Ontogeny of thyroid peroxidase activity in perinatal rats

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Abstract. The ontogeny of thyroid peroxidase (TPO) activity was investigated in rat foetuses and neonates. From day 19 to 22 of gestation in intact pregnant rats, the TPO activity in their foetuses increased with foetal age. Following maternal treatment with propylthiouracil (PTU), the TPO activity markedly increased in foetuses on and after day 20 of gestation. The TPO activity in encephalestomized foetuses increased as markedly as that in intact littersmates, whereas that in hypophysectomized littersmates failed to increase. Newborn rats nursed by mothers treated with PTU had a TPO activity similar to that in controls of untreated mothers. There was also no difference in the TPO activity between hypophysectomized adult females and intact adult ones. These observations show that in foetal rats, TPO activity increases disproportionately to the thyroid weight, but not in newborn and adult rats, and suggest that preferential synthesis of this enzyme occurs in addition to cell hypertrophy during foetal life.

Thyroid peroxidase is needed in the process of thyroid hormone synthesis (Taurog 1976; Deme et al. 1978). Thyrotrophin (TSH) controls the TPO activity in adult rats (Yamamoto & DeGroot 1974; Nagataki et al. 1973; Nagasaka & Hidaka 1976). There is a positive relationship between TPO activity and serum TSH concentration in animals with goitre induced by treatment with propylthiouracil (PTU) or a low iodine diet (Nagataki et al. 1973; Nataf et al. 1978). The foetal hypophysis regulates the growth and function of the foetal thyroid in the rat, since the growth of the foetal thyroid is retarded by foetal hypophysectomy, and since retardation can be prevented by injection of TSH into hypophysectomized foetuses (Hwang & Wells 1959; Sethra & Wells 1951; Eguchi et al. 1971). Strum et al. (1971) reported that the TPO activity can be detected histochemically on the surface of apical microvilli of follicular cells in rat foetuses and the activity increases with foetal age.

All these studies have indicated that the thyroid hormone synthesis begins to occur already during the prenatal life in the rat. However, it is still obscure to what extent TSH participates in the prenatal development of TPO activity.

The present study was designed to elucidate the ontogeny of the TPO activity in foetuses of intact or goitrous pregnant rats. The participation of the hypothalamus and/or the hypophysis in this activity was also investigated using encephalestomized or hypophysectomized foetuses. Furthermore, changes in the TPO activity were observed in the neonatal young reared by intact or goitrous mother rats.

Materials and Methods

Animals

Rats of the Jcl:SD strain, purchased from CLEA Japan, Inc., were used. They were maintained in an air-conditioned room at 25 ± 1°C with a relative humidity of 55 ± 10% with a lighting schedule of 12 h light (08.00–20.00) and 12 h darkness, and given a commercial diet (CA-1 pellets, CLEA Japan, Inc.) and tap water.
both ad libitum. The day on which mating was detected by the presence of sperm in the vaginal smear was regarded as day 1 of gestation.

Procedure of measurement of TPO activity
The thyroids were quickly removed under a dissecting microscope and weighed. To measure the thyroid peroxidase (TPO) activity, each thyroid was laid on a piece of saline-moistened paper and stored at −20°C until determination by the method established in our laboratory (Harauchi & Yoshizaki, in press). Briefly, each thyroid was homogenized in ice-cold 0.25 M sucrose in a final volume of 3.0 ml (foetus) or in a final volume of 5.0 ml (adult). Each homogenate was centrifuged at 800 × g for 10 min. The supernatants were re-centrifuged at 100 000 × g for 60 min. The sediments were suspended in 1.0 ml of 0.2 M Na-K buffer at pH 7.4, and 0.5 ml of each suspension was used to measure the TPO activity. Another portion was used for measurement of the protein content. The suspension of the TPO activity measurement was mixed in 0.25 M Na-K buffer at pH 7.4, 1% sodium oxalate and 0.05 M guaiacol. Each mixture was pre-incubated at 37°C for 3–4 min, then mixed with 0.1 ml of 8.8 mM H_{2}O_{2} and incubated at 37°C for 60 seconds, and the reaction was stopped by adding 0.1 ml of 6 N HCl. Then 0.5 g NaCl and 5.0 ml cyclohexane were added to each mixture followed by shaking for 10 min. The extracts were used for fluorescent assay with Ex at 300 nm and Em at 340 nm. A 10 μM solution of 2,2'-dihydroxydiphenyl was used as a standard solution corresponding to 10 μU of peroxidase activity. The activity was expressed in per cent to corresponding controls.

Procedure of histological examination
Some thyroids were fixed in Bouin’s fluid and dehydrated in a graded series of alcohol, embedded in paraffin and sectioned at 7 μm. The sections were stained with PAS-haematoxylin. The average height of follicular cells in each thyroid was determined by measuring 20 cells in the largest section per gland with an ocular micrometer. Follicles located in the peripheral portion of the gland were selected for measurements.

The data obtained were statistically analyzed by Student’s t-test.

Design of experiments

Experiment 1. Foetuses of the intact pregnant rats were obtained by Caesarean section on each day from day 18 to day 22 of gestation in order to study the normal development of their thyroid peroxidase (TPO) activity.

Experiment 2. Forty mg of propylthiouracil (PTU) was suspended in 1 ml of 0.5% aqueous acacia. Pregnant rats were given 1 ml of this PTU suspension (experimental) or 1 ml aqueous acacia alone (control) by stomach tube for two consecutive days, 16–17, 17–18, 18–19, 19–20 or 20–21 of gestation. The rats were autopsied on the third day, and foetuses were delivered by Caesarean section. The TPO activities of the foetuses and mothers were determined. Histological observation of the thyroid was performed.

Experiment 3. Pregnant rats were subjected to midventral laparotomy under ether anaesthesia for foetal encephalectomy or hypophysectomy on day 20 of gestation. A needle (30 mm long and 0.6 mm in diameter for encephalectomy and 0.8 mm for hypophysectomy) attached to a glass syringe was inserted through the uterine wall to pierce the forehead towards the centre of the foetal skull. For encephalectomy, the foetal brain was aspirated and the hypophysis was left intact in situ. For hypophysectomy, the needle excavated the sella turcica, and the hypophysis was aspirated together with brain tissues. Completion of surgery was confirmed histologically. Incomplete specimens were discarded. Operated pregnant rats were given 40 mg PTU on days 20 and 21 of gestation, autopsied on next day (day 22), and subjected to histological evaluation of the thyroids and determination of the TPO activity in their intact, hypophysectomized or encephalactomized foetuses.

Experiment 4. Post-partum mother rats were given 1 ml of 0.5% aqueous acacia (control) or 40 mg PTU (experimental) daily for 3 days from the 7th to 9th day of lactation. Their neonates were autopsied on the day after the last treatment (the 10th day of post-natal age) for determination of the TPO activity.

### Table 1

<table>
<thead>
<tr>
<th>Foetal age at autopsy</th>
<th>No. of foetuses</th>
<th>TPO activity (μU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PTU</td>
</tr>
<tr>
<td>Foetus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>9 (4)a</td>
<td>34.2 ± 13.8b</td>
</tr>
<tr>
<td>20</td>
<td>8 (4)</td>
<td>92.6 ± 28.6</td>
</tr>
<tr>
<td>21</td>
<td>13 (6)</td>
<td>180.1 ± 50.8</td>
</tr>
<tr>
<td>22</td>
<td>14 (7)</td>
<td>254.6 ± 58.0</td>
</tr>
<tr>
<td>Adult**</td>
<td>8</td>
<td>539.0 ± 101.6</td>
</tr>
</tbody>
</table>

*: Values differ significantly from the corresponding control group at the 5% level. **: Ten week old females were given daily 40 mg PTU po for 2 consecutive days and were autopsied on the next day. Thyroid glands were used for the measurement of TPO activity, which was calculated from the fluorescent intensity of 2,2'-dihydroxydiphenyl. a: One determination was performed for each litter using 2 or 3 littermates. b: Mean ± sd.
Experiment 5. Adult female rats (10 weeks old) were anaesthetized ip with 13 mg sodium pentobarbital. Hypophysectomy was done by the external auditory approach. The rats were autopsied 2 days later for determination of the TPO activity. Sham-operated rats served as controls.

Results

TPO activity in foetuses from intact pregnant females

The TPO activity became detectable in 19 day old foetuses. The activity increased linearly thereafter. In 22 day old foetuses, the activity was seven times that in 19 day old ones (Table 1).

TPO activity in rat foetuses from mothers treated with PTU

The TPO activity in foetuses from mothers treated with PTU drastically increased on and after day 20 of gestation, compared with the control (Table 1). Histological observations on the foetal thyroids showed an increased height of the follicular cells and an increased number of colloid droplets within a follicular cell. The maternal TPO activity of the PTU-treated groups was comparable to that of the control.

TPO activity in encephaleectomized or hypophysectomized foetuses from mothers treated with PTU

In encephaleectomized foetuses and their intact littermates from mothers treated with PTU, their TPO activities as well as thyroid weights markedly increased to about a similar extent, but the activity in hypophysectomized littermates failed to increase (Fig. 1). Histological inspection on the thyroid clearly showed that the height of the follicular cells and the number of colloid droplets within a folli-

Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of foetuses</th>
<th>Thyroid weight (mg)</th>
<th>Height of follicular cells (µm)</th>
<th>Follicular diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>Foetus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>No</td>
<td>40 (10)</td>
<td>1.66 ± 0.76b</td>
<td>16.6 ± 1.3b</td>
</tr>
<tr>
<td>PTU**</td>
<td>No</td>
<td>10 (5)</td>
<td>1.64 ± 0.47*</td>
<td>17.0 ± 0.6*</td>
</tr>
<tr>
<td>PTU**</td>
<td>H</td>
<td>10 (5)</td>
<td>0.80 ± 0.13</td>
<td>10.1 ± 2.5</td>
</tr>
<tr>
<td>PTU**</td>
<td>E</td>
<td>10 (5)</td>
<td>1.35 ± 0.35*</td>
<td>17.2 ± 2.8*</td>
</tr>
</tbody>
</table>

PTU: Treatment with 40 mg PTU po on day 20 and 21 of pregnancy. No: No treatment. H or E: Hypophysectomy or encephaleectomy on day 20 of pregnancy. All foetuses were autopsied on day 22 of pregnancy. *: Values differ significantly from the corresponding to hypophysectomized foetuses at the 5% level. **: These mothers have two hypophysectomized foetuses, two encephaleectomized foetuses and the other intact foetuses, respectively. a: Number of litters is given in parentheses. b: Mean ± sd.
Fig. 2.
Column and bar: mean ± sd. Open column: control (intact) newborn, C. Shaded column: newborn from mothers treated with PTU, P. (a): TPO activity. (b): thyroid weight. * Significant difference from the control at the 5% level. The total number of foetuses is shown at the bottom of each column.

Fig. 3.
Column and bar: mean ± sd. Open column: control (sham operation) female rats, C. Shaded column: hypophysectomized females rats, H. (a): TPO activity. (b): thyroid weight. The total number of females is shown at the bottom of each column.
cular cell in encephalotomized and intact litters from mothers treated with PTU increased significantly, compared with those in hypophysectomized littersmates (Table 2).

**TPO activity in newborn rats nursed by mothers treated with PTU**

In newborn rats nursed by mothers treated with PTU, the TPO activity was comparable to that of the control of untreated mothers, although the thyroid was significantly heavier than that of the control (Fig. 2).

**TPO activity in hypophysectomized adult female rats**

The TPO activity in hypophysectomized adult females was not different from that in the sham-operated control (Fig. 3).

**Discussion**

Remy et al. (1980) demonstrated cytochemically that foetal TPO first appears on day 17 of rat pregnancy. Day 17 in their study corresponds to day 18 in our dating method. Preliminarily we did detect TPO activity in foetuses on day 18 by the present method, but the level was near the limit of detectability and too low to allow calculation of the value, suggesting that TPO is present but has no physiological function in foetuses on day 18 of pregnancy. TPO activity in foetuses first appeared clearly on day 19 of pregnancy in the present study. These findings agree with those of Strum et al. (1971). Remy et al. (1980) used the Wistar strain, while Strum et al. (1971) and we used the Sprague-Dawley strain. The mean pregnant period of the Wistar strain is about one day shorter than that of the Sprague-Dawley strain. The difference of foetal maturation on the same day of pregnancy between the strains may partly explain the discrepancy between the results of Remy et al. (1980) and ours.

TPO activity increased with foetal age and was about seven times higher in foetuses of the last day of pregnancy (day 22) than in 19 day old foetuses. These results also parallel the histochemical observations of Strum et al. (1971). TPO activity is indispensable for thyroid hormone synthesis (Taurog 1976; Deme et al. 1978), which is initiated on days 18 to 19 of pregnancy in rat foetuses (Gorbman & Evans 1943; Geloso 1961). The results of Experiment 1 regarding the ontogeny of the foetal TPO activity also suggest that the thyroid hormone synthesis is initiated in 19 day old rat foetuses.

With PTU treatment, the TPO activity of foetuses markedly increased on and after day 20 of pregnancy, and the accompanying marked increases in the foetal thyroid weight and the height of the follicular cells indicate an increased concentration of TSH in circulation, as reported in the adult rat (Greer et al. 1967). Tonooka & Greer (1978) also reported that PTU given to pregnant rats induced a significant increase in plasma TSH in foetuses. Our results appear to be in accord with theirs and also agree with our previous report that the reciprocal relationship between the pituitary and the thyroid is established in foetuses on approximately days 19–20 of rat pregnancy (Eguchi et al. 1980).

In the late period of foetal life in rats, the hypothalamic-thyroid system is not fully under the control of the hypothalamus (Fujita et al. 1970). Tonooka & Greer (1978) also pointed out that control of foetal TSH secretion is independent of both the foetal and the maternal hypothalamis. According to the results of Experiment 3, the development of foetal TPO activity appears to be independent of the foetal hypothalamus as well, because the TPO activity of encephalotomized foetuses increased as markedly as that of their intact littermates, whereas that of the hypophysectomized littermates failed to increase.

The TPO activity of newborn rats nursed by mothers treated with PTU was comparable to that of the control of untreated mothers, although the neonatal thyroids in the PTU group were significantly heavier than those in the control (Experiment 4). Therefore, in the neonatal stage the increase of thyroid weight is not necessarily accompanied by an increment of physiological function although PTU from milk produces an increase in thyroid weight. In Experiment 5, the TPO activity of adult female rats, which were hypophysectomized and autopsied 2 days later, showed no difference from that of the control. Thus, with PTU treatment, the TPO activity increases significantly in foetuses but not in newborn rats although the weight of the thyroids increases significantly in both foetuses and newborn rats. The foetal hypophys has the capacity to augment TSH secretion in response to chemical thyroidectomy induced by
PTU (D’Angelo 1967; Tonooka & Greer 1978), but this is not the case in early post-natal life (D’Angelo 1967). The changes we observed in the TPO activity with PTU treatment in perinatal life are quite in line with these earlier TSH observations.

In conclusion, our results show that TPO activity is present in 19 day old foetuses, in parallel with the reported presence of thyroid hormone synthesis (Strum et al. 1971), and that the foetal TPO activity is controlled chiefly by the foetal hypophysis itself. The control in foetuses seems to be stronger, with PTU treatment, than in newborn or adult rats.

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References


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