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17β-Hydroxysteroid Dehydrogenase Type 1 and 2 Expression in the Human Fetus*

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ABSTRACT

The present study investigates the expression patterns of 17β-hydroxysteroid dehydrogenase (17βHSD) isozymes in human fetal tissues to understand how estrogenic activity is regulated in the human fetus. Using enzyme assay, high 17βHSD activity was detected in the placenta and liver, and low levels of 17βHSD activity were also present in the gastrointestinal tract and kidney. After Northern blot analysis, we detected the messenger ribonucleic acid for 17βHSD type 1 (17βHSD1) only in the placenta, whereas that for 17βHSD type 2 (17βHSD2) was detected in the placenta, liver, gastrointestinal tract, and urinary tract at 20 gestational weeks. In RT-PCR analysis of the messenger ribonucleic acid transcripts, 17βHSD1 was predominantly expressed in the placenta, brain, heart, lung, and adrenal, whereas 17βHSD2 expression was predominantly detected in the liver, gastrointestinal tract, and kidney. In addition, we detected 17βHSD2 immunoreactive protein in surface epithelial cells of the stomach, absorptive epithelial cells of the small intestine and colon, hepatocytes of the liver, and interstitial cells surrounding the urinary tubules of the renal medulla. 17βHSD2 in these tissues may be functioning in the prevention of *in utero* exposure of the fetus to excessive estradiol from the maternal circulation and amniotic fluids. (*J Clin Endocrinol Metab* **85:** 410–416, 2000)

THE FETUS IS exposed to large quantities of estrogens *in utero*. In normal pregnancy, large quantities of estrogens are produced by the syncytiotrophoblast, are secreted into the intervillous space, and then transferred into both the fetal and maternal circulations. Maternal levels of circulating estrogens rise continuously throughout pregnancy (1), and the increase in the plasma concentrations of all estrogens has been demonstrated to correlate with fetal weight (2). In addition to estrogens entering the fetal circulation through the umbilical vein (3), large quantities of estrogens are present in the amniotic fluid and may enter the fetal circulation by passive diffusion through the fetal skin or by swallowing and absorption from the gastrointestinal tract. The levels of estrogens in amniotic fluid are also significantly elevated as pregnancy progresses (4). Therefore, estrogens appear to play some crucial roles in maintaining pregnancy and fetal development. The feto-placental unit is known to produce and secrete large quantities of biologically active estrogens during pregnancy. The fetus must, however, be protected from excessive exposure to these estrogens. It has been reported that excessive exposure of the fetus to estrogens *in utero* occasionally has deleterious effects on fetal development, as demonstrated in the offspring of women administered diethylstilbestrol during pregnancy (5). Therefore, studying how estrogens are metabolized in fetal tissues is important in understanding estrogen actions in the fetus.

17β-Hydroxysteroid dehydrogenase (17βHSD) isozymes regulate the relative concentrations of potent 17β-hydroxy and biologically weak 17β-keto forms of estrogens and androgens locally at the target tissue level. The type 1 isozyme of 17βHSD (17βHSD1), which is associated with a high specificity for C_{18}-steroids, primarily converts the inactive C_{18}-steroid estrone (E_1) to the biologically active estrogen estradiol (E_2) (6–8). In contrast, attenuation of E_2 action is achieved by expression of the type 2 isozyme of 17βHSD (17βHSD2), an enzyme that inactivates E_2 to E_1 (8–10). 17βHSD2 is also active on androgens, and therefore, this enzyme is generally considered to play important roles in the peripheral inactivation of androgens and estrogens and in the maintenance of steady state levels of these steroids in target tissues. In addition, 17βHSD2 catalyzes the 20α-oxidation of the C_{21}-steroid, 20α-dihydroprogesterone, to the active progestin, progesterone (9–11).

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We have previously reported the immunolocalization of 17βHSD1 and 17βHSD2 protein in the human placenta (12). 17βHSD1 immunoreactive protein is localized to the syncytiotrophoblast, where the active estrogen, E_2, is produced and secreted into the maternal circulation. On the other hand, 17βHSD2 protein is detected in the endothelial cells of capillaries and veins of the chorionic villi, where E_2 is converted to E_1 before entering the fetal circulation. Therefore, 17βHSD2 in the human placenta can serve as a barrier or gatekeeper to the passage of excessive, maternally derived estrogens into the fetal circulation.

The interconversion of E_1 and E_2 has been demonstrated in rat and human adult tissues (13). Moreover, the cell type-specific expression of 17βHSD2 immunoreactive protein has been detected in hepatocytes of the fetal liver (14). However, the enzyme activity and expression of 17βHSD isozymes in the human fetus have not been examined systematically. In this study, we examined the tissue distribution of 17βHSD activities using ^{14}C-labeled E_2 or E_1 as a substrate in homogenates from various human fetal tissues.

We then studied the expression of messenger ribonucleic acid (mRNA) transcripts for 17βHSD1 and 17βHSD2 using Northern blot analysis. In addition, we examined these isozymes by RT-PCR using fluorescent dye-labeled primers. Furthermore, we used immunohistochemistry to study the cellular distribution of 17βHSD2 protein in the human fetus.

TABLE 1. Oligonucleotide primer sequences used for RT-PCR analysis

mRNA	Primer	Sequence
17βHSD1	Antisense for RT	5′-CGAAAGACTTGCTTGCTGTG-3′
	Antisense for PCR	5′-CTCTGGGCTGCCCAACAC-3′
	Sense for PCR	5′-GGACGTGCTGGTGTGTAAC-3′
17βHSD2	Antisense for RT	5′-CATGCTGCTGACATTCACCA-3′
	Antisense for PCR	5′-CTTTGTGACCTCCACAGTTC-3′
	Sense for PCR	5′-GGTGTCATGCTTCCTCATGT-3′
β-Actin	Antisense for RT	5′-ACGTCACACTTCATGATGGA-3′
	Antisense for PCR	5′-CAGCGGAACCGCTCATTGC-3′
	Sense for PCR	5′-GTGATGGACTCCGGTGACGG-3′

Materials and Methods

Tissue preparation

Human fetal tissues (11–21 weeks gestational age) were obtained after elective termination in normal pregnant women at Tohoku University Hospital and Nagaike Maternal Clinic (Sendai, Japan). This research protocol was approved by the committee on the ethics of Tohoku University School of Medicine, and informed consent was obtained from these pregnant women before elective termination. The ages of the fetuses were estimated by the last menstrual date, body weight, or crown-rump length. Human fetal tissues of 26, 30, 36, and 38 weeks of age were obtained at the time of autopsy at Tohoku University Hospital. The time elapsed from fetal death to the removal of the tissues ranged from 0.5–1 h in the case of elective termination and from 2–6 h in autopsy cases. These samples did not have any significant histopathological abnormalities. The specimens for enzyme assays and RNA isolation were snap-frozen and stored at −80 C, and those for immunohistochemistry were fixed in 10% neutral formalin for 18 h at 4 C and embedded in paraffin. Due to the availability of the specimens, enzyme assay, Northern blotting, and RT-PCR analysis could not be performed in the same fetal specimen. Among the cases examined for enzyme assay, Northern blotting, and RT-PCR analysis, one of the three cases (21 weeks) in which enzyme assay was analyzed and one of the two cases (20 weeks) in which RT-PCR analysis was performed were also evaluated by immunohistochemistry.

Enzyme assay

The specimens used in this study were obtained from fetuses aged 20 weeks (male), 20 weeks (female), and 21 weeks (female). Human fetal tissues were homogenized at 4 C in phosphate buffer (100 mmol/L KCl, 10 mmol/L KH_2PO_4, 10 mmol/L Na_2HPO_4, and 1 mmol/L ethylenediamine tetraacetate, pH 7.5) using a Potter-Elvehjam glass-Teflon homogenizer and centrifuged for 15 min at 1000 × *g*. Aliquots of the supernatant were incubated for 30 min at 37 C in 0.5 ml phosphate buffer containing 1 μmol/L ^{14}C-labeled E_2 or E_1 and 10 mmol/L NAD^+, NADH, $NADP^+$, or NADPH. Incubations were terminated and extracted with 2 ml diethyl ether. The organic phase was evaporated, and the residue was subjected to TLC; the developing solvent was cyclohexane-ethyl acetate (1:1, vol/vol). The chromatoplates were autoradiographed for 18 h at room temperature, and the radioactivity signals were detected and quantitated using a GS-250 Molecular Imager (Bio-Rad Laboratories, Inc., Hercules, CA). The protein content of the tissue homogenates was measured by the method of Lowry (15) using BSA as a standard. The differences in 17βHSD activity among various fetal organs were statistically evaluated using Bonferroni's test. The cofactor preference for the oxidative and reductive reactions of 17βHSD was also

TABLE 2. 17βHSD activities in human fetal tissues

	SA (pmol/mg protein · min)			
	$E_2 \rightarrow E_1$ (oxidation)		$E_1 \rightarrow E_2$ (reduction)	
	NAD^+	$NADP^+$	NADH	NADPH
Placenta	283.7 ± 16.4[a]	254.3 ± 52.7[a]	254.3 ± 22.3[a]	307.3 ± 20.6[a]
Liver[b]	209.3 ± 10.7[a]	92.9 ± 17.0[a]	206.3 ± 17.6[a]	89.4 ± 10.3[a]
Stomach[b]	7.2 ± 0.6	2.6 ± 0.6	4.8 ± 0.3	1.5 ± 0.4
Small intestine[b]	32.5 ± 1.5[c]	12.5 ± 1.2	30.3 ± 2.5[c]	12.3 ± 0.9
Colon[b]	11.9 ± 0.6	5.7 ± 0.7	10.4 ± 0.4	2.7 ± 0.3
Kidney[b]	17.4 ± 3.2	8.1 ± 1.3	16.2 ± 1.7	7.8 ± 0.7
Brain	1.2 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
Adrenal gland	1.3 ± 0.3	1.1 ± 0.1	1.1 ± 0.3	1.0 ± 0.2
Heart	—[d]	—[d]	—[d]	—[d]
Lung	—[d]	—[d]	—[d]	—[d]

Each value represents the mean ± SE of three independent experiments.

[a] 17βHSD activity in the placenta and liver differed significantly from other tissues ($P < 0.0001$), and significantly higher in the placenta than in the liver ($P < 0.0001$).

[b] 17βHSD activity in the liver, stomach, small intestine, colon, and kidney demonstrated significant preference for NAD(H) ($P < 0.001$).

[c] 17βHSD activity in the small intestine is significantly higher than that in the brain, adrenal gland, heart, and lung ($P < 0.01$).

[d] No kinetic parameters could be obtained.

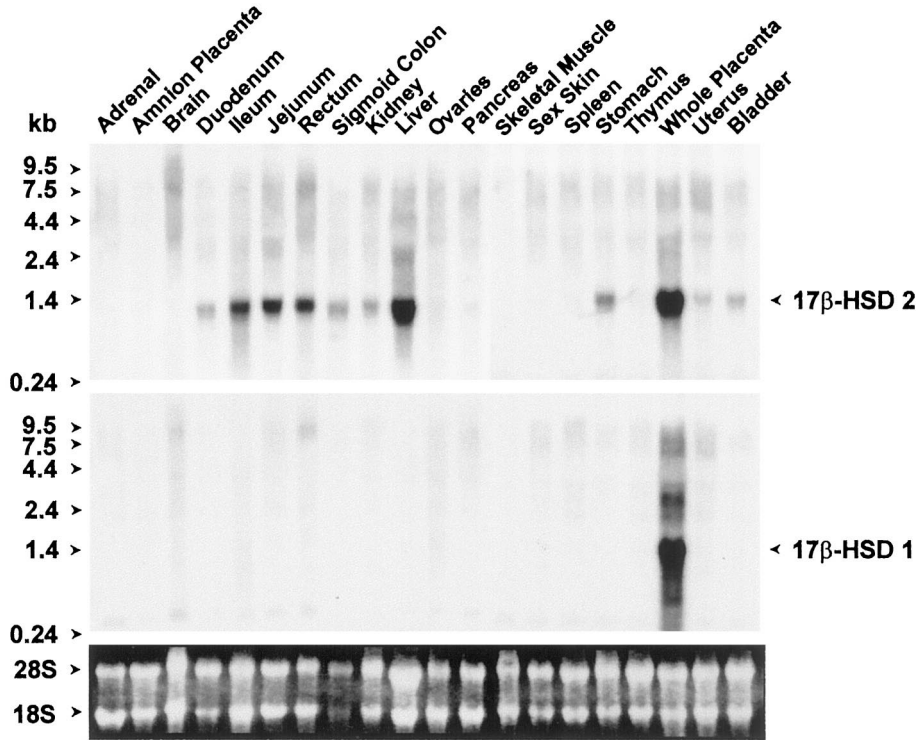

FIG. 1. Northern blot analysis of 17βHSD2 (*above*) and 17βHSD1 (*below*) in human fetal tissues (20 gestational weeks). Ten micrograms of total RNA from the indicated tissues were used to study mRNA expression of 17βHSDs by Northern blot analysis. The ethidium bromide-stained gel is presented in the *bottom panel*. The positions to which RNAs of known size migrated are shown on the *left*. The mRNA for 17βHSD1 was detected only in the placenta, whereas that for 17βHSD2 was observed in the placenta, liver, gastrointestinal tract, and urinary tract.

studied and evaluated with Student's *t* test. $P < 0.01$ was considered significant.

Northern blot analysis

The RNA used in this study was extracted from a female fetus (20 weeks gestation). The procedures employed in this study were previously described in detail by us (10). In brief, total RNA was purified from frozen tissue using the RNA STAT-60 System (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. Ten micrograms of total RNA were size fractionated by electrophoresis, transferred to a nylon membrane, and hybridized with complementary DNA (cDNA) probes under high stringency conditions as described.

Analysis of the mRNAs for 17βHSD types 1 and 2 by RT-PCR with a fluorescent primer

RNA was extracted from fetuses aged 13 weeks (male) and 20 weeks (female). Semiquantitative analysis of 17βHSD1, 17βHSD2, and β-actin mRNAs was carried out in the total RNA fractions from various human fetal tissues by RT-PCR using a specific pair of fluorescent dye-labeled primers and their standard RNAs as previously described (16, 17). Oligonucleotide primers for RT-PCR were synthesized as shown in Table 1. The coding sequence between the two PCR primer sites is located 5′ upstream of the RT primer site in each cDNA for 17βHSD1, 17βHSD2, and β-actin. The standard RNAs for 17βHSD1, 17βHSD2, and β-actin were synthesized *in vitro* with T7 RNA polymerase using their cDNA as templates, purified, and then spectrophotometrically quantitated. Total RNA (0.2–2 μg) and standard RNAs (0.02–2 amol) were subjected to RT with Superscript II reverse transcript (Life Technologies, Inc., Grand Island, NY) and a specific antisense primer at 47 C for 45 min. The resulting cDNAs were amplified by PCR using a specific pair of fluorescent dye-labeled sense primers and antisense primers for 23–28 cycles. The fluorescent PCR products were analyzed on a 2% agarose gel with a Gene Scanner 362 Fluorescent Fragment Analyzer (Perkin-Elmer Corp., Foster City, CA). Relative amounts for 17βHSD1, 17βHSD2, and β-actin mRNAs in the tissue RNA were calculated from the peak areas of the fluorescent PCR products of a specific mRNA and its standard RNA.

Primary antibody

The monoclonal antibody, mAb-C2–12 (subclass IgG1/κ), was produced by immunizing mice with a synthetic carboxyl-terminal peptide corresponding to amino acids 375–387 of 17β-HSD2. The detailed characterization of this monoclonal antibody has been previously reported (14).

Immunohistochemistry

The gestational age of the fetus examined ranged from 11–38 weeks. Paraffin-embedded tissues (3.0-μm sections) were mounted onto clean silane-coated glass slides (Matsunami Co. Ltd., Tokyo, Japan). After deparaffinization, the sections were pretreated by hydrated autoclaving in 10 mmol/L sodium citrate buffer (pH 6.0) at 120 C for 5 min to retrieve the antigenicity (18). The slides were then immersed in methanol containing 0.3% hydrogen peroxidate for 30 min to block endogenous peroxidase activity. The immunohistochemical staining procedure employed in this study was the streptavidin-biotin amplified method. We used peroxidase-conjugated streptavidin from a Histofine Kit (Nichirei Co., Tokyo, Japan) and visualized the antigen-antibody complex with 3,3′-diaminobenzidine. Normal rabbit serum was incubated for 30 min at room temperature to decrease nonspecific staining. Sections were then incubated with the primary antibody for 17β-HSD2 at a concentration of 1:5 for 18 h at 4 C. After washing, the sections were incubated with biotinylated rabbit antimouse Ig and then with peroxidase-conjugated streptavidin, followed by incubation in a solution containing 0.05% hydroxychloride (pH 7.6), 0.06 mmol/L 3,3′-diaminobenzidine, and 2 mmol/L hydrogen peroxide. Immunoreactive sections were counterstained with hematoxylin and mounted with a glycerol-gelatin water-soluble medium. As negative controls for immunostaining, the sections were incubated with normal mouse IgG instead of primary antibodies. No specific immunoreactivity was detected in these sections.

Results

Enzyme assay

The levels of estrogenic 17βHSD activity measured in various human fetal tissues are demonstrated in Table 2. The

TABLE 3. Quantitative analysis of 17βHSD1 and 17βHSD2 mRNAs

	17βHSD1/β-actin	17βHSD2/β-actin
Placenta		
13W	147.25	27.48
20W	146.04	36.99
Brain		
13W	2.26	0.32
20W	2.79	0.39
Heart		
13W	3.60	0
20W	3.42	0
Lung		
13W	1.11	0.15
20W	0.94	0.23
Adrenal		
13W	0.88	0
20W	0.77	0
Liver		
13W	0	30.85
20W	0.98	185.57
Kidney		
13W	1.15	2.22
20W	1.05	18.92
Esophagus		
13W	—[a]	—[a]
20W	0.16	2.19
Stomach		
13W	0.85	4.00
20W	0.18	9.05
Small intestine		
13W	1.42	21.2
20W	0	58.61
Colon		
13W	—[a]	—[a]
20W	0.27	13.13

Data are expressed on the basis of β-actin mRNA levels.
[a] Not available.

rates of estrogenic 17βHSD activity detected in the placenta and liver were statistically higher than those in other fetal tissues ($P < 0.0001$). 17βHSD activity in placenta was significantly higher than that in liver ($P < 0.0001$). Low, but significant, levels of 17βHSD activity were also detected in the stomach, small intestine ($P < 0.01$ compared to 17βHSD activity in brain, adrenal gland, heart, and lung), colon, and kidney. 17βHSD activity in the brain and adrenal gland was extremely low. The specimens from heart and lung showed no detectable 17βHSD activity under the experimental conditions used. For cofactor preference, the enzyme present in the liver, gastrointestinal tract, and kidney responsible for 17βHSD activity significantly preferred NAD(H) to NADP(H) ($P < 0.001$).

Northern blot analysis

Expression of the 1.3-kb mRNA for 17βHSD1 was detected only in the placenta (Fig. 1). On the other hand, the 1.5-kb mRNA for 17βHSD2 was markedly detected in the placenta, liver, ileum, jejunum, and rectum (Fig. 1). In addition, relatively low levels of 17βHSD2 mRNA hybridization signals were detected in the stomach, duodenum, sigmoid colon, uterus, kidney, and urinary bladder.

TABLE 4. Summary of 17βHSD2 immunoreactivity in human fetal tissues

Weeks gestation	Stomach	Small intestine	Colon	Liver	Kidney
11	−	+++	−	+++	−
14	−	+++	−	+++	−
16	−	+++	−	+++	−
18	−	+++	+	+++	+
20	−	+++	+	+++	+
21	−	+++	++	+++	+
26	+	+++	+++	+++	+++
30	+	+++	+++	+++	+++
36	+++	+++	+++	+++	+++
38	+++	+++	+++	+++	+++

Intensity of staining was graded subjectively on a scale of − representing no reactivity to +++ representing very intense staining.

Analysis of the mRNAs for 17βHSD1 and 17βHSD2 using RT-PCR

Results of the semiquantitative analysis of 17βHSD1 and 17βHSD2 mRNAs are summarized in Table 3. All quantitated data are expressed on the basis of β-actin mRNA levels. The greatest amount of 17βHSD1 mRNA was detected in the placenta, whereas the brain, lung, heart, and kidney expressed relatively lower amounts of this gene. 17βHSD1 expression in the adrenal gland, liver, and gastrointestinal tract was extremely low. On the other hand, a relatively large amount of 17βHSD2 mRNA was detected in the placenta, liver, kidney, and gastrointestinal tract. The expression of the 17βHSD1 gene was predominant in the placenta, brain, heart, lung, and adrenal gland, whereas that of 17βHSD2 was much more dominant in the liver, gastrointestinal tract, and kidney.

Immunohistochemistry

Results are summarized in Table 4. In small intestine and liver, immunoreactivity of 17βHSD2 was marked from early gestational weeks and throughout the course of gestation. In the stomach, colon, and kidney, 17βHSD2 immunoreactivity was not detected in the early stages of gestation; it was detected and subsequently increased in intensity from mid to late stages of gestation. 17βHSD2 immunoreactivity was detected in surface epithelial cells of the stomach, but not in gastric glands (Fig. 2a). In the small intestine and colon, 17βHSD2 immunoreactivity was detected in absorptive epithelial cells (Fig. 2, b and c). No immunoreactivity was seen in the lamina propria, muscularis mucosae, or the submucosal layers of the stomach and intestine. Hepatocytes in the fetal liver demonstrated marked immunoreactivity for 17βHSD2 (Fig. 2d). The immunolocalization of 17βHSD2 in the kidney was exclusively present in interstitial cells surrounding the urinary tubules of the renal medulla (Fig. 2, e and g), but not in the urinary tubules and glomerulus (Fig. 2f). Immunoreactivity for 17βHSD2 was analyzed in skin at 20 gestational weeks and was detected only in sebaceous glands, not in the epidermis (Fig. 2h).

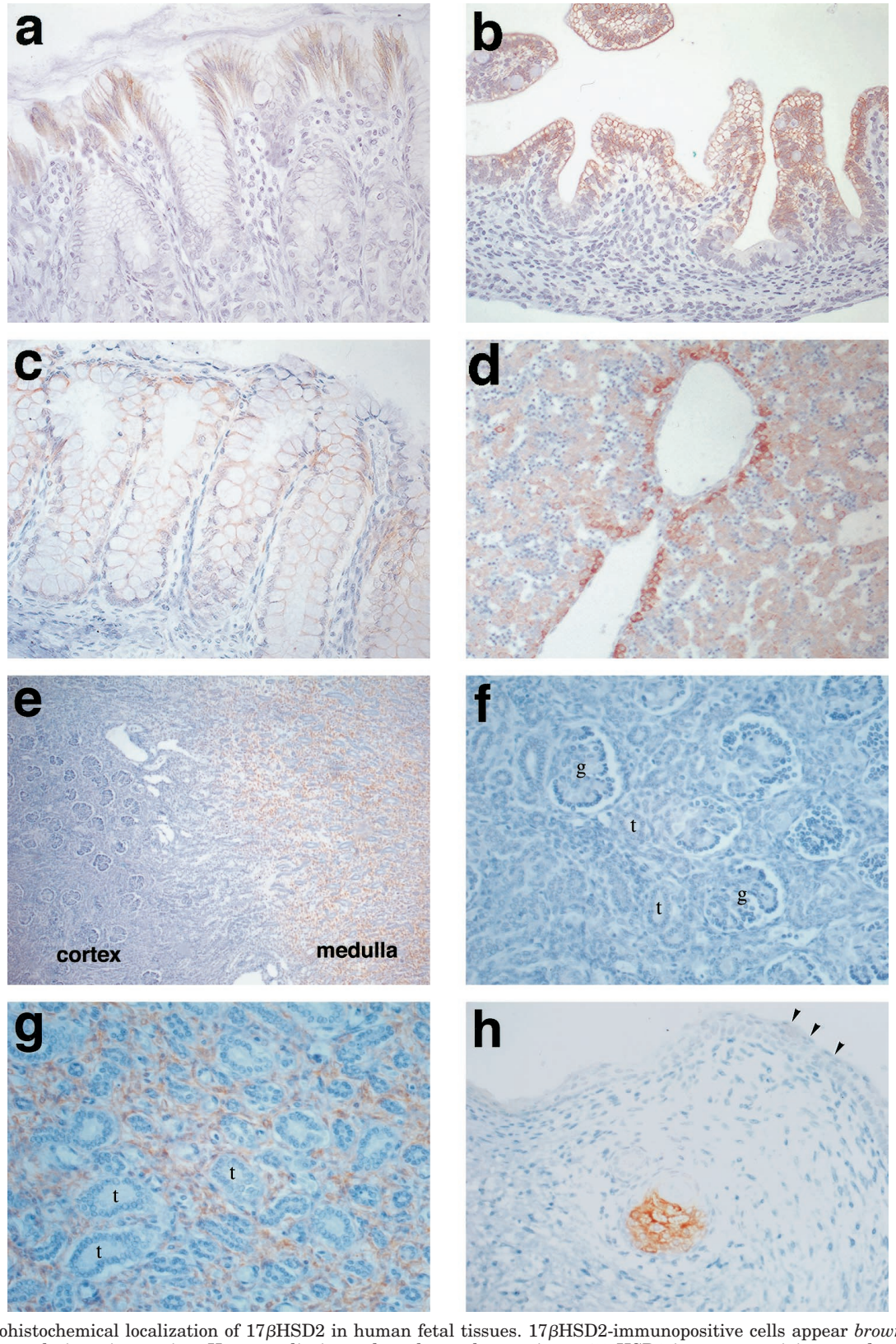

FIG. 2. Immunohistochemical localization of 17βHSD2 in human fetal tissues. 17βHSD2-immunopositive cells appear *brown* as a result of diaminobenzidine colorimetric reaction. Hematoxylin is used as the nuclear stain. a, 17βHSD2 immunoreactivity was detected in surface epithelial cells of the stomach (36 gestational weeks). b and c, Absorptive epithelial cells of the small intestine (11 gestational weeks; b) and colon (36 gestational weeks; c) demonstrated marked immunoreactivity for 17βHSD2 protein. d, 17βHSD2 was immunolocalized in hepatocytes, with intense staining around the portal veins (11 gestational weeks). e, In kidney (36 gestational weeks), marked 17βHSD2 immunoreactivity

Discussion

17βHSD1, the predominant isozyme of 17βHSD responsible for estrogen production, preferentially reduces E_1 to E_2 using NADPH as a cofactor (19). 17βHSD2 preferentially oxidizes potent C_{19}- and C_{18}-steroids to weaker estrogens and androgens at an alkaline pH using NAD^+ as a cofactor (9, 19). Expression patterns for 17βHSD1 and 17βHSD2 are suggested to play important roles in the regulation of estrogenic activity at target tissue levels. The present study demonstrated that the 17βHSD oxidative reaction was predominant in the liver, gastrointestinal tract, and kidney of the fetus, and the mRNA transcript for 17βHSD2 was detected in these tissues by Northern blot analysis, although these were not examined in the same fetal specimens. These observations suggest that the type 2 isozyme was expressed at both the mRNA and protein levels, and in addition, *in situ* estrogenic activity in these tissues was predominantly regulated by the inactivation of E_2 to E_1. We also detected the activity for 17βHSD1 enzyme, but could not detect the mRNA for 17βHSD1 in Northern blot analysis. It has been reported that the activity catalyzed by each type of 17βHSD is almost exclusively unidirectional in intact cells, whereas these 17βHSDs can drive the interconversion of the product and substrate in cell homogenate (19). Enzyme activity in intact cells depends upon the availability of endogenous cofactors, whereas cellular homogenates are supplied with an excess concentration of cofactor. The cellular compartmentalization and intracellular pH gradient may be disrupted in homogenates as well. Therefore, the possible discrepancy between the detection of type 1-like activity by direct assay and the absence of mRNA by Northern blot analysis requires further investigation, including analysis in the same fetal cases.

In this study we spectrophotometrically examined the mRNA for 17βHSD1 and 17βHSD2 in various human fetal tissues by RT-PCR using fluorescent dye-labeled primers for semiquantitation of the transcripts. We could not examine them in the same fetal tissues, but 17βHSD1 and -2 transcripts that appeared weak or absent on Northern blot analysis were detected by RT-PCR. For instance, 17βHSD1 mRNA, which could not be detected by Northern blot analysis, was observed in almost all tissues examined by RT-PCR. 17βHSD1 was predominantly expressed in the brain, heart, lung, and adrenal. These results suggest that estrogens are locally activated in these tissues and possibly play crucial roles in the control of growth and/or differentiation. On the other hand, relatively high levels of 17βHSD2 expression were detected in the liver, gastrointestinal tract, and kidney, which is consistent with the results from our Northern blot analysis. However, the number of cases examined in the mRNA study was limited, and further investigations are required for clarification.

We also characterized 17βHSD2 localization in human fetal tissues using immunohistochemistry. Among gastrointestinal tract tissues in which 17βHSD2 mRNA transcripts were detected, 17βHSD2 immunoreactivity was detected in surface epithelial cells of the stomach and absorptive epithelial cells of the small intestine and colon. 17βHSD2 enzyme in epithelial cells of the gastrointestinal tract has been suggested to be involved in the inactivation of E_2 before its absorption (20). Intense 17βHSD2 immunoreactive protein was detected in the small intestine in early stages of gestation. 17βHSD2 in the small intestine may be involved in the inactivation of E_2 in swallowed amniotic fluid. In the stomach and large intestine, relative 17βHSD2 immunointensity increased from mid to late stages of gestation as the concentrations of estrogens in amniotic fluid increased (4). Hepatocytes in the liver from early stages of gestation demonstrated marked 17βHSD2 immunoreactivity, which probably plays a major role in E_2 inactivation from the fetal portal circulation. We previously reported that 17βHSD2 was immunolocalized in the endothelial cells of capillaries in chorionic villi, which prevent excessive E_2 from entering into the fetal circulation (12). Therefore, large quantities of E_2 derived from the maternal circulation and amniotic fluid are inactivated by 17βHSD2 present in the placenta, stomach, small intestine, colon, and liver before entering the fetal systemic circulation and targeting various fetal tissues.

In the kidney, the expression of 17βHSD2 immunoreactive protein was present in interstitial and stromal cells surrounding the urinary tubules, but not in the tubules or glomerulus. The possible physiological role of 17βHSD2 in the human fetal kidney may include the inactivation of E_2, possibly leaking from the urinary tubules because of their immaturity. However, further investigations are required to clarify the biological significance of 17βHSD2 in this tissue. In the skin, 17βHSD2 was present only in sebaceous glands, not in the epidermis. Therefore, estrogens in amniotic fluid may enter the fetal circulation by diffusion through fetal skin. 17βHSD2 is highly expressed in sebaceous glands of adult skin and regulates sebum production (21). The present results indicate that sebum production occurs in human fetal skin from as early as 20 weeks gestation.

At present, six isozymes of 17βHSD have been identified. 17βHSD type 3 is predominantly expressed in the testis and converts the inactive C_{19}-steroid androstenedione to the active androgen testosterone (22). 17βHSD type 4, which is expressed in many tissues, with the highest levels in liver, heart, prostate, and testis, has been shown to convert E_2 to E_1 and androstenediol to dehydroepiandrosterone (23). 17βHSD type 5 is an aldoketoreductase that preferentially reduces androgens and estrogens using NADPH as a cofactor (24). 17βHSD type 6, isolated from rat ventral prostate, preferentially oxidizes 3α-androstanediol to androsterone using NAD^+ as a cofactor and oxidizes testosterone and estradiol (25). It has been previously reported that 17βHSD type 1, 2, 4, and 5 isozymes were detected by Northern blots of whole mouse embryos, and each of the enzymes showed

was detected in the renal medulla, but not in the cortex. f, The urinary tubules (t) and glomerulus (g) in the renal cortex did not show any immunoreactivity. g, 17βHSD2 immunoreactive protein was found in the interstitial cells surrounding the urinary tubules (t) of the renal medulla. h, In the skin, 17βHSD2 immunoreactivity was detected in sebaceous glands, but not in epidermis (*arrowheads*). Original magnification: ×40 (e), ×200 (a, c, and h), and ×400 (b, d, f, and g).

a unique pattern of expression (26). Further investigations of 17βHSD isozymes are required to clarify the regulation of various sex steroids in human fetal tissues.

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Erratum

In the article "Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects" by Metin Ozata, I. Caglayan Ozdemir, and Julio Licinio (*The Journal of Clinical Endocrinology & Metabolism* **84:**3686–3695), the term "hyperleptinemia" was misspelled as "hyperglycemia" in the first and second lines of the second column of the abstract. The printer regrets the error.

Also, Dr. Licinio wishes to correct his affiliation. He is with the Department of Psychiatry at the University of California—Los Angeles.