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Ontogeny of Iodothyronine Deiodinases in Human Liver*

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ABSTRACT

The role of the deiodinases D1, D2, and D3 in the tissue-specific and time-dependent regulation of thyroid hormone bioactivity during fetal development has been investigated in animals but little is known about the ontogeny of these enzymes in humans. We analyzed D1, D2, and D3 activities in liver microsomes from 10 fetuses of 15–20 weeks gestation and from 8 apparently healthy adult tissue transplant donors, and in liver homogenates from 2 fetuses (20 weeks gestation), 5 preterm infants (27–32 weeks gestation), and 13 term infants who survived up to 39 weeks postnatally. D1 activity was determined using 1 μM [3′,5′-^{125}I]rT_3 as substrate and 10 mM dithiothreitol (DTT) as cofactor, D2 activity using 1 nM [3′,5′-^{125}I]T_4 and 25 mM DTT in the presence of 1 mM 6-propyl-2-thiouracil (to block D1 activity) and 1 μM T_3 (to block D3 activity), and D3 activity using 10 nM [3,5-^{125}I]T_3 and 50 mM DTT, by quantitation of the release of $^{125}I^-$. The assays were validated by high performance liquid chromatography of the products, and kinetic analysis [Michaelis-Menten constant (K_m) of rT_3 for D1: 0.5 μM; K_m of T_3 for D3: 2 nM]. In liver homogenates, D1 activity was not correlated with age, whereas D3 activity showed a strong negative correlation with age (r −0.84), with high D3 activities in preterm infants and (except in 1 infant of 35 weeks) absent D3 activity in full-term infants. In microsomes, D1 activities amounted to 4.3–60 pmol/min/mg protein in fetal livers and to 170–313 pmol/min/mg protein in adult livers, whereas microsomal D3 activities were 0.15–1.45 pmol/min/mg protein in fetuses and <0.1 pmol/min/mg protein in all but one adult. In the latter sample, D3 activity amounted to 0.36 pmol/min/mg protein. D2 activity was negligible in both fetal and adult livers. These findings indicate high D1 and D3 activities in fetal human liver, and high D1 and mostly absent D3 activities in adult human liver. Therefore, the low serum T_3 levels in the human fetus appear to be caused by high hepatic (and placental) D3 activity rather than caused by low hepatic D1 activity. The occasional expression of D3 in adult human liver is intriguing and deserves further investigation. (*J Clin Endocrinol Metab* **83:** 2868–2874, 1998)

THE bioactivity of thyroid hormone is regulated importantly by enzymatic deiodination in peripheral tissues (1–4). The prohormone T_4 is converted by outer ring deiodination (ORD) to the active hormone T_3. Both T_4 and T_3 are inactivated by inner ring deiodination (IRD) to rT_3 and 3,3′-diiodothyronine (3,3′-T_2), respectively. The latter metabolite is also produced by ORD of rT_3. The three iodothyronine deiodinases involved in these processes have been characterized as homologous transmembrane selenoproteins that require thiols as cofactor (1–4). The type I deiodinase (D1) has both ORD and IRD activity. It is located in liver, kidney, and thyroid and is important for plasma T_3 production. rT_3 is the preferred substrate for D1, although the deiodination of other iodothyronines is greatly accelerated by sulfate conjugation of their 4′-hydroxyl group (1–5). D1 shows Michaelis-Menten constant (K_m) values for its substrates in the micromolar range, and the enzyme is potently inhibited by 6-propyl-2-thiouracil (PTU) (1–4). The type II deiodinase (D2) catalyzes only ORD of iodothyronines. D2 activity is found in brain, pituitary, and brown adipose tissue, whereas D2 messenger RNA (mRNA) has recently also been detected in human heart, skeletal muscle, and thyroid (6–8). D2 appears particularly important for local T_3 production in these tissues. It shows preference for T_4 over rT_3 as the substrate, with K_m values in the nanomolar range, and is insensitive to PTU inhibition (1–4). The type III deiodinase (D3) has only IRD activity; it is present among other tissues in brain, skin, and intestine (see also below). It shows preference for T_3 over T_4 as the substrate, with K_m values in the nanomolar range, and is not inhibited by PTU (1–4). The expression of these enzymes is regulated by thyroid state. In general, D1 and D3 activities are increased and D2 activity is decreased in hyperthyroidism, whereas the opposite changes are observed in hypothyroidism (1–4).

The role of the deiodinases in the tissue-specific and time-dependent regulation of thyroid hormone bioactivity during fetal development has been investigated in experimental animals (9–11), but little is known about the ontogeny of these enzymes in human development. In animals and humans, fetal serum T_3 is low and increases only at the end of gestation and in the neonatal period (11–13). Conversely, fetal serum rT_3 is high and decreases in the late fetal and early neonatal period (11–13). High levels of iodothyronine sul-

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fates, such as T_4S, T_3S, rT_3S and 3,3′-T_2S, have been documented in human and sheep fetal serum (11, 12, 14–18). Because hepatic D1 is important for production of serum T_3 and for clearance of serum rT_3 and iodothyronine sulfates (1–5), and because hepatic D1 activity is only expressed towards the end of fetal development, at least in rats and sheep (9, 11), it is generally believed that the low fetal serum T_3 and high fetal serum rT_3 and sulfates reflect a low hepatic D1 activity. However, high D3 activity has been detected in human, rat, and guinea pig placenta (19–24); fetal rat brain (25, 26) and intestine (27); and embryonic chicken liver (28, 29). Therefore, the low T_3 and high rT_3 levels in human fetal serum may also be caused by rapid degradation of serum T_3 and production of serum rT_3 in placenta (23, 24) and possibly other tissues, such as liver. The purpose of the present study was to obtain a better understanding of the ontogeny of D1 and D3 activities in human liver. The results indicate a more important role for hepatic D3 activity in the regulation of thyroid hormone bioactivity during human fetal development than previously assumed.

Materials and Methods

Materials

Nonradioactive iodothyronines were obtained from Henning Berlin (Berlin, Germany); [3,5-^{125}I]T_3 (≈35 Ci/mmol) was obtained from Dr. R. Thoma of Formula (Berlin, Germany) courtesy of Dr. G. Decker of Henning Berlin. [3′,5′-^{125}I]T_4, [3′-^{125}I]T_3, and [3′,5′-^{125}I]rT_3 (≈2000 Ci/mmol) were obtained from Amersham (Little Chalfont, UK) or prepared in our laboratory by radioiodination of T_3, 3,5-T_2, and 3,3′-T_2, respectively, as described previously (30). [^{125}I]T_3 could be used without further purification, but [^{125}I]T_4 and [^{125}I]rT_3 were purified on Sephadex LH-20 before each experiment (30). N-bromoacetyl-[3′-^{125}I]T_3 (BrAc[3′-^{125}I]T_3) and BrAc[3′,5′-^{125}I]T_4 were prepared as described previously (31). Dithiothreitol (DTT) and PTU were obtained from Sigma (St. Louis, MO); electrophoresis grade SDS-PAGE reagents, protein markers, and protein assay reagent from Bio-Rad (Richmond, IL); Sephadex LH-20 from Pharmacia (Uppsala, Sweden); and Coomassie brilliant blue R-250 from Merck (Darmstadt, Germany).

Tissues

Liver tissue was obtained from 10 fetuses (F1–F10) of 15–20 weeks gestation as well as from 8 apparently healthy tissue transplant donors (A1–A8): 4 males (29–46 yr of age) and 4 females (34–52 yr of age). Microsomes were prepared by differential centrifugation as previously described (32), suspended in 100 mM phosphate (pH 7.2), and 2 mM EDTA (P100E2), containing 1 mM DTT, at a protein concentration of ≈10 mg/ml. Liver tissue was also obtained at autopsy from 2 fetuses of 20 weeks gestation, 5 preterm infants of 27–32 weeks gestation, and 13 term infants who survived up to 39 weeks postnatally. Postmortem time varied from 1–48 h. These tissues were homogenized in 10 vol P100E2, containing 10 mM DTT, yielding protein concentrations of ≈10 mg/mL. Aliquots of microsomes and homogenates were snap-frozen on dry ice and stored at −80 C until further analysis. Protein concentration was determined using Bio-Rad protein assay reagent and BSA as the standard. Approval for this study was obtained from the Tayside Committee on Medical Research Ethics.

Deiodinase assays

Deiodinase activities were determined by analysis of the release of radioiodide by ORD of outer ring-labeled T_4 or rT_3 or by IRD of inner ring-labeled T_3 during incubation for 30–60 min at 37 C with liver homogenates or microsomes and DTT in 0.2 mL P100E2 (32). D1 activity was assayed using 1 μM (10^5 cpm) [3′,5′-^{125}I]rT_3 and 10 mM DTT, D2 activity using 1 nM (10^5 cpm) [3′,5′-^{125}I]T_4 and 25 mM DTT in the presence of 1 mM PTU (to block D1 activity) and 1 μM T_3 (to block D3 activity), and D3 activity using 10 nM (10^5 cpm) [3,5-^{125}I]T_3 and 50 mM DTT. Reactions were stopped at 0 C by addition of 0.1 mL 5% (wt/vol) BSA in water followed by addition of 0.5 mL 10% (wt/vol) trichloroacetic acid in water. Precipitated ^{125}I-labeled iodothyronines were removed by centrifugation, and the $^{125}I^-$ released was further purified from the supernatant on Sephadex LH-20 microcolumns (bed volume ≈0.25 mL), equilibrated and eluted with 0.1 M HCl (32).

The assays were validated by high performance liquid chromatography (HPLC) analysis of the deiodination products of T_4, T_3, and rT_3. For this, microsomes were incubated with a) 1 μM [3′,5′-^{125}I]rT_3 and 10 mM DTT, b) 1 nM [3′,5′-^{125}I]T_4 and 25 mM DTT in the absence or presence of 1 mM PTU and/or 1 μM T_3, or c) 10 nM [3,5-^{125}I]T_3 and 50 mM DTT in 0.2 mL P100E2. The reactions were stopped by addition of 0.2 mL ice-cold methanol. After centrifugation, 0.2 mL of the supernatant was mixed with 0.3 mL 0.02 M ammonium acetate (pH 4), and 0.1 mL of the mixture was applied to a 250 × 4.6 mm Symmetry C18 column (Waters, Etten-Leur, The Netherlands) connected to an Alliance HPLC system (Waters) and eluted isocratically with a mixture of acetonitrile and 0.02 M ammonium acetate (33:67, vol/vol) at a flow of 1.2 mL/min. Fractions of 0.3 min were collected and counted for radioactivity.

Affinity labeling

BrAc[^{125}I]T_3 or BrAc[^{125}I]T_4 (0.1 μCi) was reacted for 20 min at 37 C with 0.1 mg microsomal protein in 0.1 mL P100E2 containing 1 mM DTT (30). The reaction was stopped by addition of 50 μL SDS-sample buffer containing 30% β-mercaptoethanol and treatment for 5 min at 100 C. Proteins were separated overnight by SDS-PAGE in a 16-cm 10% polyacrylamide gel. Gels were stained with Coomassie brilliant blue R-250 at 60 C, dried at 80 C under vacuum, and autoradiographed at −70 C using Fuji RX film (Fuji Medical Systems, Houten, The Netherlands). Apparent molecular mass (M_r) was determined by interpolation with protein markers.

Results

Deiodinase activities were determined in liver homogenates from 2 fetuses of 20 weeks gestation, 5 preterm infants of 27–32 weeks gestation who died soon after birth, and 13 term infants who survived up to 39 weeks postnatally. Causes of death included congenital defects, infection, metabolic storage disease, sudden death, and prematurity. The tissues were obtained at autopsy with a postmortem time of 1–48 h. Figure 1A shows the D1 activities of these liver homogenates as a function of gestational/postnatal age. Considering the varying conditions of the tissues, the appreciable scatter of the data was not surprising. However, it is clear that hepatic D1 activity demonstrated little or no dependence on age in the range studied, with activities at 20 weeks gestation being very similar to those observed after 20 weeks postnatally. D1 activity was not correlated with postmortem time (not shown). Figure 1B shows the hepatic D3 activities in these same tissue homogenates as a function of age. In spite of the considerable scatter, there was clearly a decrease in hepatic D3 activity with age. In general, high D3 activities were observed during fetal development, which virtually disappeared after birth in term infants. Hepatic D3 activity was unexpectedly low for age in 1 infant born at 27 weeks gestation who died from intrauterine infection. Interestingly, D3 activity was unexpectedly high for age in a term infant with GM1 gangliosidosis who died at 35 weeks of age from pneumonia. Hepatic D3 activity was not correlated with postmortem time (data not shown). D2 activities in these liver homogenates were negligible (data not shown).

Figure 2 shows the microsomal D1 and D3 activities in five representative fetal liver samples obtained at 15–20 weeks

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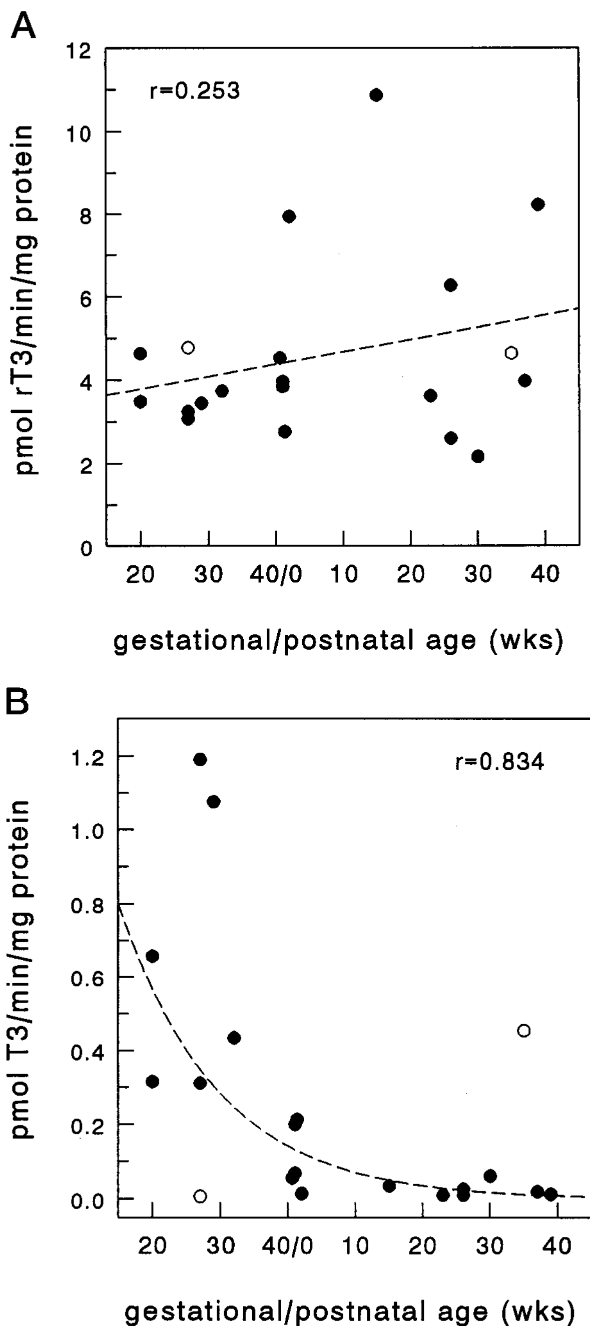

FIG. 1. D1 (A) and D3 (B) activities in human liver homogenates as function of gestational/postnatal age. Reaction conditions: 1 μM [3′,5′-^{125}I]rT_3 and 10 mM DTT (A) or 10 nM [3,5-^{125}I]T_3 and 50 mM DTT (B), 0.25 mg protein/mL, and 30 min incubation. ○, Outliers with strongly deviating D3 activities.

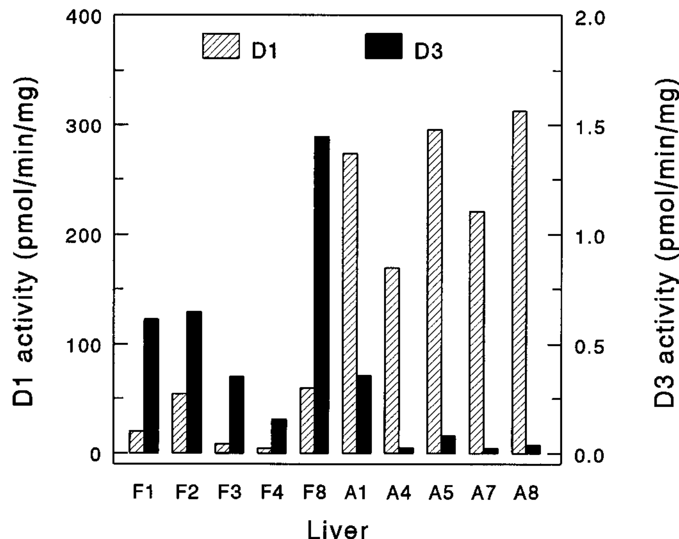

FIG. 2. Microsomal D1 and D3 activities in representative fetal (F) or adult (A) livers. Reaction conditions: D1, 1 μM [3′,5′-^{125}I]rT_3, 10 mM DTT, and 50 μg (F) or 25 μg (A) protein/mL. D3, 10 nM [3,5-^{125}I]T_3, 50 mM DTT, and 50 μg (F) or 250 μg (A) protein/mL. Incubation time was 30 min.

gestation as well as in five representative adult liver samples. Again, there was considerable scatter in both D1 and D3 activities in the fetal samples. There was no significant relationship of either D1 or D3 activity with fetal age (data not shown). However, D1 and D3 activities were significantly correlated (r = 0.85; data not shown), suggesting that the variation in both deiodinase activities was related to the quality of the tissues. Hepatic D1 activity in the adult tissue transplant donors showed a relatively narrow range of variation. D1 activity in fetal liver amounted up to 24% of that in adult liver. In seven of the eight adult liver samples D3 activity was very low (Fig. 2 and data not shown). However, significant hepatic D3 activity was detected in the remaining adult tissue sample (A1), which amounted to 56% of the mean fetal hepatic D3 activity. D2 activity was negligible in both fetal and adult liver (data not shown).

The different deiodinase assays were validated by HPLC analysis. The results demonstrated equivalent production of radiolabeled iodide and 3,3′-T_2 from [3′,5′-^{125}I]rT_3 in the D1 assay as well as from [3,5-^{125}I]T_3 in the D3 assay (data not shown). Deiodination of [3′,5′-^{125}I]T_4 by fetal liver microsomes in the absence of PTU or unlabeled T_3 resulted in the formation of radioactive rT_3, 3,3′-T_2, and iodide, suggesting IRD of T_4 by D3 to rT_3 and subsequent ORD of rT_3 by D1 to 3,3′-T_2 and iodide (Fig. 3A). Some labeled 3′-T1 was also produced presumably by IRD of 3,3′-T_2 by D3. Addition of PTU did not affect the IRD of T_4 but partially inhibited the further ORD of rT_3 (Fig. 3B). In the presence of 1 μM unlabeled T_3, conversion of [^{125}I]T_4 to [^{125}I]rT_3 was completely inhibited, and a very small amount of [^{125}I]T_3 was found to accumulate, apparently caused by inhibition of its degradation by D3 (Fig. 3C). Addition of both PTU and unlabeled T_3 resulted in the complete inhibition of T_4 metabolism (Fig. 3D).

Figure 4 shows the double-reciprocal plots of the deiodination rates of rT_3 and T_3 by fetal and adult liver microsomes as a function of substrate concentration. Although the maximum velocity (V_{max}) value for rT_3 deiodination estimated from these plots was higher in adult than in fetal liver, the apparent K_m value for rT_3 amounted to 0.5 μM with both representative tissue samples (Fig. 4A). The deiodination of rT_3 was completely inhibited by 1 mM PTU in both fetal and adult liver (not shown). The deiodination of T_3 by a representative fetal liver sample and by adult liver sample A1 was characterized by very similar Km values, *i.e.* 1.4 and 2.5 nM,

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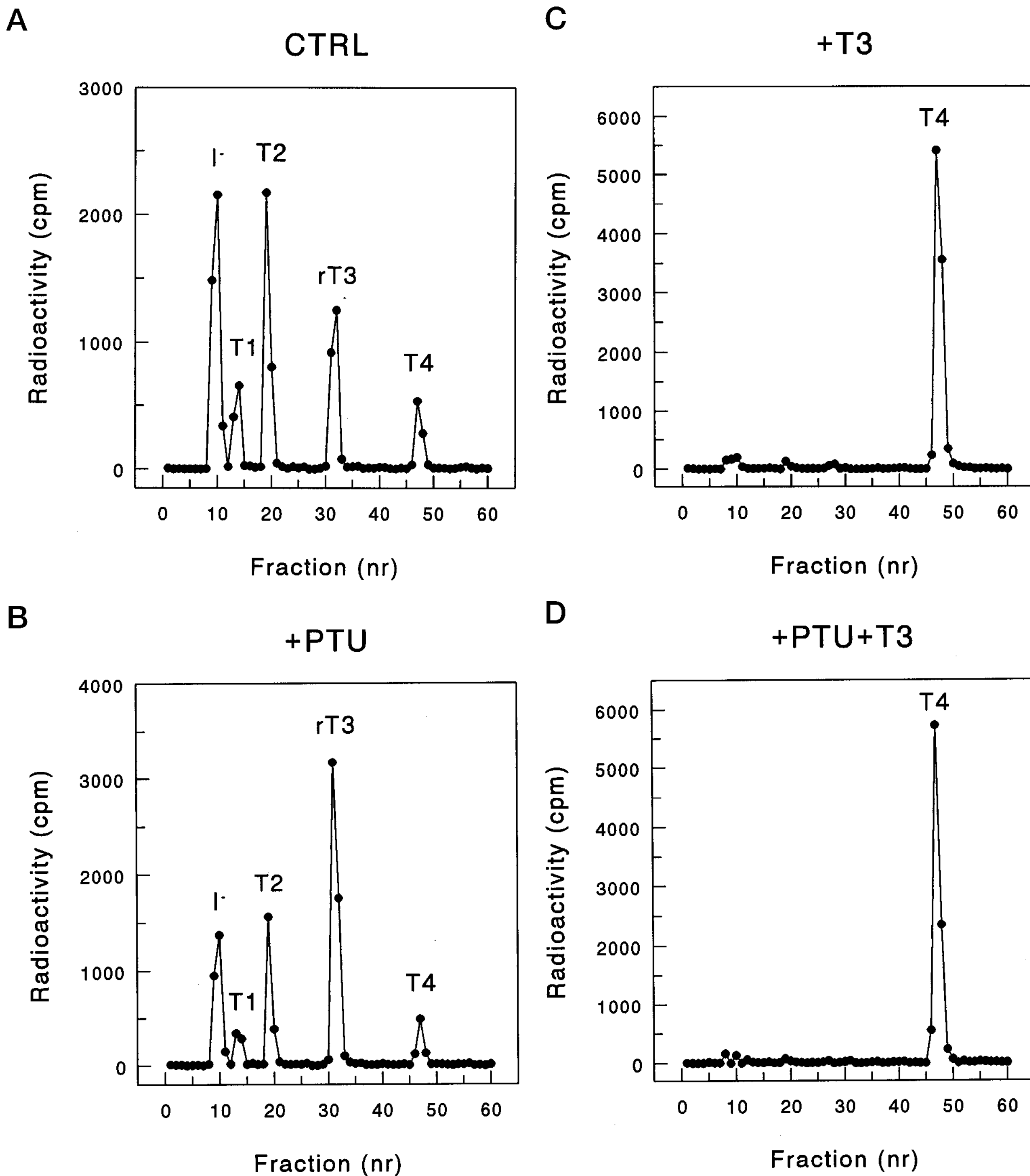

FIG. 3. HPLC analysis of deiodination products of T_4 formed during incubation with fetal human liver microsomes and DTT in absence or presence of PTU and/or unlabeled T_3. Reaction conditions: 1 nM [3′,5′-^{125}I]T_4, 25 mM DTT and 250 μg protein/mL, without PTU or T_3 (A), with 1 mM PTU (B), with 1 μM T_3 (C), or with 1 mM PTU and 1 μM T_3 (D). Incubation time was 30 min. Extraction and HPLC analysis was done as described in *Materials and Methods*.

respectively (Fig. 4B). In both cases, deiodination of T_3 was not affected by the addition of 1 mM PTU (not shown).

Figure 5 shows the results of the affinity labeling of fetal and adult human liver microsomal proteins by BrAc[^{125}I]T_4 or BrAc[^{125}I]T_3. As demonstrated previously (30), the radioactive band of ≈27 kDa represents the affinity labeling of D1. These findings, therefore, suggest similar levels of D1 protein in fetal and adult human liver. No evidence was obtained for affinity labeling of D3 with either BrAc[^{125}I]T_3 or BrAc[^{125}I]T_4, which is in agreement with our previous failure to identify D3 in rat placenta and embryonic chicken liver using these affinity labels (33).

Northern analysis of RNA isolated from two fetal and two adult human liver samples using human D1 and D3 com-

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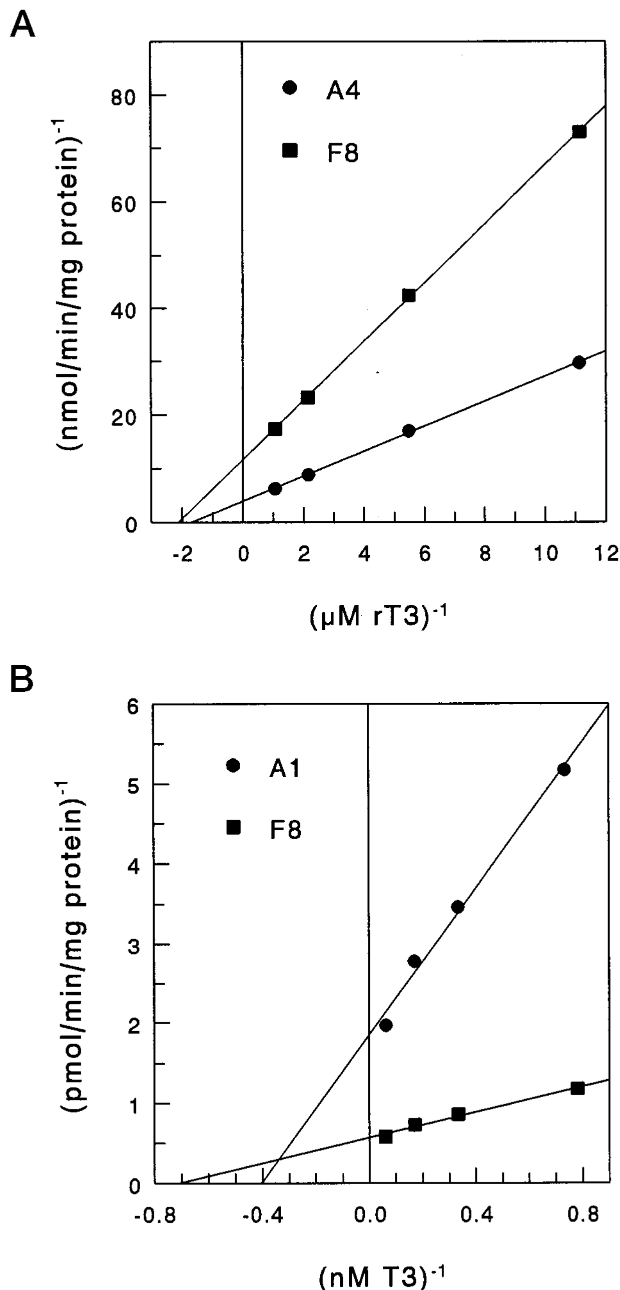

FIG. 4. Double-reciprocal plots of deiodination of rT_3 (A) or T_3 (B) by fetal (F8) or adult (A1 and A4) human liver microsomes as function of substrate concentration. Reaction conditions: A, 0.1–1 μM [3′,5′-^{125}I]rT_3, 10 mM DTT, and 50 μg (F8) or 20 μg (A4) protein/mL; B, 1.5–15 nM [3,5-^{125}I]T_3, 50 mM DTT, and 25 μg (F8) or 100 μg (A1) protein/mL. Incubation time was 30 min.

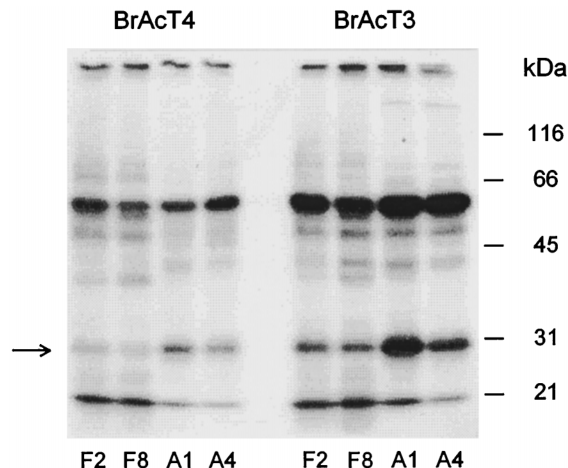

FIG. 5. Affinity labeling of fetal and adult liver microsomal proteins with BrAc[^{125}I]T_4 or BrAc[^{125}I]T_3. Position of marker proteins and their M_r values as well as labeled D1 protein (*arrow*) are indicated. Reaction conditions: 0.1 μCi BrAc[^{125}I]T_4 or BrAc[^{125}I]T_3, 0.1 mg protein, 1 mM DTT in 0.1 mL P100E2, and 20 min incubation. Electrophoresis and autoradiography were done as described in *Materials and Methods*.

plementary DNA probes indicated extensive degradation of RNA and suggested expression of both D1 and D3 mRNA in fetal liver and expression of only D1 mRNA in adult liver (data not shown).

Discussion

Thyroid hormone bioavalability in the human fetus is dependent on, among other things, a) supply of maternal hormone through the placenta, b) hormone production by the fetal thyroid gland, c) the activities of thyroid hormone-metabolizing enzymes such as deiodinases and sulfotransferases in fetal tissues, d) the activities of plasma membrane transporters that mediate uptake of iodothyronines from serum into the tissues, e) exchange (uptake and excretion) of hormone with the amniotic fluid, and f) return of fetal hormone via the placenta to the mother (11–13).

T_4 production by the human fetal thyroid gland has been demonstrated after 10–12 weeks of gestation (11–13). Before this period all T_4 in the fetal circulation is derived from maternal supply across the placenta. However, maternal supply probably remains an important source of fetal thyroid hormone after development of the fetal thyroid gland, as evidenced by substantial T_4 levels in newborns with thyroid agenesis or a complete hormone synthesis defect (34). In view of the available T_4 levels, serum T_3 is low and serum rT_3 is high in the human fetus, in particular during the first two trimesters (11–13, 35). This can be explained by the well-documented high D3 activity in the placenta, which presumably converts a large proportion of T_4 to rT_3 and of T_3 to 3,3′-T_2 during placental transfer (19–24). In addition, findings of high D3 activities in fetal rat brain (25–27) and intestine (27) and in embryonic chicken liver (28, 29) have suggested that significant D3 activity may also be expressed in fetal human tissues. Furthermore, in analogy with the ontogeny of D1 expression in rat liver (9, 27), it has been generally assumed that hepatic T_3 production and rT_3 clearance remain low until D1 starts to be expressed towards the end of human fetal development. This is supported by the high levels of T_4S, T_3S, rT_3S, and 3,3′-T_2S in human fetal serum (14–18), because these conjugates are cleared importantly by hepatic D1 (5).

Because very little is known about the potential role of hepatic D1 and D3 in the regulation of thyroid hormone bioactivity during human fetal development, we carried out the present study. Although the autopsy samples were ob-

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tained from sick infants, and the condition of the tissues, when they were collected, was in some cases suboptimal, the results clearly indicate that D1 activity already is expressed in the second trimester at levels similar to those observed in infants who survived up to 39 weeks postnatally. Another remarkable finding was the high D3 activity in liver homogenates from preterm infants, which was not detectable in livers from term born infants with one exception (see below). These findings were confirmed by comparing microsomal deiodinase activities in fetal livers of 15–20 weeks gestation with those determined in liver samples from apparently healthy adult tissue, showing that D1 activity in fetal liver amounted to at least 20% of that in adult liver. Furthermore, in contrast to the high D3 activities in fetal liver, D3 activity was absent in adult liver with one exception (see below). These findings strongly suggest that the low serum T_3 and high serum rT_3 levels in the human fetus are not so much caused by low hepatic D1 activity but rather to high D3 activity in the liver in addition to the placenta and possibly other tissues. The relative importance of the D3 activity in the fetal liver *vs.* that in the placenta is difficult to assess. The specific activity of the enzyme is similar in these tissues, but because of the difference in tissue weight, the total amount of enzyme is larger in placenta than in fetal liver.

The expression of hepatic D1 and D3 activities during human fetal development is remarkably different from that in rats (9, 27) but resembles the ontogeny of these enzymes in the chicken liver (28, 29, 36). In the chicken, hepatic D1 activity and mRNA level gradually increase until the end (day 20) of embryonic development (E20) (29, 36). In contrast, hepatic D3 activity and mRNA level strongly increase until E17, which is followed by a steep decline after E18 to almost undetectable levels at internal pipping on E20 (29, 36). This fall in hepatic D3 activity is associated with a dramatic increase in serum T_3 levels, suggesting that serum T_3 in the chicken embryo is determined to an important extent through regulation of its degradation by hepatic D3 activity (29, 36). It has been demonstrated that GH plays an essential role in the down-regulation of D3 expression in chicken liver at the end of embryonic development (37). Interestingly, Darras *et al.* (38) have also shown an acute and profound decrease in hepatic D3 activity after administration of dexamethasone to E18 chicken embryos. This was accompanied by a marked increase in serum T_3 and a marked decrease in serum rT_3 (38). It is tempting to speculate that part of the beneficial effect of the antenatal administration of glucocorticosteroids to mothers in case of an imminent premature delivery on the postnatal development of the infant (39) is caused by this down-regulation of hepatic D3 activity and consequent increase in serum T_3 levels.

It is generally believed that the high D3 activity in placenta, fetal liver, and possibly other fetal tissues protects the fetus during critical stages of development against active thyroid hormone (11–13, 23). D3 is an important enzyme for the irreversible degradation of thyroid hormone, because the products generated from T_4 and T_3 by this enzyme, *i.e.* rT_3 and 3,3′-T_2, have very little affinity for the nuclear T_3 receptor (TR) nor can they be converted to TR-binding ligands (40). T_3 stimulates the differentiation of cells, and premature exposure of growing tissues to active hormone may thus result in congenital abnormalities. Sulfation is another pathway by which thyroid hormone is inactivated, because T_3S has lost its affinity for TR (41). Furthermore, in adult subjects, sulfation represents the first step in a pathway leading to the irreversible degradation of thyroid hormone, because IRD of T_4S and T_3S by D1 is greatly accelerated compared with the deiodination of the nonsulfated iodothyronines (5). The importance of D1 for the clearance of serum T_4S, T_3S, rT_3S, and 3,3′-T_2S is indicated by the marked increases in the serum levels of these conjugates after inhibition of D1 by PTU and, in particular, iopanoic acid (14, 42–44). The high levels of the iodothyronine sulfates in human fetal serum have also been explained by the reduced clearance of these conjugates presumably caused by low hepatic D1 expression, although evidence has also been reported that production of the sulfates is increased in fetal sheep (11, 45). It has been speculated that T_3S represents a reservoir of inactive hormone from which active T_3 may be released by sulfatases expressed in different tissues (5, 46). Our results show that hepatic D1 activity may be somewhat lower in the human fetus than in the adult but not to the extent that would explain the strongly increased T_4S, T_3S, rT_3S, and 3,3′-T_2S levels in fetal serum. This suggests that additional mechanisms contribute to the elevation of serum iodothyronine sulfate levels in human fetal serum, such as decreased expression of plasma membrane transporters involved in tissue uptake of these conjugates (3).

Another surprising finding of our study is the occasional expression of hepatic D3 in livers from older subjects. If this represents a defect in the mechanism that normally shuts off D3 gene expression in the liver after birth or, more likely, reexpression of the gene under pathological conditions, remains to be explored. In this respect, it should be mentioned that high D3 activity has been detected in a monkey hepatocarcinoma cell line (47). It is also tempting to speculate that, like other fetoproteins, hepatic D3 expression may be stimulated by cytokines (48). If this is the case, changes in peripheral thyroid hormone metabolism in the low T_3 syndrome associated with nonthyroidal illness (49) may include up-regulation of hepatic D3 activity.

D2 activity was undectable in both fetal and adult human liver samples. We cannot exclude, however, that D2 activity may be expressed in human fetal liver but is lost caused by postmortem inactivation (50).

In conclusion, we have shown that expression of both D1 and D3 in human fetal liver is higher than previously assumed. This suggests that the low T_3 and high rT_3 levels in human fetal serum is not so much caused by low hepatic D1 expression but rather to high D3 activity in fetal human liver in addition to placenta and perhaps other fetal tissues. The exact role of D3 expression in the tissue-specific and stage-dependent regulation of thyroid hormone bioactivity during human fetal development remains to be fully investigated.

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