Modulation of T4 5'-Monodeiodination in Rat Anterior Pituitary and Liver Homogenates by Thyroid States and Fasting

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Abstract

In order to clarify the role of the pituitary conversion of L-thyroxine (T4) to 3, 5, 3'-L-triiodothyronine (T3) in regulating thyrotropin (TSH) secretion, the effect of altered thyroid states and fasting on intrapituitary T3 generation was investigated by a paperchromatographic procedure using the anterior pituitary homogenates. Hepatic T3 generation was also studied for comparison.

The rate of pituitary and hepatic T3 generation in normal rats averaged 25.2±12.4 (mean±SE) fmoles T3/mg protein/min and 33.8±12.7 fmoles T3/mg protein/min, respectively. T4 treatment raised the hepatic T3 generation from T4 (46.7±3.1 fmoles T3/mg protein/min) and lowered the intrapituitary T3 generation (4.5±0.2 fmoles T3/mg protein/min). On the contrary, thyroidectomy slowed the hepatic T4 5'-deiodination (11.0±2.8 fmoles T3/mg protein/min), and accelerated the pituitary T4 5'-deiodination (64.3±1.4 fmoles T3/mg protein/min). In 48 h fasted rats, serum T4, T3 and TSH concentrations were all lower than those in fed rats, and both pituitary and hepatic T3 generations were also suppressed.

Thus, altered thyroid states cause an opposite effect on pituitary and liver 5'-monodeiodination, whereas fasting causes similar changes. The findings suggest the existence of an autoregulatory mechanism for thyroid hormone activation within the target tissues.

Recent studies have demonstrated in vitro 3, 5, 3'-L-triiodothyronine (T3) generation from L-thyroxine (T4) in rat anterior pituitary fragments (Silva et al., 1978a) and homogenates (Kaplan, 1980). The T4 5'-monodeiodination in the pituitary was temperature- and pH-dependent and thiol sensitive, suggesting that the activity is enzymatic in nature (Kaplan, 1980).

Recently, we investigated the age-related changes in T4 5'-monodeiodination in rat pituitary and liver homogenates, and observed the difference between maturational patterns in rat anterior pituitaries and livers (Naito et al., 1980). Moreover, a reciprocal relation between serum thyrotropin (TSH) levels and intrapituitary T3 generating activity from T4 was demonstrated in the perinatal rats, suggesting an important role of the local T3 production in regulating pituitary TSH secretion. These results essentially agree with those observed by El-Zaheri et al. (1980) and Cheron et al. (1980).

In order to explore the further role of the pituitary T3 generation from T4, the effect of the thyroid state and fasting on intrapituitary T3 production was examined in the present experiment.

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Materials and Methods

Male Wistar rats, weighing about 300-400 g, were used in all experiments. To study the effect of altered thyroid states, rats were either thyroidectomized or treated with sc injection of T4 (10 μg/100 g BW) for 5 days. The thyroidectomized rats were maintained on 0.5% CaCl2 water ad libitum for at least 2 weeks before the experiment, and the T4 treated rats were used for the experiments 24 h after the last injection. In fasting studies, rats were deprived of food for 48 h with free access to water. Control rats were sacrificed at the same time as treated rats and the controls for T4-treated rats were injected with the drug vehicle. All rats were maintained in a temperature- and light-controlled room (12 h of light and 12 h of dark).

L-[3', 5'-125I]-T4 was prepared by the chlolamine T method (Burger and Ingbar, 1974) and its specific activity was 580–850 μCi/μg. Radioactive T4 was free from appreciable contaminants other than approximately 6% iodide as tested by paperchromatography (Bellabarba et al., 1968) and electrophoresis (Inada and Sterling, 1967). The rats were sacrificed by decapitation and the anterior pituitary and liver were dissected. Trunk blood was collected to determine serum T4, T3 and TSH concentrations. Details of preparation of homogenates and incubation procedures were described in the previous paper (Naito et al., 1981). In brief, homogenates of the tissues were prepared in 15 vol. (wt/vol) of cold 0.05 M Tris-sucrose buffer, pH 7.6 and incubated with 125I-T4 (approximately 3–5 pmoles), 0.1 μM unlabeled T4 and Tris-sucrose buffer, pH 7.6 to yield a final volume of 0.5 ml in the presence of dithiothreitol (DTT). The DTT concentrations employed in the present study were 5 mM for the liver homogenates and 50 mM for the pituitary homogenates. After the incubation, ethanol extraction of the incubation mixture was carried out. An aliquot of the ethanol extract was applied to paper, with a carrier amount of iodide, T4 and T3, and subjected to descending paperchromatography in the t-amylalcohol-2N ammonia-hexane (10:11:1) solvent (Bellabarba et al., 1968). The amount of T3 formed was calculated from the conversion ratio from T4 to T3 and T4 amount in the incubation mixture. Protein content was determined by the method of Lowry et al. (1951), and T3 generation was expressed as fmoles T3/mg protein/min.

As shown in Table 1, serum T4 and T3 concentrations in rats injected with T4 were elevated, being 200% and 136%, respectively, of the control values, whereas serum TSH concentrations were undetectable. In contrast, the thyroidectomized rats had definitely low serum T4 and T3 concentrations with markedly elevated serum TSH levels (Table 1).

The effect of altered thyroid states on T3 generation in rat anterior pituitary and liver is shown in Fig. 1. The rates of pituitary and hepatic T3 generation averaged 25.5 ± 12.4 fmoles T3/mg protein/min and 33.8 ± 12.7 fmoles T3/mg protein/min, respectively, in normal rats. In T4-treated rats, however, the elevation of hepatic T3 generation from T4 (46.7 ± 3.1 fmoles T3/mg protein/min) was found, whereas intrapituitary T3 generation was markedly decreased (4.5 ± 0.2 fmoles T3/mg protein/min, Fig. 1). On the other hand thyroidectomized rats had slower hepatic T4 5'-monodeiodination (11.0 ± 2.8 fmoles T3/mg protein/min), and accelerated intrapituitary T4 5'-monodeiodination (64.3 ± 1.4 fmoles T3/mg protein/min, Fig. 1) than the controls, showing opposite changes in hyper- and hypothyroid rats (Fig. 1).

As shown in Table 2, 48 h-fasted rats had lower serum T4 (3.4 ± 0.6 μg/dl), T3 (70.0 ± 12.5 ng/dl) and TSH (38 ± 12.3 ng/ml) levels, compared with fed rats. Table 2 also shows that fasting for 48 h led to a 75% decrease in T4 to T3 conversion activity in the liver (p<0.01). Similarly, fasting resulted in a 74% reduction in pituitary T3 generation even in the presence of 50 mM DTT (p<0.05).
Table 1. Serum concentrations of T₄, T₃ and TSH in thyroid hormone excess and deficiency.

<table>
<thead>
<tr>
<th></th>
<th>T₄ (µg/dl) mean±S.E.</th>
<th>T₃ (ng/dl) mean±S.E.</th>
<th>TSH (ng/ml) mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. T₄ treated rats (4)</td>
<td>6.3±0.6</td>
<td>127±15.3</td>
<td>under limit of determination</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.025</td>
<td></td>
</tr>
<tr>
<td>b. thyroidectomized rats (4)</td>
<td>1.3±0.4</td>
<td>67.3±15.5</td>
<td>1300±550</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.005</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>c. normal rats (4)</td>
<td>3.1±0.3</td>
<td>93.7±17.0</td>
<td>159±54</td>
</tr>
</tbody>
</table>

Numbers in parenthesis represent the number of rats in each group.
P: Probability that the values in T₄ treated rats or thyroidectomized rats are identical to the corresponding values in normal rats.

Table 2. Effect of 48h starvation on serum concentrations of T₄, T₃ and TSH and on T₃ generation from T₄ in rat anterior pituitary and liver.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum concentration</th>
<th>T₃ generation from T₄ (fmoles T₃/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₄ (µg/dl) mean±SE</td>
<td>T₃ (ng/dl) mean±SE</td>
</tr>
<tr>
<td>Fed (5)</td>
<td>4.4±0.9</td>
<td>91.8±20.9</td>
</tr>
<tr>
<td>Fasted (5)</td>
<td>3.4±0.6</td>
<td>70.0±12.5</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Numbers in parenthesis represent the number of rats in each group.
P: Probability that the values in fasted rats are identical to the corresponding values in fed rats.

Discussion

T₄ 5'-monodeiodinating activity has been demonstrated in rat liver (Visser et al., 1976; Chopra, 1978; Kaplan and Utiger, 1978), Kidney (Chiraseveenuprapund et al., 1978; Leonard and Rosenberg, 1978), anterior pituitary (Silva et al., 1978a; Kaplan, 1980), thyroid (Erickson et al., 1981) and brain (Kaplan and Yaskoski, 1980) and in human thyroid (Ishii et al., 1981). These studies have shown that the T₄ 5'-deiodinating activity is enzymatic in nature. It has also been demonstrated that some physiological alterations or pharmacological agents alter T₃ generation from T₄ in different ways in various tissues (Larsen et al., 1981). In hypothyroid rats, hepatic and renal T₃ generation was decreased (Larson et al., 1955; Grussendorf and Hufner, 1977; Harris et al., 1978; Balsam et al., 1978; Ka-
plan and Utiger, 1978), while the rates in the pituitary (Kaplan, 1980) and brain (Kaplan and Yaskoski, 1980) were increased. On the other hand, the opposite changes occurred in hyperthyroid rats. The present study confirmed these previous observations, using rat liver and anterior pituitary homogenates. These findings suggest the presence of an autoregulatory mechanism of thyroid hormone activation within the target tissues.

In the previous study, we observed the reciprocal relationship between the pituitary $T_3$ generation and serum TSH level in perinatal and young adult rats (Natio et al., 1981). This agrees with the postulate that the local $T_3$ production in rat anterior pituitary plays an important role in regulating TSH secretion (Silva and Larsen, 1977; Larsen et al., 1979; Obregon et al., 1980). In the present paper, however, pituitary $T_3$ generation rather increased in thyroidectomized rats which had markedly elevated TSH levels in serum, while they decreased in $T_4$-treated rats with suppressed TSH levels. It has been generally accepted that $T_3$ exerts its effect by first binding to specific receptors located in the cell nucleus (Oppenheimer et al., 1976). Since nuclear $T_3$ in the anterior pituitary is derived from two sources, circulating $T_3$ and intracellular $T_3$ generated from $T_4$, Silva et al. (1978b) estimated the relative contributions of $T_3$ from two sources to nuclear $T_3$. They concluded that approximately 50% of specifically bound nuclear $T_3$ is derived from intrapituitary $T_4$ 5′-monodeiodination in normal rats. In the present study, serum $T_3$ concentrations in the thyroidectomized rats were almost two thirds those in normal rats and $T_3$ generation in the pituitary was accelerated, while serum TSH levels were elevated. On the other hand, 135% elevation of serum $T_3$ along with a decreased pituitary $T_4$ concentration was associated with low serum TSH levels in hyperthyroid rats. Although the contribution of circulating $T_3$ to nuclear $T_3$ in rat anterior pituitary was not estimated in thyroid hormone excess and deficiency, the results mentioned above suggest that circulating $T_3$ in thyroidectomized and $T_4$-treated rats might contribute to nuclear $T_3$ more than $T_3$ generated locally from $T_4$. An alternate explanation is that altered TRH secretion from the hypothalamus might change the threshold of the negative feedback effect of $T_3$ on TSH secretion in hyper- and hypothyroid states.

It has been known that fasting causes a decrease in hepatic and pituitary $T_3$ generation from $T_4$ (Kaplan and Utiger, 1978; Campbell et al., 1977; Harris et al., 1978; Kaplan, 1980). Serum TSH levels as well as $T_3$ and $T_3$ concentrations were decreased in fasted rats (Kaplan and Utiger, 1978, Campbell et al., 1977; Harris et al., 1978). It is difficult to explain why serum TSH levels do not increase, despite decreased pituitary $T_3$ generation from $T_4$ and low serum $T_3$ and $T_4$ levels. Harris et al. (1978) and Campbell et al. (1979) observed that the responsiveness of TSH secretion to exogeneous TRH was maintained in fasted rats. On the basis of these observations, Gardner et al. (1979) suggested the decreased set point of TSH secretion in fasted rats. In other words, the critical level of serum thyroid hormone to induce an increase in TSH secretion is lowered by fasting. Our observation that decreased $T_3$ generation in pituitary in fasted rats is associated with low TSH levels can be explained by the decreased set point hypothesis, although the exact mechanism responsible for such a change is unknown.

The present study demonstrates a difference between the anterior pituitary gland and liver in the response of $T_3$ 5′-monodeiodination to hypothyroid or hyperthyroid state. On the other hand, changes in pituitary and hepatic $T_3$ generation from $T_4$ were similar in fasted rats. The elucidation of factors responsible for such divergent changes will be of benefit in under-
standing the nature of the feedback regulation of thyroid hormones on pituitary TSH secretion and clarifying characteristics of local T₄ 5′-monodeiodination in the anterior pituitary gland.

References


Silva, J. E., T. E. Dick and P. R. Larsen (1978b). The contribution of local tissue thyroxine mono-deiodination to the nuclear 3, 5, 3'-triiodothyronine in pituitary, liver and kidney of euthyroid rats.

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