Maturation of renal and hepatic monodeiodination of thyroxine to triiodothyronine and post-natal changes of serum thyroid hormones in young rats

Yukichi Suzuki, Kyoko Kita, Yasuko Uchigata, Ikuro Takata and Tamotu Sato

Department of Paediatrics, School of Medicine, Kanazawa University, Kanazawa, Japan

Abstract. Maturational changes of renal and hepatic 5'-monodeiodination of thyroxine (T₄) and post-natal changes of serum thyroid hormone levels were investigated in young rats under 35 days of age. Renal T₃ generation in the 1-day-old rats was low, rose progressively to a level of more than 200% of the adult rats on days 21 and 28 and declined thereafter. In contrast, hepatic T₃ generation increased from an initial low activity to a plateau after 7 days of age, which was 1½ times higher than that of adult rats. Because of the extremely low value of serum T₄ in the neonatal period, T₃/T₄ and rT₃/T₄ ratios were elevated on day 7. The ratio of rT₃/T₄ decreased gradually and became stable after 21 days of age, while the T₃/T₄ ratio increased reciprocally to a peak on days 21 and 28, corresponding to the period of maximal activity of renal T₄ monodeiodination. These results indicate that in addition to elevated hepatic T₃ monodeiodination, renal conversion of T₄ to T₃ may play a significant physiological role during the period of enhanced T₃ requirement for maturation.

Since Braverman et al. (1970) demonstrated that thyroxine (T₄) was converted to 3,3',5-triiodothyronine (T₃) in athyreotic human subjects, many studies on peripheral monodeiodination of T₄ to T₃ and T₄ to 3,3',5'-triiodothyronine (rT₃) have been reported (Cavalleri et al. 1977; Chiraseveenuprampund et al. 1978; Chopra 1977; Gavin et al. 1977; Hesch et al. 1975; Höffken et al. 1977; Hüfner et al. 1977; Kaplan & Utiger 1978; Maciel et al. 1979). Approximately 70% of serum T₃ is generated from peripheral monodeiodination of T₄ to T₃ (Inada et al. 1975) and the metabolic pathway of thyroid hormones was clarified recently (Sakurada et al. 1978). However, studies of T₄ to T₃ monodeiodination have mainly used liver homogenates. Moreover, there is at present little information regarding the effect of age on the monodeiodination of T₄ to T₃ and T₄ to rT₃ (Harris et al. 1978b). In spite of the fact that kidney is one of the major sites of in vivo T₄ to T₃ monodeiodination (Chopra 1977), no report of the effect of age on renal T₃ production has been found. Our experiment aimed to compare the maturational change of renal monodeiodination with that of hepatic monodiodination of T₄ to T₃ in young rats and to investigate the relationship between the converting system of T₄ to T₃ and post-natal changes in serum thyroid hormone concentrations.

Materials and Methods

Breeding of pregnant Wistar strain rats was started on the 10th day of gestation. They were maintained on ordinary rat chow and tap water ad libitum. Pups from pregnant rats were sacrificed by cardiac puncture under light ether anaesthesia on days 1, 7, 14, 21, 28 and 35. Kidneys, livers and blood samples were withdrawn. With regard to kidneys, livers and blood samples from neonatal rats aged 1–14 days, 20 specimens were gathered into 5 samples from each age group and used, but in the 21-day and older rats, kidneys, livers and blood samples from 5 rats were used in each experiment. Two male rats weighing approximately 250 g were sacrificed with each neonatal age group and served as controls. A total of 12 control rats was in the experiment.
Kidneys and livers were washed or perfused with ice-cold 0.1 M phosphate buffer, pH 7.0 and homogenized with 2 vol of the same ice-cold buffer, using a Teflon tissue grinder. The homogenate was centrifuged at 7000 x g for 15 min at 4°C and the protein content of the supernatant was determined after solubilization in 0.1 N KOH by the method of Lowry et al. (1951).

The incubation mixture consisted of 0.5 ml of 0.1 M phosphate buffer containing 5 mM EDTA, pH 7.0 (Chirasevenuprapund et al. 1978), 0.5 ml of the supernatant and 0.1 ml (1.0 µg) of L-T4 (3,3',5,5'-tetraiodo-L-thyronine, Sigma Chemical Co., USA). The mixture was incubated at 37°C in 10 x 75 mm glass tubes for 60 min. Before and after the incubation, withdrawal of 0.2 ml of the incubation mixture was done and precipitation with 0.4 ml of ice-cold 99.5% ethanol (v/v) was immediately carried out. After centrifugation at 2,000 r.p.m. for 10 min, T3 concentration in ethanol extracts was assayed in duplicate by double antibody radioimmunoassay (RIA) kit (Eiken Chemical Co., Ltd., Tokyo). The percentage of recovery of T3 in ethanol extraction was determined by adding [125I]T3 to the mixture just before the addition of ethanol and ranged from 80 to 82%. Cross-reactivity of T3 with L-T4 was 0.02%. After subtracting T3 concentration of the zero time tube from that of the incubated tube and correcting for protein content of the supernatant, the increment of T3 concentration was referred to as T3 production (pg T3 generated/mg protein) during the incubation. Renal and hepatic T3 generation in young rats was expressed as a percentage of renal and hepatic T3 generation by control animals.

Post-natal changes of serum T4, T3 and rT3 concentrations were measured by commercially available kits, T4 by GammaCoat RIA kit (Clinical Assays, Division of Travelen Laboratories, Inc., USA), T3 by T3-RIA kit (Dainabot Radioisotope Laboratories, Tokyo) and rT3 by rT3 kit (Dainabot Radioisotope Laboratories, Tokyo).

Statistical analysis was done by Student's t-test and all values are presented as mean ± SD. P < 0.05 is considered significant.

Results

Effect of age on renal T3 generation (Fig. 1)

In the 1-day-old rats, renal T3 generation was very low (25.7 ± 2.2%, n = 5, P < 0.01) compared with control rats (100 ± 19.8%, n = 12). Renal T3 generation increased slowly and the values in the 7- and 14-day-old rats were below those of adult male rats. However, a sharp and significant increase of renal T3 production was recognized in the 21-day-old rats (198.6 ± 25.8%, n = 5, P < 0.01 vs control) and in the 28-day-old rats (256.8 ± 46.6%, n = 5, P < 0.01 vs control). These two values were also significantly higher than those on days of 1, 7, 14 and 35 (P < 0.01). On day 35, T3 production in rat kidney fell to the level of adult controls.

Effect of age on hepatic T3 generation (Fig. 2)

As compared with adult male rats (100 ± 14.5%, n = 12), hepatic T3 generation in the 1-day-old rats
was markedly depressed (49.6 ± 11.7%, n = 5, P < 0.01). However, an increase of hepatic T₃ generation was recognized on day 7. The values of hepatic generation on days of 7, 14, 21 and 28 were 147.2 ± 27.7% (n = 5, P < 0.05 vs control), 130.5 ± 3.2% (n = 5, P < 0.05 vs control), 135.2 ± 12.4% (n = 5, P < 0.05 vs control) and 152.1 ± 15.6% (n = 5, P < 0.01 vs control), respectively. In the 35-day-old rats, the value (144.1 ± 30.6%) was not significantly high compared with that of controls.

Post-natal changes of serum T₄ (Fig. 3), T₃ (Fig. 4) and rT₃ (Fig. 5) concentrations

Serum T₄ level on day 1 was not detected by RIA. A gradual increase in serum T₄ was noticed through the experimental period and the serum T₄ level was 3.5 ± 0.4 μg/100 ml on day 35. But, serum T₄ of young rats under 35 days of age did not exceed that of adult male rats (5.5 ± 0.5 μg/100 ml, n = 12).

Serum T₃ levels were also undetectable in the 1-day-old rats. On day 21, serum T₃ reached its maximal level (112 ± 14 ng/100 ml, n = 5) which was significantly higher than those of adult male rats (87 ± 6 ng/100 ml, n = 12, P < 0.05), 7-day-old (51 ± 17 ng/100 ml, n = 5, P < 0.01) and 14-day-old rats (69 ± 10 ng/100 ml, n = 5, P < 0.01).

Serum rT₃ concentration in the neonatal period was low (0.8 ± 0.9 ng/100 ml, n = 5). On day 14, serum rT₃ reached its maximum (8.4 ± 1.4 ng/100 ml, n = 5), significantly higher than on day 1 (P < 0.01), day 21 5.4 ± 0.7 ng/100 ml, n = 5, P < 0.05) and day 28 (5.1 ± 0.6 ng/100 ml, n = 5, P < 0.05).

The ratios of T₃/T₄ and rT₃/T₄ are shown in Table 1. Although these values had wide variations on day 7, the wide values of sd disappeared after 14 days of age. T₃/T₄ ratios peaked on day 21 when the values were significantly high compared with those on days 14 and 35. On the other hand, rT₃/T₄ ratios were fairly stable after 14 days of age.

![Figure 3](image-url)  
Post-natal change of serum T₄ concentration (mean ± sd).

![Figure 4](image-url)  
Post-natal change of serum T₃ concentration (mean ± sd).

![Figure 5](image-url)  
Post-natal change of serum rT₃ concentration (mean ± sd).
Table 1.
Post-natal changes of $T_3/T_4$ and $rT_3/T_4$ ratios.

<table>
<thead>
<tr>
<th>Day</th>
<th>No</th>
<th>$T_3/T_4$ ratio</th>
<th>$rT_3/T_4$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>181.3 ± 215.6</td>
<td>11.9 ± 10.0</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>27.4 ± 4.8*</td>
<td>2.6 ± 0.6*</td>
</tr>
<tr>
<td>21</td>
<td>5</td>
<td>38.3 ± 3.8</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>28</td>
<td>5</td>
<td>32.3 ± 4.4</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>35</td>
<td>5</td>
<td>24.8 ± 2.7**</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>adult</td>
<td>12</td>
<td>16.1 ± 1.4**</td>
<td>1.5 ± 0.4</td>
</tr>
</tbody>
</table>

The values on day 1 can not be calculated, since serum $T_4$ on day 1 is not measured by RIA. All values shown are mean ± SD. Statistical analysis is carried out compared to the values on day 21. * and ** mean $P < 0.05$ and $P < 0.01$ vs data on day 21, respectively.

Discussion

As regards renal $T_3$ generation, our experiment revealed that the post-natal change of renal $T_3$ production had an exaggerated peak 3–4 weeks after birth and did not resemble the post-natal change in hepatic conversion. The effect of age on hepatic $T_3$ generation was similar but hepatic $T_3$ generation reached a plateau on day 7 (Harris et al. 1978b). It has been shown that the post-natal (or maturational) change of $T_3$ generation is associated both with the activity of a deiodinating enzyme and with the presence of certain cofactor – reduced glutathione and NADPH – which concomitantly activate the monodeiodinating enzyme system. Harris et al. (1978a) reported that decreased hepatic $T_3$ generation in foetal and neonatal rats was secondary both to a decrease in sulphydryl (SH) groups and a decrease in the deiodinating enzyme, whereas Chopra (1978) found that low conversion of $T_4$ to $T_3$ in foetal sheep liver was related more to the status of SH groups than to a deficiency of the monodeiodinating enzyme.

Chopra (1977) showed that kidney homogenates appeared more potent than liver homogenates in conversion of $T_4$ to $T_3$. Deiodinase activities in rat liver are associated with the endoplasmic reticulum (Auf dem Brinke et al. 1979) while, in kidney, plasma membranes are the predominant site of $T_4$ to $T_3$ conversion (Leonard & Rosenberg 1978). It is not clear why the post-natal change of renal $T_3$ generation was different from that of hepatic monodeiodination. Increased activity of 5′-deiodinase or suppressed activity of 5-deiodinase in kidney might be associated with a transient and exaggerated rise of $T_3$ generation for 3–4 weeks after birth. In a preliminary experiment we found that there was a difference in the dose-response of $T_3$ generation to exogenous L-$T_4$ between kidney and liver homogenates. Renal $T_3$ generation increased linearly in response to the L-$T_4$ dose, while hepatic $T_3$ generation was found to have its optimal L-$T_4$ dose and was suppressed at higher doses. These results lead to the hypothesis that post-natal changes of renal and hepatic monodeiodination progress independently.

Regarding the post-natal change of serum thyroid hormone concentrations, our data showed the same tendency as that found by van der Heide & Ende-Visser (1980), although thyroid hormone levels in our data were slightly lower. Before 14 days of age, functional activity of the thyroid was relatively immature and derangement of thyroid hormone secretion and metabolism had to be estimated, since $T_3/T_4$ ratios showed wide variations until day 14. After 14 days of age, the wide variations in the ratios stabilized and a reciprocal change in which serum $T_3$ (or $T_3/T_4$ ratio) rose and serum $rT_3$ (or $rT_3/T_4$ ratio) fell was observed. This indicates that $T_4$ is predominantly deiodinated via the $T_3$ pathway, not via $rT_3$, in this period. In humans, a similar reciprocal change is recognized 24–72 h after birth (Carella et al. 1977). These results are explained by the greater metabolic clearance rate of $rT_3$ compared to that of $T_3$ (Balsam et al. 1978; Gavin et al. 1977).
However, the reciprocal relationship found in rats after 14 days of age seems to be related to a prominent increase in renal T₃ generation noted during this period, because changes in serum T₃ levels after 14 days of age can not be explained by post-natal changes of hepatic monodeiodination alone. In accordance with the changes, most of the thyroid hormone-dependent maturational processes including development of the central nervous system and induction of several enzyme activities (Balázs et al. 1971; Hamburgh & Flexner 1957; Schapiro 1968) become manifest in this period. These findings suggest that the kidney may play a significant role in the converting system of T₄ to T₃ during maturation when the metabolic requirement for T₃ is high. The definite evidence for the physiological role of the kidney in T₄ monodeiodination, however, awaits kinetic studies on renal T₃ production rate.

References


Maciel M, Ozawa Y & Chopra I J (1979): Subcellular localization of thyroxine and reverse triiodothyronine...

Received on March 27th, 1981.