0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 6
Printed in U.S.A.

Maternal to Fetal Thyroxine Transmission in the Human Term Placenta Is Limited by Inner Ring Deiodination*

R. H. MORTIMER, J. P. GALLIGAN, G. R. CANNELL, R. S. ADDISON, AND M. S. ROBERTS

Conjoint Endocrine Laboratory (R.H.M., G.R.C., R.S.A.) and the Department of Chemical Pathology (J.P.G.), Royal Brisbane Hospital, Brisbane, Queensland 4029, Australia; and the Departments of Obstetrics and Gynecology (R.H.M.) and Medicine (M.S.R.), University of Queensland, Queensland 4072, Australia

ABSTRACT

Placental deiodination of T_4 to rT_3 has been proposed as the factor controlling materno-fetal transmission of T_4. We investigated T_4 transfer in the isolated perfused human placental lobule with and without addition of the deiodinase inhibitor, iopanoic acid. T_4 (150 nmol/L) in protein-free medium was added to the maternal circuit. Without iopanoic acid, the appearance of T_4 in the fetal circuit was very low, with fetal T_4 levels reaching only 4.1 ± 0.84 pmol/L at 6 h. Levels of rT_3 rose progressively in both circuits, reaching 28.8 ± 5.5 nmol/L in the maternal and 12.4 ± 3.2 nmol/L in the fetal circuit by 6 h. No T_3 could be measured in either circuit. Addition of 0.5 mmol/L iopanoic acid to maternal perfusate, however, resulted in significant reduction in the appearance of rT_3 [maternal levels, 0.58 ± 0.06 nmol/L (2% of control values); fetal levels, 0.33 ± 0.03 mmol/L (2.7% of control values)] and a major (~2700-fold) increase in T_4 appearance in the fetal circuit, with fetal T_4 levels reaching 10.1 ± 3.4 nmol/L at 6 h. These results support the hypothesis that placental inner ring (type III) deiodination is a major factor controlling placental transmission of maternal T_4. (*J Clin Endocrinol Metab* **81:** 2247–2249, 1996)

THERE IS CONTINUING debate about the degree of transfer of maternal T_4 to the human fetus (1). Early studies (2, 3) suggested that some maternal hormone reached the fetus, and this has been supported by the more recent description (4) of significant levels of thyroid hormone in plasma of neonates who were athyreotic or whose ability to synthesize thyroid hormone was abolished by a complete organification defect. The amount of transfer appears insufficient to prevent the high blood TSH levels and delayed bone age seen in such infants (5). Deiodination of T_4 to the biologically inactive rT_3 by a type III (inner or tyrosyl ring) deiodinase in placenta has been proposed as the mechanism that limits transfer of maternal thyroid hormone to the fetus (6). Studies in the pregnant guinea pig, however, suggested that placental conversion of maternal T_4 to rT_3 is small (7).

The isolated perfused human placental lobule offers a unique model with which to examine T_4 transfer and metabolism in human tissue independent of the contributions of maternal and fetal thyroid hormone secretion and metabolism. The purpose of this investigation was, using the isolated perfused term human placental lobule, to determine the amount of transfer of T_4 from the maternal to the fetal circuit, examine the extent of type III deiodination, and assess the effect of the deiodinase inhibitor, iopanoic acid, on deiodination and T_4 transfer.

Received August 4, 1995. Revision received December 29, 1995. Accepted January 5, 1996.

Address all correspondence and requests for reprints to: Robin Mortimer, MB.BS., Department of Endocrinology, Royal Brisbane Hospital, Herston, Queensland 4029, Australia.

* This work was supported in part by the National Health and Medical Research Council.

Materials and Methods

Perfused human placental lobule

These studies were approved by the Royal Women's Hospital research ethics committee. The placentas used were from normal women without a history of drug ingestion delivered at term by repeat cesarian section. Perfusions were established within 20 min of delivery.

The perfusion techniques, materials, conditions, and the viability of the preparation have been described previously (8). The maternal perfusate was recirculated at 25 mL/min, and the fetal perfusate at 3 mL/min. Perfusion of the placental tissue was continued, while in a duplicate circuit that bypassed the placental lobule, T_4 (150 nmol/L) without (n = 4) or with (n = 3) 0.5 mmol/L iopanoic acid was recirculated for 15 min before the start of the experiments. Separate experiments (data not shown) indicated that in the absence of placental tissue, perfusate T_4 levels fell by about 20% during the first 10 min of recirculation in the duplicate circuit, but then remained constant, indicating saturation of tubing with T_4. At time zero, perfusate in the duplicate circuit was switched to flow through the placental lobule. Samples were taken then and for the succeeding 6 h from the maternal and fetal circuits at 10- to 60-min intervals for measurements of T_4, T_3, and rT_3. At the end of the perfusion period, the perfused portion of the lobule was dissected from the unperfused portion, blotted dry, weighed, and frozen at −20 C for later measurement of tissue levels of T_4, T_3, and rT_3.

Hormone assays

Perfusate assays. Perfusate levels of T_4 and rT_3 were measured by modifications of commercially available RIAs. Pure T_4, T_3, and rT_3 were obtained from Sigma Chemical Co. (St. Louis, MO). As matrix effects were observed when perfusate was assayed for each of the analytes assay standards were prepared in 0.05 mol/L phosphate-buffered saline (PBS), pH 7.5, containing 0.3 g/100 mL BSA (Sigma), and 1.0 g/100 mL bovine serum γ-globulin (Calbiochem Corp., La Jolla, CA). For each assay, equal parts of buffer standard and blank perfusate and sample perfusate plus PBS buffer were taken to give an identical matrix. Iopanoic acid was added to standards when relevant.

T_4 levels in maternal perfusate and fetal perfusate from placentas perfused with iopanoic acid were measured using reagents from the Magic T_4 kit (Ciba Corning Diagnostic Corp., Medfield, MA). The assay

sensitivity was 3.0 nmol/L, and the interassay variability was 5.3% at 57 nmol/L, 5.0% at 107 nmol/L, and 9.0% at 182 nmol/L. Cross-reactivity was 4.0% with T_3 and 2.7% with rT_3. T_4 levels in fetal circuit perfusates from control experiments were measured with the Amerlex-MAB Free T_4 kit (Kodak Clinical Diagnostics, Amersham, UK), yielding a sensitivity of 0.5 pmol/L and interassay variabilities of 6.9% at 6.0 pmol/L, 5.5% at 18.4 pmol/L, and 4.6% at 61 pmol/L. Cross-reactivity was 1.2% with T_3 and less than 0.01% with rT_3.

rT_3 levels were measured using a kit from Biodata (Guidonia Montecelio, Italy). A sensitivity of 0.03 nmol/L was achieved with interassay variations of 10.9% at 0.13 nmol/L, 5.1% at 0.35 nmol/L, and 3.6% at 0.45 nmol/L. Cross-reactivity with T_4 was 0.09%, and that with T_3 was less than 0.01%. T_3 was measured by T_3 RIABEAD (Abbott Laboratories Diagnostic Division, North Chicago, IL). Sensitivity was 0.2 nmol/L, and interassay variability was 9.1% at 1.1 nmol/L, 7.1% at 2.8 nmol/L, and 6.4% at 4.7 nmol/L. Cross-reactivity with T_4 was 0.4%, and that with rT_3 was 0.04%. Sensitivities and degrees of cross-reactivity of assays were not significantly altered by coincubation with iopanoic acid. Iopanoic acid had no effect on specific or nonspecific binding.

Tissue assays. Perfused placental tissue was minced with scissors in 10 mL PBS, homogenized with a motorized Teflon pestle (Parvalux Electric Motors, Bournemouth, UK) and glass tube for 2 min on ice, and centrifuged for 30 min at 3000 rpm. Supernatant (200 μL) was added dropwise to 800 μL fractionally redistilled ethanol in a fractionally redistilled methanol washed tube and dried under vacuum. This was then made up to the appropriate volume with PBS-albumin-γ-globulin buffer and assayed for T_4, rT_3, and T_3.

Statistical analysis

Data are expressed as the mean ± SD. Means were compared by Student's t test. T_4 and rT_3 levels in maternal and fetal perfusates from control and iopanoic acid-treated placental perfusions were compared by repeated measures ANOVA, using thyronine levels in control and iopanoic acid perfusions as dependent variables repeated over time. A banded covariance structure and a small sample correction were employed (9). $P < 0.05$ was accepted as statistically significant.

The initial and terminal half-lives of disappearance of T_4 from the maternal circuit were estimated by fitting T_4 levels to the equation: $T_4 = A \times \exp(-a \times t) + B \times \exp(-b \times t)$, where t is time and by calculating the initial half-life as $0.693/a$ and terminal half life as $0.693/b$.

Results

Maternal perfusate T_4 levels fell in control experiments (Fig. 1) from an initial 129.5 ± 9.1 nmol/L to a nadir of 35.0 ± 2.12 nmol/L at 6 h. The fall was biexponential, with an initial half-life of 16.7 ± 2.2 min and a terminal half-life of disappearance of 1299 ± 756 min. rT_3 levels rose progressively in the maternal and fetal circuits to 28.8 ± 5.5 and 12.4 ± 3.2 nmol/L, respectively, at 6 h. T_4 was detectable in the fetal circuit by 20 min, reaching a peak of 4.1 ± 0.84 pmol/L at 6 h. No T_3 could be detected in either circuit, and no T_4, T_3, or rT_3 could be detected in placental tissue at the end of the perfusion.

Addition of iopanoic acid to the maternal circuit had no significant effect on T_4 levels in the maternal circulation, which fell from 108.7 ± 9.1 nmol/L to a nadir of 31.3 ± 3.3 nmol/L at 6 h (Fig. 2). The initial half-life of disappearance of T_4 (14.2 ± 7.7 min) did not significantly differ from that in control perfusions. The terminal rate constant for disappearance of maternal T_4 fell significantly ($P = 0.041$) to zero, suggesting inhibition of placental metabolism. This was supported by major reductions in rT_3 levels, which by 6 h reached 0.58 ± 0.06 nmol/L, ($P = 0.004$) in the maternal and 0.33 ± 0.03 nmol/L, ($P = 0.011$) in the fetal circuit. This inhibition of deiodination was associated with striking increases in fetal circuit T_4 levels, which rose to a plateau ranging from 10.0 ± 2.6 nmol/L at 20 min to 10.1 ± 3.4 nmol/L at 6 h ($P = 0.002$). Placental tissue T_4 and rT_3 levels were 106 ± 6.0 and 2.35 ± 0.05 pmol/g wet wt.

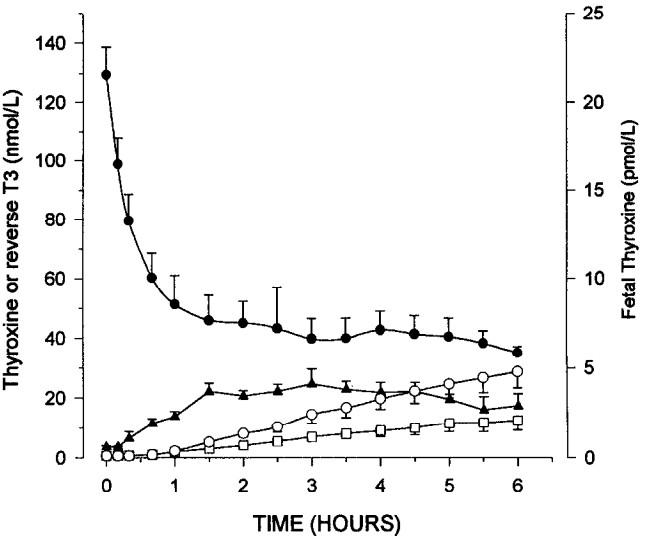

FIG. 1. Perfusion of the maternal circuit with 150 nmol/L T_4, showing maternal T_4 (●), maternal rT_3 (○), fetal T_4 (▲), and fetal rT_3 (□) levels over the 6-h perfusion. Note the picomolar scale for fetal T_4.

Discussion

In these experiments we added to the maternal circuit amounts of T_4 (150 nmol/L) approximating total plasma levels seen in pregnancy. As the perfusate contained no added protein, this resulted in grossly supraphysiological levels of unbound T_4. Despite this, only picomolar amounts of T_4 could be detected in the fetal circuit, leading to a fetal/maternal perfusate T_4 ratio at equilibrium of approximately 8×10^{-5}. The demonstration of relatively high amounts of rT_3 in both circuits suggested that there had been active inner ring deiodination of T_4 to rT_3 and possibly to di- and monoiodothyronines. An inner ring (type III) deiodinase has been described (10, 11) in homogenates of human placenta, with an apparent K_m for T_4 of 1.2×10^{-7} mol/L (12). The rapid disappearance of nanomolar amounts of T_4 from the maternal circuit and the absence of measurable tissue T_4 at the end of perfusion suggest that the clearance of T_4 from the maternal circuit and very low transfer of T_4 to the fetal circuit result from T_4 metabolism by a deiodinase with a K_m of similar order to that described in placental homogenates, rather than tissue binding of T_4 alone. We were not able to demonstrate T_3 in either circuit or in placental tissue, suggesting no significant outer ring (type II) deiodination. A type II deiodinase has been described in human placental homogenate fractions, but has an apparent K_m in the low nanomolar range (13).

These results differ somewhat from *in vivo* studies of T_4 clearance by the guinea pig placenta (7). As in our studies, no significant outer ring deiodination of T_4 to T_3 was detected, and there was active inner ring deiodination to rT_3, but there was a much greater transfer of maternal T_4 and a much lower transfer of rT_3. Thus, although the guinea pig,

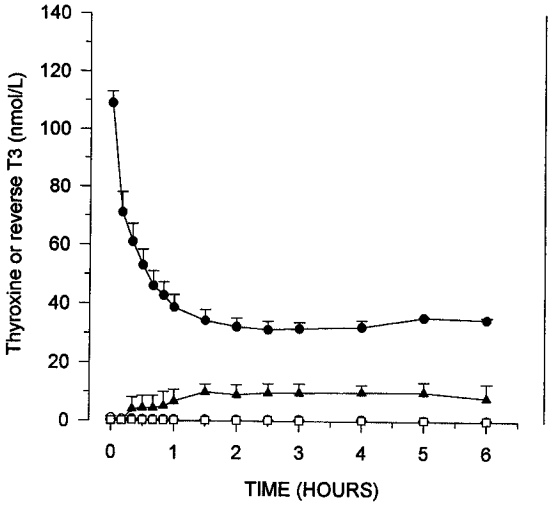

FIG. 2. Perfusion of the maternal circuit with 150 nmol/L T_4 and 0.5 mmol/L iopanoic acid, an inhibitor of type III deiodinase, showing maternal T_4 (●), maternal rT_3 (○), fetal T_4 (▲), and fetal rT_3 (□) levels over the 6-h perfusion. Maternal rT_3 symbols are obscured by fetal rT_3 symbols. Fetal T_4 levels were significantly greater (by repeated measures ANOVA, $P = 0.002$) and maternal and fetal rT_3 levels were significantly lower ($P = 0.004$ and $P = 0.011$, respectively) than control values.

in common with man, has a hemochorial placenta, placental transfer and deiodination of T_4 appear to be quantitatively different.

Iopanoic acid, an iodine-containing radiographic contrast agent, inhibits inner and outer ring deiodination of thyroid hormone in many tissues, including placenta (14). In *in vitro* studies, significant inhibition of T_3 inner ring deiodination was achieved in guinea pig placenta with 1 mmol/L iopanoic acid (15). We found that the addition of 0.5 mmol/L iopanoic acid to the maternal circuit caused major inhibition of tissue type III deiodinase, with reduction of rT_3 levels to 2.0% of those seen in the maternal and 2.6% in the fetal circuits in control experiments. This was associated with a major increase (~2700-fold) in the appearance of T_4 in the fetal circuit, where concentrations reached 30% of those in the maternal circuit. Addition of iopanoic acid also resulted in measurable levels of T_4 and rT_3 in placental tissue at the end of the 6-h perfusion.

Our data suggest that in the human placenta at term, transfer of maternal T_4 to the fetal circulation is very low, with fetal circuit levels 0.008% of those in the maternal circuit. The present studies were performed using a gross excess of free T_4 in the maternal circuit. If these results are extrapolated to the maternal free T_4 levels found at term (on the order of 10–20 pmol/L) (16), only femtomolar amounts of maternal T_4 would be expected to appear in the fetal circulation. Several clinical studies suggest, however, that considerably more than this crosses from the maternal to the fetal circulation (1–3, 17–19)

There is, therefore, a difference between the hypothyroid near-term fetus to which significant amounts of maternal thyroid hormone are transferred and the perfused placental lobule in which very limited amounts of T_4 are transferred from maternal to fetal circuits. Possible explanations for this include an alternate pathway of transfer of maternal thyroid hormones across the extraplacental chorio-allantoic membranes (1) or inhibition of placental type III deiodinase by fetal hypothyroidism. Although brain type III deiodinase is inhibited by hypothyroidism (20), maternal and fetal hypothyroidism do not inhibit inner ring deiodination in the rat (21).

Acknowledgments

We thank Winthrop Laboratories, Division of Sterling Winthrop (Ermington, Australia), for their kind gift of iopanoic acid, and Ms. Razeena Hassan for technical assistance. We also thank the patients and midwives of the Royal Women's Hospital, without whose assistance this study could not have been performed.

References

1. **Burrow GN, Fisher DA, Larsen PR.** 1994 Mechanisms of disease: maternal and fetal thyroid function. N Engl J Med. 331:1072–1078.
2. **Carr Jr EA, Beierwaltes WH, Raman G, et al.** 1959 The effect of maternal thyroid function on fetal thyroid function and development. J Clin Endocrinol Metab. 19:1–18.
3. **Raiti S, Holzman GB, Scott RL, Blizzard RM.** 1967 Evidence for the placental transfer of triiodothyronine in human beings. N Engl J Med. 277:456–459.
4. **Vulsma T, Gons MH, De Vijlder JJM.** 1989 Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to total organification defect or thyroid agenesis. N Engl J Med. 321:13–16.
5. **Sack J, Kaiserman I, Siebner R.** 1993 Maternal-fetal T_4 transfer does not suffice to prevent the effects of *in utero* hypothyroidism. Horm Res. 39:1–7.
6. **Roti E, Fang SL, Green K, Emerson CH, Braverman LE.** 1981 Human placenta is an active site of thyroxine and 3,3',5-triiodothyronine tyrosyl ring deiodination. J Clin Endocrinol Metab. 53:498–501.
7. **Cooper E, Gibbens M, Thomas CR, Lowy C, Burke CW.** 1983 Conversion of thyroxine to 3,3',5'-triiodothyronine in the guinea pig placenta: *in vivo* studies. Endocrinology. 112:1808–1815.
8. **Cannell GR, Kluck RM, Hamilton SE, Mortimer RH, Hooper WD, Dickinson RG.** 1988 Markers of physical integrity and metabolic viability of the perfused human placental lobule. Clin Exp Pharmacol Physiol. 15:837–844.
9. **Dixon WJ, Merdian K.** 1992 ANOVA and regression with BMDP 5V. Los Angeles: Dixon Statistical Associates.
10. **Banovac K, Bzik L, Tislaric T, Selso M.** 1980 Conversion of thyroxine to triiodothyronine and reverse triiodothyronine in human placenta and fetal membranes. Horm Res. 12:253–259.
11. **Roti E, Fang S-L, Green K, Emerson CH, Braverman LE.** 1981 Human placenta is an active site of thyroxine and 3,3',5-triiodothyronine tyrosyl ring deiodination. J Clin Endocrinol Metab. 53:498–501.
12. **Fay M, Roti E, Fang SL, Wright G, Braverman LE, Emerson CH.** 1984 The effect of propylthiouracil, iodothyronines, and other agents on thyroid hormone metabolism in human placenta. J Clin Endocrinol Metab. 58:280–286.
13. **Kaplan MM, Shaw EA.** 1984 Type II iodothyronine 5'-deiodination by human and rat placenta *in vitro*. J Clin Endocrinol Metab. 59:253–257.
14. **Leonard JL, Visser TJ.** 1986 Biochemistry of deiodination. In: Hennemann G, ed. Thyroid hormone metabolism. New York, Basel: Marcel Dekker; 189–229.
15. **Castro MI, Braverman LE, Alex S, Wu CF, Emerson CH.** 1985 Inner-ring deiodination of 3,5,3'-triiodothyronine in the *in situ* perfused guinea pig placenta. J Clin Invest. 76:1921–1926.
16. **Wiersinga WM, Vet T, Berghout A, Endert E.** 1991 Serum free thyroxine during pregnancy: a meta-analysis. In: Beckers C, Reinwein D. eds. The thyroid and pregnancy, Stuttgart, New York: Schattauer; 79–94.
17. **Kearns JE, Hutson W.** 1963 Tagged isomers and analogues of thyroxine (their transmission across the human placenta and other studies). J Nucl Med. 4:453–461.
18. **Fisher DA, Lehman H, Lackey C.** 1964 Placental transfer of thyroxine. J Clin Endocrinol Metab. 24:393–400.
19. **Dussault JH, Row VV, Lickrich G, Volpe R.** 1968 Studies of serum triiodothyronine concentration in maternal and cord blood: transfer of triiodothyronine across placenta. J Clin Endocrinol Metab. 29:595–603.
20. **Kaplan MM, McCann UD, Yaskoski KA, Larsen PR, Leonard JL.** 1981 Anatomical distribution of phenolic and tyrosyl ring iodothyronine diodinases in the nervous system of normal and hypothyroid rats. Endocrinology. 109:397–402.
21. **Emerson CH, Bambini G, Alex S, Castro MI, Roti E, Braverman LE.** 1988 The effect of thyroid dysfunction and fasting on inner ring deiodinase activity in the rat. Endocrinology. 122:809–806.