Effect of Changes in Thyroid State on Metabolism of Thyroxine by Rat Placenta

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Abstract

We studied the effect of the state of the thyroid on T_4 monodeiodination in the rat placenta, and it was compared with those in the liver and kidney. The tissues, maternal serum, and amniotic fluid were obtained from pregnant rats. The tissues were homogenized in cold 50 mM Tris-HCl buffer, pH 7.5. The homogenate (1 mg protein) was incubated at 37°C for 60 min with 1 μ g T_4 in the presence of 5 mM DTT. The T_3 and reverse T_3 generated in the reaction mixture were extracted into cold ethanol and measured by RIAs. The conversion of T_4 to reverse T_3 in rat placenta was not significantly changed in MMI-induced hypothyroidism or T_4 induced hyperthyroidism. On the other hand, conversion of T_4 to T_3 in the liver and kidney were changed in parallel with the thyroid state. The concentration of reverse T_3 in the amniotic fluid was increased in accordance with the increase in the maternal serum T_4 concentration. These results indicate that the placental T_4 inner ring deiodination is not affected by the thyroid state, and that the change in the amniotic fluid reverse T_3 concentration in this study is mainly dependent upon the change in maternal thyroid function.

Studies in man and animals have demonstrated that changes in the thyroid state have an influence on the metabolism of thyroxine (T_4) in peripheral tissues such as the liver, kidney (Balsam *et al.*, 1978; Kaplan and Utigar, 1978; Harris *et al.*, 1978), brain (Kaplan and Yoskoski, 1980) and pituitary gland (Kaplan, 1980; Maeda and Ingbar, 1982). Recently it was reported that rat (Roti *et al.*, 1982b), human (Roti *et al.*, 1981; Suzuki *et al.*, 1983) and guinea pig (Cooper

et al., 1983) placentas actively deiodinate the inner ring of T_4 to 3, 3', 5'-triiodothyronine (reverse T_3) and this might be one of the causes of placental impermeability of thyroid hormone. There has been little information, however, about the effect of the thyroid state on the deiodination of T_4 to reverse T_3 in the placenta. In the present paper, we describe the effect of the thyroid state on T_4 monodeiodination in rat placental homogenate, as compared with those in the liver and kidney.

Materials and Methods

Treatment protocol

Pregnant Sprague-Dawley rats were obtained from Charles River Japan Inc. (Kanagawa, Japan). Animals were mated at the supply house. Pregnant rats were housed in separate cages with free access to Funabashi laboratory chow (Funabashi Farm Inc., Chiba, Japan) and water in a temperature and light controlled room. Treatment protocols for various thyroid states are summarized in Table 1. In experiment (exp) 1, rats were administered 0.05% methylmercaptoimidazole (MMI) in drinking water from the 11th day to the 17th day of gestation. In addition, Group I and II were prepared by a subcutaneous injection of 15 μ g or 1.5 μ g T_4 /100 g body weight (BW)/day in 0.01 N NaOH, respectively, and Group III by a subcutaneous injection of the vehicle. In exp 2, Group I was prepared by subcutaneous injection of 10 μ g T_4 /100 g BW/day and Group III by 0.05% MMI in drinking water, respectively, from the 10th day to 16th day of gestation. In exp 3, Group I was prepared by the same method for exp 2 from the 14th day to the 20th day of gestation. Group II of each exp is a control group. Furthermore, to evaluate the effect of fetal hyperthyroidism on placental T_4 to reverse T_3 conversion, 10 μ g T_4 was injected into the amniotic sacs of 6 rats on the 17th day of gestation because T_4 injection into the amniotic fluid is known to be an effective method of increasing the fetal serum T_4 concentration (Klein *et al.*, 1978). One hundred μ l of 0.01 N NaOH was injected into each amniotic sac of the control group.

Tissue preparation and incubation procedure

On the 18th day (exp 1), 17th day (exp 2) or 21st day (exp 3) of gestation, the rats were killed by decapitation and trunk blood was collected. The abdomens were rapidly opened and the placentas, livers and kidneys were removed. In exp 2, besides these tissues, the amniotic fluid was obtained by needle aspiration from each amniotic sac. All amniotic fluids from each rat were pooled. Not all amniotic fluids were available for the measurement of reverse T_3 . Sera and amniotic fluids were stored at -20°C after centrifugation until use. The placentas of the intraamniotic fluid T_4 injected rats were removed on the 20th day of gestation. There was insufficient sera available in fetuses to permit thyroid hormone measurement.

The homogenates of placentas, kidneys and livers were prepared with 5 vol of cold 50 mM Tris-HCl buffer, pH 7.5. One mg protein of each tissue homogenate was incubated with 1 μ g T_4 in the presence of 5 mM dithiothreitol (DTT) for 60 min at 37°C , and thereafter the net 3, 5, 3'-triiodothyronine (T_3) and reverse T_3 generated were extracted into cold ethanol and measured by the radioimmunoassays (RIAs) already reported (Yoshida *et al.*, 1984). The net T_4 to T_3 conversion in the placenta and the net T_4 to reverse T_3 conversions in the liver and kidney were negligible. There was no measurable reverse T_3 degradation when 4 ng reverse T_3 was added to rat placental homogenates, and incubations were carried out for 60 min (Yoshida *et al.*, 1984). The amniotic fluid reverse T_3 concentration (Yoshida *et al.*, 1985) and maternal serum T_4 (Sakurada *et al.*, 1976) and T_3 (Sakurada *et al.*, 1973) concentrations were also measured by RIAs. T_4 to reverse T_3 conversion in the placenta and

Table 1. Treatment Protocol

Exp No.	Duration of T_x	Group	No.	T_x
1	Gestational day 11 to 17	I	(n=8)	MMI*, T_4 15 μ g/100 g BW/day SC
		II	(n=9)	MMI*, T_4 1.5 μ g/100 g BW/day SC
		III	(n=8)	MMI*, NaOH SC**
2	Gestational day 10 to 16	I	(n=8)	—, T_4 10 μ g/100 g BW/day SC
		II	(n=6)	—, NaOH SC**
		III	(n=7)	MMI*, NaOH SC**
3	Gestational day 14 to 20	I	(n=5)	—, T_4 10 μ g/100 g BW/day SC
		II	(n=6)	—, NaOH SC**

* 0.05% MMI in drinking water

** 0.01 N NaOH 0.1 ml/100 g BW/day SC

T₄ to T₃ conversion in the liver and kidney were not affected by *in vitro* addition of 1×10^{-5} M MMI.

L-T₄, L-T₃ and DTT were obtained from Sigma Co., St. Louis, Mo. and L-reverse T₃ from Henning Co., Berlin. Labelled T₄, T₃ and reverse T₃ were kindly donated by Dainabot Co., Japan and anti-reverse T₃ serum was presented by Dr. Braverman, Worcester, MA. All other reagents were of the highest grade commercially available. The protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical analyses were conducted by Student's unpaired *t*-test. The results are described as the mean $\pm$ SD. Correlations between two different variables were calculated by linear regression analyses.

Results

Maternal serum thyroid hormone concentrations

Table 2 shows maternal serum T₄ and T₃ concentrations. The results indicate that the treatment protocols described above were sufficient to induce an abnormal thyroid state.

T₄ monodeiodination in tissue homogenates

The results are shown in Table 3. T₄ to reverse T₃ conversion in the placentas of Groups I and III was not significantly different from that of Group II in all 3 experiments. In the liver and kidney, T₄ to

T₃ conversion of Group I was significantly greater than the corresponding Group II in all experiments, and that of Group III declined though there was no statistical significance between Group III and II kidneys in exp 2.

Table 4 shows the results of the net T₄ to reverse T₃ conversions of placentas ob-

Table 2. Maternal Serum T₄ and T₃ Concentrations

Exp No.	Group No.	Serum T ₄ (μ g/dl)	Serum T ₃ (ng/dl)
1	I (n=8)	$2.3 \pm 0.6^{a***}$	$129 \pm 18^*$
	II (n=9)	1.6 ± 0.4	90 ± 12
	III (n=8)	$0.6 \pm 0.2^*$	$64 \pm 7^*$
2	I (n=8)	$1.9 \pm 0.4^{a***}$	128 ± 49
	II (n=6)	1.4 ± 0.3	95 ± 11
	III (n=7)	$0.6 \pm 0.2^*$	$66 \pm 7^*$
3	I (n=5)	2.3 ± 0.7	$141 \pm 34^{**}$
	II (n=6)	1.6 ± 0.5	79 ± 17

a mean $\pm$ SD

* $p < 0.001$, ** $p < 0.01$ and *** $p < 0.05$ versus control Group II of each exp.

Table 4. Effect of Intraamniotic Fluid T₄ Injection on T₄ to reverse T₃ Conversion in Placenta

Group	T ₄ to rT ₃ conversion in placenta (ng/tube)
Intraamniotic Fluid	
T ₄ Injection (n=6)	9.9 ± 4.5
Control (n=6)	7.2 ± 3.9

Table 3. Effect of Thyroid Status on Iodothyronine Metabolism in Rat Tissue Homogenates

Exp No.	Group No	T ₄ to rT ₃ conversion in placenta (ng/tube)	T ₄ to T ₃ conversion in liver (ng/tube)	T ₄ to T ₃ conversion in kidney (ng/tube)
1	I (n=8)	22.4 ± 3.3^a	$20.2 \pm 6.8^{**}$	—
	II (n=9)	21.8 ± 3.8	10.6 ± 3.2	—
	III (n=8)	21.2 ± 3.1	$3.0 \pm 0.8^{**}$	—
2	I (n=8)	17.2 ± 2.8	$47.0 \pm 10.8^*$	$22.8 \pm 6.4^*$
	II (n=6)	20.3 ± 3.0	13.8 ± 1.4	7.8 ± 1.2
	III (n=7)	17.9 ± 4.1	$10.0 \pm 3.0^{**}$	7.4 ± 1.8
3	I (n=5)	6.3 ± 2.1	—	$16.1 \pm 3.1^{***}$
	II (n=6)	7.2 ± 2.7	—	12.7 ± 0.6

a mean $\pm$ SD

* $p < 0.001$, ** $p < 0.01$ and *** $p < 0.05$ versus control Group II of each exp.

Amniotic Fluid rT_3 Concentration and Maternal
Serum T_4 Concentration*

Fig. 1. Correlation of reverse T_3 concentration in amniotic fluid with maternal serum T_4 concentration in experiment 2.

*Exp 2

tained from the rats which had T_4 or vehicle injected into the amniotic sacs. No statistically significant differences were observed.

Amniotic fluid reverse T_3 concentrations

There is a significant correlation between the concentration of reverse T_3 in amniotic fluid and the maternal serum T_4 concentration (Fig. 1), but not between the former and the maternal serum T_3 concentration (data not shown).

Discussion

As for the effect of the change in the thyroid state on the peripheral conversion of thyroid hormone, Kaplan *et al.* (1978) reported that T_4 administration enhanced and thyroidectomy inhibited the T_4 deiodination to T_3 in rat liver and kidney homogenates. Balsam *et al.* (1978) demonstrated that the T_4 metabolism in slices of rat liver and kidney was greatly affected by both thyroidectomy and hypophysectomy and that these effects were apparently due to the lack

of thyroid hormone since they could be recovered by the administration of thyroid hormone. Our results in rat liver and kidney are in good concordance with those previously reported.

As far as we know, only one study on the effect of the thyroid state on the placental T_4 to reverse T_3 conversion in the rat has been reported (Roti *et al.*, 1982a). They reported that placental inner ring deiodinating activity was not significantly decreased in thyroidectomized rats. In the present study the conversion of T_4 to reverse T_3 in rat placenta is not significantly changed by maternal and fetal hypothyroidism induced by MMI which can pass through the placenta (Roti *et al.*, 1983). It is reported that maternal and fetal hypothyroidism is induced by the addition of 0.05% MMI to the drinking water (El-Zaheri *et al.*, 1981), therefore we think that fetal as well as maternal hypothyroidism was present in Group III in expts 1 and 2, though we did not measure the fetal thyroid hormone concentration. Furthermore, neither maternal hyperthyroidism induced by overphysiological

dose of T_4 nor fetal hyperthyroidism induced by intraamniotic fluid T_4 injection can affect the placental T_4 inner ring deiodinating activity. These effects of thyroid status on placental deiodinating activity seemed to be placenta specific, because there were significant changes in T_4 to T_3 deiodination in the liver and kidney in pregnant rats in the present study as well as in the previous reports on non-pregnant rats (Balsam *et al.*, 1978; Kaplan *et al.*, 1978; Harris *et al.*, 1978). In human placenta, we also reported that there was no significant change in the placental deiodinating activity in hyperthyroid patients (Yoshida *et al.*, 1985).

There are no data available on the quantitative contribution of placenta to daily extrathyroidal T_4 metabolism. It is likely that placental T_4 inner ring deiodination is related to the placental impermeability of thyroid hormones.

El-Zaheri *et al.* (1981) described how the maternal thyroid state plays a dominant role in maintaining amniotic fluid reverse T_3 in rat. They postulated that maternal T_4 is deiodinated to reverse T_3 in the placenta and this reverse T_3 is directly transferred to the amniotic fluid. If so, the rat amniotic fluid reverse T_3 concentration is influenced by both maternal T_4 and placental specific deiodinating activity. In man, we reported that amniotic fluid reverse T_3 concentration is influenced by both the placental T_4 deiodinating activity and maternal thyroid function (Yoshida *et al.*, 1985). In this study, the conversion rate of T_4 to reverse T_3 in the placenta was neither influenced by MMI induced hypothyroidism nor by an over-physiological dose of T_4 administration and intraamniotic fluid T_4 injection. The change in amniotic fluid reverse T_3 concentration paralleling those of maternal thyroid status observed in the present study can be accounted for solely by the increase in the substrate, maternal T_4 , but not by the change in the specific activity of the placental deiodinase.

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