Effects of sodium ipodate and propylthiouracil in athyreotic human subjects, role of triiodothyronine and pituitary thyroxine monodeiodination in thyrotrophin regulation

G. Schaison, P. Thomopoulos, D. Leguillouzic, G. Thomas and M. Moatti

Department of Endocrinology, Hôpital Bicêtre, 78, rue de Général Leclerc, 94270-Le Kremlin-Bicêtre, France

Abstract. To investigate the respective role of triiodothyronine (T₃) and thyroxine (T₄) in the regulation of TSH secretion, we studied the action of sodium ipodate and propylthiouracil (PTU) in 11 athyreotic patients. The 1.5 replacement dose was adjusted to obtain, in each patient, a normal basal TSH level and a normal TSH response to TRH. In the 5 ipodate-treated patients (single 6 g oral dose), the mean serum T₃ level fell by 64% below the baseline value and serum T₄ rose 180% above the baseline. The free T₄ index (FT₄I) did not change whereas the mean serum TSH concentration increased 280% above baseline values. In the 6 PTU-treated patients (250 mg orally every 6 h for 10 days), serum T₃ levels fell 33%, serum T₄ increased up to 82% and the FT₄I did not change. The mean serum TSH concentration increased 68% above the baseline value. Thus, the mean percentage increase in serum TSH was less in PTU- than in ipodate-treated patients (68% vs 280%). Statistical analysis of the correlation between the serum T₃ decrease (∆T₃) and the serum TSH (∆TSH) increase demonstrated that for the same T₃ diminution, the ipodate-treated group displayed higher increase of TSH than the PTU-treated patients. In the rat, PTU interferes with the 5'-deiodination of T₄ in the liver and kidney but not in the pituitary, while ipodate appears to have the same effect in all tissues. If this holds true for human subjects, our data strongly suggest that circulating T₄ (through its intrapituitary conversion to T₃) shares with serum T₃ the capacity to regulate TSH secretion in man.

Previous studies in animals and in humans have clearly shown that the circulating 3,5,3'-triiodothyronine (T₃) is an important regulator of pituitary thyrotrophin (TSH) secretion (Silva & Larsen 1977; Larsen et al. 1981; Larsen 1982). However, elevated serum TSH levels, normal serum T₃ concentrations and reduced serum thyroxine (T₄) levels may be found in iodine deficiency and in patients with early primary hypothyroidism. These situations lacking a correlation between serum T₃ and TSH levels suggest an important role for T₄ in TSH regulation. The oral cholecystographic agents, iopanoic acid and ipodate have been shown to inhibit T₄-5'-monodeiodination in all tissues that have been studied to date including pituitary and brain (Larsen et al. 1979; Kaplan & Utiger 1978; Crantz & Larsen 1980). 6-n propylthiouracil (PTU) is also a well known inhibitor of T₄ to T₃ conversion (Oppenheimer et al. 1972). However, its failure to decrease the intrapituitary T₃ concentration suggests that in vivo 5'-deiodination of T₄ proceeds in this tissue via a PTU insensitive pathway (Silva et al. 1982).
The present study was carried out to compare the effect of ipodate and PTU in athyreotic T4 maintained euthyroid patients. The aim was to further investigate the relative importance of the concentration of plasma T3 and of T3 generated locally from T4 within the pituitary, in the regulation of TSH secretion. Our data demonstrate that circulating T3 concentration and serum T4 through its intrapituitary conversion are both important in TSH regulation.

Patients and Methods

After informed consent was obtained, 11 patients, 7 women and 4 men (aged 23 to 58 years) with well established primary hypothyroidism were included in the protocol (Table I). All were selected because of their complete thyroid deficiency with plasma T4 levels originally below 1 µg/dl. At the time of the study, they had been taking 0.1 to 0.2 mg per day of L-thyroxine for more than 6 months. The L-T4 replacement dose was adjusted to obtain in each patient a basal TSH level below 10 µU/ml and a TSH response to TRH of more than 100% with a peak increase in TSH above 10 µU/ml. In this study, two TRH tests were performed at intervals of up to 30 days by administering a single iv bolus of 200 µg TRH at 08.00 h after an overnight fast. Blood was sampled immediately before and 15, 30, 60, 90 and 120 min after the TRH injection. Plasma TSH was measured by a double antibody RIA as previously described (Schaison et al. 1981). The sensitivity of the method permits the detection of 0.6 µU/ml and the range of values found in the plasma of normal controls was 0.5—11 µU/ml. The inter-assay coefficient of variation was less than 7%. Blood samples were also analyzed by RIA for T4 (clinical assay Cambridge, MA, USA). The free T4 index (FT4I) represents the product of the serum T4 concentration and the T3 resin uptake (Pharmacia Uppsala, Sweden). T3 and 3,3',5'-triiodothyronine (rT3) were measured by RIA using materials supplied by respectively Behring (Berlin, W-Germany) and Biodata Serono (Chavannes de Bogia, Switzerland). All plasma samples for a given individual were assayed concurrently.

Table 1.
Clinical and biochemical data of 11 hypothyroid patients before and after L-T4-treatment.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Aetiology</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FT4I µg/dl</td>
<td>TSH µU/ml</td>
</tr>
<tr>
<td>Ipodate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>23</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>50</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>33</td>
<td>0.5</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>58</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>30</td>
<td>0.5</td>
<td>72</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>39</td>
<td>0.45</td>
<td>79.6</td>
</tr>
<tr>
<td>PTU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>31</td>
<td>0</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>57</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>44</td>
<td>0.5</td>
<td>66</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>30</td>
<td>0.25</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>44</td>
<td>0.9</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>33</td>
<td>0.6</td>
<td>78</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>40</td>
<td>0.37</td>
<td>69</td>
</tr>
</tbody>
</table>
and in duplicate for each hormone. Normal ranges for control subjects in this laboratory are 4.5–11.5 µg/dl for T4, 0.81–1.14 for T3 resin uptake, 3.6–13.1 for FT4I, 70–220 ng/dl for T3 and 9–35 ng/dl for rT3. During the study, the 11 patients were continuing l-thyroxine therapy. They were divided into 2 groups in a nonrandom fashion. The 2 groups were however comparable and studied during the same period (Table 1).

**Ipodate-treated patients**

Five patients were given a single 6 g oral dose of sodium ipodate (Oragrafin) at 08.00 h. Blood was drawn from each patient, immediately before and on days 1, 2, 3, 4, 5, 7 and 10 after ipodate administration and was analyzed for FT4I, T3, rT3 and TSH. In these patients, total plasma iodine was measured by a classic chemical method (Piette et al. 1966).

**PTU-treated patients**

Six patients received PTU (250 mg orally every 6 h) for 10 days. Blood was drawn before and during the treatment period for measurement of FT4I, T3, rT3 and TSH.

Student’s t-test was used to evaluate the hormone level changes from pre-treatment values. In order to assess whether the ΔTSH difference between ipodate- and PTU-treated patients was due only to the difference in T3, or in part to a differential effect of treatment on the pituitary, linear regression lines of TSH vs ΔT3 within each group were calculated by the least square method, and compared by analysis of covariance1 (Snedecor & Cochran 1967).

### Results

Table 2 shows the sequence of the changes in TSH and iodothyronines and total iodine concentration in ipodate-treated patients.

After ipodate administration, the mean serum T3 concentration significantly decreased from the pre-treatment (day 0) level of 100 ± 3 ng/dl to 36 ± 3 ng/dl by day 4 (P < 0.001). Ten days later, the serum T3 values returned to a mean level of 79 ± 14 ng/dl, not significantly different from the initial level. The mean serum rT3 level increased

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1 Comparison of regression lines by analysis of covariance consists of 3 successive steps: 1) test for the significance of correlation between TSH and T3 within each group, 2) test for the difference between slopes of regression lines, 3) test for the difference between intercepts of regression lines.
significantly \( (P < 0.001) \) from the pre-treatment level of \( 24 \pm 3 \) ng/dl to \( 67 \pm 7 \) ng/dl on day 3 and then gradually declined. However, the value was still higher than the initial level on day 7 and even on day 10. The serum \( T_4 \) and \( FT_4I \) increased slightly, but the change in the mean value was too small to be significant. The serum TSH concentration increased significantly from the pre-treatment level of \( 4.2 \pm 2.0 \) µU/ml to \( 16.1 \pm 4.5 \) µU/ml by day 7 \( (P < 0.05) \). From day 5 to day 10, the change in the plasma TSH mean value was not significant. On day 10, total plasma iodine was still elevated \( (511 \pm 204 \) µg/dl). 

Table 3 illustrates the results in PTU-treated patients. The mean serum \( T_3 \) concentration decreased significantly \( (P < 0.001) \) from the pre-treatment value of \( 91 \pm 6 \) ng/dl to \( 61 \pm 3 \) ng/dl by day 4 and thereafter varied minimally.

The mean serum \( rT_3 \) increased from \( 27 \pm 5 \) ng/dl and reached a peak of \( 49 \pm 5 \) ng/dl on day 3. Afterwards \( rT_3 \) gradually declined returning to the pre-treatment level by day 10.

The serum \( T_4 \) and \( FT_4I \) remained unchanged during the study period.

The serum TSH concentration increased from the pre-treatment level of \( 6.2 \pm 1.9 \) µU/ml to \( 10.2 \pm 1.6 \) µU/ml by day 5. This slight but significant TSH increase \( (P < 0.05) \) was the same on days 5 and 10.

Thus, after sodium iodate administration, the maximum per cent decrement in \( T_3 \) \( (64\%) \) and the maximum per cent increment in \( rT_3 \) \( (180\%) \) were greater than those in the PTU-treated patients \( (33\% \) and \( 82\%, \) respectively). Likewise, the enhanced TSH secretion was greater in the iodate-treated \( (280\%) \) than in the PTU-treated patients \( (68\%) \). The comparison of iodate- and PTU-treated patients by analysis of covariance showed:

1) a significant correlation between TSH and \( T_3 \) in the iodate \( (r = -0.30, \ P < 0.01)^2 \) and PTU \( (r = -0.05, \ P < 0.01)^2 \) groups; 2) no significant difference between slopes but 3) a significant difference between intercepts of the two regression lines\(^3 (P < 0.01)\) (Fig. 1). In other words, the lower serum \( T_3 \) levels in iodate-treated patients did not fully account for their higher serum TSH levels. For the same \( \Delta T_3 \) decrease, a higher \( \Delta TSH \) increase was observed in these patients.

\(^2 \) \( r \) = standard correlation coefficient.

\(^3 \) by Fisher's F-test with 1 and 78 degrees of freedom \( (F_{78} = 10.2) \).

Table 3:

<table>
<thead>
<tr>
<th>Days during PTU treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_3 ) ng/dl</td>
<td>91 ± 6</td>
<td>72 ± 4*</td>
<td>67 ± 3**</td>
<td>70 ± 2**</td>
<td>61 ± 3**</td>
<td>51 ± 2*</td>
<td>65 ± 3**</td>
<td>68 ± 3**</td>
</tr>
<tr>
<td>( rT_3 ) ng/dl</td>
<td>27 ± 5</td>
<td>46 ± 3**</td>
<td>49 ± 5**</td>
<td>49 ± 3**</td>
<td>46 ± 3**</td>
<td>39 ± 3*</td>
<td>33 ± 2</td>
<td>67 ± 3**</td>
</tr>
<tr>
<td>( FT_4I ) µg/dl</td>
<td>10.1 ± 0.4</td>
<td>9.6 ± 0.7</td>
<td>9.8 ± 0.5</td>
<td>11.2 ± 0.6</td>
<td>10.1 ± 0.5</td>
<td>10.3 ± 0.5</td>
<td>10.3 ± 0.5</td>
<td>10.2 ± 1.6*</td>
</tr>
<tr>
<td>( TSH ) µg/ml</td>
<td>6.2 ± 1.9</td>
<td>7.1 ± 0.4</td>
<td>8.5 ± 0.4</td>
<td>9.1 ± 0.9</td>
<td>9.5 ± 1.2*</td>
<td>9.4 ± 1.3*</td>
<td>9.4 ± 1.3*</td>
<td>10.4 ± 1.5*</td>
</tr>
</tbody>
</table>

Asterisks indicate a significant difference from the values at day 0 \( (* P < 0.05; ** P < 0.01; *** P < 0.001) \).
Discussion

The aim of our study was to evaluate the relative importance of circulating T₃ and T₄ in the regulation of TSH secretion in man.

It has now been clearly demonstrated that circulating T₃ is an important regulator of TSH secretion. Clinical observations indicate, however, that circulating T₄ might also play a significant role: thus, in iodine deficiency and in the early stages of thyroid failure, a low serum T₄ concentration is associated with an increased level of TSH and a normal serum T₃ level (Larsen 1982).

In the present study, we chose to investigate hypothyroid patients in order to avoid any interference with thyroid hormonal secretion. In addition, the replacement doses of T₄ were carefully adapted for each patient so that the basal and TRH stimulated TSH levels were within the normal range. Our data shows that PTU-treated patients have a smaller decrease in serum T₃ levels and a smaller increase in TSH levels than the ipodate-treated group. It has been reported that PTU was less potent than ipodate in inhibiting the conversion of T₄ to T₃ (Sharp et al. 1981; Wu et al. 1982). Therefore, in order to compare the two protocols, we attempted to find out if the serum TSH values were similar in both groups of patients for the same serum T₃ levels.

The shape of the dose-response curves for T₃ and TSH is not well known. However, Silva & Larsen (1978) have shown that, 3 h after iv injection of increasing doses of T₃ into hypothyroid rats, the plasma T₃ concentration correlated with the change in TSH. There was a linear relationship between the nuclear occupancy by T₃ and TSH
release in rats. Moreover, for a plasma T3 level between 20 and 200 ng/dl, the TSH suppression was linearly related to the increase in plasma T3. Therefore, for both groups in the present study, we assumed a linear relationship between the decrease in serum T3 and the rise in TSH levels. If this assumption is not completely valid, however, analysis of covariance has been shown to be robust with respect to deviation from linearity (Sneden & Cochran 1967).

The totality of our data shows that for a similar decrease in serum T3, the rise in serum TSH was higher in the ipodate-treated group than in the PTU-treated patients. The TSH increase and the T3 decrease ratio through day 4 was similar in both groups. However, after day 4, while plasma T3 was already returning to normal in the ipodate-treated group, plasma TSH was not decreasing. This discrepancy argues against a simple relationship between TSH secretion and circulating T3 at that time. It is clear from the assays of total plasma iodine that ipodate was still present on day 7. It can be hypothesized that its effect on the pituitary persisted for a longer period than on peripheral deiodination. A more satisfactory explanation might be that the role of the locally produced T3 on TSH regulation in the pituitary is overpowered by the presence of very low circulating T3 levels. This is the case during the first 4 days for the ipodate-treated group. The pituitary T3 produced by deiodination of T4 becomes important for TSH regulation when plasma T3 increases towards normal levels. This would occur during the last 6 days. This hypothesis would be valid if 50% of the intrapituitary T3 in man originates from plasma T3 and 50% from local production as is the case in rats (Larsen 1982). Regardless of the mechanism, the fact that from day 5 to day 7, serum TSH did not respond to increasing T3 levels, is a strong argument against plasma T3 being the exclusive regulator of TSH production.

In man, previous studies have attempted to assess the role of T3 and T4 in TSH regulation, with partially conflicting conclusions. Thus, Wu et al. (1978) studied the effects of ipodate on normal subjects and hypothyroid patients on T3 replacement therapy. No increase in serum TSH was measurable in either group. However, the replacement doses of T4 were probably too high. Other investigators have reported increased serum TSH levels in hypothyroid patients on T4 replacement therapy, when their serum T3 levels were lowered by inhibition of T4 deiodination, either by administration of sodium iopanoate (Bürgi et al. 1976) or by PTU (Saberi et al. 1975; Geffner et al. 1975). Serum T4 values did not change. Several of these patients were undertreated, however, as evidenced by the basal TSH levels. Overall, the results indicate that circulating T3 is one of the factors regulating TSH secretion. Finally, Kleinmann et al. (1980) and Suzuki et al. (1981) showed that, in normal subjects, the iopanoic acid-induced decrease in serum T3 is accompanied by an increase in serum TSH. Furthermore, in both studies, administration of T3 sufficient to restore almost normal serum T3 levels prevented or reversed the effects of iopanoic acid on TSH. T4 replacement was ineffective. However, the authors stress that no definite conclusion can be drawn concerning the relative importance of extrapituitary T3 sources and intrapituitary T3 generation in the regulation of TSH secretion in patients receiving iopanoic acid (Kleinmann et al. 1980). These results are in accordance with our present study using PTU administration and suggest that circulating T3 is the main regulator of TSH secretion. However, if sodium ipodate inhibits the intrapituitary T4 to T3 conversion in humans as it does in rodents, the augmented TSH levels obtained with this drug for the same diminution in circulating T3 levels strongly suggests a role for circulating T3 in the regulation of TSH secretion. This conclusion would be in agreement with the data obtained from earlier animal studies.

In animals, Silva & Larsen (1977), Larsen et al. (1979), Crantz & Larsen (1980), and Silva & Larsen (1978) measured the plasma concentrations of TSH, T3 and T4 as well as intracellular levels and nuclear receptor bound iodothyronines in the pituitary and peripheral tissues of the rat. These parameters were compared in controls and in hypothyroid animals before and after treatment with T3 or T4. The authors also made use of the specific actions of propylthiouracil and of iopanoic acid on the conversion of T4 to T3. They showed that PTU interferes with the 5'-deiodination of T4 in the liver and kidney but not in the pituitary (Silva et al. 1978). 5'-deiodination of T4 proceeds via a PTU insensitive pathway in the central nervous system and the pituitary (Silva et al. 1982). On the other hand, iopanoic acid inhibits T4 5'-mono-deiodination in all tissues and in particular blocks the enzyme activity in the rat anterior pituitary (Obregon et al. 1980). These studies demonstrated...
that TSH secretion is regulated by the level of nuclear-receptor bound T₃ in the pituitary. Only 40–50% of nuclear T₃ in this organ originates from plasma T₃. Conversion of T₄ to T₃ within pituitary tissue accounts for the remainder (Larsen 1982). On the contrary, nearly all the nuclear T₃ present in the liver and kidney is derived from plasma T₃. Thus, the mixed origin of T₃ in the pituitary explains how TSH secretion can be a function of by both plasma T₃ and T₄ (Crantz et al. 1982).

In conclusion, the effects of PTU or ipodate administration on the serum TSH and iodothyronine levels in hypothyroid patients on T₄ replacement therapy were investigated. For the same decrease in serum T₃, ipodate treatment elicited a higher rise in the serum TSH than PTU. If the specific properties of these drugs are the same in animals and humans, our data suggest that circulating T₃, as well as circulating T₄, as a result of its intrapituitary deiodination to T₃, are important in the regulation of TSH secretion in man.

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