Propylthiouracil Inhibits the Conversion of L-Thyroxine to L-Triiodothyronine

AN EXPLANATION OF THE ANTITHYROXINE EFFECT OF PROPYLTHIOURACIL AND EVIDENCE SUPPORTING THE CONCEPT THAT TRIIODOTHYRONINE IS THE ACTIVE THYROID HORMONE

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ABSTRACT 6-n-propylthiouracil (PTU) administered to male Sprague-Dawley rats maintained on 2 and 5 μg L-thyroxine (T₄)/100 g body weight resulted in a marked reduction in the rate of conversion of L-thyroxine to L-triiodothyronine (T₃). These effects could not be ascribed to induced hypothyroidism since the group maintained on 5 μg T₄/day had normal levels of liver mitochondrial alpha glycerophosphate dehydrogenase. In confirmation of previous studies, PTU also reduced the fractional rate of deiodination of T₄. These observations provide a possible explanation of the many published observations indicating that PTU antagonizes the tissue effects of T₄ but not of T₃. The data suggest that monodeiodination of T₄ but not of T₃ is essential before hormonal effects can be manifested at the cellular level.

INTRODUCTION
In addition to its well recognized effects on intrathyroidal iodine metabolism, 6-n-propylthiouracil (PTU) inhibits the peripheral hormonal manifestations of injected T₄. Thus, in rats PTU and many related thiouracil-type compounds reduce the capacity of a given dose of T₄ to increase the oxygen consumption of the whole animal (1-6) and isolated tissues (7, 8), to raise the activity of mitochondrial alpha glycerophosphate dehydrogenase (α-GPD) in heart, liver, and kidney (5, 8-10), to inhibit the pituitary release of TSH (7, 11-14), and to decrease the heart rate (15). The peripheral tissue effects of PTU have been attributed to the capacity of this drug to inhibit T₄ deiodination (7, 15-20). Curiously, however, PTU does not antagonize the peripheral effects of injected T₃; a variety of studies show either no change, or in fact a slight increase in the tissue effects of T₃ when administered to PTU-treated animals (4-6, 10, 14, 21). Although the biological effects of T₃ are not decreased, PTU inhibits its deiodination as effectively as it does the deiodination of T₄ (15, 17, 20).

The recent demonstration that T₃ is converted to T₄ under physiological conditions both in man (22-24) and in the rat (25) afforded a possible explanation of these findings. Quantitative considerations based on the biological potency ratio of T₄ to T₃ and the extent of T₄ to T₃ conversion in the rat raised the possibility that all of the hormonal effect of injected T₄ may be derived from its peripheral conversion to T₃ (25). Thus, if PTU inhibited the monodeiodination of T₄ to form T₃, a marked
Table 1

Effect of PTU on Conversion of T₄ to T₃

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Observed</th>
<th>Corrected</th>
<th>λ₄</th>
<th>K</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTU + 2 μg T₄/100 g body wt.</td>
<td>12</td>
<td>0.0262±0.0030</td>
<td>0.0224±0.0031</td>
<td>0.0344±0.0013</td>
<td>0.0026±0.0004</td>
<td>7.51±0.90</td>
</tr>
<tr>
<td>PTU + 5 μg T₄/100 g body wt.</td>
<td>6</td>
<td>0.0174±0.0041</td>
<td>0.0153±0.0070</td>
<td>0.0456±0.0170</td>
<td>0.0028±0.0008</td>
<td>6.20±1.76</td>
</tr>
<tr>
<td>Control†</td>
<td>11</td>
<td>0.1000±0.0038</td>
<td>0.0873±0.0038</td>
<td>0.0492±0.0020</td>
<td>0.0083±0.0008</td>
<td>16.9±1.0</td>
</tr>
</tbody>
</table>

Explanation of symbols: n, number of animals in each group; T₄/T₃, the ratio of isotopic T₃ to T₄ in the carcass; λ₄, fractional removal rate of T₄; K, fractional rate of nonradioactive T₄ to T₃ conversion; CR, conversion ratio = (K/λ₄) × 100.

* Statistically different from control group at P < 0.01 level (Student’s t test). Conversion ratios for 2 μg and 5 μg T₄ groups were not significantly different from each other.

† Control data has previously been published (25). Mean ±SE are indicated.

Reduction in T₄ hormonal effects would be anticipated. On the other hand, inhibition of deiodination of T₃ would not be expected to result in a diminution in hormonal effect. The following studies were therefore undertaken to determine whether in fact PTU inhibits the conversion of T₄ to T₃.

Methods

Methods for quantitating the conversion of T₄ to T₃ in PTU-treated male Sprague-Dawley rats (100-150 g) were identical to those described in a previous communication dealing with this phenomenon in untreated animals (25). In brief, animals were killed 48 hr after the injection of tracer [¹³¹I]-T₄. The carcass was homogenized and extracted with ethanol. After addition of authentic [¹³¹I]-T₃, T₃ in the extract was isolated and purified by serial chromatography in three paper and one thin-layer systems. The final eluate of the T₃ region was chromatographed on paper in three different solvent systems. The ratio of [¹³¹I]-T₃ to [²⁵¹I]-T₃ was determined and the content of [¹³¹I]-T₃ in the carcass calculated. Account was taken of artifactual in vitro T₃ to T₄ conversion by repeating similar procedures in animals killed within 1 min after the intravenous injection of tracer [²⁵¹I]-T₄. The fractional removal rate of T₃ (λ₃), was determined from the residual T₃ in the carcass. The fractional removal rate of T₃ (λ₄), was measured in a separate series of paired animals as previously described (26). It was possible to calculate K, the fractional rate of nonradioactive T₄ to T₃ conversion, and the conversion ratio (CR), the percentage of nonradioactive T₄ ultimately converted to T₃, by appropriate substitution of carcass [²⁵¹I]-T₄/ [¹³¹I]-T₄, λ₃ and λ₄, and t = 48 hr into the following equation derived in reference 25,

\[
CR = \frac{K(100)}{\lambda_4} = \frac{200 \left[ \frac{1}{(1 - e^{-0.05})^t} \right]}{(1 - e^{-0.05})^4}.
\]

In some animals, fecal and urinary radioactive content were also measured. The fraction of total radioactive disposed of via the deiodinative pathways, Fₐ, and the fraction of total radioactivity disposed via the fecal route, Fₙ, were calculated as previously described (26). The fractional fecal excretion rate (λₜ), and the fractional deiodinative removal rate (λₐ) were calculated respectively from the product of λ and Fₐ, and λ and Fₙ.

Animals were maintained on 0.10% PTU in their drinking water from 3.5 to 8 wk. During this period, T₃ was administered daily by subcutaneous injection in 0.01 N NaOH solution (2 and 5 μg/100 g body weight). The animals were in good health during this period and gained weight normally. Since the present series of studies followed immediately upon our analysis of T₄ to T₃ transformation in untreated animals and since an identical strain of rats and identical techniques were used, the results of the previously published study were considered adequate as controls in determining the T₄ to T₃ transformation under the influence of PTU. The level of the mitochondrial enzyme α-GPD was determined by the method of Lee and Lardy (27).

Results

PTU effects a marked reduction in the fractional conversion of T₄ to T₃ (Table 1). In both groups of PTU-treated animals, the conversion ratio was reduced to at least 40% of the control value. There was no statistical difference between the conversion ratio of the PTU-treated group supplemented with 2 μg T₄/100 g body weight and those PTU-treated animals supplemented with 5 μg T₄/100 g body weight.

Since PTU is known effectively to block thyroidal synthesis of T₃ and T₄, the theoretical possibility arises that the lower T₃ content in the PTU-treated animals is attributable to a more effective thyroidal blockade provided by the PTU than by the 1 mg NaI alone administered daily to the control group. This explanation, however, is not tenable since less than 0.06% of the radioactivity in the injected [²⁵¹I]-T₄ dose was found in the thyroid of control animals 48 hr after injection. The release rate of total thyroidal radioactivity from the gland in animals treated with a high iodine diet is small, less than 20% per day. Thus, the amount of free T₃ in the gland or in the carcass derived from recycled labeled iodine, is several orders of magnitude less than the [²⁵¹I]-T₄ actually observed.

The possibility that the results obtained were due to a differential extraction of T₄ in control and PTU-treated animals was considered. Successive extractions were performed in two PTU and two control animals sacrificed within 2 min after the injection of a combined dose of [²⁵¹I]-T₄ and [¹³¹I]-T₃. The extraction characteristics were the same for both iodothyronines and there was no difference between control and PTU-treated animals.
with 5 μg/100 g body weight. As indicated in Table II, administration of 2 μg T4/100 g body weight, however, was insufficient to maintain the animals in the euthyroid state. This was inferred from the reduced activity of hepatic mitochondrial α-GPD, an enzyme which has been shown to be a sensitive index of the thyroidal state of tissues (27). Since the daily dose of T4 required to maintain the euthyroid state is probably 2 μg/100 g body weight or less (8, 28), the finding of a low α-GPD is probably an example of the well documented antagonism between PTU and T4 which prompted this study. Since the administration of 5 μg T4/100 g body weight led to a normal liver activity of α-GPD, the reduction in the fractional conversion of T4 to T3 in this group cannot be ascribed to hypothyroidism.

Table III summarizes kinetic data obtained in those groups which were studied primarily in order to determine the fractional removal rate of T3 (λ3), in the treated animals. As pointed out above, this value is necessary in the calculation of T3 to T4 conversion. In confirmation of previous studies (15, 17, 20), PTU effected a marked reduction in the fraction of labeled iodine from T3 and T4 which was disposed of via deiodinative pathways (Ff). PTU caused a modest reduction in the fractional removal rate of T3 (λ3). In the case of T4, PTU appeared to reduce slightly the fractional removal in the animals treated with 2 μg/100 g body weight and to increase slightly the fractional removal rate in the animals treated with 5 μg/100 g body weight. In all cases, however, the fractional rate of deiodination (λ3) was markedly reduced in PTU-treated animals. Of interest was the slight but consistent increase in the fractional rate of fecal excretion (λf) in all groups. An increase in the fractional disposition via the fecal route offsets the decreased fractional rate of deiodination, thus stabilizing the overall fractional removal rate (λ = λ3 + λf). It is entirely probable that the increase in the fraction of hormone iodine excreted via the fecal pathway (Ff) is related to the increase in biliary clearance of T3 and T4 previously reported by Lang and Premachandra (29). A decrease in distribution space, especially in the case of T4 was noted. Unfortunately, no plasma binding studies were performed in order to assess the contribution of alterations in plasma binding to the reduction of distribution volume. Increased binding of rat plasma proteins associated with hypothyroidism (30), however, could not explain the reduced distribution volume of T3 since this change was also found in the group of animals treated with 5 μg T4/100 g body weight, a dosage regimen which maintained animals in the euthyroid condition.

**DISCUSSION**

The most important result of these studies is the demonstration that PTU causes a marked reduction in the fractional conversion of T4 to T3. No information is currently available regarding the site of inhibition, nor is it known where monodeiodination of T3 occurs. In this connection, however, it is of interest to evaluate the ratio K/λu,

### TABLE II

<table>
<thead>
<tr>
<th>Mitochondrial α-GPD in PTU-Treated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-GPD</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>PTU + 5 μg T4</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>PTU + 2 μg T4</td>
</tr>
<tr>
<td>Thyroidectomized</td>
</tr>
</tbody>
</table>

ΔOD/mg per min: Normal = 0.1355 ± 0.0135; PTU + 5 μg T4 = 0.1294 ± 0.0087; Normal = 0.0981 ± 0.0106; PTU + 2 μg T4 = 0.0483 ± 0.0028; Thyroidectomized = 0.0396 ± 0.0038.

Mean ± SE are indicated. Statistical comparisons are made with simultaneously run untreated intact rats.

### TABLE III

**Effect of PTU on Tracer T3 and T4 Metabolism**

<table>
<thead>
<tr>
<th>n</th>
<th>VT</th>
<th>F0</th>
<th>Ff</th>
<th>λ3</th>
<th>λu</th>
<th>λf</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml/100 g body wt.</td>
<td>hr⁻¹</td>
<td>hr⁻¹</td>
<td>hr⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 1H1-T3

- **Control**: 10 213.0 ± 29.2 0.49 ± 0.03 0.51 ± 0.03 0.122 ± 0.0020 0.0600 ± 0.0039 0.0626 ± 0.0031
- **PTU + 2 μg T4**: 10 107.8* ± 8.1 0.16* ± 0.01 0.84* ± 0.01 0.0982* ± 0.0036 0.0158* ± 0.0011 0.0823* ± 0.0037
- **PTU + 5 μg T4**: 8 119.7* ± 16.3 0.22* ± 0.02 0.78* ± 0.02 0.1384 ± 0.0092 0.0294* ± 0.0036 0.1090* ± 0.0061

### 1H1-T4

- **Control**: 10 21.2 ± 1.0 0.40 ± 0.02 0.60 ± 0.02 0.0461 ± 0.0013 0.0183 ± 0.0012 0.0278 ± 0.0010
- **PTU + 2 μg T4**: 10 18.1* ± 0.4 0.13* ± 0.01 0.87* ± 0.01 0.0398* ± 0.0016 0.0050* ± 0.0003 0.0347* ± 0.0015

* Significant difference from control at P < 0.01 level.

**Explanation of symbols**: n, number of animals in group; VT, volume of distribution; F0, fraction of labeled hormone iodine excreted in urine; Ff, fraction of labeled hormone iodine excreted in feces; λ, fractional removal rate of hormone (per hour); λu = (λ)(F0); λf = (λ)(Ff). Mean ± SE indicated.

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where K is fractional rate of conversion of T\textsubscript{s} to T\textsubscript{s} and λ\textsubscript{s}, the fractional deiodinative removal rate of T\textsubscript{s}. From the data in Tables I and III, one can calculate that in control animals K/λ\textsubscript{s} = 0.45 and in PTU-treated animals, 0.52. These results suggest that one-half of all T\textsubscript{s} which is deiodinated is converted to T\textsubscript{s}. Recent data from our laboratory are compatible with the idea that iodine atoms are detached from both the inner and outer ring of T\textsubscript{s} in a random fashion (31). If deiodination occurs more rapidly than transformation of other parts of the thyroxine molecule, random deiodination would result exactly in a 50% conversion of T\textsubscript{s} to T\textsubscript{s}. These considerations raise the interesting possibility that T\textsubscript{s} formation is an obligatory step in all biological deiodination of T\textsubscript{s} regardless of the tissue involved.

If one assumes that the entire hormonal potency of injected T\textsubscript{s} is derived from its conversion to T\textsubscript{s}, it is possible to provide an estimate of the degree of inhibition of T\textsubscript{s} activity expected to result from the concurrent administration of PTU. Within a restricted range, hormonal tissue effects appear to be grossly proportional to the exchangeable hormonal tissue pool (32). Since only 5% of total body T\textsubscript{s} in the rat is bound to plasma protein, and the rest is contained within the cells (26), the total exchangeable body pool of T\textsubscript{s} can be regarded as a first approximation of the tissue pool. Thus, the following equation can be written:

$$\Delta E = \alpha P_s = \frac{\alpha (CR)S_i}{100\lambda_s}$$

where ΔE is the increment in a given hormonal tissue effect over base line hypothyroid levels produced by S\textsubscript{i}, the daily dose of injected T\textsubscript{s}; α, a proportionality constant; P\textsubscript{s}, the exchangeable body pool of T\textsubscript{s}; CR, the conversion ratio expressed in per cent; and λ\textsubscript{s}, the fractional rate of T\textsubscript{s} removal. It is assumed that in this system, the only source of T\textsubscript{s} is the injected T\textsubscript{s}. If PTU exerts its effects by inhibiting the conversion of T\textsubscript{s} to T\textsubscript{s}, it follows that

$$\frac{\Delta E_{\text{PTU}}}{\Delta E_{\text{CON}}} = \frac{(CR)_{\text{PTU}}}{(CR)_{\text{CON}}} \cdot \frac{\lambda_s}{\lambda_{\text{PTU}}}$$

where the subscripts refer to control and PTU-treated groups.

If we substitute in the expression the average value for the conversion ratio in PTU-treated (6.9%), control animals (16.9%), and the corresponding average λ\textsubscript{s} in control animals (0.122/hr) and PTU-treated animals (0.118/hr), the predicted value for ΔE\textsubscript{PTU}/ΔE\textsubscript{CON} would be 0.42. Experimentally, the value for ΔE\textsubscript{PTU}/ΔE\textsubscript{CON} can be estimated best from the data of Hoffman, Richert, and Westerfield (5) in which the effect of PTU on the increase in tissue mitochondrial α-GPD is measured at various doses of injected T\textsubscript{s}. For liver, ΔE\textsubscript{PTU}/ΔE\textsubscript{CON} is 0.37 and for kidney, ΔE\textsubscript{PTU}/ΔE\textsubscript{CON}, 0.47. The anticipated decrease in T\textsubscript{s} activity brought about by PTU is thus in general agreement with the experimentally observed values. Because the effect of PTU on the fractional turnover of T\textsubscript{s} is not marked, it is apparent that PTU would be expected to have no major effect on the effectiveness of injected T\textsubscript{s}. In fact, a slight slowing of the fractional turnover might explain the enhanced effectiveness of T\textsubscript{s} reported in some of the published studies.

The internal consistency of this formulation lends additional credence to the concept that most, if not all, of the hormonal effect of T\textsubscript{s} is due to its conversion to T\textsubscript{s}. This proposal was first advanced by Gross and Pitt-Rivers (33) and was supported specifically by the studies of Maclagan, Sprott, and Wilkinson (34) and Wilkinson and Maclagen (35). Nevertheless, the report by Lassiter and Stanbury negating T\textsubscript{s} to T\textsubscript{s} conversion in man (36) led to an abandonment of the concept that T\textsubscript{s} was the active thyroid hormone. Renewed interest in the problem was aroused by the demonstration by Braverman, Ingbar, and Sterling (22) that T\textsubscript{s} to T\textsubscript{s} conversion did in fact occur in man and that the earlier conclusion by Lassiter and Stanbury was premature. Additional evidence favoring the concept that T\textsubscript{s} is the active hormone has been the finding of limited capacity anterior pituitary sites for T\textsubscript{s} but not T\textsubscript{s} (37) and the demonstration of stereospecific binding of T\textsubscript{s} but not T\textsubscript{s} by rat hepatic and renal nuclei (38).

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REFERENCES


