Structure of Two in Tandem Human 17β-Hydroxysteroid Dehydrogenase Genes

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Two human 17β-hydroxysteroid dehydrogenase (17β-HSD) genes (h17β-HSDI and h17β-HSDII) included in tandem within an approximately 13 kilobase pair fragment were isolated from a genomic λEMBL3 DNA library using cDNA encoding human 17β-HSD (hpE$_2$DH216) as probe. We have determined the complete exon and intron sequences of the two genes as well as their 5′ and 3′-flanking regions. Human 17β-HSDII contains six exons and five short introns for a total length of 3250 base pairs. The exon sequence of h17β-HSDII is identical to the previously reported hpE$_2$DH216 cDNA while the overlapping nucleotide sequences of the corresponding exons and introns of h17β-HSDI and h17β-HSDII show 89% homology. In addition, we have used the hpE$_2$DH216 cDNA to demonstrate the widespread expression of 17β-HSD mRNAs in steroidogenic and peripheral target tissues. These new findings provide the basis for a better understanding of the molecular mechanisms involved in 17β-HSD deficiency and peripheral sex steroid metabolism. (Molecular Endocrinology 4: 268–275, 1990)

INTRODUCTION

Estradiol 17β-dehydrogenase (EC1.1.1.62) (1, 2), also known as 17β-hydroxysteroid dehydrogenase (17β-HSD) (3), catalyzes the interconversion of the following 17-ketosteroids and their 17β-hydroxysteroid counterparts: estrone and 17β-estradiol, dehydroepiandrosterone and 5-androstene-3β,17β-diol, androstenedione and testosterone, androstane-3,17-dione and 5α-dihydrotestosterone, androsterone and androstane-3α, 17β-diol, as well as 3-epiandrosterone and androstane-3β,17β-diol. The enzyme 17β-HSD is present in a series of human tissues, including the placenta (4), testis (4, 5), endometrium (6), vaginal mucosa (7), lung (8), liver (9), ileum (5), adipose tissue (10), skin (7), red blood cells (11), breast cancer cells (12), and prostatic cancer cells (13). Previously, we have isolated and characterized cDNAs for human placental 17β-HSD (2). The existence of two mRNA species which differ only in their 5′-noncoding regions was then demonstrated and a unique primary structure of 327 amino acid residues has been deduced from the nucleotide sequence. Using 17β-HSD cDNA (clone hpE$_2$DH216) as a probe, we have isolated, sequenced and characterized two in tandem 17β-HSD genes which reside within a 13 kilobase pair (kbp) genomic DNA fragment.

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RESULTS AND DISCUSSION

Cloning and Nucleotide Sequencing

A human genomic DNA library constructed in λ EMBL3 phage vector was screened with a ^{32}P-labeled human placental 17β-HSD cDNA (hpE$_2$DH216) as probe (2). Three positive clones were obtained from 5 × 10^5 recombinants. Clones λ17β-HSD29 and λ17β-HSD14 possess identical inserts of approximately 13 kbp while the third clone, λ17β-HSD2, consists of an overlapping genomic fragment of approximately 7 kbp contained within the longer clones (Fig. 1A). The fragments resulting from digestion of λ17β-HSD29 with *Sac*I, *Pst*I, and *Bam*HI were purified and subcloned into the Bluescript SK vector and sequenced in both directions (Fig. 1A). The merged sequence contains two 17β-HSD genes (h17β-HSDI and h17β-HSDII) located in tandem within approximately 13 kbp human genomic fragment. The h17β-HSDII gene consists of 6 exons and 5 introns (Fig. 1B) and its exonic sequence is identical to the hpE$_2$DH216 cDNA sequence previously reported (2). The complete nucleotide sequence of the human 17β-HSDII gene and the deduced amino acid sequence of its coding region are presented in Fig. 2. The nucleotide sequences adjoining the splice junctions are consistent with the recognized consensus sequence (14).

Comparison of the nucleotide sequence of h17β-HSDI with that of the exons and introns of the h17β-HSDII gene (Fig. 2) shows 89% homology. In analogy

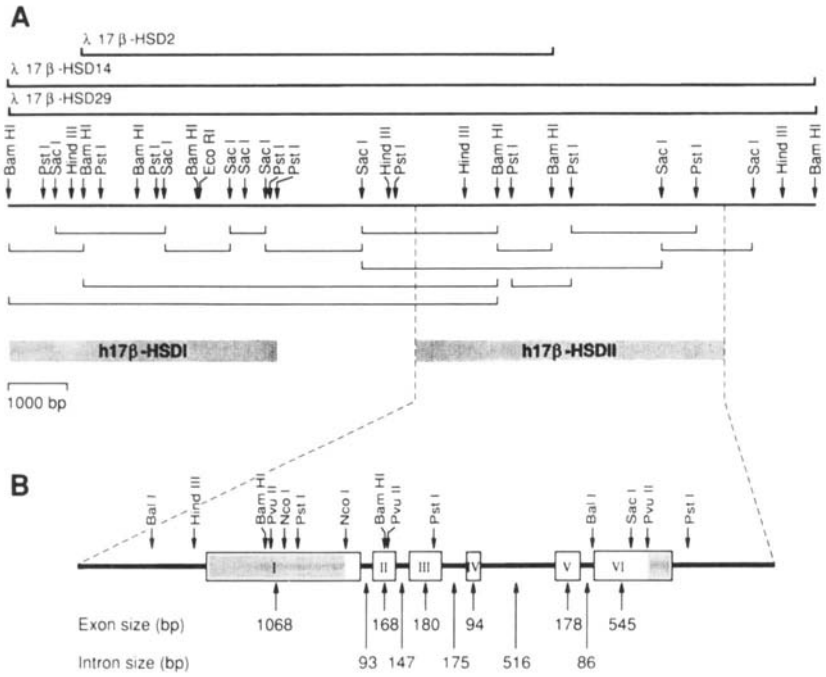

Fig. 1. Structure and Restriction Map of Human h17β-HSD Genes

A, Restriction map of an approximately 13 kbp human genomic fragment containing h17β-HSDI and h17β-HSDII genes. Sizes of fragments isolated from three λEMBL3 recombinant clones are indicated above while *Sac*I, *Bam*HI, and *Pst*I restricted fragments subcloned in Bluescript SK as well as the sequenced regions (*shadowed boxes*) corresponding to h 17β-HSDI and h17β-HSDII genes are shown below the map. B, Exon-intron organization and restriction map of h17β-HSDII gene. Exons are represented by *boxes* and numbered from I to VI. *Shaded box areas* indicate noncoding regions. Nontranscribed regions are represented by the *solid line*. Exon and intron sizes are indicated below the schematic h17β-HSDII gene.

with gene h17β-HSDII, gene h17β-HSDI could be potentially transcribed, spliced at the same exon-intron junctions and translated from the first corresponding in-frame ATG codon position. However, due to a change from a G to a T, thus creating a TAA stop codon rather than encoding for the amino acid Gln at position 218, gene h17β-HSDI potentially encodes a protein of 214 amino acids (including the first Met).

Southern blot analysis (Fig. 3) was performed using the ^{32}P-labeled hpE$_2$DH216 cDNA fragment (nucleotides 0 - 1892) as probe. The restriction patterns obtained with *Bam*HI shows three fragments of about 1, 5, and 9.4 kb which could well correspond to the fragments found in the human genomic fragment of the two h17β-HSD genes (Fig. 1). The difference between the 4 kb DNA fragments obtained by restriction mapping of λ17β-HSD29 and the approximately 9.4 kb band observed by Southern blot analysis suggests that the *Bam*HI site at the 3′-end of the genomic clone is generated by cloning since human genomic DNA was partially digested with *Sau*3AI before insertion in the *Bam*HI site of λEMBL3. The digestion pattern of *Pst*I also yields the two strongly hybridizing (~1 and 2 kb) fragments expected from restriction mapping of the h17β-HSDII gene. An additional faint band at approximately 0.9 kb and another band of approximately 1.7 kb obtained after a longer exposure time (data not shown) could well correspond to the *Pst*I fragments found in the h17β-HSDI gene. Furthermore, digestion with EcoRI which cuts once in h17β HSDI gave rise to two doublets of 18 kb and 15.5 kb. In addition, digestion with *Hind*III which cuts once in the 5′ and 3′-flanking region of both h17β-HSDI and h17β-HSDII produced a doublet of approximately 5.1 and 5.3 kb. Our recent demonstration of the unique chromosomal assignment of 17β-HSD gene to the q12 band of chromosome 17 (2) coupled with the data from Southern blot analysis thus agree with the existence of the two genes described in Fig. 2. In addition, digestion of human genomic DNA with *Xba*I indicates that the two genes are included in one fragment of greater than 23 kb.

Two mRNA species have been identified in poly(A) RNA from human placenta, namely a major species of 1.3 kb and a minor one of 2.2 kb (2). S_1 analysis indicates that the major mRNA starts nine nucleotides upstream of the starting codon while the minor mRNA species contains approximately 971 nucleotides upstream from the in frame ATG initiating codon (Fig. 4).

Primer extension analysis (2) confirmed the position of the cap site of the short mRNA as determined by S1 nuclease protection experiment (Fig. 4). Since the transcription start site of the long mRNA agrees with an observed mRNA size of 2.2 kb (2) and no AG consensus sequence for an intron-exon splice site is found up to 65 bp upstream from the potential cap site, it is unlikely that the S_1 nuclease-protected fragment ends at an

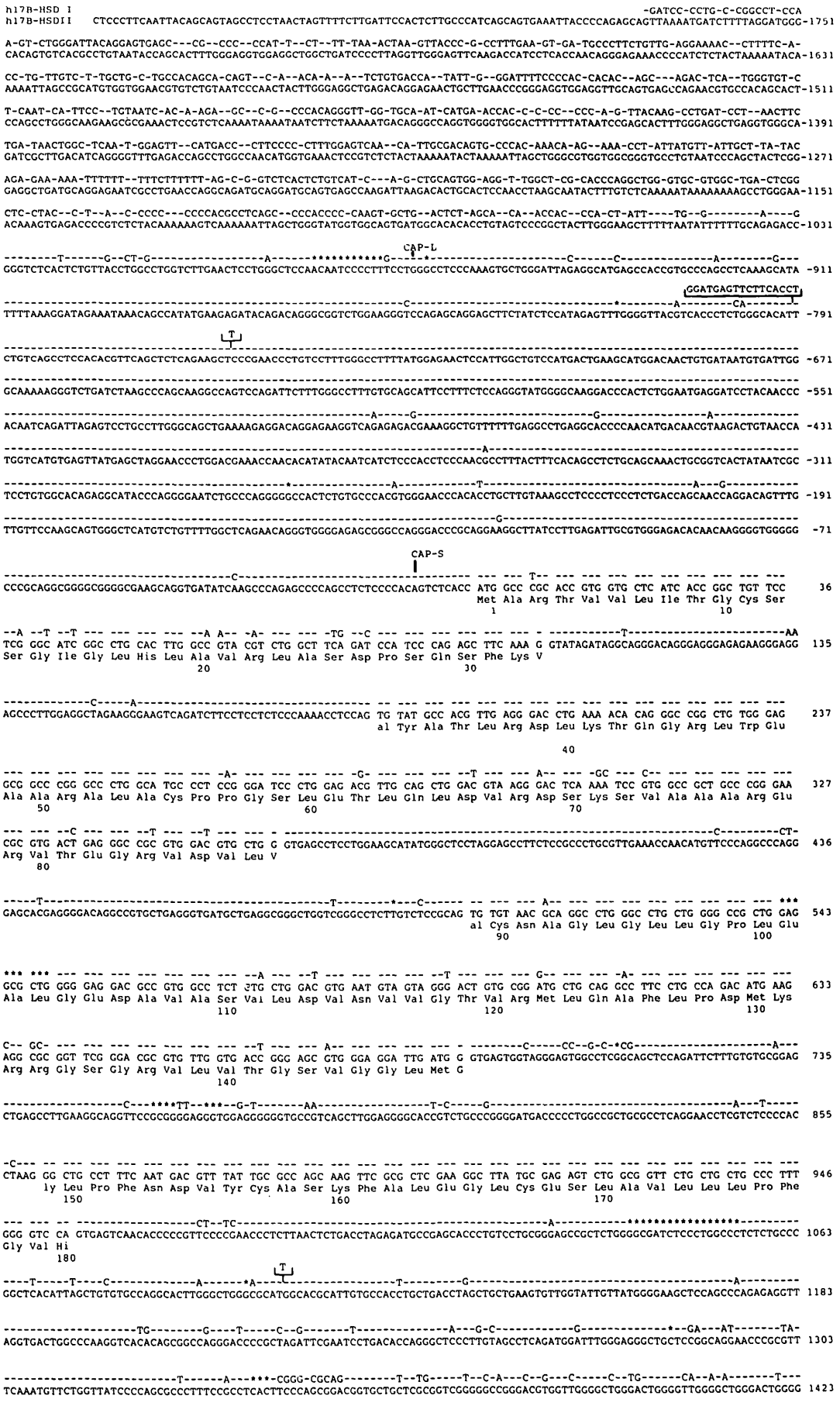

Fig. 2. *(continued next page)*

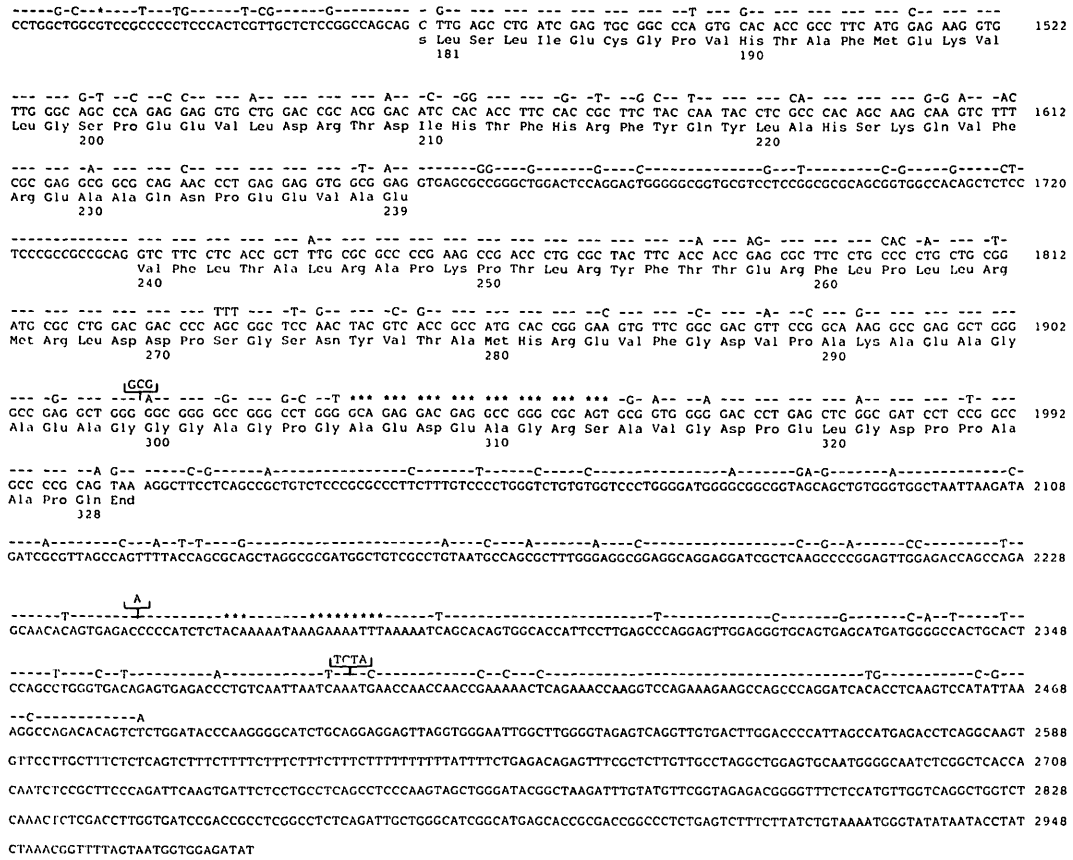

Fig. 2. Alignment of Nucleotide Sequences of the h17β-HSDI and h17β-HSDII Genes

Numbers on the right correspond to nucleotide positions in h17β-HSDII beginning with the translation initiation codon labeled as +1. Amino acid residues are shown below the nucleotide sequence and numbered below the sequence. CAP sites for the long (L) and short (S) mRNA transcripts of h17β-HSDII gene are indicated. The nucleotide sequence of h17β-HSDI is indicated above that of h17β-HSDII. Dashed line (– – –) and *asterisks* (***) indicate identical and missing nucleotides, respectively. Additional nucleotides are indicated in *brackets*.

exon-intron junction. As observed in the nucleotide sequences of the 5′ noncoding region of placental 17β-HSD cDNAs (2), it can be seen that the cap site for the short mRNA is preceeded upstream by a TATA box-like motif (ATATCAA) (15, 16), a GC box (GGGGCGGGGC) (17, 18) and a putative inverse CAAT box (GATTG) (19) at 32, 52, and 93 nucleotides, respectively.

We subsequently studied the distribution of 17β-HSD mRNAs in a series of human tissues. All of the tissues studied contain the 2.2 kb mRNA species (Fig. 5). As mentioned above, this transcript has been shown to contain an extended 5′-noncoding region as evidenced by the signal resulting from hybridization of the placental poly(A)* RNA with the ^{32}P-labeled *Eco*RI-*Nco*I fragment which corresponds to the 5′-noncoding region of hp-E_2DH216 cDNA (2). The 2.2 kb mRNA species is predominant in myometrial, endometrial, prostatic, and abdominal fat tissues. The 1.3 kb 17β-HSD mRNA, on the other hand, is very abundant in the placenta and ovary while it is present to a lesser extent in testicular tissue. Shorter transcripts are detectable in endometrial, mammary, testicular and prostatic tissues as well as ZR-75-1 and LNCaP cancer cells.

As expected, 17β-HSD mRNAs are expressed in known steroidogenic tissues such as the ovary, testis, and placenta. More interestingly, the appreciable level of expression of 17β-HSD mRNAs in peripheral tissues such as the uterus, breast, prostate, and fat indicates that 17β-HSD could play an important role in regulating the activity of sex steroids in target tissues by locally synthesizing active androgens and estrogens from inactive adrenal precursors.

It is thus likely that 17β-HSD plays a major role in breast and prostate development and functions as well as in the growth of breast and prostate cancer (20–22). While h17β-HSDII encodes an enzyme having preferential activity for estrogens (2), h17β-HSDI could possibly encode the proposed androgen-specific form of 17β-HSD (3) or simply be a pseudogene.

Cloning of the 17β-HSD genes offers the possibility of studying in detail the interaction between transcriptional factors and cis-acting elements responsible for the tissue specificity and hormonal regulation of 17β-HSD expression. It also offers the possibility of studying the molecular basis for the genetic deficiency of 17β-HSD in the human (23).

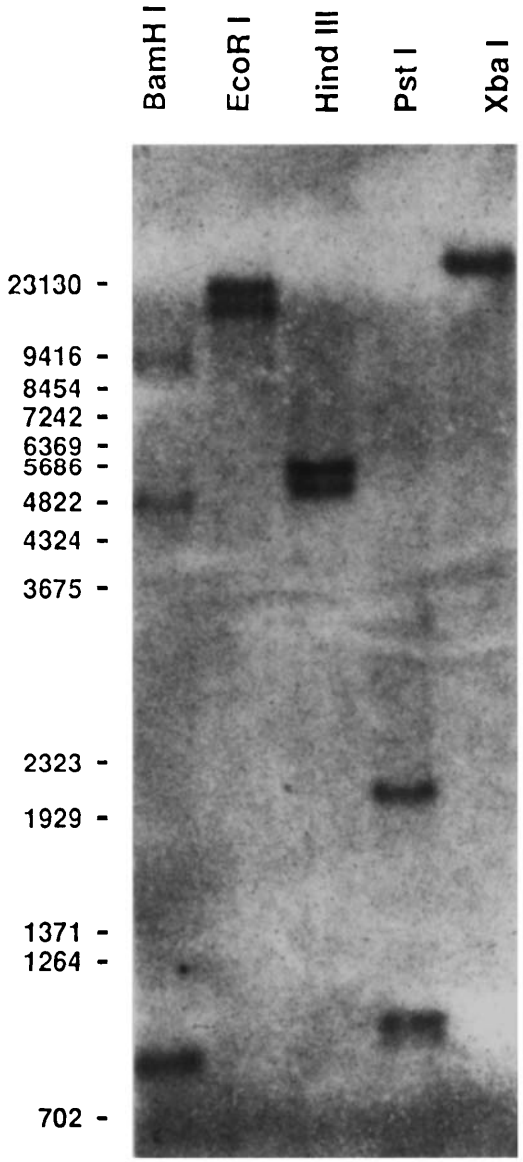

Fig. 3. Southern-Blot Hybridization Analysis of Human Genomic DNA with Human 17β-HSD cDNA Probe

Ten micrograms of genomic DNA isolated from venous blood were digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, or *Xba*I and subjected to electrophoresis in 0.7% agarose gel. Southern blot analysis was performed as described in *Materials and Methods* using the ^{32}P-labeled *Eco*RI *Pvu*II fragment (nucleotides 0-1892) of clone hpE$_2$DH216 (2). *Bst*EII-digested λ DNA as well as the two larger *Hin*dIII-digested λ DNA fragments were used as molecular size markers.

MATERIALS AND METHODS

Screening of the Human Genomic DNA Library

The human leucocyte genomic DNA λEMBL3 library (amplified once) was obtained from Clontech Laboratories Inc. (Palo Alto, CA). Screening was performed with the hpE$_2$DH 216 cDNA probe (2). Hybridization and washing conditions were as described (2, 24).

DNA Sequencing

Fragments were subcloned in the Bluescript SK vector (Stratagene, San Diego, CA) and synthetic oligonucleotides were used as sequencing primers. The cDNA sequences were determined using the dideoxy chain termination method (25) with modified T7 DNA polymerase (26) (Sequenase kit, United States Biochemical Corp., Cleveland, OH).

S$_1$ Nuclease Analysis

The indicated amount of human placental poly(A)$^+$ RNA was hybridized with the indicated 5′-end-labeled probe in 20 μl buffer containing 80% formamide, 0.4 M NaCl, 40 mM 1,4-piperazinediethanesulfonic acid (pH 6.4), and 1 mM EDTA (27). End labeling was performed by incubating the synthetic oligonucleotide primer with polynucleotide kinase and [γ-^{32}P]ATP (4000 Ci/mmol, Amersham, Arlington Heights, IL) while the probe was prepared by synthesizing the complementary strand of the 17β-HSD DNA template with the Klenow fragment of *Escherichia coli* polymerase 1. The synthesized single strand probe was separated from the template plasmid and free nucleotides by electrophoresis in 1.2% low-melting temperature agarose gel in alkaline buffer. After overnight hybridization at 30 C, the sample was diluted with 300 μl ice-cold buffer containing 50 mM sodium acetate (pH 4.5), 4.5 mM $ZnSO_4$, 280 mM NaCl, 10 μg/ml tRNA, and digested with 400 U S$_1$ nuclease for 1 h at 30 C. Protected fragments were resolved by electrophoresis in polyacrylamide urea denaturing gel and visualized by autoradiography.

Southern Blot Analysis

Human DNA prepared from venous blood as described (28) was digested with a series of restriction endonucleases under the conditions specified by the supplier. The resulting DNA fragments were separated by electrophoresis in 0.7% agarose gels overnight at 3 V/cm gel length and transferred to nylon membranes (Hybond N, Amersham) as described (29). Filters were then prehybridized overnight at 42 C in 50% (vol/vol) formamide, 5× SSPE (1× SSPE being 0.18 M NaCl, 10 mM NaH_2PO_4, pH 7.4, 1 mM EDTA, 0.1% (wt/vol) sodium dodecyl sulfate (SDS), 0.1% (wt/vol) BSA, 0.1% (wt/vol) Ficoll, 0.1% polyvinyl-pyrrolidone, 200 μg/ml denatured salmon testis DNA, 200 μg/ml yeast transfer RNA, and 20 μg/ml poly (A). The hpE$_2$DH216 cDNA fragment ranging from the *Eco*RI cloning site to the last *Pvu*II restriction site (0-1892) fragment was labeled with [^{32}P]dCTP (3000 Ci/mmol; Amersham) by the random primer method (30) to a specific activity of 1×10^9 dpm/μg. The cDNA fragment was treated at 100 C for 5 min and 2×10^6 cpm [^{32}P]cDNA probe(s)/ml were added to fresh prehybridization buffer containing 4% (wt/vol) dextran sulfate. After 18 h of hybridization at 42 C, the filters were washed twice (30 min each) in 2× SSC with 0.1% SDS at 25 C and twice (30 min each) at 65 C in 0.1× SSC and 0.1% SDS. Autoradiography was performed at −80 C using XAR-5 films in the presence of two Quanta III intensifying screens for 3 days.

RNA Blot Analysis

Term placental tissue, as well as normal ovaries, myometrium, endometrium, mammary tissue, and abdominal fat were removed from otherwise healthy adult women during routine surgical procedures. Prostatic tissue was obtained from men undergoing prostatectomy for benign prostatic hyperplasia while testicular tissue was removed for the treatment of prostate cancer. ZR-75-1 human breast cancer cells and LNCaP human prostatic cancer cells were grown as described (12, 13). Total RNA was extracted by the guanidinium isothiocyanate cesium chloride centrifugation method (31) and

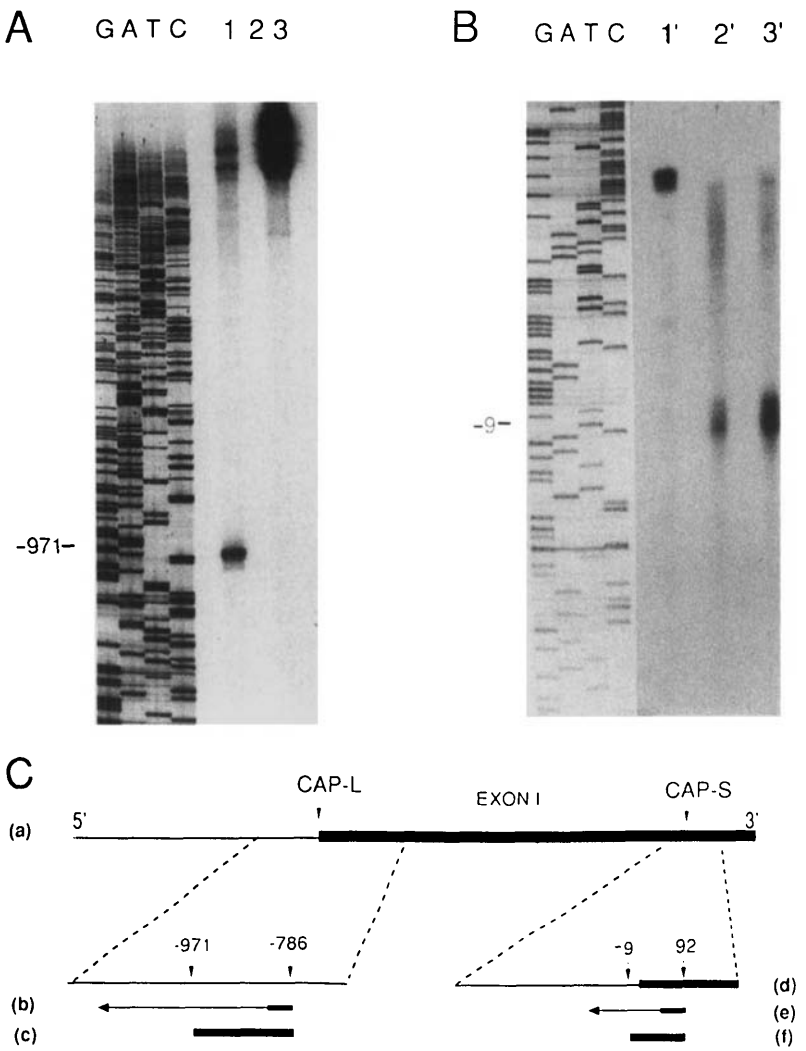

Fig. 4. S_1 Nuclease Analysis

Poly(A)$^+$ RNA (3 μg in A and 2 or 5 μg in B) from human placenta was used to protect the single strand probe (synthesized according to *Materials and Methods*) from S_1 nuclease digestion. Probe using primer starting at position −786 to −810 (A), Probe using primer starting at position +92 to +76 (B). Lane 1, protected fragment; lane 2, digested probe; lane 3, nondigested probe; lanes GATC, sequence of the template clone using the same primer used to synthesize the probe. B, Probe using primer starting at position +92 to +76. Lane 1′, nondigested probe; lane 2′, protected fragment using 2 μg poly(A$^+$) mRNA; lane 3′-protected fragment using 5 μg poly(A$^+$) mRNA. C, Detailed scheme of S_1 nuclease analysis. (a), fragment of the first exon (*thick line*) and its 5′-flanking region (*thin line*) indicating localization of S_1 nuclease-protected fragments; (b) Probe synthesized by extension of the ^{32}-P labeled 25 bp primer starting at position −786; (c) fragment protected by the long mRNA species; (d) localization of the S_1 fragment protected by the short mRNA; the coding sequence is represented by the *thick line* while the *thin line* indicates the untranslated region; (e) probe synthesized by extension of the 17-nucleotide primer starting at position +92; (f) protected fragment of the short mRNA species.

poly(A)$^+$ RNA was purified by two cycles of chromatography on oligo(dT)-cellulose columns (32).

Samples of poly(A)$^+$ RNA were electrophoresed on a 1.2% agarose/2.2 M formaldehyde gel alongside the BRL 0.24-9.5 kb RNA ladder, transferred to a nylon membrane (Hybond-N, Amersham), and hybridized to the random-primer (30) ^{32}P-labeled cDNA fragment corresponding to the *PstI-SacI* region of the hpE$_2$DH216 cDNA (2). The stringent hybridization and washing conditions were as described (2). Autoradiography was carried out at −80 C with two intensifying screens for up to 1 week.

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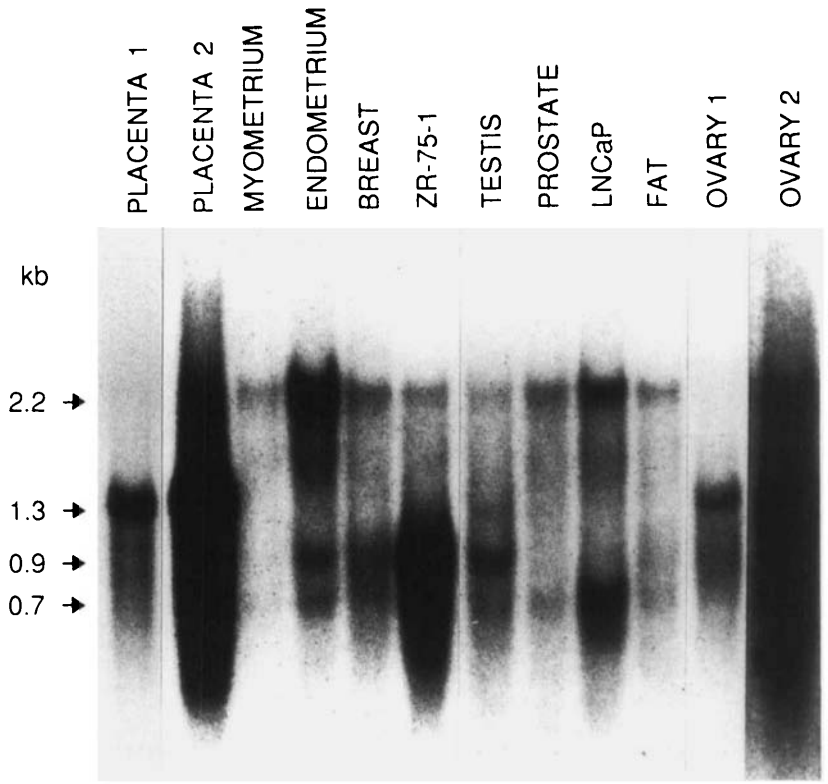

Fig. 5. Tissue Distribution of Human 17β-HSD mRNAs

Poly(A)$^+$ RNA purified from the indicated human tissues was probed with the ^{32}P-labeled *Pst*I-*Sac*I fragment corresponding to the coding region of hpE$_2$DH216 cDNA. With the exception of placenta 1 and 2 (2 μg) and ovary 1 (5 μg), all the lanes contained 20 μg poly(A)$^+$ RNA. The blot was exposed to x-ray film for 1 week with the exception of placenta 1 which was exposed for 1 h.

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