Inhibition of Placental Thyroxine 5-Deiodinase Activity Decreases Amniotic Fluid Concentration of 3,3',5'-Triiodothyronine in Rat

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Abstract. We investigated whether the inhibition of placental T4 5-deiodinase (5-D) activity would decrease the amniotic fluid (AF) concentration of rT3. Iopanoic acid (IOP) was used to inhibit placental T4 5-D activity. From gestation days 14 to 17, a group of rats received IOP (10 mg/100 g bw/day, sc) once daily in experiment (exp) 1, and received it (40 mg/100 g bw/day) four times daily in exp 2. In exp 2, control dams received propylthiouracil (PTU; 2 mg/100 g bw/day) instead of IOP. Methimazole and T4 were also given to all dams in exp 1 and 2. On day 17 of gestation, we collected the liver, placenta, blood, and AF of each animal. In the IOP-treated group in both experiments, the serum T4 concentration was significantly increased. Hepatic T4 5'-deiodinase activity was significantly decreased by either PTU or IOP administration. In both experiments placental T4 5-D activity was significantly decreased in the IOP-treated group. The concentration of rT3 in AF was significantly decreased in the IOP-treated group in exp 2 (1.71 vs. 0.75 nmol/l, P<0.01) despite a higher serum T4 concentration. There was a significant positive correlation between placental T4 5-D activity and the concentration of rT3 in AF in exp 2 (r=0.62, P<0.05). These observations indicate that the inhibition of placental T4 5-D activity decreases the concentration of rT3 in rat AF, and that placental T4 5-D and the T4 concentration in maternal serum plays important roles in maintaining the concentration of rT3 in rat AF.

Key words: T4 5-deiodinase, Thyroid hormone, Placenta, Amniotic fluid, Iopanoic acid.

THE PLACENTA of human and rat contains type III T4 5-deiodinase (5-D) which converts T4 to rT3 [1–6]. Placental T4 5-D activity is abundant in the chorion rather than the amnion in human [7]. At the subcellular level, it is abundant in microsomal and mitochondrial fractions [3]. Unlike type I and type II T4 5'-deiodinase [8, 9], placental T4 5-D activity is reported to be independent of either the maternal or fetal thyroid state [5, 6, 10, 11]. Iopanoic acid (IOP), which inhibits the activity of T4 5'- and 5-deiodinase in the liver [8], kidney [12, 13], brain [14, 15], pituitary gland [9, 16], and other organs, also inhibits placental T4 5-D activity in vitro [17].

Although the physiological role of placental T4 5-D is unclear, it may limit the maternal-fetal transfer of T4. It might also be related to the production of rT3 in amniotic fluid (AF) [5, 7, 10]. It is unclear, however, whether a change in placental T4 5-D activity leads to a change in the rT3 concentration in AF. Moreover there is little
information on the effects of IOP administered in vivo on placental T4 5-D activity and on the concentration of rT3 in AF. In the present study, we administered IOP to pregnant rats in vivo to evaluate its effect on placental T4 5-D activity and the concentration of rT3 in AF.

**Materials and Methods**

**Reagents**

L-T4, L-T3, L-rT3, propylthiouracil (PTU), and dithiothreitol (DTT) were obtained from Nacalai Tesque Inc. (Kyoto, Japan), and IOP from Sigma Chemical Co. (St. Louis, MO). Methimazole (MMI) was a gift from Chugai Pharmaceutical Co. (Tokyo, Japan). Outer ring 125I-labeled rT3 and T3 were obtained from Dainabot Co. (Tokyo, Japan). All other reagents were of analytical grade.

**Animal treatment**

Pregnant Sprague-Dawley rats (200–300 g), mated at the supply house, were obtained from Charles River Japan Inc. (Kanagawa, Japan), and were fed Funabashi laboratory chow (Funabashi Farm Inc., Chiba, Japan) and tap water ad libitum in a light-dark and temperature-controlled room. The study protocol is as follows. 0.05% MMI was administered in the drinking water from days 14 to 17 of gestation in experiment (exp) 1, and from days 13 to 17 of gestation in exp 2. In exp 1, T4 (10 µg/100 g bw/day), IOP (10 mg/100 g bw/day; IOP-treated group) and vehicle (alkaline saline solution; control group) were administered by subcutaneous injection once daily from days 14 to 17 of gestation. In exp 2, however, T4 (10 µg/100 g bw/day), PTU (2 mg/100 g bw/day; control group), and IOP (40 mg/100 g bw/day; IOP-treated group) were administered by subcutaneous injection four times daily every 6 h from days 14 to 17 of gestation. Control animals (group A) were from the same shipment as the treated rats (group B) in each experiment.

**Preparation of samples**

On day 17 of gestation, animals were anesthetized with ether and sacrificed by decapitation 90 min after the last subcutaneous injection of reagents. Trunk blood was collected, and the liver and placenta were removed from each animal. AF was collected from the amniotic sac of each fetus by amniocentesis and pooled for each litter. Following centrifugation, sera and AF were stored at −50°C until used.

All the subsequent tissue preparations were performed at 4°C. Placentas were weighed, washed, minced, and homogenized in 5 vol (wt/vol) 250 mM sucrose, 25 mM Tris-HCl, pH 7.5 (sucrose-Tris buffer) using a Polytron homogenizer (Kinematica, Switzerland). The homogenates were centrifuged at 800 × g for 10 min. The supernatants were then centrifuged at 100,000 × g for 60 min. The pellets were then resuspended in 0.05 M Tris-HCl, pH 7.5 (Tris buffer). This is referred to as the mitochondrial-microsomal fraction (M fraction). The M fractions of the placenta were used in determining placental T4 5-D activity. The livers were homogenized in Tris buffer with a Polytron homogenizer. Supernatants obtained following centrifugation at 800 × g for 10 min are referred to as homogenates, and were used as the source of type I T4 5’-deiodinase (5’-DI).

**Deiodinase assay**

The deiodinase assay was performed as previously described [4–6]. The placental M fractions (500 µg protein each) were incubated with T4 (2 ng/tube, the final concentration 12.9 nM) for 60 min at 37°C in Tris buffer, containing 10 mM DTT. The final volume of the reaction mixture was 200 µl. The reaction was stopped by adding 400 µl of 95% ethanol. The mixture was allowed to stand overnight at 4°C. The rT3 present in the ethanol extract was determined by RIA as previously described [4, 5]. Blanks (zero incubation) for each sample preparation were treated as described, except that unlabelled T4 was added just before ethanol extraction. The net concentration of rT3 produced was calculated by subtracting the values for the rT3 concentration obtained in blanks from those measured in test samples.

For the assay of hepatic T4 5’-DI activity, the liver homogenates (1 mg protein each) were incubated with T4 (0.5 µg/tube, the final concentration 0.64 µM) for 30 min at 37°C in Tris buffer, containing 1 mM DTT. The subsequent procedure for the determination of the net T3 production from T4 in the liver homogenates was the
same as described above [4, 18].

The protein concentration was measured by the method of Lowry et al. [19], using bovine serum albumin as the standard.

Iodothyronines in serum and AF

The concentration of rT₃ in maternal serum and AF were determined as previously described [5, 6]. A 300 µl aliquot of serum and of AF was mixed with 600 µl of 99% ethanol in a vortex mixer and centrifuged at 800 × g for 10 min. A 200 µl aliquot of the supernatant was used directly for determination of rT₃ by RIA. The serum concentration of T₃ was also measured by RIA [18]. The serum concentration of T₄ was measured with either a RIA kit or an enzyme immunoassay kit (Dainabot Co., Tokyo, Japan).

Statistical analyses

Assays were performed in duplicate or triplicate. Values were reported as the mean ± SD. Statistical analyses were performed by Student's unpaired t-test and Wilcoxon's rank-sum test. Correlations between two different variables were calculated by linear regression analyses and Spearman's rank correlation test. A level of P<0.05 was accepted as statistically significant.

Results

Maternal serum T₄, T₃, and rT₃ concentration (Table 1)

In exp 1, the concentrations of T₄ and rT₃ in maternal serum were significantly increased, while the T₃ concentration was significantly decreased in the IOP-treated group. In exp 2, the concentrations of T₄ and rT₃ in maternal serum were increased, while those of serum T₃ were very low in both groups. There was no significant difference in the serum concentrations of T₃ and rT₃ in either group, but that of serum T₄ in the IOP-treated group significantly exceeded that of the control group (P<0.001).

Hepatic T₄ 5'-DI activity

IOP almost completely inhibited hepatic T₄ 5'-DI activity in exp 1 (Fig. 1): there was 14.29±2.93 pmol T₃/mg protein·h in the control group vs. 0.34±0.25 pmol T₃/mg protein·h in the IOP-treated group (P<0.001). In exp 2, the hepatic T₄ 5'-DI activity of both groups was significantly suppressed. That of the control (PTU-treated) group, 0.15±0.25 pmol T₃/mg protein·h, was slightly, but significantly, lower than that of the IOP-treated group, 0.63±0.45 pmol T₃/mg protein·h (P<0.05). In exp 1, there was a significant positive correlation between hepatic T₄ 5'-DI activity and the maternal serum concentration of T₃ (r=0.92, P<0.001). There were significant negative correlations between hepatic T₄ 5'-DI activity and the maternal serum concentrations of T₄ and rT₃ (r=-0.72, P<0.02, in both T₄ and rT₃, data not shown).

Placental T₄ 5-D activity

The results of the in vitro kinetic study performed with the placentas from the control group in exp 1 indicated that IOP is a competitive inhibitor of the conversion of T₄ to rT₃ (Fig. 2). In the absence of IOP, the apparent Michaelis constant (Km) for T₄ was 12.7 nM.

### Table 1. Maternal serum and amniotic fluid concentrations of iodothyronines

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Serum T₄ (nmol/l)</th>
<th>Serum T₃ (nmol/l)</th>
<th>Serum rT₃ (nmol/l)</th>
<th>Amniotic fluid rT₃ (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A(6)</td>
<td>231.7±25.7</td>
<td>1.69±0.31</td>
<td>4.55±0.39</td>
<td>0.66±0.18</td>
</tr>
<tr>
<td></td>
<td>B(4)</td>
<td>404.1±115.8*</td>
<td>0.39±0.09**</td>
<td>7.72±2.33*</td>
<td>0.41±0.34</td>
</tr>
<tr>
<td>2</td>
<td>A(6)</td>
<td>187.9±18.0</td>
<td>&lt;0.23</td>
<td>9.69±2.94</td>
<td>1.71±1.41</td>
</tr>
<tr>
<td></td>
<td>B(6)</td>
<td>289.6±39.9**</td>
<td>&lt;0.23</td>
<td>7.02±1.33</td>
<td>0.75±0.13*</td>
</tr>
</tbody>
</table>

Group A was the control and group B was treated with IOP in each experiment. Values are the mean±SD. *P<0.01 and **P<0.001 vs. group A in each experiment. Numbers in parentheses are the numbers of dams.
Preliminary studies revealed that IOP maintained its inhibitory effect on placental T4 5-D activity for at least 4.5 h, but lost it 24 h after the injection. Therefore, in exp 2, we administered T4, IOP, and PTU four times (every 6 h) daily to maintain the inhibitory effect of IOP for as long as possible.

IOP administered in vivo strongly inhibited placental T4 5-D activity in both experiments (Fig. 3). The placental T4 5-D activity of the IOP-treated group was 37% of the control group in exp 1 (1.72±0.31 vs. 0.63±0.26 pmol rT3/mg protein·h, \( P<0.001 \)) and 34% of the control group in exp 2 (5.31±0.91 vs. 1.80±0.40 pmol rT3/mg protein·h, \( P<0.001 \)).

AF rT3 concentration

The concentration of rT3 in AF of the IOP-treated group in exp 1 was decreased, but not to a significant extent (Table 1). However, in exp 2, the concentration of rT3 in AF of the IOP-treated group was significantly decreased (1.71±1.41 vs. 0.75±0.13 nmol/l, \( P<0.01 \)). In the latter experiment there was a significant positive correlation between placental T4 5-D activity and the concentration of rT3 in AF (r=0.62, \( P<0.05 \), Fig. 4).
Discussion

It has been clearly documented that the placenta of human and rat possesses type III T4 5-D activity [1-7, 10, 11]. It is suggested that placental T4 5-D plays an important role in maintaining the rT3 concentration in AF for the following reasons: 1) placental T4 5-D converts T4 to rT3 [1, 2]; 2) Age-dependent changes in placental T4 5-D activity occur, and may affect the rT3 concentration in AF [5, 10]; 3) The AF concentration of T4 and T3 is much lower, and that of rT3 is much higher, than the corresponding values in maternal serum [20]. It has not been shown directly, however, whether a change in placental T4 5-D activity leads to a change in the concentration of rT3 in AF.

Previous studies have revealed that placental T4 5-D activity is independent of the maternal thyroid state [5, 6, 10, 11], and that it differs significantly from type I T4 5'-deiodinase in the liver and kidney [8, 21], and from type II T4 5'-deiodinase in the brain [14] and pituitary gland [9]. We are not aware of any information about the way the placental T4 5-D is activated. IOP is known to inhibit the activity of iodothyronine deiodinase in various tissues. A previous study with an in situ perfused guinea pig placenta model showed that IOP inhibited the deiodination of T3 [22]. We therefore administered IOP in vivo to pregnant rats to determine whether the inhibition of placental T4 5-D activity would decrease the concentration of rT3 in AF. T4 was also administered to dams in order to increase the rT3 concentration in AF and to emphasize its fall.

In this study, the administration of IOP in vivo caused a rise in serum concentrations of T4 and rT3 and a fall in serum concentration of T3 in rats,
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as previously described in humans and rats [23–26]. It is known that, in such peripheral tissues as the liver and kidney, T₄ is deiodinated to T₃, and rT₃ is deiodinated to 3,3′-T₂ by type I 5′-deiodinase [27]. We demonstrated that hepatic T₄ 5′-DI activity is completely inhibited by IOP. We found a positive correlation between hepatic T₄ 5′-DI activity and the maternal serum concentration of T₃, and negative correlations between hepatic T₄ 5′-DI activity and the maternal serum concentrations of T₄ and rT₃ in exp 1. These observations suggest that the inhibition of type I 5′-deiodinase activity by IOP reduces the rate of degradation of T₄ and of rT₃ and the rate of production of T₃ from T₄ as previously reported [8, 12]. In exp 2, the administration of PTU also produced a complete inhibition of hepatic T₄ 5′-DI activity [8, 12].

Fay et al., using human placental homogenates, demonstrated that IOP inhibited placental T₄ 5′-D activity in vitro [17]. They also performed a kinetic study and showed the apparent Km of human placental T₄ 5′-D to be 1.2 × 10⁻⁷M. We demonstrated that the apparent Km of rat placental T₄ 5′-D was 1.3 × 10⁻⁸M, similar to that of rat brain type III deiodinase for T₄ described by Kaplan et al. [28]. Differences between the apparent Km values may be due to differences in experimental conditions. In this study, IOP was shown to be a competitive inhibitor of the conversion of T₄ to rT₃ in the rat placenta. IOP has been reported to act as a competitive inhibitor of type I deiodinase [29].

The administration of IOP in vivo produced a considerable inhibition of placental T₄ 5′-D activity in both of our experiments. The inhibition rate of placental T₄ 5′-D activity was 63% in exp 1 and 66% in exp 2. This similarity in the inhibition rate might be due to the same dose of IOP injected 90 min before sacrifice. In spite of the considerable inhibition of placental T₄ 5′-D activity, the concentration of rT₃ in AF of the IOP-treated group significantly exceeded the control value in exp 2, the concentration of rT₃ in AF of the IOP-treated group was significantly decreased. There was a significant positive correlation between placental T₄ 5′-D activity and the concentration of rT₃ in AF in exp 2. These observations indicate that the inhibition of placental T₄ 5′-D activity decreases the concentration of rT₃ in AF. It seems that IOP administered every 6 h in exp 2 caused the persistent inhibition of placental T₄ 5′-D activity. The concentration of rT₃ in maternal serum was significantly higher in the IOP-treated group in exp 1, while no difference was observed in exp 2. Our results agree with those of a previous report which indicated that the rise in the maternal serum concentration of rT₃ was not related to the rise in the rT₃ concentration in AF [30].

The physiological and clinical significance of a high concentration of rT₃ in AF remains to be elucidated. The majority of rT₃ in AF is derived from inner ring deiodination of T₄ in the placenta. And the amount of rT₃ entering AF due to fetal urinary excretion and filtration from maternal serum is very small [20, 30]. Although placental T₄ 5′-D also converts T₄ derived from the fetal circulation to rT₃, its contribution to the maintenance of rT₃ in AF seems to be negligible [30]. A previous report has demonstrated that the levels of rT₃ in AF were not useful in predicting fetal hypothyroidism [32]. The entry of rT₃ into the fetal circulation from AF due to fetal swallowing [20] may be important in maintaining appropriate metabolism of thyroid hormone. The activity of type II 5′-deiodinase in the rat brain and pituitary
gland has been shown to be inhibited by rT3 administered *in vivo* [33], and therefore the high concentration of rT3 in fetal serum may modulate the activity of iodothyronine deiodinases.

In conclusion, the present study indicates that the inhibition of placental T4 5-D activity decreases the concentration of rT3 in rat AF, and that the placental T4 5-D, as well as the maternal serum concentration of T4, plays important roles in maintaining the rT3 concentration in rat AF.

References

22. Castro MI, Braverman LE, Alex S, Wu C-F, Emerson CH (1985) Inner-ring deiodination of


