Modulation of T_4 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 (T_4) to 3, 5, 3'-L-triiodothyronine (T_3) in regulating thyrotropin (TSH) secretion, the effect of altered thyroid states and fasting on intrapituitary T_3 generation was investigated by a paperchromatographic procedure using the anterior pituitary homogenates. Hepatic T_3 generation was also studied for comparison.

The rate of pituitary and hepatic T_3 generation in normal rats averaged 25.2 ± 12.4 (mean $\pm$ SE) fmoles T_3 /mg protein/min and 33.8 ± 12.7 fmoles T_3 /mg protein/min, respectively. T_4 treatment raised the hepatic T_3 generation from T_4 (46.7 ± 3.1 fmoles T_3 /mg protein/min) and lowered the intrapituitary T_3 generation (4.5 ± 0.2 fmoles T_3 /mg protein/min). On the contrary, thyroidectomy slowed the hepatic T_4 5'-deiodination (11.0 ± 2.8 fmoles T_3 /mg protein/min), and accelerated the pituitary T_4 5'-deiodination (64.3 ± 1.4 fmoles T_3 /mg protein/min). In 48 h fasted rats, serum T_4 , T_3 and TSH concentrations were all lower than those in fed rats, and both pituitary and hepatic T_3 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 (T_3) generation from L-thyroxine (T_4) in rat anterior pituitary fragments (Silva *et al.*, 1978a) and homogenates (Kaplan, 1980). The T_4 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 T_4 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 reci-

procal relation between serum thyrotropin (TSH) levels and intrapituitary T_3 generating activity from T_4 was demonstrated in the perinatal rats, suggesting an important role of the local T_3 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 T_3 generation from T_4 , the effect of the thyroid state and fasting on intrapituitary T_3 production was examined in the present experiment.

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 T_4 ($10 \mu\text{g}/100 \text{ g BW}$) for 5 days. The thyroidectomized rats were maintained on 0.5% CaCl_2 water ad libitum for at least 2 weeks before the experiment, and the T_4 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 T_4 -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).

$L-[3', 5'-^{125}\text{I}]-T_4$ was prepared by the chloramine T method (Burger and Ingbar, 1974) and its specific activity was $580\text{--}850 \mu\text{Ci}/\mu\text{g}$. Radioactive T_4 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 T_4 , T_3 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 $^{125}\text{I}-T_4$ (approximately 3–5 pmoles), 0.1 μM unlabeled T_4 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, T_4 and T_3 , and subjected to descending paperchromatography in the *t*-amylalcohol-2N ammonia-hexane (10:11:1) solvent (Bellabarba *et al.*, 1968). The amount of T_3 formed was calculated from the conversion ratio from T_4 to T_3 and T_4 amount in the incubation mixture. Protein content was determined by the method of Lowry *et al.* (1951), and T_3 generation was expressed as fmoles $T_3/\text{mg protein}/\text{min}$.

TSH levels in rat serum were determined with a radioimmunoassay kit which was generously supplied by the NIAMDD of the NIH, USA (Naito *et al.*, 1981). T_4 and T_3 levels in rat serum were also measured by radioimmunoassay kits provided by Dainabot Radioisotope Laboratories, Chiba, Japan (Naito *et al.*, 1981).

All experiments were made in duplicate. Results are expressed as the mean $\pm$ S. E. Comparison between groups were performed using Student's *t*-test for unpaired values.

Results

As shown in Table 1, serum T_4 and T_3 concentrations in rats injected with T_4 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 T_4 and T_3 concentrations with markedly elevated serum TSH levels (Table 1).

The effect of altered thyroid states on T_3 generation in rat anterior pituitary and liver is shown in Fig. 1. The rates of pituitary and hepatic T_3 generation averaged 25.5 ± 12.4 fmoles $T_3/\text{mg protein}/\text{min}$ and 33.8 ± 12.7 fmoles $T_3/\text{mg protein}/\text{min}$, respectively, in normal rats. In T_4 -treated rats, however, the elevation of hepatic T_3 generation from T_4 (46.7 ± 3.1 fmoles $T_3/\text{mg protein}/\text{min}$) was found, whereas intrapituitary T_3 generation was markedly decreased (4.5 ± 0.2 fmoles $T_3/\text{mg protein}/\text{min}$, Fig. 1). On the other hand thyroidectomized rats had slower hepatic T_4 5'-monodeiodination (11.0 ± 2.8 fmoles $T_3/\text{mg protein}/\text{min}$), and accelerated intrapituitary T_4 5'-monodeiodination (64.3 ± 1.4 fmoles $T_3/\text{mg protein}/\text{min}$, 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 T_4 ($3.4 \pm 0.6 \mu\text{g}/\text{dl}$), T_3 ($70.0 \pm 12.5 \text{ ng}/\text{dl}$) and TSH ($38 \pm 12.3 \text{ ng}/\text{ml}$) levels, compared with fed rats. Table 2 also shows that fasting for 48 h led to a 75% decrease in T_4 to T_3 conversion activity in the liver ($p < 0.01$). Similarly, fasting resulted in a 74% reduction in pituitary T_3 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.

	T ₄ (μ g/dl) mean $\pm$ S.E.	T ₃ (ng/dl) mean $\pm$ S.E.	TSH (ng/ml) mean $\pm$ S.E.
a. T ₄ treated rats (4)	6.3 $\pm$ 0.6	127 $\pm$ 15.3	under limit of determination
P	<0.05	<0.025	
b. thyroidectomized rats (4)	1.3 $\pm$ 0.4	67.3 $\pm$ 15.5	1300 $\pm$ 550
P	<0.005	<0.05	<0.005
c. normal rats (4)	3.1 $\pm$ 0.3	93.7 $\pm$ 17.0	159 $\pm$ 54

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

Treatment	Serum concentration			T ₃ generation from T ₄ (fmoles T ₃ /mg protein/min)	
	T ₄ (μ g/dl) mean $\pm$ SE	T ₃ (ng/dl) mean $\pm$ SE	TSH (ng/ml) mean $\pm$ SE	Liver mean $\pm$ SE	Pituitary mean $\pm$ SE
Fed (5)	4.4 $\pm$ 0.9	91.8 $\pm$ 20.9	134 $\pm$ 41.8	46.5 $\pm$ 4.9	35.7 $\pm$ 2.2
Fasted (5)	3.4 $\pm$ 0.6	70.0 $\pm$ 12.5	38 $\pm$ 12.3	34.9 $\pm$ 0.3	26.6 $\pm$ 5.5
P	<0.05	<0.05	<0.01	<0.01	<0.05

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

Fig. 1. Effect of thyroid hormone excess and deficiency on T₃ generation from T₄ in the rat anterior pituitary and liver. Each bar represents the mean $\pm$ S.E. The DTT concentrations in the incubation mixtures were 50 mM for the pituitary homogenates and 5 mM for the liver homogenates.

Euthyroid: normal rats

Hyperthyroid: T₄-treated rats

Hypothyroid: thyroidectomized rats

* P<0.01

** P<0.05

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_4 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 exogenous 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_4 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.

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