17β-Hydroxysteroid Dehydrogenase Type 2: Chromosomal Assignment and Progestin Regulation of Gene Expression in Human Endometrium

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Abstract

The cDNAs for two separate human 17β-hydroxysteroid dehydrogenases (17β-HSD) have been isolated and sequenced. The well-studied human placental cytosolic 17β-HSD (also referred to as estradiol dehydrogenase) preferentially catalyzes the reduction of estrone to estradiol-17β and the reduction of the C-20-ketone of progesterone to 20α-dihydroprogesterone. This isoform of the enzyme has been referred to as 17β-HSD type 1 and localized to chromosome 17. A second 17β-HSD isoform (referred to as type 2) is localized in the endoplasmic reticulum of human trophoblast and is characterized by the preferential oxidation of the C-17β-hydroxyl group of C_{18}- and C_{19}-steroids and the C-20α-hydroxyl group of 20α-dihydroprogesterone. In this study, we determined the chromosomal localization of human 17β-HSD type 2, the expression of this gene in human endometrium, and the tissue distribution of the mRNA. We found that the human 17β-HSD type 2 gene is localized on chromosome 16, 16q24. 17β-HSD type 2 mRNA (~ 1.5 kb) was identified in human endometrial tissues by Northern analysis of total RNA (10 μg). The highest levels of 17β-HSD type 2 mRNA were found in endometrial tissues obtained during the mid- to late secretory phase of the ovarian cycle (i.e., during the time of high plasma levels of progesterone). 17β-HSD type 2 mRNA levels were much greater in glandular epithelium than in the stromal cells isolated from secretory phase endometrium. The levels of 17β-HSD type 2 mRNA in secretory phase endometrium were approximately one-tenth that in villous trophoblast tissue from human placenta. We did not detect 17β-HSD type 1 mRNA in endometrial tissue by Northern analysis of total (10 μg) RNA. These findings are consistent with the view that the progestin-regulated 17β-HSD of the glandular epithelium of the human endometrium is primarily, if not exclusively, the product of the 17β-HSD type 2 gene. 17β-HSD type 2 mRNA was present in human placenta, liver, and small intestine; much smaller amounts, barely detectable by Northern analysis of poly(A)$^+$ RNA, were present in prostate, kidney, pancreas, and colon, but not in heart, brain, skeletal muscle, spleen, thymus, ovary, or testis. (*J. Clin. Invest.* 1994. 94:2135–2141.) Key words: endometrium • 17β-hydroxysteroid dehydrogenase • progesterone • estradiol-17β • chromosome 16

Introduction

It is established that estradiol-17β, and not estrone, is the principal estrogen secreted by the human placenta (see reference 1), ovary (see reference 2), and testis (3). Also, testosterone (and not androstenedione) is secreted by the adult human testis (3), and progesterone (and not 20α-dihydroprogesterone) is the primary C_{21}-steroid secreted by human placenta (1) and corpus luteum (4). The adrenal cortex, and most tumors of the adrenal, secrete androstenedione (but not testosterone) and small amounts of progesterone but little or no 20α-dihydroprogesterone (5, 6). Contrarily, many extraglandular tissues preferentially convert estradiol-17β to estrone, testosterone to androstenedione, and progesterone to 20α-dihydroprogesterone (5, 7–9). It is unlikely that this anatomical distribution of 17β-hydroxysteroid (17β-HSD)[1] and 20α-hydroxysteroid oxidation/reduction activities, favoring C-17 and C-20 reduction in some tissues and oxidation in others can be accounted for by the redox potential of a given cell. Rather, it is much more likely that this difference in sex steroid hormone synthesis and metabolism is attributable to tissue-specific expression of isozymes that catalyze the 17β-oxidation-reduction of C_{19}- and C_{18}-steroids and the 20α-oxidation-reduction of progesterone, in a manner similar to that now recognized for the 3β-hydroxysteroid dehydrogenase family of enzymes (10). A number of investigators have deduced, therefore, that there must be multiple 17β-HSDs, some of which also are capable of C-20α-oxidoreduction.

In human placenta, the cytosolic isoform of 17β-HSD, most recently referred to as 17β-HSD type 1 (11), is most efficient in the reduction of estrone to estradiol-17β and much less effective in 17β-oxidation-reduction of C_{19}-steroids. A cDNA for this enzyme has been isolated and sequenced by several groups of investigators (12–14). Thus, the placental cytosolic 17β-

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1. *Abbreviation used in this paper:* 17β-HSD, 17β-hydroxysteroid dehydrogenase.

HSD enzyme (also referred to as estradiol-17β dehydrogenase) preferentially catalyzes the reduction of estrone to give the biologically potent estradiol-17β and the reduction of 16α-hydroxyestrone to give estriol. Estradiol-17β and estriol are the principal estrogens secreted from the syncytiotrophoblast into the maternal circulation during human pregnancy (see reference 1). In addition, a microsomal 17β-HSD has been identified in human placenta; this membrane-bound enzyme preferentially catalyzes the oxidation of the 17β-hydroxyl group of estradiol-17β and testosterone as well as the 20α-hydroxyl group of 20α-dihydroprogesterone (15). Recently, Andersson and associates (11) cloned the cDNA for this microsomal form of 17β-HSD and referred to this isozyme as 17β-HSD type 2.

The most striking structural difference between the predicted amino acid sequences of 17β-HSD types 1 and 2 is an extended hydrophobic amino terminus of the microsomal 17β-HSD type 2 enzyme, which is believed to correspond to the transmembrane signal anchor. The amino acid sequence of the lumenal portion of 17β-HSD type 2 shares 23% homology and 45% similarity with 17β-HSD type 1 (11). The human 17β-HSD type 1 has been mapped by Winqvist and colleagues (16) to chromosome 17 (wherein a pseudogene is localized in tandem with the gene). Herein we report that human 17β-HSD type 2 gene is on chromosome 16, 16q24.

There has been considerable controversy concerning the particular type(s) of 17β-HSD in endometrium and other extraglandular tissues. This is an important issue from both the biochemical and physiological perspectives. Gurpide and colleagues (17–22) were the first to show that progesterone and synthetic progestins act in vivo and in vitro to cause a striking increase in the enzymatic oxidation of estradiol-17β to estrone in human endometrium and in selected human endometrial carcinoma cells in culture. These findings have been confirmed by several other groups of investigators (23–25). This enzyme activity was localized particularly in the glandular epithelium of the endometrium (23, 26). From these findings, it has been reasoned that one action of progesterone as an antiestrogen in endometrial glands is effected by facilitating the oxidation of estradiol-17β (a potent estrogen) to the biologically weak or inactive estrone. Moreover, the progestin-induced increase in 17β-HSD activity should serve to preserve progesterone action in endometrium by favoring the C-20-ketone configuration rather than the reduction of progesterone to inactive 20α-dihydroprogesterone. Similar findings with respect to progestin stimulation of 17β-HSD activity have been described in studies of human breast cancer cells (27, 28). The identity of the 17β-HSD(s) in endometrium or breast, however, was not certain. In this investigation, we evaluated the levels of mRNAs for 17β-HSD types 1 and 2 in human endometrial tissue and in separated endometrial glandular epithelium and stromal cells in monolayer culture. By Northern analysis of total RNA (10 μg) with cDNA probes for the coding regions, we found that 17β-HSD type 2, but not type 1, mRNA was present in high levels and that 17β-HSD type 2 mRNA levels increased strikingly with progesterone secretion in vivo. In addition, we surveyed other human adult tissues for 17β-HSD type 2 mRNA.

Methods

Endometrial and placental tissues. Endometrial tissues were obtained after hysterectomy, conducted for reasons other than endometrial disease, from the uteri of ovulatory women. Informed consent for the use of tissues was obtained in writing from each woman before the surgical procedure. The consent form and protocol used were approved by the Institutional Review Board of this university. The day of the endometrial cycle was estimated from the woman's menstrual history and by histological examination of the endometrium. In addition, the concentration of progesterone in plasma of the women from whom endometrial tissue was obtained was determined. From the plasma concentration of progesterone, we normalized the estimated day of the cycle for each endometrial sample to an idealized 28-d interval.

Within 1 h of hysterectomy, endometrial tissues were frozen in liquid N_2 (for isolation of RNA) or else placed in culture medium (for separation of glands and stroma) and transported to the laboratory. Endometrial glandular epithelium and stromal cells of some endometrial tissue samples were separated as described (29). Briefly, endometrial tissue was minced into small pieces ($\sim$ 1 mm^3), and the minced tissue was incubated at 37°C for 20–40 min in Hanks' balanced salt solution that contained Hepes (25 mM), penicillin (200 U/ml), streptomycin (200 mg/ml), collagenase (1 mg/ml; 134 U/mg), and DNAse (0.08 mg/ml; 1,950 Kunitz units/ml). The dispersed endometrial stromal cells were separated from endometrial glands by filtration through a wire sieve (75 μm). The separated glandular epithelium and stromal cells (in the filtrate) were washed and pelleted by centrifugation (400 g, 10 min). Villous trophoblast tissue was obtained from a placenta of a normal human pregnancy delivered at term.

Northern analyses. Total RNA was purified from endometrial tissues or separated cells by the method of Chirgwin et al. (30). The tissues or cells were homogenized in guanidinium isothiocyanate (4 M) and the homogenates were centrifuged at 238,000 g for 18 h at 25°C over CsCl (5.7 M). The total RNA was size fractionated by electrophoresis on formaldehyde-agarose (1%) gels (5–10 μg/lane) and transferred electrophoretically to a nylon membrane. The RNA was cross-linked to the nylon membranes by ultraviolet irradiation. RNA from human tissues [2 μg poly(A)$^+$ RNA/lane] on a nylon membrane (Clontech Laboratories, Inc., Palo Alto, CA) also was probed for 17β-HSD type 2. The membranes were prehybridized for 24 h at 42°C in prehybridization buffer comprised of formamide (50%, by volume), NaH_2PO_4 (250 mM, pH 7.2), NaCl (250 mM), SDS (7%, wt/vol), and denatured sheared salmon sperm DNA (100 μg/ml). Hybridizations were conducted for 16 h at 42°C in the same buffer that contained 17β-HSD type 2 cDNA probe (11) or a 17β-HSD type 1 cDNA (12) probe (2×10^6 cpm/ml) radiolabeled with [α-^{32}P]dCTP using random hexanucleotide primers and Klenow. The 17β-HSD type 1 cDNA was kindly provided to us by Dr. Michael Gast (Washington University, St. Louis, MO). After hybridization, the blots were washed with 2×SSC and SDS (0.1%, wt/vol) for 15 min at room temperature, twice with 0.1×SSC and SDS (0.1%, wt/vol) for 20 min at room temperature, and two to four times with 0.1×SSC and SDS (0.1%, wt/vol) for 30 min at 55°C. The membranes were blotted on filter paper, sealed in a plastic bag, and exposed to film with intensifying screens for autoradiography at −80°C. The presence of equal amounts of total endometrial tissue RNA in each lane was verified by visualization of 18S and 28S rRNA subunits or by analysis of β-actin using a specific cDNA purchased from Clontech Laboratories, Inc.

Genomic library screening and fluorescence in situ hybridization. A human genomic library in the Lambda Fix II vector was purchased from Stratagene (catalog no. 946205; Stratagene, La Jolla, CA). Screening in 50% formamide-containing buffers was conducted by use of standard procedures (31) with random hexanucleotide primed ^{32}P-labeled probes derived from the full-length human 17β-HSD type 2 cDNA (11). The $\sim$ 13-kb DNA insert from the hybridization-positive bacteriophage lamda 49-24 was subcloned into pBluescript II (Stratagene) and analyzed by double-stranded DNA sequencing. The lambda 49-24 clone contained a segment identical to nucleotides 1–350 in the 17β-HSD type 2 cDNA, flanked by unknown sequences. Fluorescence in situ hybridization (BIOS Laboratories, New Haven, CT) was performed using the method of Lichter et al. (32) with minor modifications. The plasmid was labeled with digoxigenin-11-dUTP by nick-translation, and the labeled probe was combined with sheared human DNA and hybrid-

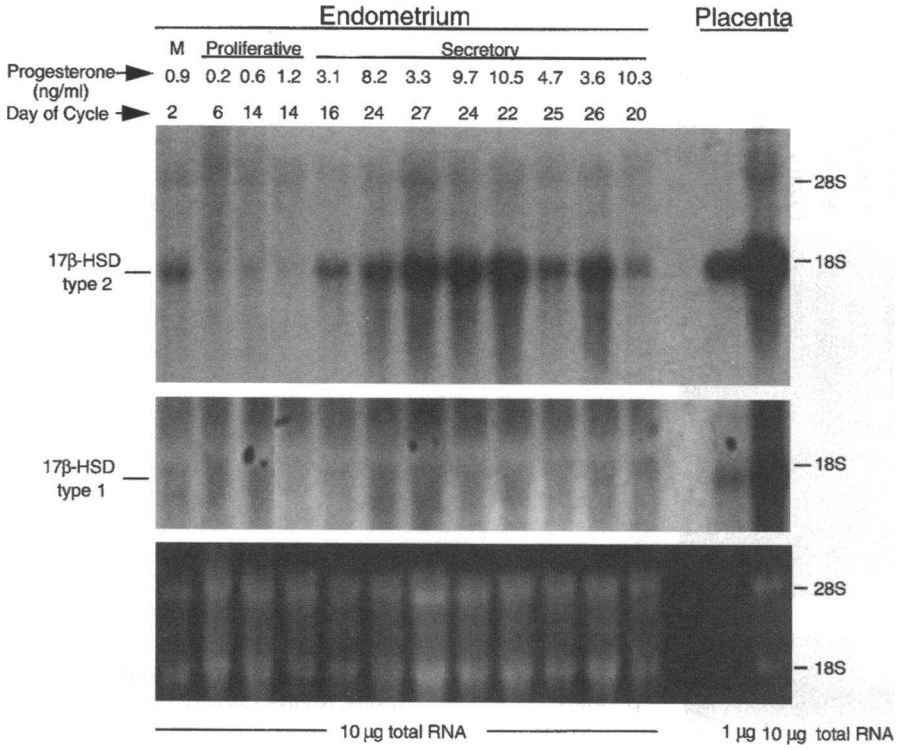

Figure 1. Northern analyses of 17β-HSDs mRNAs in human endometrial tissue. The levels of 17β-HSD type 2 mRNA (*top*) and 17β-HSD type 1 mRNA (*middle*) mRNA were evaluated by Northern analysis of total RNA (10 μg/lane); the ethidium-stained blot is presented in the bottom panel. Endometrial tissues were obtained from the menstrual (*M*), proliferative, or secretory phases of the cycle. The day of the cycle is indicated as is the plasma level of progesterone on the day the tissue was obtained. For reference total RNA (1 and 10 μg) from villous placental tissue also is included on the blot.

ized to normal human metaphase chromosomes in a solution containing formamide (50%, vol/vol), 2×SSC, and dextran sulfate (10%, vol/vol). Specific hybridization signal was detected with FITC-conjugated anti-digoxigenin antibodies and the chromosomes were then counterstained with propidium iodide.

Results

17β-HSD type 2 mRNA was readily detected by Northern analysis of total RNA (10 μg) prepared from human endometrium. The cDNA probe for 17β-HSD type 2 hybridized with a species of mRNA ~ 1.5 kb in length. The level of 17β-HSD type 2 mRNA was greater in secretory than in proliferative endometrium (Fig. 1) and was highest during the mid to late secretory phase of the cycle, when plasma progesterone levels are maximum and progesterone action is greatest. The level of 17β-HSD type 2 mRNA in mid-secretory endometrium was approximately one-tenth of that in placenta (Fig. 1). When this blot was probed for 17β-HSD type 1 mRNA, an mRNA species of ~ 1.3 kb was detected in placental tissue but not in endometrial tissues.

17β-HSD type 2 mRNA was localized principally in the glandular epithelium of the endometrium (Fig. 2). In three different endometrial tissues obtained during the secretory phase, the level of 17β-HSD type 2 mRNA was much greater in the glands than in the stroma and much greater than that in either cell type of three endometrial tissue samples obtained during the proliferative phase of the cycle. A small amount of 17β-HSD type 2 mRNA was present in stroma of two of the secretory endometrial tissues. As it is not possible to separate completely the two cell types, we suspect that this may be accounted for by a small number of epithelial cells in the stromal preparation. Using cytokeratin as a marker of epithelial cells, we estimated the extent of epithelial cell contamination of the stromal cell preparation to vary from 2 to 17% (33).

17β-HSD type 2 mRNA (~ 1.5 kb) also was detected in human placenta, liver, pancreas, kidney, and small intestine by Northern analysis of poly(A$^+$) RNA (1 μg) (Fig. 3). In placenta, a ~ 1.3-kb species of mRNA also was detected with the 17β-HSD type 2 cDNA probe. On longer exposure (90 h) of this blot of poly(A)$^+$ RNA, a faint signal, corresponding to a size of ~ 1.5 kb, was detected in colon and prostate. The level of 17β-HSD type 2 mRNA detected among these human tissues was greatest in placenta, liver, and small intestine, with the levels in kidney, pancreas, colon, and prostate being much lower. Also, on long exposure (90 h), there was a faint signal in with poly(A)$^+$ RNA from peripheral blood leukocytes, but the size of this mRNA appears to be slightly greater (~ 1.6 kb)

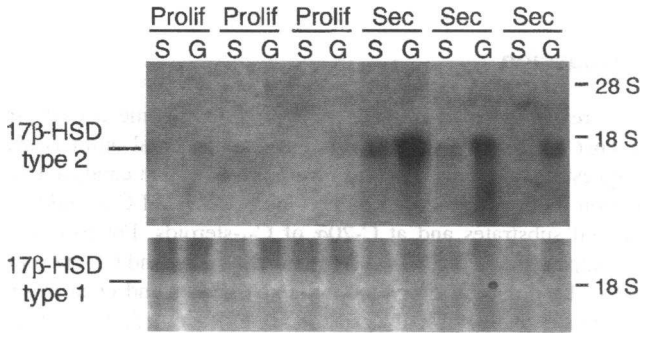

Figure 2. Northern analyses of 17β-HSD type 2 and type 1 mRNAs in separated glands and stroma isolated from human endometrial tissue. The stromal (*S*) cells and glands (*G*) in endometrial tissues obtained during the proliferative (n = 3) or secretory (n = 3) phase were separated and RNA was prepared. Total RNA (5 μg RNA) was evaluated by Northern analyses for 17β-HSD type 2 (*top*) and 17β-HSD type 1 (*bottom*).

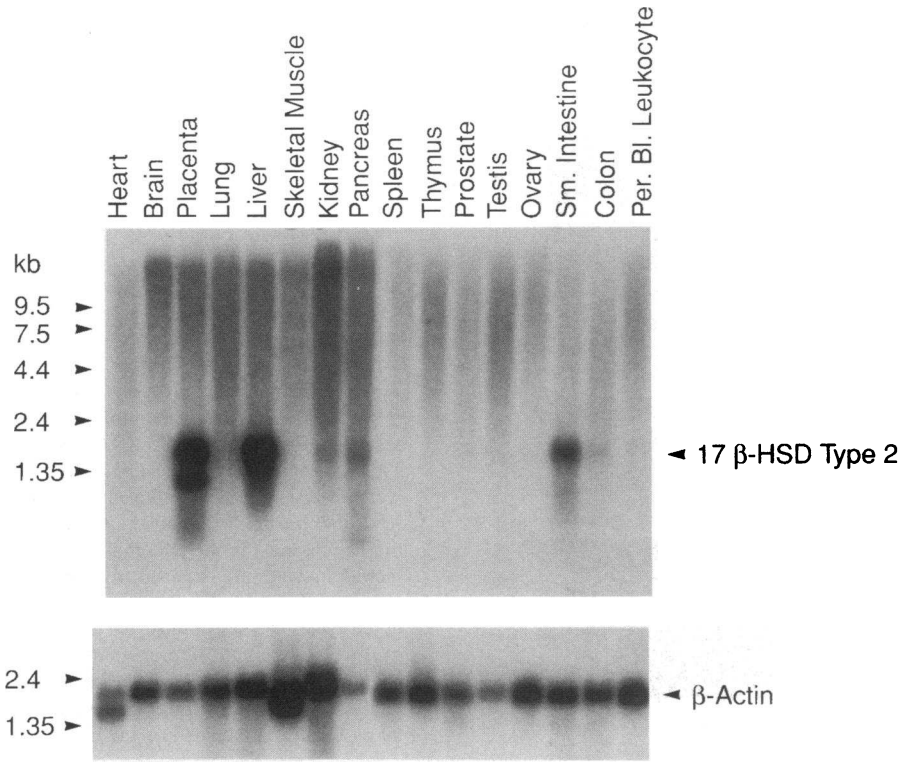

Figure 3. 17β-HSD type 2 mRNA in human tissues. Northern analysis of poly(A)$^+$ RNA (2 μg/lane; membrane purchased from Clontech Laboratories, Inc.).

than that in endometrium, liver, and small intestine. Even with poly(A)$^+$ RNA and long exposure (90 h), there was no evidence of 17β-HSD type 2 mRNA in heart, brain, skeletal muscle, spleen, thymus, or testis.

Using fluorescence in situ hybridization with human metaphase chromosomes, the 17β-HSD type 2 gene was localized to the distal long arm of chromosome 16 (Fig. 4). The identity of chromosome 16 was confirmed by cohybridization studies using the 17β-HSD type 2 probe and a chromosome 16–specific centromere probe, D16Z2 (34) (Fig. 4). From measurements taken in 20 chromosome 16s, it was determined that the 17β-HSD type 2 gene is located 89% of the distance from the centromere to the telomere of 16q. This area corresponds to 16q24. Specific signal was detected on chromosome 16 in 57 of 75 metaphase cells evaluated.

Discussion

The results of many studies of 17β-HSD enzyme activity in a host of tissues from humans and experimental animals are suggestive that there must be several proteins that catalyze oxidation-reduction reactions of steroids at C-17β of C_{18}- and C_{19}-steroid substrates and at C-20α of C_{21}-steroids. For example, the apparent K_ms of 17β-HSD(s) for C_{18}-, C_{19}-, and C_{21}-steroids differ by orders of magnitude in some tissues and even in selected subcellular fractions of the same tissue (15, 35, 36). In addition to steroid substrate specificity, the oxidative pathway of the reaction catalyzed by 17β-HSD is dominant in some tissues (in the absence of added co-factor [e.g., with intact cells or tissue slices]), whereas reduction is more prevalent in others (37–40). Furthermore, whereas the reduction pathway is important in the synthesis of the bioactive C_{18}- and C_{19}-steroids, estradiol-17β and testosterone, respectively, the C-20α reduction of progesterone serves, in the human at least, to inactivate this steroid hormone. Contrarily, the preferential oxidation of the C-17β-hydroxy group gives rise to biologically inactive estrone and androstenedione from estradiol-17β and testosterone, respectively; but, the oxidation of 20α-dihydroprogesterone gives bioactive progesterone. These differences in steroid C-17β and C-20α oxidation-reduction cannot be explained singularly by assuming that a given isozyme is localized in steroidogenic tissues and another isozyme is localized in extraglandular tissues. This obtains because the bioactive products of the reduction pathway, e.g., estradiol-17β, and the oxidative pathway, e.g., progesterone, are preferentially secreted by the same steroidogenic cells in some tissues, e.g., the luteinized granulosa cells of the corpus luteum and the syncytiotrophoblast of the placenta (41). Similarly, we cannot deduce that extraglandular metabolism by the oxidative pathway results singularly in steroid hormone inactivation because the oxidation of 20α-dihydroprogesterone gives rise to bioactive progesterone.

The findings of this study are indicative that (*a*) human 17β-HSD type 2 is localized on chromosome 16, 16q24 and (*b*) 17β-HSD type 2 is the progestin-responsive gene expressed in endometrial glandular epithelial cells. The levels of mRNA for this gene in endometrial glands are increased in vivo during the secretory phase of the cycle with increased progesterone secretion. By Northern analysis of total RNA, we could not detect transcripts for the 17β-HSD type 1 gene in either endometrial tissue or in separated endometrial cells by Northern analysis of total RNA. This finding is consistent with the finding of others that immunoreactive 17β-HSD is not demonstrable in endometrium using an antibody prepared against purified placental cytosolic 17β-HSD (42). Other investigators detected 17β-HSD type 1 mRNA in low levels in endometrium when large amounts (20 μg) of poly(A)$^+$ RNA (43) or RNase protection assays (44) were used.

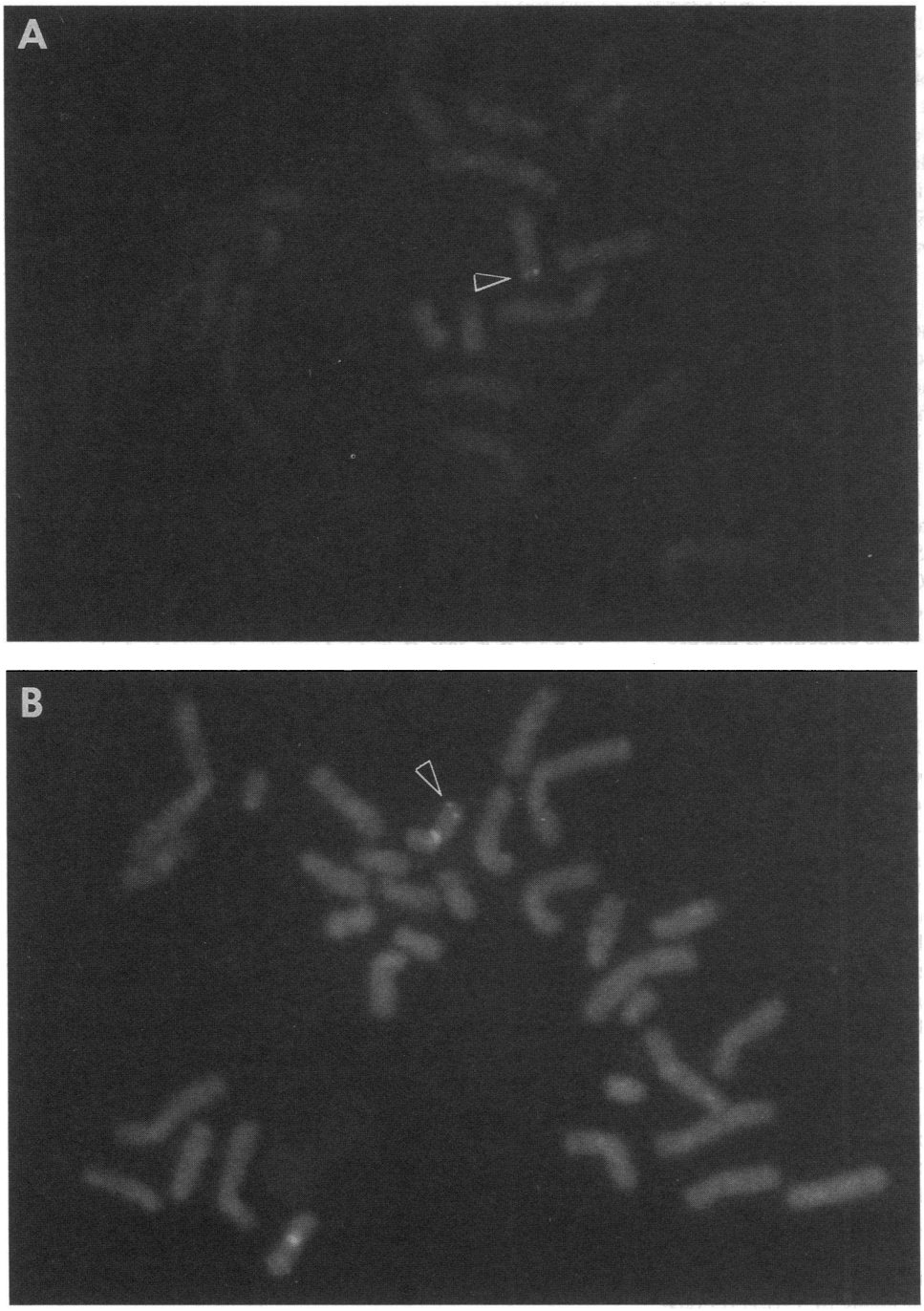

Figure 4. Fluorescence in situ hybridization of a genomic probe for 17β-HSD type 2 with human metaphase chromosomes. The 17β-HSD type 2 gene was localized to the distal long arm of chromosome 16 (*A* and *B*, *arrows*), which was identified using a chromosome 16–specific centromere probe, D16Z2 (34) (*B*).

As characterized by Andersson and colleagues (11), 17β-HSD type 2 isozyme preferentially catalyzes the oxidation of estradiol-17β to estrone, presumably resulting in a tissue-specific decrease in estrogenic activity. This enzyme also catalyzes the oxidation of testosterone to androstenedione and 20α-dihydroprogesterone to progesterone. These enzyme characteristics are consistent with the proposition that 17β-HSD type 2 functions in selected extraglandular tissues to diminish estrogen and androgen action but to preserve the action of progesterone. In the studies of Tseng and Gurpide (19), the preferential oxidation of 17β-hydroxy C_{19}- and C_{18}-steroids and C-20α-hydroxy steroids was demonstrated in endometrial tissues. Moreover, this enzyme activity was localized in the glandular epithelium and not in the stroma and the specific activity of 17β-HSD was increased in vivo and in vitro by treatment with progestin (17, 19, 26). These findings, together with those of this investigation, are supportive of the conclusion that the progestin-stimulated 17β-HSD of the glandular epithelium of human endometrium is the type 2 isozyme. In addition, progestins act in human endometrium to increase estrone sulfotransferase activity and to decrease the synthesis of estrogen receptors (45–47). Collectively, these findings are suggestive that progestins act in a coordinated manner to attenuate estrogen action in endometrium, as suggested by Gurpide.

The human placenta is characterized by the secretion of very large amounts of estradiol-17β (10–15 mg/24 h) and

estriol (60–150 mg/24 h) but very little or no secretion of estrone into the maternal compartment (see reference 1). Similarly, the human placenta secretes phenomenal amounts of progesterone (200–600 mg/24 h) but very little 20α-dihydroxyprogesterone (37, 48). These considerations, together with the fact that the human placenta uses plasma-borne C_{19}-steroids for estrogen formation (49, 50), give rise to a reasonable explanation for the actions of both 17β-HSD types 1 and 2 in trophoblast. The plasma-borne C_{19}-steroids used for placental estrogen synthesis, dehydroepiandrosterone sulfate (49, 50) and 16α-dehydroepiandrosterone sulfate, are converted by placental sulfatase activity to the respective nonconjugated steroids. Before aromatization, dehydroepiandrosterone must be converted to a Δ^4-3-ketone-C_{19}-steroid, namely androstenedione. Androstenedione is aromatized to estrone, which thence is converted to estradiol-17β through the action of 17β-HSD type 1. Excessive conversion of androstenedione to testosterone in trophoblast could result in the virilization of female fetus as ~ 15% of steroids synthesized in placenta enter the fetal circulation (see reference 1). The action of 17β-HSD type 2 in placenta may serve to maintain the 17-ketone of C_{19}-steroids to attenuate the formation of biologically potent androgen and to maintain the C-20-ketone of progesterone to ensure the bioaction of this progestation steroid hormone.

Using total RNA prepared from villous trophoblast isolated from human placenta and the 17β-HSD type 2 cDNA probe, we detected a major species of mRNA at ~ 1.5 kb. When the blot purchased from Clontech Laboratories, Inc. was probed with this same cDNA, a minor species at ~ 1.3 kb was detected in addition to the major species at ~ 1.5 kb. Previously, when placental RNA was probed with the same cDNA probe, a signal at ~ 2.2 kb was detected in addition to the major mRNA species at ~ 1.5 kb (11). This difference may be attributable to higher stringency of probing in this study. Indeed, it may be that alternative splicing gives rise to a longer mRNA as is the case for 17β-HSD type 1. Two forms of 17β-HSD type 1 mRNAs are present in most tissues: the major form in placenta is 1.3 kb, and a 2.2-kb mRNA with an extended 5′-noncoding region is present in lower levels in placenta. In addition, it is possible that minor species of mRNA (~ 2.2 and 1.3 kb) are present in another extraembryonic, non-villous trophoblast tissue (e.g., umbilical cord, amnion, chorion laeve), which may have been present in the tissue/RNA preparation that was used in those studies.

Andersson and colleagues (11) could not detect 17β-HSD type 2 mRNA in human prostate and the data obtained in this study also are indicative of very low levels of 17β-HSD type 2 mRNA in prostate. This is suggestive that an alternate form of 17β-HSD is expressed in prostate or else that testosterone inactivation in prostate is accomplished primarily by way of the 6α- or 7α/β-hydroxylation of 5α-androstan-3β,17β-diol (11).

The anatomic distribution of 17β-HSD isozymes appears to be important in the synthesis of bioactive (17β-reduced) C_{19}- and C_{18}-steroids and thence in the inactivation (17-oxidation) of these steroids; 17β-HSD type 1 (placenta) catalyzes the biosynthesis of the potent estrogen, estradiol-17β; 17β-HSD type 2 (endometrium) catalyzes the inactivation of estradiol to estrone; 17β-HSD type 1 provides for the synthesis of estradiol-17β in placenta and 17β-HSD type 2 may ensure that there is little or no net synthesis of testosterone in this tissue. It now seems reasonable to suspect that yet other 17β-HSD isoenzymes will be discovered to account for testosterone synthesis in testis and to account for the conversion of plasma androstenedione to plasma testosterone and of plasma estrone to plasma estradiol-17β in extragonadal tissues.

Acknowledgments

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