with a decrease in pituitary 5′D-II activity (15), perhaps due to the increased serum T_4 concentrations, as this isoenzyme is directly related to thyroid status, although 5′D-II might also be a selenoenzyme (26), as has been reported for inner ring or 5D (27). In contrast, PTU administration does not affect 5′D-II activity (28), yet serum TSH increased only in the PTU-treated rats despite the similar small decreases in serum T_3 values in PTU-treated and Se^- rats. This observed dichotomy requires further study.

In conclusion, inhibition of peripheral liver and kidney 5′D-I activities by either selenium deficiency or PTU treatment in athyreotic T_4-replaced rats results in modest decreases in serum T_3 values and T_3 production. These findings

are consistent with the recent report that serum T_3 concentrations are not reduced in mice with inherited deficiency of 5'D-I (29). They are also similar to studies in athyreotic humans receiving T_4 replacement. In these patients, PTU treatment causes a modest decline of about 20–30% in serum T_3 concentrations, but unlike the findings in the rat, serum T_4 concentrations did not increase (30, 31). In the rat and perhaps also in the human, 5'D-I-mediated T_4 to T_3 conversion, at least in the liver and kidney, does not account for a substantial fraction of the circulating T_3 that arises from peripheral T_4 to T_3 conversion. Perhaps other tissues, such as muscle and heart, might contribute to maintaining serum T_3 concentrations by generating T_3 from T_4 by PTU-insensitive pathways, by PTU not reaching sufficient concentrations to inhibit 5'D-I, or by releasing a large pool of tissue T_3 that is not mobilized unless there is a threat to the maintenance of normal plasma T_3 levels.

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