Effects of graded doses of triiodothyronine on TSH synthesis and secretion rates in hypothyroid rats

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Abstract. The effects of a graded low dose of T3 on plasma TSH, anterior pituitary TSH content (AP-TSH) and on TSH synthesis and secretion rates, were studied in adult male rats previously treated for 7 days with 0.01% propylthiouracil (PTU). Pituitary TSH content, plasma T3, T4 and TSH levels were measured by RIA at 0, 6, 12, 24 h after the injection of T3 ranging from 0.1 to 0.75 μg/100 g b.w. Saline treated normal rats served as control. The 7-day PTU treated rats displayed low but still detectable thyroid hormone plasma levels; high plasma TSH levels were observed, but with no further increase after TRH injection as the AP-TSH content was depleted. Injections of T3 increased plasma T3 in proportion with the dose given. Plasma T4 remained low and there was no significant decrease in plasma TSH until doses of 0.2 μg/100 g b.w. T3 was given. Then the transient TSH depression was dose-dependent from 0.2 to 0.75 μg/100 g b.w. The AP-TSH content increased regularly from 0.2 μg/T3 onwards, overlapping the transient inhibition of TSH secretion resulting in a 3-fold increase in the AP-TSH content, suggesting a positive action of T3 on TSH synthesis. In addition, TSH response to TRH was observed at every time studied after T3 injection. Then, the different groups were injected with [125I]rTSH in order to estimate metabolic clearance rates, TSH secretion and synthesis rates. The half-life of [125I]rTSH (22 min) and its metabolism clearance rates (16 ± 0.4 ml/h/100 g b.w.) were found similar in all groups. Whereas the TSH secretion rates was highly reduced in the normal rats receiving 0.3 μg T3 (156 ± 9 vs 408 ± 22 μg/24 h in normal rats), the PTU group displayed a 3-fold increased secretion rate (1222 ± 44 μg/24 h) which was not modified by the injection of 0.1–0.2 μg T3 and decreased to 868 and 472 μg/24 h with 0.3 μg and 0.75 μg T3, respectively. TSH synthesis rates were found highly increased in the PTU group (1222 ± 44 μg/d vs 408 ± 22 μg/d in normal rats) and was neither increased nor reduced in the 7-day PTU rats receiving 0.1–0.3 μg T3 but a slight reduction was observed only in the 0.75 μg T3 group.

These data show that 0.1–0.2 μg/100 g b.w. T3 changed neither TSH secretion nor its synthesis rates while 0.3 μg more or less a replacement dose inhibited TSH secretion without changing TSH synthesis rates, resulting in a AP-TSH replenishment. Therefore, no direct positive effect of low doses of T3 on TSH synthesis could be demonstrated over 24 h while higher doses are capable of inhibiting first secretion and then synthesis of TSH.

Secretion of TSH by the pituitary depends on two opposite factors: the thyrotrophin-releasing hormone (TRH) stimulates both TSH secretion and synthesis, and thyroid hormones above a certain level, exert a negative feedback on pituitary TSH secretion. Whether low levels have a positive effect to maintain TSH synthesis is still controversial. Indeed in hypothyroid rats, Bakke & Lawrence (1964) suggested that T4 may stimulate TSH synthesis, and Van Rees (1966) in thyroidectomized animals has raised the question of whether or not thyroid hormones exert separate effects on TSH synthesis and release. D’Angelo et al. (1976) when studying the pituitary TSH rebound phenomenon, observed that high doses of T4 inhibited both TSH synthesis and release whereas low doses suppressed TSH release, but appeared to increase TSH synthesis. Sellers & Schönbaum (1965) as well as...
Griessen (1970) have observed a larger goitre when small amounts of T₄ were added to the propylthiouracil (PTU) treatment than with PTU alone, suggesting a positive interaction of T₄ on the pituitary or hypothalamic centres. In addition, Hervas et al. (1975) and Aranda et al. (1976) have shown a stimulatory effect of low doses of T₄ or T₃ on the pituitary growth hormone and LH content, respectively, in thyroidectomized rats. Moreover, our previous study on goitre induced by low-iodine diet (LID) in rats also speaks in favour of a positive effect of T₃ on the pituitary TSH content (Berthier & Lemarchand-Béraud 1978), since both pituitary TSH content and plasma TSH were maintained within the normal range only in the presence of normal T₃ concentrations. Finally, Gershengorn (1978) has shown that low physiological levels of T₃ or T₄ stimulate, and high levels inhibit, the production of TSH when added to a monolayer culture of dispersed mouse thyrotrophin pituitary tumour cells.

The purpose of the present study was to investigate further whether or not low levels of T₃ have a positive effect on TSH synthesis and secretion. Seven-day PTU treated rats received graded, low doses of T₃ and the variations in plasma TSH and pituitary TSH content were measured as well as TSH synthesis and release rates within the first 24 h following T₃ and [¹²⁵I]rTSH injections. This also allowed us to establish the minimal effective dose of T₃ which would inhibit TSH secretion. It was found that 0.1–0.15 μg/100 g b.w. of T₃ did not change neither the secretion nor the synthesis rates of TSH. Therefore, no positive effect of low doses of T₃ could be demonstrated.

Materials and Methods

Male Sprague-Dawleys rats, weighing 180 g on arrival, were fed a standard Purina diet, maintained for at least 1–2 weeks in light-control and air condition (23°C) before beginning the studies. They weighed 225–275 g at the time of experiment (mean: 247 ± 4 g; n = 325). The animals were rendered hypothyroid by treatment with 0.01% propylthiouracil (PTU) given in distilled drinking water for 7 days, which corresponded to 2.3–2.9 mg PTU/rat/24 h.

The experiments were conducted as follows:

After 7 days of PTU treatment, rats were injected ip with saline or graded doses of T₃ from 0.1 to 0.75 μg/100 g b.w. They were killed by decapitation in groups of 6–8 rats at times 0, 6, 12 and 24 h after the T₃ injection and still under PTU treatment.

The rats were divided into one control group, one group of 7-day PTU-treated rats (PTU group representing time 0 and dose 0) and for each concentration of T₃ tested, three groups of 7-day PTU-treated rats killed after 6, 12 and 24 h, respectively (PTU + T₃). The 7-day PTU treated rats were selected, as in a previous work (Berthier & Lemarchand-Béraud 1978) it had been shown that these rats presented an almost depleted AP-TSH content which was no more capable of responding to TRH injection. This situation appeared therefore particularly suitable for studying the effect of T₃ on TSH synthesis.

To carry out the TRH test, 200 ng of TRH/100 g b.w. was injected into a caudal vein, and blood was drawn by cardiac puncture under light ether anaesthesia before and 7 to 10 min after the iv injection. The rats were killed by decapitation, the pituitaries removed and the pars nervosa discarded, the anterior pituitary (AP) weighed and homogenized in 1 ml of 0.15 m NaCl. Plasma and pituitary homogenates were stored at −20°C until the hormone measurements were performed.

Hormone measurements

TSH was measured in plasma and pituitary by the double-antibody radioimmunoassay, using the homologous rat system kindly provided by NIAMDD-NIH. Rat TSH T₃ was iodinated with the chloramine T method of Greenwood et al. (1963) and purified on a Sephadex G-75 column just before use. The rT₃ T₃ antiserum was used at a final dilution of 1/30 000 and incubation performed for 3 days at 4°C followed by another 24 h after adding a goat-guinea pig anti-γ globulin antiserum for the second precipitation. The results were expressed as μg of the rat TSH RP standard reference containing 0.22 units USP/mg or 220 μU/μg. Plasma T₃ was measured by a double-antibody RIA developed in our laboratory, using an antiserum kindly donated by Dr. Burger (Burger et al., 1975), and plasma T₄ by RIA with the Beckman commercial kit.

Estimate of TSH synthesis and release rates

[¹²⁵I]rTSH was injected iv into normal rats, 7-day PTU-treated rats and PTU + T₃-treated rats (0.3 μg T₃/100 g b.w.). Blood was removed 5, 6, 9, 12, 15, 30, 45, 60, 75 and 120 min after the TSH injection. The [¹²⁵I]rTSH immunoprecipitate and the [¹²⁵I]TICA (trichloroacetic acid) precipitate were counted and expressed as %/ml of the injected dose. A minimum of 3–4 rats was used each time and only 3–4 blood samples per rat were taken.

The secretion rates were calculated by using the metabolic clearance rates (MCR), determined from the disappearance curves of the injected [¹²⁵I]rTSH, using the single compartment model (Odell et al. 1967) and a non-compartmental one (integral) as initially described.
by Tait (1963) and modified for thyroid hormones by Oppenheimer et al. (1975).

For the single compartment, MCR = k · V = ml/min, where the fractional removal rate (= k) was assumed to correspond to the slope of plasma disappearance rate 
\[
\frac{1}{T_{1/2}} = \frac{\ln 2}{120 \text{ min}}
\]
Distribution volume (= V) was assumed to be 
\[
\frac{R}{\text{Co}},
\]
where R is the total radioactivity injected (TCA or immunoprecipitated) and Co, the concentration of radioactive TSH at t = 0, as determined from extrapolation of the terminal plasma disappearance rate.

For non-compartmental kinetics, MCR = \[\frac{R}{\int \text{c} \ast \text{d}t}\] ml/min, where R is the total radioactivity injected (TCA precipitated) expressed as 100%, and \[\int \text{c} \ast \text{d}t\] represents the integral of the disappearance curve where c* is the TCA or immunoprecipitated radioactive expressed as %/ml of total radioactivity at any time (dt).

The secretion rate per hour is MCR · plasma TSH \(\mu g/ml \cdot 60 = \mu g/h\). Immunoprecipitation was performed by adding to 100 μl of plasma, 100 μl of a 1/800 antiserum rTSH Ss; the mixture was incubated for 24 h at room temperature and the complex formed was precipitated by adding 0.2 ml of a second antibody (rabbit goat anti-γ globulin) and 1.0 ml of 8% polyethylene glycol (PEG). Sixty percent of the total radioactivity was immunoprecipitated, while 85 to 90% were precipitated with TCA (addition of 1.0 ml of 10% TCA).

In a preliminary experiment, the plasma [\(^{125}\text{I}\)]rTSH disappearance curve and MRC were established in a group of normal rats, using immuno- and TCA-precipitation. With both methods, the plasma disappearance curve showed a plateau 90 min after the injection. Therefore, the lines were drawn according to least-square regression analysis of the semi-log plots from 5 to 90 min after the injection. Since non-significant differences were obtained between TCA precipitable and

**Table 1.**

Effects of increasing doses of T₃ on TSH and thyroid hormone concentrations in 7-days PTU-treated and normal rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time h</th>
<th>No. of rats</th>
<th>Basal TSH (\mu g/ml)</th>
<th>(\Delta TSH) post-TRH (\mu g/ml)</th>
<th>AP-TSH (\mu g/mg AP)</th>
<th>(T_3) ng/ml</th>
<th>(T_4) ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day PTU</td>
<td>0</td>
<td>53</td>
<td>1.30 ± 0.04a</td>
<td>0.20 ± 0.03a</td>
<td>26.7 ± 1.4a</td>
<td>0.37 ± 0.01a</td>
<td>14.9 ± 0.6a</td>
</tr>
<tr>
<td>7-day PTU + T₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 (\mu g)</td>
<td>0.1</td>
<td>6</td>
<td>1.33 ± 0.10</td>
<td>-</td>
<td>23.3 ± 1.6</td>
<td>0.50 ± 0.07b</td>
<td>22.0 ± 4.0c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.27 ± 0.13</td>
<td>-</td>
<td>23.1 ± 1.4</td>
<td>0.56 ± 0.05b</td>
<td>17.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.39 ± 0.12</td>
<td>0.33 ± 0.02c</td>
<td>24.5 ± 2.8</td>
<td>0.30 ± 0.04</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>0.15 (\mu g)</td>
<td>0.15</td>
<td>6</td>
<td>1.00 ± 0.27</td>
<td>-</td>
<td>27.3 ± 1.9</td>
<td>0.94 ± 0.14b</td>
<td>22.0 ± 2.0c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.16 ± 0.13</td>
<td>-</td>
<td>42.5 ± 8.7c</td>
<td>0.57 ± 0.06b</td>
<td>20.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.44 ± 0.11</td>
<td>0.55 ± 0.08b</td>
<td>31.2 ± 1.2</td>
<td>0.36 ± 0.03</td>
<td>14.3 ± 1.4</td>
</tr>
<tr>
<td>0.2 (\mu g)</td>
<td>0.2</td>
<td>6</td>
<td>0.89 ± 0.07b</td>
<td>-</td>
<td>38.2 ± 4.9</td>
<td>1.43 ± 0.12b</td>
<td>12.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.29 ± 0.10</td>
<td>-</td>
<td>58.4 ± 4.3b</td>
<td>0.87 ± 0.05b</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.40 ± 0.13</td>
<td>-</td>
<td>37.7 ± 7.4</td>
<td>0.44 ± 0.02</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>0.3 (\mu g)</td>
<td>0.3</td>
<td>6</td>
<td>0.49 ± 0.05b</td>
<td>0.53 ± 0.06c</td>
<td>48.0 ± 2.0b</td>
<td>1.98 ± 0.07b</td>
<td>15.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.10 ± 0.08</td>
<td>0.92 ± 0.14b</td>
<td>66.5 ± 4.0b</td>
<td>1.09 ± 0.05b</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.15 ± 0.06</td>
<td>0.51 ± 0.14c</td>
<td>84.2 ± 6.4b</td>
<td>0.64 ± 0.04b</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>0.75 (\mu g)</td>
<td>0.75</td>
<td>6</td>
<td>0.18 ± 0.01b</td>
<td>0.17 ± 0.02</td>
<td>70.6 ± 4.0b</td>
<td>3.74 ± 0.16b</td>
<td>20.0 ± 1.0c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>0.24 ± 0.07b</td>
<td>0.72 ± 0.17b</td>
<td>98.4 ± 6.6b</td>
<td>1.70 ± 0.15b</td>
<td>18.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>0.85 ± 0.24c</td>
<td>0.59 ± 0.14c</td>
<td>99.5 ± 9.4b</td>
<td>0.90 ± 0.07b</td>
<td>15.0 ± 0.2</td>
</tr>
<tr>
<td>Normal rats</td>
<td></td>
<td>39</td>
<td>0.44 ± 0.02</td>
<td>1.44 ± 0.13</td>
<td>99.8 ± 3.3</td>
<td>0.99 ± 0.02</td>
<td>47.5 ± 0.9</td>
</tr>
<tr>
<td>Normal rats + T₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 (\mu g)</td>
<td>0.3</td>
<td>6</td>
<td>0.08 ± 0.01a</td>
<td>0.53 ± 0.04a</td>
<td>149.1 ± 13.8a</td>
<td>1.70 ± 0.09a</td>
<td>48.4 ± 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>0.12 ± 0.06a</td>
<td>1.51 ± 0.10</td>
<td>190.7 ± 24.7a</td>
<td>0.97 ± 0.07</td>
<td>42.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>0.21 ± 0.03a</td>
<td>1.07 ± 0.09a</td>
<td>160.4 ± 19.9a</td>
<td>0.92 ± 0.04</td>
<td>34.3 ± 4.9a</td>
</tr>
</tbody>
</table>

*a: P < 0.001 vs. normal rats.  b: P < 0.001 vs. 7-day PTU group.  c: P < 0.05 vs. 7-day PTU group.*
immunoprecipitable material, the TCA precipitate was used for studying the kinetics of $[^{125}I]$rTSH in the other group so as to spare rTSH antisera for TSH determination by RIA.

As far as TSH synthesis is concerned, it was assumed that normal rats were under steady conditions, i.e. that TSH synthesis rates/24 h were equal to TSH release rates/24 h. The 7-day PTU rats were also under steady conditions as 6- and 8-day PTU rats presented similar plasma TSH and AP-TSH contents. In the other groups, not under steady conditions, release and synthesis rates were calculated for each period examined: 0–6, 6–12, 12–24 h, the total of which representing release and synthesis rates for 24 h.

TSH synthesis rates were established by adding to secretion rate at time $t_2$ the difference between AP-TSH content at time $t_2-t_1$ ($t_1 =$ time preceding $t_2$), and addition of synthesis from 0–6, 6–12 and 12–24 h will yield the total TSH synthesis for 24 h.

Drugs

L-thyroxine was purchased from Sigma (USA), TRH (TRF) from Roche (Switzerland), L-triiodothyronine from Glaxo Laboratories (England) and 4-propyl-2-thiouracil from Bender-Hobein (Switzerland).

Results

Effects of PTU

Hypothyroidism induced in rats by a 7-day treatment with 0.01% PTU resulted in a rapid decrease in both plasma T3 and T4 levels, increased plasma TSH levels and diminished anterior pituitary TSH content (AP-TSH). Moreover, the TRH injected into these PTU rats did not induce any further

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**Fig. 1.**

The action of graded doses of T3 on plasma T3, T4, TSH concentrations and pituitary TSH content in 7-day PTU treated rats are shown. Dose zero represents hormone levels in 7-day PTU treated rats. The effect of one single injection of T3 (0.1, 0.15, 0.2, 0.3 and 0.75 µg/100 g body weight 6 and 24 h after the injection) is illustrated.
The action of 0.3 µg/100 g body weight on plasma T3, T4, TSH concentrations and pituitary TSH content in 7-day PTU-treated rats are shown before, 6, 12 and 24 h after the injection.

**Fig. 2.**

There was a significant increase in plasma TSH in contrast to control rats (Table 1). This experimental model was chosen for its depleted TSH pituitary content allowing to better study the TSH synthesis.

**Effects of a single low dose of T₃ in 7-day PTU rats over 24 h** (Table 1, Fig. 1)

The effects of graded low doses of T₃ over 24 h (from 0.1 to 0.75 µg/100 g b.w.) are represented in Fig. 1 and Table 1. The mean values obtained in the 7-day PTU rats served as dose and time zero.

Six hours after the T₃ injection, its plasma concentration increased in proportion to the dose given, but exerting its inhibitory action on TSH secretion only with doses of 0.2 µg and over. Indeed, plasma TSH gradually decreased with 0.2 to 0.75 µg, reaching lower values than the control ones, with a concomitant progressive increase in pituitary TSH content. Plasma T₄ remained constantly low.

Twelve hours after the T₃ injection, the results ranged between those found at 6 h and 24 h as shown in Table 1. Plasma TSH levels were significantly lower than those of PTU and of control only with the dose of 0.75 µg T₃, whereas plasma T₄ remained higher than that of PTU group with all doses. With the 0.15 µg T₃ dose and over, pituitary TSH content increased regularly, reaching complete replenishment with 0.75 µg.

Twenty-four hours after the T₃ injection, plasma T₃ levels returned to the initial low values with the doses of 0.1–0.2 µg, but they did not with the higher ones (0.3 and 0.75 µg). Except with the 0.75 µg dose, plasma TSH levels returned to the initial high values. With the 0.1–0.2 µg doses, the pituitary TSH content was similar to that found before the injection, but it further increased with the higher doses, resulting in an almost 4-fold increase in 24 h (370%).

The alterations in TSH and thyroid hormones occurring in the course of 24 h after an injection of 0.3 µg T₃ in 7-day PTU-treated rats are illustrated in Fig. 2.

Six hours after the T₃ injection, plasma T₃ levels had increased 5-fold and plasma TSH levels were similar to the levels observed in normal rats. It shows that TSH secretion is not completely suppressed in spite of these high plasma T₃ levels. Moreover, the TSH response to TRH was positive, whereas it was negative before the injection (Table 1). Twelve hours after T₃, plasma TSH returned to its high initial values in spite of normal T₃ levels.
and the TSH response to TRH was even more important. Twenty-four hours after T3, plasma T3 decreased further to levels lower than normal, but still remained higher than the initial low value. Plasma TSH remained high and similar to the value found at 12 h and the TSH response to TRH was still positive. The pituitary TSH content progressively increased, reaching more than three times its initial value within 24 h; T4 remained low and unaltered.

**Effect of 0.3 µg T3 in normal rats** (Table 1)
The effect of the dose of 0.3 µg T3/100 g b.w. was also studied in normal rats 6, 12 and 24 h after the injection: plasma T3 increased moderately at 6 h and had returned to its initial value at 12 h. Plasma TSH is almost suppressed at 6 h and remained low up to 24 h. Plasma T4 significantly fell after 24 h. The TSH response to TRH was positive although reduced at 6 h and was back to its initial response at 12 and 24 h. Pituitary TSH content doubled in 12 h.

**TSH turnover studies and estimate of TSH synthesis and secretion rates** (Table 2, Fig. 3)
The half-time disappearance of the injected [125I]-rTSH (t½) was found similar to control, PTU and PTU + 0.3 µg T3 groups (Fig. 3).

The metabolic clearance rate (MCR), calculated by means of the single and non-compartmental methods was also non-significantly different in the various groups with a mean of 15.99 ± 0.4 ml/h/100 g. This value was used for calculation of all groups.

**Table 2.**
Effect of T3 on TSH secretion and synthesis rates in normal rats and in 7-day PTU treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time h</th>
<th>No. of rats</th>
<th>TSH secretion rate</th>
<th>AP-TSH</th>
<th>TSH synthesis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg/h</td>
<td>µg/24 h</td>
<td>µg/24 h</td>
</tr>
<tr>
<td>Normal rats</td>
<td>39</td>
<td>17.0 ± 0.9</td>
<td>408 ± 22</td>
<td>745 ± 19</td>
<td>408 ± 22</td>
</tr>
<tr>
<td>Normal rats + T3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 µg</td>
<td>0–6</td>
<td>9</td>
<td>3.02 ± 0.2</td>
<td>156</td>
<td>953 ± 58</td>
</tr>
<tr>
<td></td>
<td>6–12</td>
<td>5</td>
<td>4.53 ± 0.5</td>
<td>1067</td>
<td>478</td>
</tr>
<tr>
<td></td>
<td>12–24</td>
<td>12</td>
<td>7.93 ± 0.6</td>
<td>1225</td>
<td></td>
</tr>
<tr>
<td>7-day PTU</td>
<td>53</td>
<td>50.95 ± 1.9</td>
<td>1222 ± 44</td>
<td>213 ± 12</td>
<td>1222 ± 44</td>
</tr>
<tr>
<td>7-day PTU + T3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 µg</td>
<td>0–6</td>
<td>5</td>
<td>52.13 ± 7.0</td>
<td>1266</td>
<td>168 ± 19</td>
</tr>
<tr>
<td></td>
<td>6–12</td>
<td>6</td>
<td>49.78 ± 5.1</td>
<td>127 ± 14</td>
<td>1252</td>
</tr>
<tr>
<td></td>
<td>12–24</td>
<td>10</td>
<td>54.48 ± 4.9</td>
<td>127 ± 14</td>
<td>1252</td>
</tr>
<tr>
<td>0.15 µg</td>
<td>0–6</td>
<td>5</td>
<td>39.19 ± 4.5</td>
<td>1180</td>
<td>324 ± 66</td>
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<tr>
<td></td>
<td>6–12</td>
<td>6</td>
<td>45.46 ± 4.4</td>
<td>163 ± 8</td>
<td>1129</td>
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<tr>
<td></td>
<td>12–24</td>
<td>10</td>
<td>56.44 ± 4.9</td>
<td>163 ± 8</td>
<td>1129</td>
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<tr>
<td>0.2 µg</td>
<td>0–6</td>
<td>6</td>
<td>34.88 ± 3.9</td>
<td>1172</td>
<td>477 ± 38</td>
</tr>
<tr>
<td></td>
<td>6–12</td>
<td>6</td>
<td>50.56 ± 5.2</td>
<td>278 ± 52</td>
<td>1237</td>
</tr>
<tr>
<td></td>
<td>12–24</td>
<td>6</td>
<td>54.87 ± 5.3</td>
<td>278 ± 52</td>
<td>1237</td>
</tr>
<tr>
<td>0.3 µg</td>
<td>0–6</td>
<td>19</td>
<td>13.29 ± 1.1</td>
<td>868</td>
<td>494 ± 39</td>
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<tr>
<td></td>
<td>6–12</td>
<td>21</td>
<td>44.67 ± 2.5</td>
<td>643 ± 47</td>
<td>1300</td>
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<tr>
<td></td>
<td>12–24</td>
<td>22</td>
<td>42.54 ± 2.6</td>
<td>643 ± 47</td>
<td>1300</td>
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<tr>
<td>0.75 µg</td>
<td>0–6</td>
<td>6</td>
<td>6.68 ± 0.6</td>
<td>472</td>
<td>733 ± 44</td>
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<tr>
<td></td>
<td>6–12</td>
<td>6</td>
<td>8.91 ± 0.9</td>
<td>704 ± 50</td>
<td>963</td>
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<td></td>
<td>12–24</td>
<td>6</td>
<td>30.38 ± 4.0</td>
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Table 2 shows that PTU treatment alone induced a 3-fold increase in TSH secretion rate compared with normal rats. This high secretion rate was not modified by the injection of 0.1 to 0.2 µg of T3, but diminished with 0.3 µg T3 and reached that found in normal rats only with 0.75 µg T3. TSH synthesis rate already elevated by the 7-day PTU treatment, did not change with doses of 0.1–0.3 µg T3 and is slightly reduced with the 0.75 µg dose of T3 although remaining double than in control animals. In control animals, a dose of 0.3 µg T3 drastically reduced the secretion rates, but it did not significantly alter the TSH synthesis rates.

Discussion

Seven days of PTU treatment resulted in reduced thyroid hormone levels, however not so low as after total thyroideectomy or prolonged LID diet, and in high plasma TSH levels. The pituitary exhausts its own supply of TSH in order to maintain this high TSH secretion rate. Indeed, as confirmed by the TRH test, the pituitary is no longer capable of responding to an exogenous TRH stimulation. This particular situation of TSH-exhausted pituitary content was then selected for studying the effect of graded low doses of thyroid hormones on the pituitary TSH content, and the release and
synthesis rates. Indeed, if high T₃ or T₄ levels are shown to inhibit TSH secretion and TSH response to TRH, some experiments have suggested that, in hypothyroid rats, a given level of thyroid hormone has a positive effect on TSH synthesis and secretion rates, and this investigation was performed to examine further this aspect.

a) Effects of graded low doses of T₃ on plasma and AP-TSH content
The effect of a single dose of T₃ (lower as well as higher than the replacement dose) tested for 24 h in 7-day PTU treated rats showed that the 0.1 and 0.15 μg T₃/100 g b.w. doses did not exert any effect on plasma TSH levels as the time tested. With 0.2 μg, there was only a slight and transient decrease in plasma TSH levels, which was more important with 0.3 μg and TSH remained lower than in the PTU group for 24 h only with the highest dose given (0.75 μg). It is striking to note the rapid and important increase (2- to 4-fold) in pituitary TSH content which was observed with the T₃ doses ranging from 0.2 to 0.75 μg. This pituitary TSH replenishment appeared to be slightly higher than the transient and incomplete inhibitory effect of T₃ on TSH release (i.e. + 430 μg/24 h vs −356 μg/24 h for the 0.3 μg of T₃). It would suggest a positive effect of T₃ on TSH synthesis. A similar situation was described recently by Ridgway et al. (1979) in hypothyroid patients who showed an increased TSH response to TRH when they had been treated with subphysiological doses of T₃. However TSH secretion is probably inhibited immediately after the T₃ injection. Indeed, Fukuda et al. (1975) and Spira et al. (1979b) have shown that as soon as 15 min after the injection of similar doses of T₃ to chronically or recently hypothyroid rats, TSH secretion is reduced, but the greatest inhibition is observed between 3 and 6 h. Garcia et al. (1976) also in thyroidectomized rats reported a maximum plasma TSH suppression 4–6 h after the injection of 0.4 μg T₃/100 g b.w. Therefore the 6 h tested in the present experiment seems to correspond to the end of the maximum suppressive period.

b) TSH response to TRH
The unusual absence of TSH response to TRH was noteworthy in the 7-day PTU hypothyroid rats in spite of low T₄ and T₃ concentrations. This reflects a TSH-exhausted pituitary, though the secretion rate was high then. Similar situations – absence of TSH response to TRH with hypothyroidism – have already been described. Indeed, no response to TRH was observed in euthyroid patients after treatment for hyperthyroidism (Clifton-Bligh et al. 1974; Sanchez-Franco et al. 1974) or in rats (Harada et al. 1975; Obregon et al. 1979), suggesting a delayed TSH synthesis after a long period of inhibition or of exaggerated secretion. However, in our experiments, a few hours after T₃ was given, the pituitary was capable of responding again to TRH even when the T₃ dose did not reduce significantly TSH secretion. This would suggest a positive action of T₃ on TSH secretion. It should be noted as well that TSH responded to TRH 6 h after the 0.3 μg T₃ injection, when plasma T₃ levels were twice the normal value (1.98 ± 0.07 ng/ml vs 0.99 ± 0.02 ng/ml), but in the presence of low T₄ concentrations. Similar results were reported by Garcia et al. (1976) with an injection of 0.2 μg T₃/100 g b.w. to thyroidectomized rats, which resulted in an unexpected increased TSH response to TRH suggesting some positive action of this low concentration of T₃. For better understanding these paradoxical results, MCR and secretion rates of TSH were studied.

c) TSH turnover and evaluation of TSH synthesis and secretion rates
The important increase in pituitary TSH content observed a few hours after the T₃ injection led us to examine TSH kinetics for evaluating TSH synthesis.

Contrary to the observations of Silva & Larsen (1978b) [¹²⁵I]T₃ was injected into normal rats gave similar plasma disappearance curves whether [¹²⁵I]T₃ was TCA or immunoprecipitated, resulting in a half-time disappearance rate of 22 and 21 min, respectively. This slight discrepancy could be due to the fact that [¹²⁵I]T₃ was injected on the day after labelling and purification, and no week-old [¹²⁵I]T₃ was used. Nevertheless, with both methods, part of the injected dose (~ 0.2%) remained at a plateau from 90 min to 3 h (the animals were not tested for longer periods).

From 8 to 90 min, the precipitated radioactivity disappeared linearly, but when blood samples were taken between 2 and 8 min after the injection as well, disappearance of the precipitated activity was multi-exponential. For this reason MCR in normal and in PTU-treated rats was calculated by means of
single-compartmental and multi-compartmental kinetics. With both these methods a similar MCR was found and thereafter the single-compartmental method was used for the other groups. No significant differences in MCR were obtained in the various groups. The mean of 16.0 ± 0.4 ml/h/100 g b.w. is similar to that recently reported by Spira et al. 1979a. These investigators too did not observe differences between normal and recently thyroidectomized rats (5 days).

The absence of a prolonged half-life of TSH in the PTU groups is due, in the present study, to the short period of hypothyroidism covered (7 days) and to the use of homologous TSH instead of a bovine TSH preparation as previously reported (Griessen 1970; Pisarev & Altschuler 1968; Marceau et al. 1972). The 22 min half-life of the control group is slower than found by Pisarev & Altschuler (1968), Marceau et al. (1972) and Surks & Lifschitz (1977) but similar to that of Silva & Larsen (1978a) who used immunoprecipitation and of Spira et al. (1979a).

As expected, PTU treatment for 7 days induces a 3-fold increase in TSH secretion rate, whereas thyroid hormone injection into normal rats reduces it more than 3-fold. However, the most striking features are, first, TSH secretion rate still remaining high after injections of graded low doses of T3 (0.1 to 0.2 μg) into PTU-treated rats, and secondly, the unchanged TSH synthesis rates in these animals revealing no stimulatory effect of these low T3 levels on TSH synthesis, but without inhibiting TSH secretion.

These results are in agreement with the recent observations of Spira et al. (1979b), who did not in 5 days thyroidectomized rats find any effect of 0.5 μg T3 on TSH synthesis. Lower doses were however not tested. A rapid pituitary TSH replenishment was also observed.

It is probable that the absence of reduced TSH secretion rates in the group of rats treated with the lowest doses of T3 is due to the too short nuclear occupancy of T3 at the pituitary level. Six hours after the T3 injection, its effect may be finished. Similar results have been reported by Fukuda et al. (1975), after the injection of similar low doses of T3 to LID hypothyroid rats and by Spira et al. (1979b) in the 5 days thyroidectomized rats. In the present experiments, the PTU did not seem to play a role (Larsen et al. 1979) contrary to what was reported with T4 (Escobar del Rey et al. 1974).

In normal rats, the dose of 0.3 μg T3/100 g b.w. results in a clear inhibition of the TSH secretion rates with an increased pituitary TSH content, but with no action on TSH synthesis. It is noteworthy that in these normal rats the high T3 plasma levels observed 6 h after the hormone injection strongly reduce basal plasma TSH levels, but only partially the TRH-induced TSH response. The dose used seem to be sufficient for inhibiting the basal TSH secretion (Silva & Larsen 1978a) but not that due to exogenous TRH.

It is particularly striking to note that no decrease in plasma TSH levels occurred until more than 0.2 μg T3/100 g b.w. was injected although plasma T3 had reached normal values. This is in agreement with the recent data of Tonooka & Greer (1980) who did not find any decrease in plasma TSH concentration in thyroidectomized or PTU treated rats until 0.3 μg T3/100 g b.w./day was given. This suggests that the thyrostat is not sensitive to very low doses of T3 and that there is a narrow range of T3 concentration between the beginning of suppression and full suppression of TSH secretion. In the present study, this was observed between 0.2 and 0.3 μg T3/100 g b.w.

Finally no positive action of the smallest doses of T3 either on TSH secretion or TSH synthesis rates over 24 h could be demonstrated. The rapid TSH replenishment in the pituitary is probably due to the transient inhibitory effect of T3 on TSH release with a constant rate of TSH synthesis. Although 0.3 μg T3/100 g b.w. was capable of slightly reducing TSH secretion rates, it had no effect on TSH synthesis rate, however, it is not excluded that a study on a more prolonged period would not demonstrate a positive effect of T3. It was only when TSH secretion rate returned to normal (with 0.75 μg of T3) that TSH synthesis began to decrease. Therefore, lower doses of T3 are required to decrease TSH secretion than to reduce TSH synthesis.

In conclusion, in the 7-day PTU treated rats having a particularly low pituitary TSH content, one single injection of low doses of T3 induced a slight and transient inhibition of the TSH secretion rate resulting in a rapid replenishment of the pituitary TSH content but no effect on the TSH synthesis rate could be demonstrated over 24 h studied. Furthermore, the hypothyroid rats were insensitive to the lowest dose of T3 tested, inducing no change in TSH secretion. The thyrostat appeared to work within a narrow range of thyroid hormones.
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