The disappearance of L-thyroxine and triiodothyronine from plasma and red and white skeletal muscle after administration of one sc dose of L-thyroxine to hyperthyroid and euthyroid rats

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Abstract. Rats were made hyperthyroid by repeated sc injections of l-thyroxine (T4) in a two-week period. At several time-points after the last injection, the triiodothyronine (T3) and T4 concentrations in plasma and red and white skeletal muscle were determined by a radioimmunoassay. This was done to investigate the relationship between the change in thyroid-hormone concentration in plasma and muscle and to see whether this change was similar in both muscle types. The results show that:

1. Information about the T3 and T4 concentrations in the muscles of the hyperthyroid rats can only be obtained by direct measurement in the muscles and cannot be gathered from the plasma-T3 concentration at any time-point or from below-normal plasma-T4 concentrations.
2. In hyperthyroid rats T4 and T3 are cleared more rapidly from the plasma than from the skeletal muscle.
3. A proportionally higher T3 concentration is present in the red compared with the white skeletal muscle of hyperthyroid rats.

Hyperthyroidism can be induced in rats by the application of a daily quantity of L-thyroxine (T4) or triiodothyronine (T3). Several methods differing as to the route and frequency of administration have been described. Some investigators mix the dose of T3 (Dow 1967) or T4 (Winder 1979) through the food, whereas others prefer daily injections of T3 (Bouhnik et al. 1979; Dillman et al. 1977) or T4 (Schapiro & Percin 1966; Kaplan & Utiger 1978; Janssen et al. 1978) to induce a hypermetabolic state as in hyperthyroidism. Little information is available about the course of the injected iodothyronine or the T3 and T4 concentrations in plasma and tissues of these animals. Galton (1975) and van Hardeveld & Kassenaar (1977) have shown that 24 h after an injection of doses ranging from 1 to 20 and 20 to 80 µg T4/100 g body weight into hyperthyroid rats, the plasma T3 and T4 levels have returned to normal. But it is not known whether the disappearance of T3 and T4 from the tissues parallels the plasma disappearance, although knowledge of the thyroid-hormone levels in the tissues is of great importance because most investigators working with hyperthyroid rats are interested in effects on the tissues.

In the present study we followed the last sc dose of T4 in hyperthyroid rats for 72 h after injection. The T3 and T4 concentrations in the plasma as well as those in the red and white skeletal muscles were determined to obtain answers to the following questions:

1. What is the relationship between the changes in thyroid-hormone concentration in plasma and muscle?
2. Is the change in thyroid-hormone concentration similar in both muscle types?

In a second series of experiments we followed one dose of T4 in euthyroid rats to determine the extent to which the observed changes in plasma and muscles are due to the chronic treatment of the animals with thyroid hormone.
Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>T₄ nmol/l</th>
<th>T₃ pmol/g</th>
<th>T₄ pmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Red muscle</td>
<td>White muscle</td>
</tr>
<tr>
<td>Group A</td>
<td>67.2 ± 14.0 (19)</td>
<td>2.92 ± 0.51 (7)</td>
<td>2.38 ± 0.25 (7)</td>
</tr>
<tr>
<td>Group B</td>
<td>48.1 ± 6.7 (8)</td>
<td>3.46 ± 0.43 (8)</td>
<td>3.06 ± 0.72 (8)</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(Student’s t-test)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

Animals

Ten-week-old male Wistar rats weighing approximately 200 g were used. The animals were fed a standard laboratory diet (Hope Farms, Linschoten, The Netherlands) ad libitum and had free access to tap water. The animals were made hyperthyroid by a daily sc injection of 80 µg T₄/100 g body weight for two weeks. T₃ (Sigma Chemicals) was dissolved in 0.1 mol/l NaOH and diluted with 0.15 mol/l saline containing 0.1% bovine serum albumin (Ortho Diagnostics, Raritan, N.J., USA).

The control rats received the same vehicle without T₄, also in an amount related to the body weight. The experiments were carried out in two periods, i.e., January to March and May to July. Significant differences in thyroid-hormone levels were found between the controls for the periods January to March (Group A) and May to July (Group B). The means ± SD are presented in Table 1, with the number of animals given in brackets.

Since there were no significant differences between the T₃ or T₄ values of the standard serum in the two periods, which were determined in the same run as the samples in all T₃ and T₄ radioimmunoassays (RIA), it is very unlikely that differences between the control groups were due to technical errors in the T₃ and T₄ RIA. For the results in the control groups all data in Figs. 1–2 are expressed as a percentage of the accompanying control-group value (% control). The T₃ concentration in the plasma and the T₄ concentration in the red muscle did not differ significantly between control groups A and B and a mean value ± SD of 1.37 ± 0.26 (27) nmol T₃/g and 5.25 ± 1.98 (14) pmol T₄/g, respectively, was found.

A second series comprised euthyroid male rats, which were given a single sc injection at the same dose level (80 µg T₄/100 g body weight). The corresponding control group was given only the vehicle. No significant differences in T₄ and T₃ values were found between this control group and group B, both of which were treated in the same period.

Groups of rats were bleed totally at 8, 16, 24, 32, 40, 48, 56, 64, and 72 h after the last injection. Before isolation of the m. quadratus femoris and m. soleus (red-type skeletal muscles) and the m. caudofemoralis (white-type skeletal muscle), the hind-limb was perfused with 50 ml buffered saline to remove all residual blood from the tissues. This perfusion procedure has been described elsewhere (Janssen et al. 1979).

Thyroid-hormone determination in plasma and skeletal muscle

The thyroid hormones in plasma and red and white skeletal muscle were extracted after homogenization of the tissues with ethanol containing 10% mol/l propylthiouracil (PTU). Aliquots of these extracts were dried, and the T₃ and T₄ concentrations were determined with a Wick RIA (Janssen et al. 1979) with the following modifications. Because the turbid material present in the samples containing extracts of more than 20 mg tissue slowed down the chromatographic separation between the antibody-bound and free hormone, the RIA incubation mixture was centrifuged for 20 min at 1800 × g and 4°C. This modification gives faster chromatographic separation of the bound and free hormone and does not influence the amount of the percentage-bound hormone.

The Whatman FT-81 chromatography paper originally used in the T₃-RIA was no longer available from the manufacturer and was replaced by Whatman DE-81. The addition of 0.15 mol/l NaCl to the 0.04 mol/l sodium phosphate buffer (pH 8.0) used in the T₃-RIA, resulted in optimal separation of bound and free T₃ with the Whatman DE-81 chromatography paper.

The intra-assay variability was 8% for both assays and the inter-assay variability was 12% for the T₄-RIA and 15% for the T₃-RIA.

Results

Hyperthyroid rats

Fig. 1a,b shows the disappearance of the last sc dose of 80 µg T₄/100 g body weight administered to hyperthyroid rats. The course of the injected hormone was followed by measuring by RIA the T₄...
(Fig. 1a) and T₃ (Fig. 1b) concentrations in plasma and in red and white skeletal muscle at time-points between 8 and 72 h after injection. Fig. 1a shows that already at 28 h after the last injection the plasma-T₄ concentration lay below normal and then continued to decrease until 15% of the control value was reached after 72 h. From 8 h to 25 h, the T₄ concentration in plasma and in red and white skeletal muscle decreased in parallel. However, when the T₄ concentrations in plasma and muscles fell below the control values, the hormone concentration decreased less rapidly in both kinds of muscle than in the plasma.

The disappearance of T₃ (Fig. 1b), which is generated from T₄, shows a quite different pattern. Only at 40 h after the injection of T₄ did the plasma-T₃ concentration drop below the normal value, and it then decreased further until 45% of the control value was reached at 72 h. The T₃ concentration in the red skeletal muscle decreased to 75% of the control value, whereas in the white skeletal muscle it decreased to less than 55% of the control value. At all time-points there was a significantly higher T₃ concentration in the red than in the white muscle.

Euthyroid rats

In a second series of experiments euthyroid rats received one sc injection of 80 µg T₄/100 g body weight. Fig. 2a,b shows the disappearance of T₄ and T₃, respectively, determined at various intervals between 8 and 72 h.

The decrease of the T₄ concentration in the plasma, shown in Fig. 2a, runs parallel with the decrease in the red and white skeletal muscle. But at all time-points after injection the T₄ concentration in plasma and both muscle types was much higher than in the hyperthyroid rats. Only at 72 h did the T₄ concentration in the muscles lie slightly below the control value.

As can be seen from Fig. 2b, the disappearance of T₃, which is generated from T₄, was equal in red

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Fig. 1a and b.

Disappearance curve of T₄ (1a) and T₃ (1b) in plasma (○—○) and in red (■—■) and white (▲—▲) skeletal muscle after the last sc dose of 80 µg T₄/100 g body weight administered to hyperthyroid rats. In the insert the muscle/plasma ratio is shown for red (■) and white (▲) skeletal muscle. All data are expressed as per cent of the control values. Values are means ± SD for 6 animals. ×: P (red vs. white) < 0.05. Student's two-sample t-test.

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and white skeletal muscle, but a much higher T3 concentration, expressed as per cent control, was observed in the plasma than in either type of muscle. The T3 concentration was at all time-points higher than or equal to the control value.

Discussion

The present results show that in hyperthyroid rats, T4 and T3 are cleared more rapidly from the plasma than from skeletal muscle, and that the concentration of T3, expressed as per cent control, is higher at all time-points in the red muscle compared with the white muscle.

The initial phase of the disappearance of T3 and T4 from the plasma of hyperthyroid rats was similar to that described by Galton (1975) and van Hardeveld & Kassenaar (1977), who studied only the first 24 h after injection. During our experiments a study by Obregon et al. (1979) appeared, in which very low T3 and T4 concentrations were also found in the plasma of hyperthyroid rats 3 days after the last T4 injection. These investigators concluded that the inability of the thyroid of hyperthyroid rats to restore the low circulating T4 pool is due to a low TSH level in the pituitary. In addition, we found no 131I-uptake in the thyroid of the hyperthyroid rats 72 h after the last T4 injection (not shown). But as our results indicate, the low T4 concentration in the plasma may not be extrapolated to the thyroid-hormone concentrations in the muscles.

To find out whether the observed differences between the red and white skeletal muscle were due to the hyperthyroid state, the disappearance of T3 and T4 was also followed in euthyroid rats after a single dose of T4. Some differences were found between these two groups: firstly, both thyroid hormones disappeared more rapidly from the plasma of the hyperthyroid rats, probably due to
the elevated deiodinative and faecal clearance rates in these animals (Chung & van Middlesworth 1967; Cullen et al. 1973; Edwards et al. 1976). Secondly, the T4 concentration in the ‘euthyroid’ rats decreased proportionally in the plasma and muscles, whereas the T3 concentration, given as per cent control, was lower in the muscles than in the plasma between 8 and 50 h. This might be due to the high T4 concentration in the plasma of the ‘euthyroid’ rats, which would prevent T3-tissue uptake, as shown by Zaninovich et al. (1977). In the ‘euthyroid’ group no differences were observed between the two muscle types as to the disappearance of T4 and T3.

Since in the ‘euthyroid’ group the T4 concentrations in plasma and muscle were always higher or equal to the control values, it cannot be concluded that the higher tissue/plasma ratio of T4 at very low T4 concentrations in the plasma, which was observed in the hyperthyroid animals, is an effect of hyperthyroidism. But for T3, these results show that the proportionally higher T3 concentration of the red muscle compared with the white muscle is due to the hyperthyroid state.

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References


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