Monodeiodination of Thyroxine to 3,3',5'-Triiodothyronine in the Human Placenta

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SUZUKI, M., YOSHIDA, K., SAKURADA, T., KITAOKA, H., KAISE, K., KAISE, N., FUKAZAWA, H., YAMAMOTO, M., SAITO, S. and YOSHINAGA, K. *Monodeiodination of Thyroxine to 3,3',5'-Triiodothyronine in the Human Placenta*. Tohoku J. exp. Med., 1983, 140 (3), 311-318 — We studied the characteristics of monodeiodination of thyroxine to T_3 and reverse T_3 in the human placenta which was obtained at normal delivery. The placentas were homogenized in cold sucrose Tris-HCl buffer, pH 7.5. The microsomal fraction was incubated at 37°C in the air for 1 hr with 2 μ g of T_4 in the presence of 0.05 M DTT. The T_3 and reverse T_3 generated in the reaction mixture were extracted into cold ethanol and measured by RIA. Among the usual subcellular fractions of the placental homogenate, microsomes were most potent in deiodinating T_4 to reverse T_3 , 17.9 ng/mg protein/ μ g T_4 /60 min. In microsome, production of reverse T_3 from T_4 was dependent upon protein concentration, incubation temperature, incubation time, pH and T_4 concentration, and unstable to prior heating of the microsomal fraction. The production of T_3 from T_4 was negligible in the present system. Degradation of T_3 in the human placenta was rapid. Although addition of anti- T_3 antibody to the reaction mixture suppressed the degradation of T_3 , it had no effect on the net production of T_3 , suggesting that the obtained net T_3 production rate had not been influenced by its degradation. Degradation of reverse T_3 was negligible. These results indicate that the human placenta actively deiodinates T_4 to reverse T_3 enzymatically. This enzyme system might have some influence on the trans-placental passage of thyroid hormone from the mother to the fetus. ——— monodeiodination; thyroxine; 3,3',5'-triiodothyroxine (reverse T_3); human placenta

It has been shown that peripheral monodeiodination is an important pathway of metabolism of iodothyronine (Braverman et al. 1970). Studies in man and animals, especially in rat, demonstrate that T_4 to T_3 conversion takes place in a variety of tissues, including the liver (Visser et al. 1975), kidney (Chiraseveenuprapond et al. 1978; Yoshida et al. 1982), heart (Rabinowitz and Hercher 1971), brain (Dratman and Crutchfield 1978), pituitary (Silva and Larsen 1978) and leucocytes (Woeber 1978). It is also shown that T_4 is converted to 3,3',5'-tri-

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iodothyronine (reverse T_3) in the brain (Kaplan and Yaskoski 1980), liver (Cavalieri et al. 1977), kidney (Yoshida et al. 1982) and leucocytes (Woeber 1978). Recently, it has been reported that T_4 is metabolized in the placenta (Banovac et al. 1980; Roti et al. 1981). In the present paper, we describe the T_4 monodeiodination to reverse T_3 in vitro in the human placental homogenate and subcellular fractions obtained from the placentas at normal delivery.

MATERIALS AND METHODS

Tissue preparation. Human placentas, obtained at normal delivery, were cut off and chopped into small pieces. These pieces were homogenized in 3 ml 0.025 M Tris-HCl, 0.25 M sucrose buffer (pH 7.5)/g wet tissue, using a Polytron (Kinematica, Switzerland). This crude homogenate was filtered through four layers of gauze and then centrifuged at $800 \times g$ for 10 min. The supernatant was decanted and the pellet was homogenized again with the same buffer. The pellet of the second homogenate was homogenized once again and centrifuges as above. Final pellet was suspended in 0.025 M Tris-HCl, 0.25 M sucrose buffer (pH 7.5) and termed the "nuclear fraction". The combined supernatant was termed "homogenate" and then centrifuged at $9,000 \times g$ for 20 min, and the pellet was washed twice and resuspended in the same buffer to obtain the "mitochondrial fraction". The $9,000 \times g$ supernatant was centrifuged at $100,000 \times g$ for 60 min and resulting pellet was resuspended in 0.025 M Tris-HCl, 0.25 M sucrose buffer (pH 7.5), and termed the "microsomal fraction". The $100,000 \times g$ supernatant was referred to as the "cytosolic fraction". All procedures were carried out at $4^\circ C$.

Incubation procedure and RIA for T_3 and reverse T_3 . Except as otherwise indicated, $2 \mu g$ T_4 was incubated with the placental homogenate or subcellular fraction (1 mg protein in 0.025 M Tris-HCl, 0.25 M sucrose buffer, pH 7.5) containing 0.05 M dithiothreitol (DTT) for 60 min at $37^\circ C$ in the air. The final volume of the reaction mixture was 1 ml. After incubation, the reaction was stopped by the addition of 2 ml of 95% ethanol and the mixture was allowed to stand for 16 hr at $4^\circ C$. The amounts of T_3 and reverse T_3 present in the ethanol extract obtained after centrifugation at $800 \times g$ for 10 min were measured by radioimmunoassay using specific antibodies for T_3 (Sakurada et al. 1973) and reverse T_3 (Vagenakis et al. 1975) as follows: $50 \mu l$ of ethanol extract, $100 \mu l$ of tracer ^{125}I - T_3 or ^{125}I -reverse T_3 , and $100 \mu l$ of anti- T_3 serum (1:40,000) or anti-reverse T_3 serum (1:2,000) were added to $750 \mu l$ of 0.1 M sodium phosphate buffer, pH 7.5. The concentration of ethanol in each standard was adjusted to be the same as the samples. Reaction mixture was allowed to stand for 16 hr at $4^\circ C$. Separation of free and bound forms was achieved by the addition of $100 \mu l$ of dextran-coated charcoal. The net concentration of each iodothyronine generated from T_4 was calculated by subtracting the iodothyronine concentration in time zero incubation tubes from the one in the test tubes.

Effects of various factors. To characterize the conversion of T_4 to reverse T_3 in the human placenta, we studied the effects of the various factors on this reaction; concentrations of DTT, substrate and tissue protein, duration of incubation, incubation temperature, pH of incubation mixture and heat stability. To test the effect of changes in pH of incubation mixture on the conversion of T_4 to T_3 and reverse T_3 , four buffer systems were used as follows: 0.05 M acetate buffer for pH 4.0–5.5, 0.05 M phosphate buffer for pH 6.0–7.0, 0.05 M Tris-HCl buffer for pH 7.5–9.0 and 0.05 M glycine-NaOH buffer for pH 9.5–10.5.

Degradations of T_3 and reverse T_3 . The degradations of T_3 and reverse T_3 under the same condition as their production from T_4 were studied. Furthermore, effect of anti- T_3 serum on the degradation of T_3 was studied based on the report by Borges et al (1981).

Reagents. L - T_4 , L - T_3 and DTT were obtained from Sigma Co., St. Louis, Mo. and L -reverse T_3 from Henning, Berlin. Labeled T_3 and reverse T_3 were kind gifts of Dainabbot Co., Japan and anti-reverse T_3 serum was presented by Dr. L.E. Braverman, Worcester, Mass. 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.

Statistics. Points in all figures represent mean $\pm$ s.d. of quadruplicate assays.

RESULTS

Since no conversion of T_4 to T_3 at 37°C was detected over the pH ranges examined, we will mainly describe about the conversion of T_4 to reverse T_3 in the human placenta.

T_4 to reverse T_3 conversion in the human placental homogenate and subcellular fractions

Fig. 1. presents the data on the distribution of T_4 monodeiodinating activity

Fig. 1. Conversion of T_4 to reverse T_3 in various subcellular fractions of human placenta.

Fig. 2. Effect of DTT concentration on conversion of T_4 to reverse T_3 .

in the various subcellular fractions of the human placenta. Because microsomal fraction had the most potent activity of inner-ring monodeiodination of T_4 , this fraction was used in the following systems. The net reverse T_3 production in the microsomes was 17.9 ± 1.4 ng/mg protein/ μ g T_4 /60 min, 3.6 times as high as that in the homogenate, 5.0 ± 0.4 ng/mg protein/ μ g T_4 /60 min.

Effects of various factors

Effect of DTT. In the absence of DTT, there was observed no consistent conversion of T_4 to reverse T_3 . Since the net reverse T_3 production from T_4 increased in accordance with the increase of DTT concentration in the reaction mixture and reached a plateau at 0.05 M of DTT as shown in Fig. 2, 0.05 M of DTT was routinely added in the present system.

Concentration of tissue protein. Fig. 3 indicates the effect of changes in tissue protein concentration on the conversion of T_4 to reverse T_3 . The amount of reverse T_3 produced increased progressively to a point with increasing concentration of tissue protein up to 1 mg/tube.

Incubation time. Fig. 4 shows the effect of incubation time on T_4 conversion to reverse T_3 . An increase in the incubation time up to 120 min was associated with increased production of reverse T_3 .

Incubation temperature. The effect of the incubation temperature on the production of reverse T_3 from T_4 is shown in Fig. 5. The net production of reverse T_3 was negligible at 4°C and maximal at 37°C.

Effect of preheating. The monodeiodinating activity in microsomal fraction, when preheated at 56°C for 30 min, was completely abolished (Fig. 6).

Effect of pH. Fig. 7 shows the effect of the pH of the incubation buffer on

Fig. 3

Fig. 3. Effect of protein concentration on conversion of T_4 to reverse T_3 .

Fig. 4

Fig. 4. Effect of incubation time on conversion of T_4 to reverse T_3 .

the production of reverse T_3 from T_4 . It was clear that the conversion of T_4 to reverse T_3 was influenced considerably by pH. The net production of reverse T_3 was maximal at pH 7.0.

Concentration of substrate (T_4). Fig. 8 presents the data on the effect of substrate concentration on the reverse T_3 production from T_4 . The amount of reverse T_3 produced increased in accordance with increasing concentration of T_4 .

Degradations of T_3 and reverse T_3 . Fig. 9 shows the degradation of T_3 or reverse T_3 at pH 7.5 in microsomal fraction of the human placenta when 8 ng of

Fig. 5

Fig. 5. Effect of incubation temperature on conversion of T_4 to reverse T_3 .

Fig. 6

Fig. 6. Effect of pH on conversion of T_4 to reverse T_3 .

Fig. 7. Effect of T_4 concentration on conversion of T_4 to reverse T_3 .

Fig. 8

Fig. 8. Effect of preheating on conversion of T_4 to reverse T_3 .

Fig. 9

Fig. 9. Effect of incubation time on T_3 (●—●) and reverse T_3 (○—○) degradation.

Fig. 10

Fig. 10. Effect of the presence of T_3 -antibody on T_3 degradation.

Fig. 11

Fig. 11. Effect of the presence of T_3 -antibody on conversion of T_4 to T_3 or reverse T_3 .

T_3 or reverse T_3 is incubated with the tissue under the same condition as described above. Ninety per cent of original reverse T_3 remained after 60 min incubation, although only 8 per cent of original T_3 was measurable after the same incubation time. Fig. 10 shows the degradation of T_3 when anti- T_3 serum is added to the incubation mixture. It was clear that anti- T_3 serum could inhibit the degradation of T_3 . In fact, original T_3 could be maintained after 60 min incubation when 100 μ l of 1:20 diluted anti- T_3 serum was added. Then, we studied the production

of T_3 or reverse T_3 when anti- T_3 serum was added to the incubation mixture. As shown in Fig. 11, neither T_3 nor reverse T_3 production rate was changed.

DISCUSSION

The present study confirmed that the human placenta actively deiodinate the inner ring of T_4 to reverse T_3 as Roti et al. (1981) had shown.

There are two possibilities in the reason why the net production of T_3 from T_4 by the human placenta is negligible. First, there may actually be no production of T_3 . Second, rapid degradation of T_3 may follow the production of T_3 . As shown in Fig. 9, rapid degradation of T_3 was observed in the human placenta. Therefore, we added anti- T_3 serum to incubation medium to inhibit the degradation of T_3 . Although this "immunosequestration" technique (Borges et al. 1981) could stop almost all the degradation of this hormone as described above, the negligible production of T_3 still observed. There is a great similarity in degradation of triiodothyronines between human placenta and rat cerebrum (Dratman and Crutchfield 1978). Interestingly, the converse phenomena, rapid degradation of reverse T_3 and slow one of T_3 , were found in other tissues such as rat liver (Visser 1975; Cavalier et al. 1977) and rat (Chiraseveenuprapund et al. 1978) and human kidney (Yoshida et al. 1982).

The present study makes it clear that the conversion of T_4 to reverse T_3 by the human placenta is enzymatic in nature, since this reaction is dependent on incubation time, incubation temperature, concentration of substrate and protein concentration, and unstable to heating. The deiodinating activity is highest in microsomal fraction of the human placenta. The net production of reverse T_3 from T_4 was maximal at pH 7.0. We have reported that the optimal pH for the reaction of T_3 deiodination to reverse T_3 by the human kidney was 10.0 (Yoshida et al. 1982). The difference of these optimal pH's indicates that there might be different enzyme systems in these peripheral tissues.

Recently, Banovac et al. (1980) and Roti et al. (1981) reported about the deiodination of T_4 to T_3 and reverse T_3 by the human placental homogenate. In the former, only 0.32 per cent of stable T_4 was observed to be degraded to T_3 and reverse T_3 in a 3-hr incubation and the vast majority of the generated triiodothyronines was T_3 ; strikingly different results from ours. Although the reason for such a great difference is unknown, one possibility is the absence of DTT in the system of Banovac et al. (1980) as suggested by Roti et al. (1981).

Roti et al. (1981) reported that T_4 was actively deiodinated to reverse T_3 by the human placenta, but not to T_3 . They confirmed using paperchromatography that there is no deiodination of T_4 to T_3 nor deiodination of reverse T_3 . Our present results using anti- T_3 serum are in good concordance with their report.

It has been reported that less than 1 percent of term fetal T_4 production can be accounted for by transplacental passage of T_4 (Dussault and Coulombe 1980). Furthermore, El-Zaheri et al. (1981) have demonstrated that the dam is an important source of reverse T_3 concentrations in the fetal rat serum and amniotic

fluid, which are partially dependent upon maternal thyroid function. The present study demonstrates the presence of an active inner-ring deiodinase of T_4 in the human placenta and suggests that maternal T_4 deiodination by this enzyme may be one source of reverse T_3 in fetal serum and amniotic fluid. And this deiodinating mechanism might be related to the placental impermeability of thyroid hormones.

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