Effect of long-term iodide refeeding on the synthesis and secretion of T₃, T₄ and TSH in severe iodine deficient rats

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Abstract. For 6 months, male Wistar rats received an iodine deficient diet (LID), with propylthiouracil (PTU) for the last 2 months. At the end of this treatment (day 0), a daily iodide intake of 20 μg (group 20) and 50 μg (group 50) was supplied. Synthesis and secretion of T₃, T₄ and TSH as well as thyroid and serum PBI were studied during 140 days of iodine repletion. For the first 4 days of iodide refeeding, thyroid PBI remained low (0.4 ± 0.2 μg ¹²⁷I/gland) whilst T₃ and T₄ were undetectable during 2 days only. Thereafter, these parameters increased regularly, reaching control values on days 80, 16 and 90, respectively. During 2 weeks, the thyroid T₃/T₄ ratio was 6-fold higher and reached the control value on day 45. Plasma T₃ and T₄ levels were low, but measurable for the first 4 days (0.5 ± 0.1 and 9.9 ± 2.6 nm, respectively). T₃ rapidly reached a normal level on day 8, inducing a high plasma T₃/T₄ ratio for 16 days. This ratio decreased abruptly when T₄ secretion increased, attaining the control value on day 45. Therefore, the preferential T₃ secretion occurring during the first 16 days is directly linked to the preferential T₃ synthesis. Plasma TSH levels remained 10-fold higher than the control value for the first 8 days (490 ± 99 vs 39 ± 15 mU/ml), then decreased abruptly and reached normal level after 1 month. Pituitary TSH contents varied in an opposite manner to the plasma TSH concentrations.

In conclusion, the effect of iodide on thyroid function occurs sequentially, i.e. thyroid hormone synthesis precedes neosecretion (latency phase). In both processes, T₃ appears preferentially. This phenomenon is linked to the low thyroglobulin (Tg) iodination rate. In addition, the re-establishment of a normal thyroid hormone secretion prevailing over the iodinated Tg store constitution represents an adaptive mechanism; this leads rapidly to a maximum supply of active hormone (T₃) with iodine economy. Moreover, it takes 2–3 months for the complete thyroid function restoration. At the pituitary level, the normal plasma T₃ concentration found during the latency phase of iodide refeeding is not sufficient by itself to inhibit TSH secretion. This parameter starts to decrease only when T₄ has reached a critical level to generate adequate T₃ from T₄ deiodination. Inhibition of TSH synthesis is a less sensitive process since it occurs for more elevated T₄ concentrations. Finally, the similar pattern of thyroid function’s recovery in the two groups indicates that the 20 μg dose is a sufficient supply and that thyroid autoregulation occurs, thus avoiding a thyroid hyperfunction.

The beneficial effect of iodide treatment in the endemic goitre areas is now well established (see compilation book of Stanbury 1968; Ermans et al. 1980; Delange et al. 1982; Croxson et al. 1976). However, only a few experimental investigations
concerned the effect of iodide refeeding on the iodine depleted thyroid gland to total recovery. Indeed the effect of iodide refeeding during a few days has been studied by Studer & Greer (1965) on thyroid hormone synthesis, by Bürgi et al. (1974) on reiodination of thyroglobulin (Tg) and by Fukuda et al. (1975b) on the changes in plasma thyroid hormones and TSH concentrations in short-term low iodine diet (LID) rats. Therefore to our knowledge, no data are reported on the sequential events and progressive recovery of thyroid function during more than 1 month of iodide refeeding and studying both the thyroid and peripheral levels.

The comparison of experimental studies on the thyroid iodine deficiency obtained either with LID alone (Halmi 1954; Lachiver & Leloup 1955; Querido et al. 1957; Riesco et al. 1977; Okamura et al. 1981), or with propylthiouracil (PTU) added to LID (Astwood & Bissell 1944; Van Middlesworth et al. 1959; Van Middlesworth & Murphy 1970) shows that the thyroid function is systematically more depressed when PTU is added. An approximate situation is found in some iodine deficient areas. Ermans et al. (1980), Delange et al. (1982), indeed have pointed out that in some endemic goitre areas of Africa, the iodide deficiency was worsened by absorption of naturally occurring goitrogens (Cassava).

The aim of the present work was thus to study in severely iodine depleted rats for 6 months (LID and PTU), the effect of 20 or 50 μg daily iodide refeeding for 140 days on the sequential events and progressive reestablishment of thyroid hormones and TSH synthesis and secretion.

Materials and Methods

Animals and treatment

Two hundred and sixty Wistar male rats weighing 200 g body weight on arrival were supplied for 6 months with low iodine diet (LID) composed of distilled water and Remington diet prepared in the laboratory containing 1.39 μg of iodide/100 g and corresponding to a daily iodine intake of 0.3 μg. The animals were housed in an air conditioned room (23°C) with 8 a.m. to 8 p.m. lighting. During the last 2 months, 6 x propylthiouracil (PTU) was added to the diet in the proportion of 0.15% equivalent to about 30 mg PTU/day.

At the end of the 6 months, PTU was removed from the LID and the rats were refeed with iodide given in the drinking water at two concentrations (0.9 or 2.2 μg of 127I/ml) corresponding approximately to a daily iodide intake of 20 μg (group 20) or 50 μg (group 50). Three to 60 rats were sacrificed at 0, 1, 2, 4, 8, 12, 16, 30, 45, 80, 140 days after the beginning of the iodide refeeding. Two control groups composed of 60 rats each were used, they received a normal daily iodine intake of 20 or 50 μg for 8 months.

After a blow on the neck, the animals were exsanguinated, the blood collected in heparinized tubes and the plasma stored at −20°C for later hormone determinations. Thyroid glands were rapidly dissected and homogenized with a Potter teflon glass homogenizer in Tris HCl 0.1 m, sucrose 0.15 m at 4°C. The pituitaries were removed, the neurohypophysis discarded and the antihypophysis (AP) homogenized in 1.0 ml NaCl 0.15 m at 4°C with a Potter teflon glass homogenizer and stored at −20°C for TSH determinations.

Biochemical analysis

An aliquot of thyroid homogenates was submitted to paper electrophoresis in pyridine acetic buffer (pH 3.5) in order to estimate the total thyroid protein bound iodine (thyroid PBI) and intrathyroidal iodide as previously described (Bastiani & Simon 1977). The thyroid homogenates were centrifuged at 100,000 × g and the supernatant was again centrifuged at 90,000 × g for 5 h on a 0.1 to 20% sucrose linear gradient. The gradient fractions corresponding to the 19 S Tg were collected and pooled for the determination of its iodoaminoacid content. Hydrolysis of 19 S Tg was performed with pronase (1.2%) containing thiouracil (0.4 × 10−3 m) (Tong et al. 1963) and the hydrolysates chromatographed in two ascending systems: butanol:ethanol:ammoniac (BEA 5:1:2) and butanol:acetic:acid water (BAW 78:5:17 v/v).

The quantity of the different iodoamino acids as well as the thyroid and serum PBI were determined by the chemical ceric-arsenious reaction as modified by Bastiani & Simon (1977), in samples studied from day 0 to 8 of iodide refeeding. In samples studied from day 8 to 140, as well as for the control groups, iodine was measured by isotopic 125I equilibrium (Simon & Morel 1960). 125I was given in the drinking water with a specific radioactivity (SRA) of 0.15 μCi/μg127I starting on day 0 of iodide refeeding for the experimental rats, and for 60 days before sacrifice for the control group. Isotopic equilibrium was reached after 8 and 60 days in experimental and control rats, respectively. Both methods allowed to determine total 127I and not only its renewed fraction.

In order to study the newly synthesized hormones, injections of 100 or 200 μCi of 131I were performed 2 h before sacrifice in experimental and control rats, respectively. The radioactivity was expressed as % of the injected dose. The 125I, 131I radioactivities were measured using a two channel Packard Autogamma spectrometer and the interference of 131I with 125I was corrected.

For protein bound iodine (PBI) determinations, plasma was mixed for 3 min with Iobeads resin (Technicon...
Corporation, Tarrytown, NY, USA) in order to eliminate iodide. Plasma T₃ was determined by double antibody RIA (Berthier & Lemarchand-Béraud 1978) using a T₃ antiserum prepared by Burger et al. (1975). Plasma T₄ was determined by RIA using a commercial kit (Beckman, USA) with slight modifications.

Plasma and pituitary TSH were estimated by RIA (Berthier & Lemarchand-Béraud 1978) using homologous rat material provided by the NIAMD-NIH rTSH-RP₁ as standard, rTSH-I₃ for iodination with ¹²⁵I and rTSH-S₂ antiserum. The results are expressed in μU/ml plasma and mU/AP in terms of the standard preparation (RP₁) containing 0.22 U/mg.

The results were analyzed by Student's t-test.

Results

At the end of 6 months LID, i.e. day 0 in the present study, all rats presented a hyperplastic goitre with a mean of 80 ± 15 mg while the thyroid weight of the control group was 15 ± 2 mg (mean ± SD).

a) Evolution of the thyroid iodine parameters (Tables 1 and 2)

On day 0, the thyroid PBI was extremely low and remained practically unchanged during the first 4 days of iodide refeeding in both groups 20 and 50 (Table 1). Later on, it increased progressively, reaching values similar or slightly increased to those found in the control group at day 80 and 90, respectively, for group 20 and 50.

T₄ and T₃ thyroid contents were not measurable either by chemical or isotopic methods at day 0 and during the first 2 days of iodide refeeding, but afterwards, they increased regularly and reached control values on day 16 for T₃, but between day 45 and 90 for T₄, at which time T₄ was slightly higher.

<table>
<thead>
<tr>
<th>Iodide refeeding (days)</th>
<th>Amounts of thyroid PBI (μg ¹²⁷I/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 20</td>
</tr>
<tr>
<td>0</td>
<td>***0.40 ± 0.20 (5)</td>
</tr>
<tr>
<td>1</td>
<td>***0.30 ± 0.15 (3)</td>
</tr>
<tr>
<td>2</td>
<td>***0.60 ± 0.20 (4)</td>
</tr>
<tr>
<td>4</td>
<td>***0.71 ± 0.29 (6)</td>
</tr>
<tr>
<td>8</td>
<td>***2.10 ± 0.90 (4)</td>
</tr>
<tr>
<td>10</td>
<td>***4.50 ± 0.70 (4)</td>
</tr>
<tr>
<td>12</td>
<td>***5.00 ± 0.12 (4)</td>
</tr>
<tr>
<td>16</td>
<td>***5.20 ± 2.70 (4)</td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>***5.90 ± 2.60 (5)</td>
</tr>
<tr>
<td>45</td>
<td>**9.90 (2)</td>
</tr>
<tr>
<td>60</td>
<td>*12.30 ± 1.90 (4)</td>
</tr>
<tr>
<td>80</td>
<td>12.85 ± 4.60 NS (3)</td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>11.50 NS (2)</td>
</tr>
<tr>
<td>Control</td>
<td>14.60 ± 1.10 (6)</td>
</tr>
</tbody>
</table>

Mean ± SD.
* P < 0.05; ** P < 0.01; *** P < 0.001 statistically different from control.
Table 2.

<table>
<thead>
<tr>
<th>Iodide refeeding (days)</th>
<th>T3, T4 thyroid contents (nmol/gland)</th>
<th>Amounts of labelled hormones/gland (%00 131I injected dose)</th>
<th>T3*/T4* molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 50</td>
<td>T3</td>
<td>T4</td>
</tr>
<tr>
<td>0</td>
<td>undetectable</td>
<td>undetectable</td>
<td>undetectable</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>***0.01 ± 0.004 (5)</td>
<td>***0.02 ± 0.007</td>
<td>0.02</td>
</tr>
<tr>
<td>8</td>
<td>***0.17 ± 0.10 (7)</td>
<td>***0.25 ± 0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>16</td>
<td>0.52 ± 0.22 NS (7)</td>
<td>***0.60 ± 0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>30</td>
<td>0.56 ± 0.21 (10)</td>
<td>***1.85 ± 0.95</td>
<td>0.06</td>
</tr>
<tr>
<td>45</td>
<td>0.73 (2)</td>
<td>3.20</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>0.61 ± 0.23 (6)</td>
<td>***7.40 ± 1.48</td>
<td>0.05</td>
</tr>
<tr>
<td>140</td>
<td>0.77 (2)</td>
<td>*6.40</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ± 0.16 (11)</td>
<td>4.60 ± 0.89</td>
<td>0.29</td>
</tr>
</tbody>
</table>

than in the control group (Table 2). This delayed T4 synthesis was also seen in the evolution of the thyroid T3/T4 molar ratios as shown in Fig. 1 (upper panel). The initial high ratio (average 0.85) remained unchanged until day 16, decreased rapidly from day 16 to 45, but reached the control value (0.14) only on day 80.

In a short-term labelling experiment, a similar pattern to that of the 125I results was obtained. [131I]T3 and [131I]T4 increased in parallel from day 4 to 16 resulting in a constant high T3*/T4* ratio for the first 16 days (Table 2) at which time only T4 continued to increase leading to a progressively normal ratio.

On day 0, low plasma thyroid hormone levels were found, the T4 concentration (9.86 ± 2.6 nM) being however, lower than the T3 (5.1 ± 0.09 nM) in comparison to control animals (T4 = 67.1 ± 13.3; T3 = 150.5 ± 25 nM). During iodide refeeding, T3 increased more rapidly than T4 in both groups, resulting in normal values on day 8 for T3, but only on day 45 and 30 for T4 in group 20 and 50, respectively (Figs. 2 and 3, upper panel). This delayed increase in T4 leads to a further increase in the high initial T3/T4 ratio for the first 8 days, which decreased abruptly after the 16th day, attaining the normal range on day 45, as shown in Fig. 1, upper panel.

c) Changes in plasma TSH and pituitary TSH (Figs. 2 and 3, lower panel)

As expected, on day 0, in both iodide groups, a 10-fold increase in plasma TSH levels was observed

b) Changes in plasma PBI, T3, T4 (Figs. 1, 2 and 3)

After 4 days latency, serum PBI increased more rapidly than thyroid PBI, reaching the control value between day 16 and 30 (Fig. 1, lower panel).
Discussion

After 6 months of LID, day 0 in the present study, a large, diffuse goitre was obtained with a 40 times lower thyroid iodine content than that of the control and low serum T₄ and T₃ levels, confirming the severe iodine deficiency.

a) Thyroid and serum PBI

During iodide refeeding, thyroid and serum PBI display similar pattern with the two doses of iodide used, 20 and 50 μg/day, suggesting that 20 μg is already sufficient and that thyroid autoregulation is functioning. The more rapid return to normal values for serum PBI than for thyroid PBI indicates that the newly iodinated proteins are preferentially and immediately secreted, and that a delay of 2–3 months is necessary to reestablish the

in comparison to control groups. This high plasma TSH level did not change during the first 8 days of iodide refeeding (Figs. 2 and 3). Between days 8 and 16, TSH diminished at first abruptly and later on, more slowly, attaining the control value on day 45 for group 20 and on day 30 for group 50.

Pituitary TSH content on day 0 was not significantly different from control groups. After an initial fall during the first 8 days of iodide refeeding, it increased rapidly from day 8 to 16, revealing significantly higher levels than the controls. Afterwards, it decreased to normal concentration on day 30 (Figs. 2 and 3, lower panel).
normal thyroid iodine store. These results are compatible with the so-called 'last come, first served' model (Schneider 1964).

b) Thyroid hormone synthesis

The lack of thyroid hormone synthesis during the first 2 days of iodide refeeding may be due to the well-known PTU blocking effect on thyroid hormone synthesis which lasts for 24 h after PTU withdrawal (Studer & Greer 1967). This inhibition is also probably strengthened by the Wolff-Chai-koff effect (Wolff & Chaikoff 1948) provoked by the high thyroid iodide levels found (Rognoni 1980).

After this initial latency, T₃ and T₄ appeared in Tg, but T₃ reached control value sooner than T₄, as also demonstrated with the high T₃/T₄ ratio. These results are in agreement with previous data obtained in LID rats with a short-term labelling method (Lachiver & Leloup 1955; Bois & Larsson 1958; Studer & Greer 1965; Abrams & Larsen 1973; Riesco et al. 1976). This high thyroid T₃/T₄ ratio cannot be due to a preferential T₄ secretion, since neosecretion did not start before day 4 and concerns essentially T₃. The high molar T₃/T₄ ratios during the first 16 days correspond to an average of 85 moles of T₃ for 100 moles of T₄ vs 15 moles in the control rats. This near equimolar proportion of T₃ and T₄ results in a 6-fold T₃ enrichment of Tg. Therefore up to day 16, a preferential T₃ synthesis occurs. The possible factor responsible for this increased T₃/T₄ ratio in the iodine depleted gland may be the high TSH levels as hypophysectomy has been shown to induce a larger decrease in T₃/T₄ ratio (Studer & Greer 1965). However, in the present study, the absence of correlation between plasma TSH concentrations and the T₃/T₄ ratios in Tg suggests that low Tg iodination levels are the main factor regulating the preferential synthesis of T₃ as also reported by Taurog (1970) in enzymatically iodinated human thyroglobulin.

c) Thyroid hormone secretion

The low, but measurable, T₃ and T₄ plasma levels found on day 0 with no detectable T₃ and T₄ in the thyroid suggest that in the severe iodide deficient gland, despite the presence of PTU, the few newly synthesized hormones are instantaneously secreted. The absence of an immediate T₃, T₄ neo-secretion during the first 4 days of iodide refeeding is due to the Wolff-Chai-koff effect (Wolff & Chaikoff 1948; Yamada et al. 1963; Yamamoto et al. 1972). These results confirm previous data obtained with butanol extractable iodine (Rognoni & Simon 1980).

The extremely high plasma T₃/T₄ ratio that lasts more than 2 weeks may result either from an increased T₄ deiodination secondary to PTU withdrawal or to the high stimulation. However, on day 0 in spite of the T₄ peripheral deiodination PTU blockade (Oppenheimer et al. 1972), high plasma T₃/T₄ ratio is already observed. Moreover, the thyroid T₃/T₄ ratio remains elevated for 16 days whereas TSH decrease abruptly. Therefore, high plasma T₃/T₄ maintained during this period corresponds to a preferential secretion of T₃ directly linked to the preferential T₃ synthesis. This phenomenon lasts as long as the serum PBI takes to recover its normal value, about one month.

The preferential synthesis and secretion of T₃ as already reported by Abrams & Larsen (1973) in
iodine deficient rats and Izumi & Larsen (1977) in human Graves' disease, represents an adaptative mechanism to the low iodide supply. 25% of iodine being saved with T3 which also has a much greater biological activity than T4.

d) TSH secretion and synthesis regulation

The high initial plasma TSH levels remain unchanged during the first 8 days of iodide refeeding at which time a normal T3 plasma level is already reestablished, but with still low T4 levels. Therefore, even within a normal range, T3 by itself is not capable of inhibiting TSH secretion probably due to the low T4 concentration which cannot provide enough T3 through deiodination in the thyrotrropic cells (Larsen et al. 1979; Obregón et al. 1980). Similar situations, i.e. elevated plasma TSH levels with normal plasma T3, but reduced T4 concentration, have been reported in slightly hypothyroid patients (Chopra et al. 1973) and in LID rats (Fukuda et al. 1975a; Riesco et al. 1977; Berthier & Lemarchand-Béraud 1978). The latency in TSH secretion inhibition is not reported by Fukuda et al. (1975b) but having not added PTU to LID, they had a less severe iodine deficiency. The fact that plasma TSH levels start to decrease only when circulating T4 reaches an adequate level suggests the substantial participation of T4 as a prohormone, providing by deiodination a sufficient quantity of T3 for the regulation of TSH secretion. Indeed Spira et al. (1979b, 1981) in thyroidectomized, and Lemarchand-Béraud & Berthier (1981) in PTU treated rats have demonstrated that TSH secretion is inhibited after T3 injection only when serum T3 concentrations are markedly elevated.

At the end of the LID, day 0, the normal TSH pituitary content displays an intensive TSH synthesis as previously reported in hypothyroid rats by Bakke & Lawrence (1964), Wilber & Utiger (1967), Lemarchand-Béraud & Berthier (1981) and Spira et al. (1979a). This corresponds also to a greater number of thyrotrropic cells (Astier et al. 1980). During the first 8 days, the unexpected slight decrease in pituitary TSH content whereas plasma TSH remains high, may be due to the effect of high plasma iodide concentrations observed during this period (110 and 220 ng 127I/ml vs controls, 43 and 120 ng 127I/ml, in groups 20 and 50, respectively) (Rognoni 1980).

From days 8 to 16 of iodide refeeding, the pituitary TSH content increased in inverse correlation with the TSH secretion. Later on, pituitary TSH content decreasing more intensively than plasma TSH concentrations, TSH synthesis was blocked, due to the cumulative action of both normal T3 and almost normal T4 plasma concentrations. The inhibition of the TSH synthesis is thus a slower and less sensitive process than that of TSH secretion, as already suggested by D'Angelo et al. (1976), Spira et al. (1979b) Lemarchand-Béraud & Berthier (1981).

In conclusion, the present data show that in rats, after a long and drastic (PTU) iodide deficiency similar to that found in some endemic goitre areas (Ermans et al. 1980; Delange et al. 1982), the effect of iodide on thyroid function occurs sequentially and takes 2–3 months for complete restoration. The reestablishment of a normal thyroid secretion prevails over the iodinated Tg store constitution. Neosynthesis and neosecretion of thyroid hormones appear chronologically in an early phase (latency phase) when the negative feedback control of TSH secretion has not yet occurred. This original hormonal pattern displays that a normal plasma T3 level alone is not sufficient to inhibit TSH secretion which starts only in a later phase, when plasma T4 concentrations also reach an adequate level. The preferential T3 secretion due to preferential T3 synthesis, directly linked to the low Tg iodination rate, lasts until T4 plasma levels reach their normal value. At this time TSH synthesis is inhibited, showing that it is a slower process than the inhibition of TSH secretion. In addition, the fact that the pattern of thyroid function's recovery is similar in the two groups of iodide supply indicates that the lower dose is a sufficient supply and that thyroid autoregulation occurs thus avoiding a thyroid hyperfunction.

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