Reciprocal Relation between Serum Thyrotropin Levels and Intrapituitary 3,5,3'-L-Triiodothyronine Generating Activity from Thyroxine in Perinatal Rats

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Abstract

Thyroxine (T₄) 5'-monodeiodinating activity in rat pituitary was studied, using paper chromatography. Rat anterior pituitary homogenates were incubated with ¹³¹I-T₄, dithiothreitol and unlabeled L-T₄ at 37°C. The incubation mixture was extracted by ethanol and the extracts were subjected to descending paper chromatography. The conversion ratio of T₄ to 3, 5, 3'L-triiodothyronine (T₃) was calculated from the radioactivity of T₃ spot to total radioactivity on the paper strip. The T₃ amount generated was estimated from the T₄ concentration in the incubation mixture and the conversion ratio.

The T₃ generating activity from T₄ increased as the amount of tissue increased. It was temperature- and pH-dependent, and thiol sensitive. These results suggest the enzymatic nature of T₄ 5'-monodeiodinating activity of the anterior pituitary. A kinetic study revealed low Kₘ for T₄ (7.9±1.6 nM, Mean±SE), with Vₘₐₓ of 68.0±12.7 fmoles T₃/mg protein/min.

T₄ monodeiodinating activity was consistently, though minimally, detected in fetal rat pituitaries and increased after birth, reaching the maximum at 22 days. It declined thereafter to the young adult level. Serum TSH levels were markedly elevated in fetus. They decreased after birth, reaching the nadir at 22 days, and then increased to young adult levels. Serum T₄ and T₃ levels were markedly diminished in fetus and gradually increased after birth, reaching the young adult levels at 17 days. Thus, a reciprocal relationship was observed between intrapituitary T₃ generating activity from T₄ and serum TSH levels in developing rats. It suggests that the conversion of T₄ to T₃ in the pituitary plays a role in regulating TSH secretion.

It has been suggested that the anterior pituitary is the site of local thyroxine (T₄) conversion to 3, 5, 3'L-triiodothyronine (T₃), based on the observation that ¹³¹I-T₃ accumulated in the pituitary following the injection of ¹³¹I-T₄ (Ford and Gross, 1958a, b; Reichlin et al., 1966). More recently, Silva et al., (1978) have demonstrated T₄ 5'-monodeiodinating activity in fragments and homogenates of rat anterior pituitary, as in homogenates of the liver (Visser et al., 1975; Kaplan and Utiger, 1978) and kidney (Chirasevenuprapund et al., 1978). Utilizing radioimmunoassay for T₃, Kaplan (1980) studied the kinetics of T₄ to T₃ conversion in the pituitary and showed its enzymatic nature.

In the present experiments, the T₃ generating activity from T₄ in rat pituitary was studied by means of the paperchromatographic method. Furthermore, perinatal changes in T₄ 5'-monodeiodinating activity were investigated in relation to changes in serum T₄, T₃ and thyrotropin (TSH) levels in rats.

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Materials and Methods

Fetal Wistar rats (18–19 days of gestation) and 8-, 17-, 22-, 33- and 49-day-old male Wistar rats were used for the study on age-related changes in T₄ 5'-monodeiodinating activity in the pituitary. For other studies, male Wistar adult rats, weighing 200-350 g were used. They were maintained in a temperature and light controlled room (12 hr of light and 12 hr of dark).

L-[3', 5'-¹²⁵I]-T₄ was prepared by the chloramine T method (Burger and Ingbarg, 1974). Specific activity was 580-850 μCi/μg. Radioactive T₄ was free of 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 pituitaries and the livers were removed, and the posterior pituitaries were removed from the gland. Trunk blood was collected for determining serum T₃, T₄ and TSH concentrations. The anterior pituitaries or the livers were weighed, placed in 15 vol. (wt/vol) of cold 0.05 M Tris-sucrose buffer, pH 7.6 and homogenized in a glass homogenizer with a teflon pestle for about 10 strokes. In fetal rats, it was technically difficult to isolate the pituitary and, therefore, a small portion of the surrounding tissue was included in fetal pituitary. In 8-day-old neonatal rats, the whole pituitary was used because of the difficulty of removing the posterior pituitary. About 30-40 pituitaries of the fetal rats were pooled and homogenized. In neonatal and young rats, 5–10 rat pituitaries were pooled to prepare homogenates. Pooled livers from 5 to 10 rats in 33 days of age or younger and livers from individual older rats were homogenized, and the homogenate was then centrifuged at 800 g for 10 min at 4°C. The supernatant was used and the term liver homogenate will henceforth refer to this supernatant preparation. A small amount of the homogenate was kept for the measurement of endogenous T₄ concentration by radioimmunoassay (Larsen et al., 1973) and of protein concentration by Lowry’s method (Lowry et al., 1951). To study age-related changes in T₄ 5'-monodeiodination in the pituitary and the liver, the experiments were repeated 4 times in fetal rats, twice in 8- and 17-old rats, 3 times in 8- and 49-day-old rats and twice in 22-and 33-day-old rats.

An 150-200 μl aliquot of the pituitary homogenate or the liver homogenate was mixed with 1²⁵I-T₄ (approximately 3-5 pmoles), and 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. To study T₃ degradation during the incubation, a tracer dose of ¹²³I-T₃ was added to the ¹²⁵I-T₄ free incubation mixture for adult rats. Incubation was carried out at 37°C for 45 min under nitrogen gas after flushing each vessel with the gas and sealing tightly with parafilm. In all experiments, tissue-free mixtures and the homogenates were incubated separately and they were combined at the end of the incubation for the controls. After the incubation, 100 μl of hormone-free serum and 40 μg of T₄ and T₃ were added to the incubation mixture, followed by the addition of 2 volumes of cold ethanol. After mixing completely, all vessels were plunged into cracked ice for at least 20 minutes, followed by centrifugation at 1500×g for 20 min at 4°C. Supernatants were kept for subsequent paperchromatography. Recovery of the radioactivity in each vessel during the extraction procedure was 88-99%. An aliquot of the ethanol extract (10-20 μl) was applied to paper (Toyo filter paper No. 2), with a carrier amount of iodide, T₃ and T₄, dried in air and subjected to descending paperchromatography in the t-amyl alcohol-2N ammonia-hexane (10:11:1) solvent (Bellabarba et al., 1963). The zones containing radioactive compounds were localized by paperchromatoscanning with the Nuclear Chicago Actigraph II and the locations of T₄ and T₃ were also determined by visualization of these substances, which had been cochromatographed as carriers, under UV light. Each zone was excised and radioactivity in these zones as well as that in the intervening segments of the strips was determined with an automatic gamma counter. The counts in the T₃ zone of the experimental group were usually more than twice those in the intervening segment of the strip. However, for the experiments determining Michaelis-Menten constant and age-related T₄ 5'-deiodinating activities in the pituitary, significance of differences between the counts in the T₃ zone and those in the intervening segment was tested by Student’s paired t test, since the magnitude of the differences was small. The conversion ratio from T₄ to T₃ (CR) was calculated from the percentage of ¹²³I activity found in the T₃ zone of the paperchromatograph to the total ¹²³I activity on the paper as follows:

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CR = \frac{125I \text{ in T₃ area of chromatogram from the experimental tube}}{125I \text{ in T₄ area of chromatogram from the control tube}} \times 2
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¹²³I-T₄ molecule contains an ¹²³I atom either at the 3' or the 5' position, but not at both positions; hence deiodination of a particular ¹²³I-T₄ molecule would give an unlabeled T₃ molecule in 50% of the cases, and a labeled T₃ molecule in 50% of the cases (Chirasveenuprapund et al., 1978; Nakamura et al.,...
Thus, values for CR were corrected by multiplying the measured values by 2. However, the values might be underestimated to some extent because some of the total $^{125}$I starts out as $I^-$, and perhaps other contaminants. In the present study, the $T_3$ content of every tracer which was newly prepared was calculated to minimize underestimation. The amount of $T_3$ formed was calculated from CR and the $T_4$ amount in the incubation mixture, and $T_3$ generation was expressed as fmoles $T_3$/mg protein/min.

TSH levels in rat serum were determined with a radioimmunoassay kit generously supplied by the NIAMDD of the NIH, USA. TSH levels were expressed as weight equivalents of the corresponding NIAMDD reference preparations. $T_4$ and $T_3$ levels in rat serum were also measured with radioimmunoassay kits provided by Dainabot Radioisotope Laboratories, Chiba, Japan. Assay sensitivity was 0.75 ng/dl for $T_4$ and 25 ng/dl for $T_3$. All samples were run in duplicate in the same assay.

Comparisons between groups were performed using Student's nonpaired t test and, where appropriate, an analysis of variance.

**Results**

Fig. 1 shows radiochromatograms of the ethanol extract of the incubation mixture with $^{125}$I-$T_4$ and the homogenate of adult rat anterior pituitaries. As shown in the upper portion of Fig. 1, a small but distinct peak of radioactivity was found in the $T_3$ area of the paper chromatogram. This peak completely disappeared when the homogenate was preheated at 56°C for 30 min before incubation, as shown in the lower portion of Fig. 1. In addition, no radioactivity was found in the $T_3$ area, when incubated at 4°C. The radioactive material in the $T_3$ zone of the paper strip was eluted by methanol-ammonia (99:1) and subjected to thin layer chromatography using thin layer chromatography-plastic sheet, silica gel 60, in
the toluene: acetic acid: water solvent (2:2:1) to separate tetraiodothyroacetic acid (Surks et al., 1968). All radioactivity remained at the origin of the thin layer. The origin component was again eluted with the methanol-ammonia solution and subjected to ascending paper chromatography in butanol: dioxane: 2N ammonia solvent (4:1:5). Only a single peak of radioactivity was found in the T₃ area on the paper. The T₃ generation from T₄ was not observed with the homogenate of rat posterior pituitaries.

In an experiment with anterior pituitaries from adult rats, there was a progressive increase in the ratio of T₄ to T₃ during incubation over a period of 180 min and it was almost linear in the initial 60 min. When the homogenates of fetal, 8-old and adult rat pituitaries were incubated with ¹²⁵I-T₃ for 60 min and the ethanol extracts were subjected to descending paper chromatography, over 95% of the radioactivity was found in the T₃ area on the paper. This suggests that T₃ degradation was negligible in the homogenates of fetal, neonatal and adult rat pituitaries during the incubation for 60 min. The T₃ generation from T₄ progressively increased with the increase in the amount of anterior pituitary tissue from adult rats. It was optimal at pH 7.6. No T₃ generation was detected without DTT, and a progressive increase in T₃ generation from T₄ occurred with an increase in DTT up to 50 mM in the medium. Increasing amount of unlabeled T₄ over a range of 0.005 μM-10 μM in the presence of a constant amount of anterior pituitary tissue from adult rats resulted in increasing formation of T₃. The apparent Michaelis-Menten constant (Kₘ) for T₄ was estimated to be 7.9 ± 1.6 (SE) μM and the apparent Vₘₐₓ was 68.0 ± 12.7 fmoles T₃/mg protein/min in four experiments.

The rate of the intrapituitary T₃ generation averaged 29.8 ± 4.0 (Mean ± SE) fmoles T₃/mg protein/min in 49-day-old rats. As shown in Fig. 2, a marked diminution in T₃ generating activity was found in the fetal rat pituitaries (7.6 ± 1.8 fmoles T₃/mg protein/min). However, the values obtained in fetal pituitaries might be underestimated, because the whole pituitary with the surrounding tissue was used. By 8 days after birth, T₃ generating activity increased to the mean value of 52.0 ± 10.7 fmoles T₃/mg protein/min, which was significantly higher than those in 49-day-old rats. This increase persisted till 22 days of age, with the peak of 146 ± 1.3 fmoles T₃/mg protein/min in 22-day-old rats (Fig. 2). Although the activity decreased in 33-day-old-rats, the values were still significantly higher than those in 49-day-old rats. On the other hand, in the presence of DDT, T₃ generating activity in rat livers, which was markedly low in the fetal rats, had already increased to 36.6 ± 4.5 fmoles T₃/mg protein/min in 8-day-old rats and remained almost constant throughout 17, 22, 33 and 49 days of age (Fig. 2).

As shown in Fig. 3, serum T₄ and T₃ levels were under the limits of detection in fetal rats, but they increased rapidly after birth. Serum T₄ levels in 8-day-old rats (3.6 ± 0.7 μg/dl) were comparable to those in 49-day-old rats (4.0 ± 1.2 μg/dl), although serum T₃ levels were still lower at 8 days of age (72.5 ± 3.7 ng/dl) than at 49 days (93.0 ± 4.4 ng/dl). The serum T₃ concentration reached the level of 49 days at 17 days of age. Markedly elevated serum TSH levels in fetal rats (280 ± 37.8 ng/ml) declined after birth, reaching the nadir at 22 days (36 ± 7.3 ng/ml). Subsequently, serum TSH rose to the level of 49-day-old rats (180 ± 12.2 ng/ml). Thus, a reciprocal relationship was evident between intrapituitary T₄ 5'-monodeiodinating activity and serum TSH levels in developing rats (Fig. 4).
Discussion

In the present study, $T_4$ 5'-monodeiodinating activity was demonstrated in homogenates of rat anterior pituitary, employing the paperchromatographic procedure. This intrapituitary $T_3$ generating activity from $T_4$ in adult rats increased with the increase in the amount of tissue. Moreover, the activity increased linearly over the initial 60 min incubation and was temperature- and pH-dependent. These results suggest the enzymatic nature of $T_3$ generating activity and are consistent with a recent observation (Kaplan, 1980), in which a radioimmunoassay method was used to detect the $T_3$ produced.
It has been previously reported that T₄ 5'-monodeiodinating activity in liver and kidney homogenates were thiol-dependent (Chopra, 1978; Visser et al., 1976; Leonard and Rosenberg, 1978). The intrapituitary T₃ generation did not require the addition of DTT in intact pituitary tissue (Cheron et al., 1980a, b). However, the present study showed that no T₃ was formed in rat anterior pituitary homogenates in the absence of DTT. The T₃ generation progressively increased with the increase in the DTT concentration up to 50 mM. A rather high concentration of DTT was required to obtain T₄ 5'-monodeiodinating activity in the pituitary homogenate compared with those found in the liver and kidney.

The apparent Kₘ for intrapituitary T₄ 5'-monodeiodinating activity is much lower than those obtained in the liver and kidney by previous studies (Kaplan and Utiger, 1978; Chiraseveenuprapund et al. 1978). The estimated Kₘ for T₄ in the present study was quite almost the same as to that (8.8 nM) obtained by others (Kaplan, 1980). The results provide validation of Kₘ obtained in the present study.

It is believed that serum TSH levels are regulated by serum T₄ and T₃ concentration through the feedback mechanism. El-Zaheri et al. (1980) recently demonstrated enhanced T₃ generation from T₄ in neonatal rat pituitary and suggested that T₃ generated in the pituitary might suppress TSH release in spite of low serum T₄ and T₃ concentrations. In the present experiment, serum T₄ and T₃ levels reached the young adult level at 17 days after birth, whereas serum TSH was lowest at 22 days, when the T₄ monodeiodinating activity of the pituitary was maximal. Thereafter, serum TSH increased with the decrease in pituitary T₄ monodeiodinating activity. These age-related changes in serum TSH could not be explained by the alteration of serum T₄ and T₃ levels, and rather suggests the importance of intrapituitary generation of T₃ from T₄.

In fetal rats, serum TSH levels were elevated with low T₃ and T₄ levels. The high TSH levels in fetal rat serum were in variance with the data of previous reports (Fukuda and Greer, 1978; Kojima and Hershman, 1974), and the basis for the difference between our estimates and the

Fig. 4. Change of serum TSH levels (open circle) and intrapituitary T₄ 5'-monodeiodinating activity (closed circles) in rats of varying ages. Each bar represents the mean ± SE.
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results of previous studies (Fukuda and Greer, 1978; Kojima and Hershman, 1974) is not apparent. Any explanation for the high TSH levels must consider differences in the gestational days of fetal rats and in the method used for radioimmunoassay of TSH. Since TRH plays little role in regulating TSH secretion in fetal life (Theodoropoulos et al., 1979), elevated TSH levels might be ascribed to the decreased feedback inhibition of thyroid hormones on the pituitary. Although the T₃ generating activity of fetal rat pituitary might be somewhat underestimated by the contamination of surrounding tissue, it was less than one fourth of the activity in 49-day-old rats. This low T₃ generating activity might contribute, at least in part, to the elevated TSH level in fetus. These results are consistent with the observation that intrapituitary T₃ generation from T₄ plays a role in regulating TSH secretion (Obregon et al., 1980).

Very recently, Cheron et al. (1980a) reported the difference between T₄ 5'-monodeiodinating activity in rat pituitary and that in liver during maturation. They showed that the activity in 2-and 4-day-old rats was 3.5 times that in 72-day-old rats, and it fell to the 72-day value by 30 days. These results are at variance with the observation by us and others (El-Zaheri et al., 1980). The difference might be caused at least in part by different experimental procedures, since Cheron et al. (1980a) studied with pituitary fragments in the absence of DTT. On the other hand, they found no significant difference in hepatic T₄ 5'-monodeiodinating activity in 2-to 72-day-old rats. This agrees with our observation that in the presence of DTT, T₃ generation from T₄ in liver homogenates was not significantly different in 8-to 49-day-old rats. It is concluded that T₄ to T₃ conversion is regulated independently in the pituitary and liver during maturation, and that local conversion may play an important role in regulating pituitary function, as reported previously (Cheron et al., 1980a; El-Zaheri et al., 1980).

References


