Molecular cloning of a novel widely expressed human 80 kDa 17β-hydroxysteroid dehydrogenase IV

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Reactions of oestrogens and androgens at position C-17 are catalysed by 17β-hydroxysteroid dehydrogenases (17β-HSDs). Cloning of the cDNA of a novel human 17β-HSD IV and expression of its mRNA are described. A probe derived from the recently discovered porcine 17β-oestradiol dehydrogenase (17β-EDH) was used to isolate a 2.6 kb human cDNA encoding a continuous protein of 736 amino acids of high (84%) similarity to the porcine 17β-EDH. The calculated molecular mass of the human enzyme is 79595 Da. Other sequence similarities shared by the two enzymes are: an N-terminal sequence which is similar to that of members of the short-chain alcohol dehydrogenase family; amino acids 343–607 which are similar to the C-terminal domains of a trifunctional *Candida tropicalis* enzyme and the FOX2 gene product of *Saccharomyces cerevisiae*; amino acids 596–736 which are similar to human sterol carrier protein 2. The previously cloned human 17β-HSD I, II and III are less than 25% identical with 17β-HSD IV. mRNA for HSD IV is a single species of 3.0 kb, present in many tissues with highest concentrations in liver, heart, prostate and testes. When overexpressed in mammalian cells, the human 17β-HSD IV enzyme displays a specific unidirectional oxidative 17β-HSD activity.

INTRODUCTION

Oestrogens and androgens play a crucial role in the development, growth and function of all tissues involved in reproduction and fertility, as well as in a series of hormone-sensitive diseases [1]. The redox reactions at position C-17 of the steroid molecule strongly influence the biological properties of the hormones. The enzymes mediating such reactions, 17β-hydroxysteroid dehydrogenases (17β-HSDs), are responsible for interconversion of 17β-oestradiol and oestrone and may also play a role in the interconversion of androgens such as testosterone and Δ^4-androstenedione or Δ^5-androstene-3β,17β-diol and dehydroepiandrosterone [1,2].

The complex kinetics of the conversions in crude tissue fractions of human term placenta suggest the presence of enzymes that differ in subcellular localization, substrate specificity and oxidation/reduction rates [3]. Indeed, besides the purified [4] and cloned [5–7] soluble 17β-HSD I consisting of 327 amino acids, a microsomal enzyme (17β-HSD II) of 387 amino acids has been cloned [8]. 17β-HSD I is a typical oxidoreductase with high and equal affinities for oestradiol and oestrone but low affinity for androgens [9–11]. 17β-HSD II can accept both oestrogens and androgens with a slight preference (2-fold) for oxidation over reduction [8]. The preferred reduction of the oxo forms of androgen (Δ^4-androstenedione) and oestrogens (oestrone) was detected with another dehydrogenase (17β-HSD III) consisting of 310 amino acids and expressed exclusively in human testes [12].

Some human target tissues such as uterus and breast exhibit a 17β-HSD activity in epithelial cells with different kinetic parameters from those described for 17β-HSD I, II and III [13]. In normal uterus and breast, the oxidative reaction (which provides protection against excess oestradiol) prevails (10-fold) and is induced by progestins [13,14]. Increased reductive activity (oestradiol production) has been reported in breast cancer cells [15] but the assumed role of epithelial dehydrogenase in tumour pathogenesis is still unclear. At present, only 17β-HSD III has been unequivocally assigned to an endocrine abnormality: mutations in the 17β-HSD III gene have been identified in male pseudohermaphroditism [12].

Porcine (*Sus scrofa*) endometrial cells express oestradiol dehydrogenase (17β-EDH) activity with similar kinetics [16,17], subcellular associations and induction by progesterone [18] as the enzyme from human endometrium and breast [13]. The porcine enzyme inactivates oestradiol after nuclear passage and probably protects against an excess of the hormone. 17β-EDH has recently been purified [19] and cloned [20]. Its cDNA codes for a protein of 737 amino acids with a calculated molecular mass of 79973 Da. This 80 kDa protein is cleaved to the N-terminal 32 kDa EDH which is similar to members of the short-chain alcohol dehydrogenase family [21]. The amino acid sequence of porcine 17β-EDH is less than 25% identical with 17β-HSD I, II and III, underlining a pronounced difference. The data available on the porcine enzyme were used in the search for a human counterpart.

The present work describes the cloning and mRNA expression of a novel human 17β-HSD IV of 736 amino acids in length with a predicted molecular mass of 79595 Da.

Abbreviations used: 17β-HSD, 17β-hydroxysteroid dehydrogenase; 17β-EDH, 17β-oestradiol dehydrogenase; 1 x SSC, 0.15 M NaCl/0.015 M sodium citrate.

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The nucleotide sequence of human 17β-HSD IV cDNA has been deposited in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under accession no. X87176.

EXPERIMENTAL

Isolation, subcloning and sequencing of 17β-HSD IV cDNA clones

Molecular cloning of the 17β-HSD IV was performed with a probe of 413 bp stretching from nucleotide 271 to nucleotide 683 of porcine 17β-HSD cDNA [20]. Approx. 10^6 recombinant phages from an oligo(dT)-primed human adult liver (kindly provided by Dr. Charles de Taisnes, Mérieux, Lyon, France) and placenta λgt11 cDNA expression library were screened with a ^{32}P-labelled probe (1×10^6 c.p.m./ml). The filters were washed with 0.5 × SSC/0.1 % SDS (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate) at 60 °C for 30 min and then autoradiographed for 12 h at −80 °C. After two purification steps, phages were isolated [22] and the inserts were excised by *Eco*RI digestion. The purified cDNA inserts were subcloned into the polylinker site of pBluescript SK vector (Stratagene, La Jolla, CA, U.S.A.). Synthetic oligonucleotides, as well as T7 or T3 vector primers, and modified T7 DNA polymerase were used to sequence both strands of double-stranded plasmid DNA by the dideoxy chain-termination method with [[^{35}S]thio]ATP (USB, Cleveland, OH, U.S.A.). Sequences were confirmed using an Applied Biosystems (Foster City, CA, U.S.A.) 370A automatic system with fluorescent-dye-labelled cDNA sequence-specific primers and a *Taq* dye-primer sequencing kit (Applied Biosystems).

Protein sequence analysis

Protein sequences were screened for similarities using FASTA [23] with Swissprot release 27 and EMBL release 37 databases.

In vitro transcription and translation of human 17β-HSD IV in rabbit reticulocyte lysates

After partial digestion for 2 min at 37 °C with *Eco*RI endonuclease, the full-length 17β-HSD IV fragment was subcloned into the unique *Eco*RI restriction site of pCDNA3 expression vector (In vitrogen, San Diego, CA, U.S.A.) to produce the pCDNA3-17β-HSD IV recombinant plasmid. Then 1 μg of this plasmid or pCDNA3 plasmid was used for transcription and translation with 10 units of T7 RNA polymerase, 25 μl of rabbit reticulocyte lysate and 40 μCi of [^{35}S]methionine in the appropriate buffer as specified by the manufacturer (Promega, Madison, WI, U.S.A.) for 1.5 h at 30 °C. Translation of the full-length 17β-HSD IV protein was verified by denaturing SDS/PAGE.

RNA analysis

Two commercial human polyadenylated RNA blots were obtained from Clontech (Palo Alto, CA, U.S.A.). RNA was extracted from cell cultures by washing the cells twice with PBS (pH 7.5) and adding 2–4 ml of RNazol (Cinna-Biotex) followed by equal volumes of chloroform as described previously [22]. Extracted RNA was precipitated with 1 vol. of propan-2-ol. Total RNA (10 μg) was fractionated by electrophoresis through a 1 % denaturing agarose gel, transferred to a Hybond N membrane (Amersham, Little Chalfont, Bucks., U.K.) and UV-cross-linked as described previously [22]. Equal mRNA loading was checked using ethidium bromide staining followed by UV visualization (not shown). The membranes were prehybridized in a solution containing 250 μg/ml denatured salmon sperm DNA, 50 % formamide, 5 × Denhardt's (1 × Denhardt's is 0.02 % Ficoll 400/0.02 % polyvinylpyrrolidone/0.02 % BSA), 0.1 % SDS, 5 × SSC and 50 mM Na_2HPO_4, for 2 h at 42 °C, and hybridization was carried out in the same solution containing 5 % dextran sulphate and the ^{32}P-labelled probe for 16 h at 42 °C. The α-^{32}P-labelled *Eco*RI fragment corresponding to nucleotides 302–1753 of human 17β-HSD-IV was used as the probe. The membranes were washed sequentially in 2 × SSC containing 0.1 % SDS at 55 °C for 1 h, in 0.5 × SSC/0.1 % SDS at 60 °C for 30 min and in 0.1 × SSC/0.1 % SDS at 65 °C for 30 min. They were then exposed to X-ray films with an intensifying screen at −80 °C.

Expression of 17β-HSD IV in Hek 293 cells

The fragment containing the 17β-HSD IV open reading frame was obtained by PCR amplification using the following primers 5′-CTGCGCGGCCGCTTCATGGGCTCACCGC-3′ and 5′-AGTGGGTACCGCCCTTCAGAGCTTGGC-3′ containing a *Not*I and a *Kpn*I site adjacent to the initiation and the stop codons respectively. The PCR product was digested with *Not*I and *Kpn*I and inserted into the corresponding sites of pRep10 vector (Invitrogen) to produce the pRep10-h17β-HSD IV plasmid. Hek 293 cells were grown in Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM Hepes, 100 units of penicillin/ml and 100 μg of streptomycin sulphate/ml. Cells were plated at an initial density of 5×10^4/6 cm dish. After a 24 h incubation, medium was changed and transfections were performed by the calcium phosphate co-precipitation method [24] using 10 μg of pRep10 or pRep10-h17β-HSD IV plasmid per dish. After a 72 h culture period, cells were harvested and steroid conversion was assayed as described [19] for 30 min at 37 °C in the presence of 17β-[2,4,6,7-^{3}H]-oestradiol (3.5 TBq/mmol; NEN), [2,4,6,7-^{3}H]oestrone (3.6 TBq/mmol; NEN), Δ^5-[1,2-^{3}H]androstene-3β,17β-diol (2.1 TBq/mmol; NEN), dehydro[1,2,6,7-^{3}H]epiandrosterone (3.9 TBq/mmol; Amersham), [1,2,6,7-^{3}H]testosterone (3.4 TBq/mmol; NEN), 5α-[9,11-^{3}H]androstane-3α,17β-diol (1.7 TBq/mmol; NEN), [1,2,4,5,6,7-^{3}H]dihydrotestosterone (4.4 TBq/mmol; NEN), 20α-dihydro[1,2-^{3}H]progesterone (1.7 TBq/mmol; NEN) or [9,11-^{3}H]androsterone (1.9 TBq/mmol; NEN). Oxidation of steroids was measured in 100 mM phosphate buffer, pH 7.8, with 1 mM NAD^+ as cofactor, and reduction at pH 6.6 with 1 mM NADPH as cofactor. Steroids were quantified by HPLC and on-line radioactivity monitoring as described previously [19].

RESULTS

Isolation of human 17β-HSD type IV cDNA

A fragment of porcine 17β-HSD cDNA [20] corresponding to nucleotides 271–683 was chosen for screening of human cDNA libraries. Using the ^{32}P-labelled probe, 20 cDNAs were isolated from adult human liver and four cDNAs from placenta using 10^6 λgt11 recombinants in each library. The cDNAs were purified and size-characterized. After *Eco*RI digestion, one clone from the liver library (Li-17βIV clone) containing three fragments of 0.3, 0.8 and 1.4 kb, and one clone from the placenta library (Pl-17βIV clone) containing two fragments of 0.3 and 1.4 kb, were obtained. The fragments were subcloned into pBluescript SK vectors, amplified and the respective 5′ and 3′ regions sequenced. As both 0.3 kb fragments contained the first in-frame ATG codon similar to porcine 17β-EDH, the 0.8 kb fragment from the Li-17βIV clone was sequenced and the stop codon identified as corresponding to that of porcine 17β-EDH (Figure 1). Although the Pl-17βIV clone is 3′-truncated, it is 34 bp longer than the Li-17βIV clone in the 5′-untranslated region. The 3′-untranslated region of Li-17βIV clone is about 400 bp long. Three classical polyadenylation consensus AATAAA sites [25] were detected at

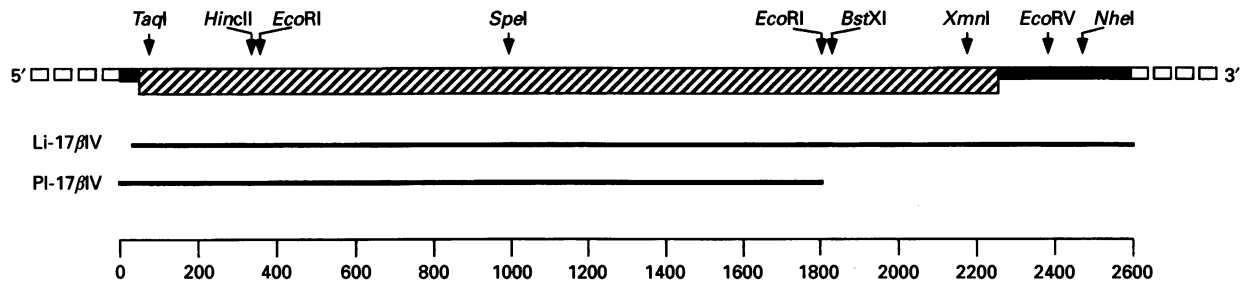

Figure 1. Restriction map of human 17β-HSD IV cDNA clones

The protein-coding region is represented by a hatched box, and the flanking 5′- and 3′-non-coding regions are shown as black boxes; the cloning vector pBluescript SK is represented by open boxes. The length and position of two 17β-HSD IV clones are given below the restriction map. The Li-17βIV clone contains the complete human 17β-HSD IV open reading frame, whereas the incomplete PI-17βIV open reading frame clone containing the longer 5′-untranslated sequence is from a human placenta cDNA library. The scale below begins at the first 5′-nucleotide of the 17β-HSD IV cDNA from the PI-17βIV clone.

19, 241 and 316 nucleotides downstream from the stop codon. However, the data obtained on the expression of this gene indicate that this clone is truncated in its 5′- or 3′-untranslated region and that the corresponding full-length cDNA is about 3.0 kb.

The human protein encoded by this new cDNA was named 17β-HSD IV.

Deduced amino acid sequence and similarity to other 17β-HSDs

The sequence of the first in-frame initiating codon, ATTCATGG (Figure 2), contains only two of the five nucleotides of the consensus sequence required for optimal initiation by eukaryotic ribosomes [26], i.e. Ca/gCCATGG. However, porcine 17β-EDH is also devoid of an optimal initiation consensus site [20]. The 48 nucleotides upstream from this first ATG are not conserved when compared with the 5′-non-coding region of porcine 17β-EDH. Fifteen in-frame ATG codons are found in the sequence. The second in-frame ATG downstream from the first is not followed by a G at position +4, which is essential for efficient translation [26]. Some 48 nucleotides of the 5′-untranslated region was obtained upstream from the first ATG. The open reading frame starting at the first ATG encodes a continuous protein of 736 amino acids of high (84%) similarity to porcine 17β-EDH. The calculated molecular mass of the product is 79595 Da. Translation of the open reading frame predicts a protein with eight cysteines, rich in glycine (11%), alanine (9%), leucine (8%) and lysine (7%). These amino acids are apparently not clustered in any particular region of the primary structure. There are three potential Asp-linked glycosylation sites, marked by asterisks in Figure 2. Hydrophobicity analysis has not identified a segment satisfying the criteria for membrane-spanning stretches (not shown).

Partially *Eco*RI-digested Li-17βIV fragment containing the full-length human 17β-HSD IV open reading frame was introduced into the pCDNA3 plasmid (pCDNA3-17β-HSD IV recombinant plasmid), transcribed and translated *in vitro* with the rabbit reticulocyte lysate system using radiolabelled methionine. The translation product migrates in SDS/PAGE with an apparent molecular mass of ≈ 80 kDa (Figure 3, lane 2). No corresponding 80 kDa band was observed when pCDNA3 plasmid alone was translated (Figure 3, lane 1).

The first 300 N-terminal amino acids are similar to several members of the short-chain alcohol dehydrogenase family. The best matches include the N-terminal regions of FOX2 protein from *Saccaromyces cerevisiae* (54% identity) and the multifunctional enzyme of *Candida tropicalis* (50% identity) [27–29]. Using FASTA software, we were not able to align the four cloned human 17β-HSDs, which are less than 25% identical with 17β-HSD IV and with each other. Only some remote similarities are seen in the conserved amino acids of the short-chain alcohol dehydrogenase family, e.g. in the cofactor-binding (G^{16}, G^{18}, G^{22}) and the catalytic (Y^{164} and K^{168}) domains [30,31].

The region corresponding to amino acids 343–607 of 17β-HSD IV shares 40% identity with the C-terminal part of the trifunctional *C. tropicalis* enzyme (Figure 4a) and 37% similarity to the FOX2 protein. The hydratase–dehydrogenase–epimerase of *C. tropicalis* and FOX2 of *S. cerevisiae* participate in peroxisomal β-oxidation of fatty acids [27,28]. The high score on alignment with porcine 17β-EDH excludes random similarity.

The C-terminal region of 17β-HSD IV (residues 596–736) exhibits 39% identity with the human sterol carrier protein 2, which has been reported to participate in intracellular transport of cholesterol and lipids [32]. Residues essential for these functions [33] are also conserved in 17β-HSD IV (L^{635}, D^{685}, K^{715} and N^{719}) and are marked by asterisks in Figure 4(b). The last three residues AKL^{736} agree with the consensus sequence for a peroxisomal targeting signal [34].

All amino acid similarities seen for 17β-HSD IV correspond to those observed for porcine 17β-EDH.

Enzymic properties of human 17β-HSD IV

As illustrated in Table 1, in enzyme assays performed on Hek 293 cells transfected with the pRep10-h17β-HSD IV plasmid, Δ^5-androstene-3β,17β-diol and oestradiol are transformed to their 17-oxo forms with K_m values of 0.90 and 0.81 μM respectively. In contrast, the other androgens tested are not substrates for human 17β-HSD IV. As a control, pRep10-transfected cells did not transform any of the substrates tested (results not shown). This oxidative 17β-HSD activity is NAD^+-dependent; the reductive NADPH-dependent activity is at background level. 17β-HSD IV is free from 3α, 3β- and 20α-dehydrogenase activities.

Northern-blot analysis of 17β-HSD IV in human tissues

Expression levels of 17β-HSD IV were analysed by Northern blotting. Polyadenylated mRNA (2 μg/lane) from different human tissues were hybridized with the α-^{32}P-labelled *Eco*RI fragment corresponding to nucleotides 302 to 1753 of human 17β-HSD IV. Using stringent conditions, a 2.8–3.2 kb mRNA

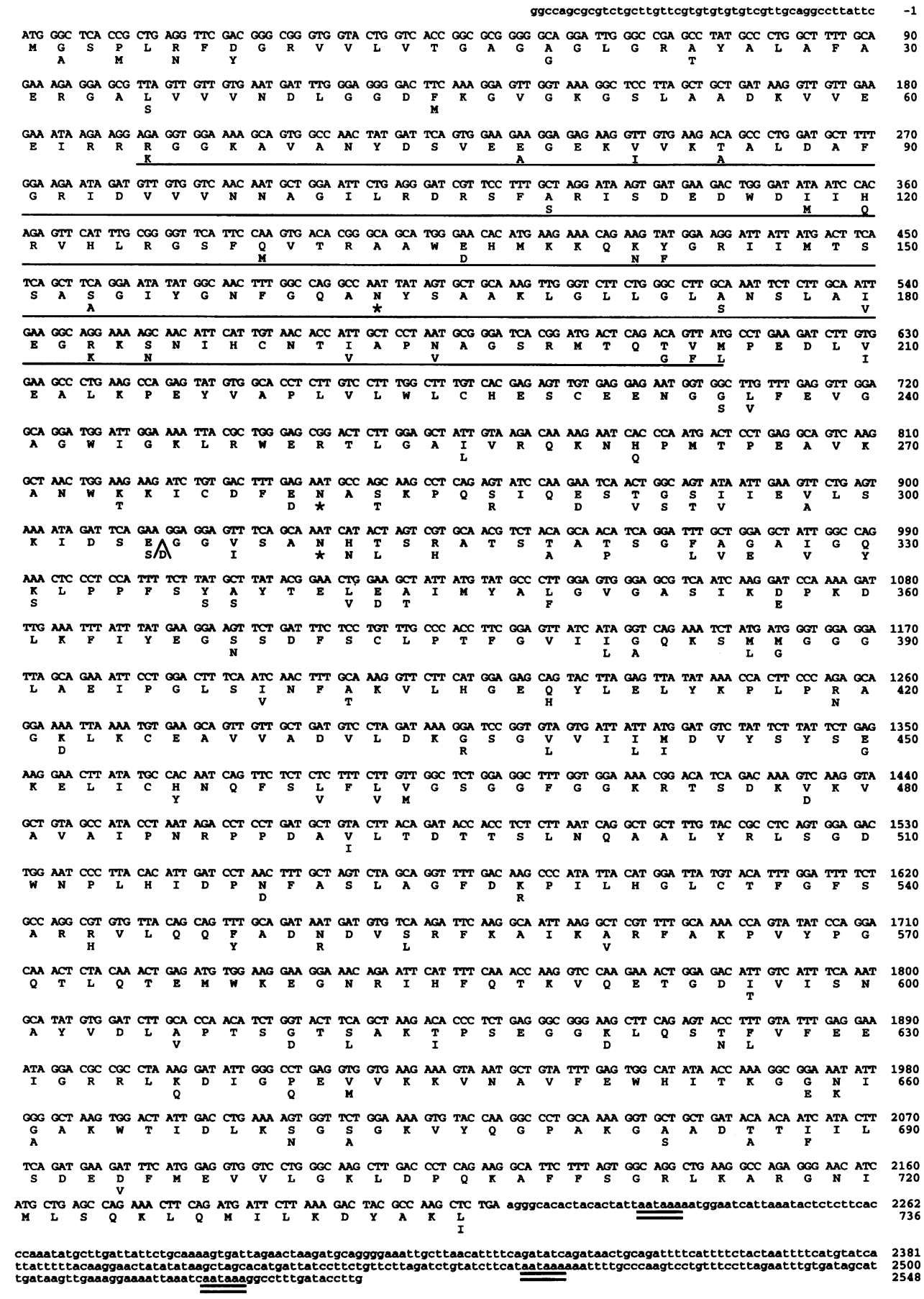

Figure 2. For legend see facing page.

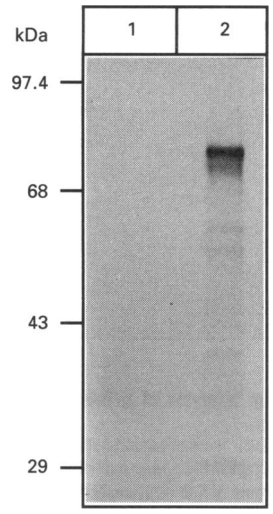

Figure 3. *In vitro* protein synthesis of human 17β-HSD IV

Synthesis of human 17β-HSD IV was performed by *in vitro* transcription from pCDNA3-Li-17βIV plasmid and using rabbit reticulocyte lysates containing [^{35}S]methionine for translation (lane 2). Lane 1 represents rabbit reticulocyte lysate translated with pCDNA3 plasmid alone. The products were separated on an SDS/6% polyacrylamide gel and visualized on X-ray film. Molecular-mass standards are indicated on the left.

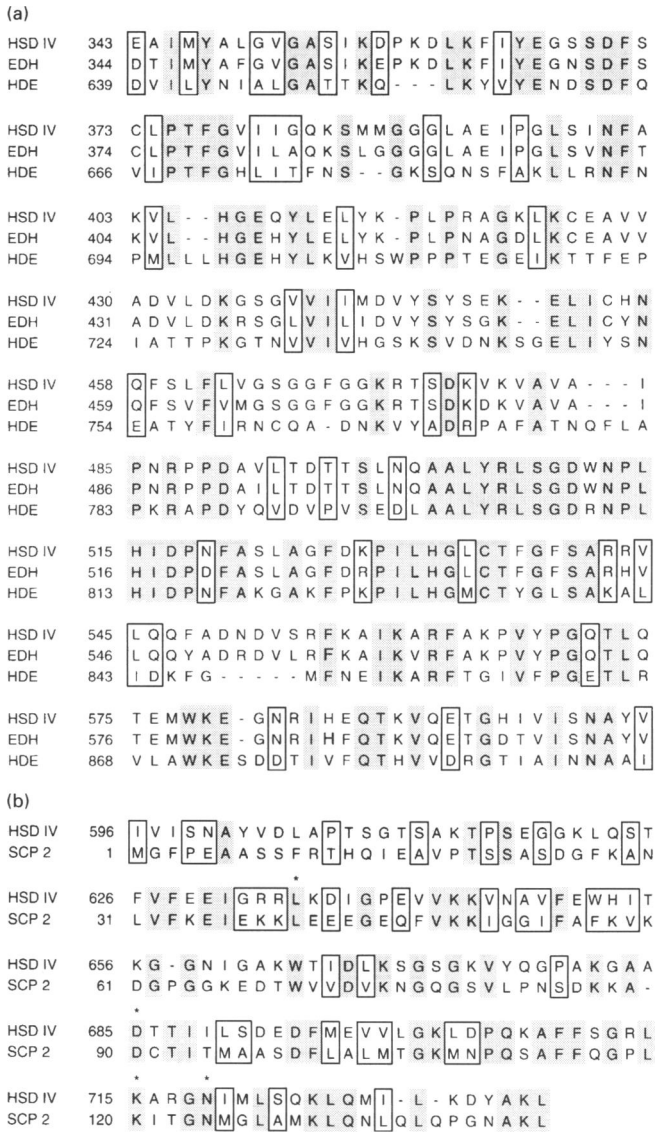

Figure 4. Alignment of protein sequences

(*a*) Similarities of the central domains of human 17β-HSD IV, porcine 17β-EDH and multifunctional enzyme (HDE) of *C. tropicalis*. (*b*) Comparison between the C-terminal parts of 17β-HSD IV and human sterol carrier protein 2 (SCP2). Asterisks indicate residues essential for SCP2 transfer activity [32]. Identical residues are indicated by a shaded background, and similar residues are boxed.

transcript was found to be expressed in virtually all human tissues examined (Figure 5). The apparently highest expression was seen in liver (Figure 5a, lane 5) followed by heart (Figure 5a, lane 1), prostate (Figure 5b, lane 3) and testis (Figure 5b, lane 4). Moderate expression was observed in lung (Figure 5a, lane 4), skeletal muscle (Figure 5a, lane 6), kidney (Figure 5a, lane 7), pancreas (Figure 5a, lane 8), thymus (Figure 5b, lane 2), ovary (Figure 5b, lane 5), intestine (Figure 5b, lane 6) and term placenta (Figure 5a, lane 3). Faint signals were produced by brain (Figure 5a, lane 2), spleen (Figure 5b, lane 1), colon (Figure 5b, lane 7) and peripheral blood lymphocytes (Figure 5b, lane 8).

As illustrated in Figure 6, 17β-HSD IV is also expressed in human cancer cell lines. The T-47D oestrogen-receptor-positive mammary cancer cell line (lane 2) expresses more 17β-HSD IV mRNA transcript than the three oestrogen-receptor-negative [BT-20 (lane 3), MDA-MB-453 (lane 4) and MDA-MB-231 (lane 5)] and other oestrogen-receptor-positive [MCF-7 (lane 1)] ones. Human cancer cell lines from other origins such as the early embryo [Tera-1 (lane 7)], the liver [HEPG2 (lane 9)] and colon [HCT-15 (lane 12)] also express significant levels of 17β-HSD mRNA. The highest level of expression was observed in the Dami megakaryocytic cell line (lane 11).

DISCUSSION

Purification and cloning of porcine 17β-EDH was a prerequisite for delineating the amino acid sequence of the closely related human enzyme 17β-HSD IV. Like the former, 17β-HSD IV has a multidomain structure (Figure 7). The first 300 amino acids of both enzymes (which in porcine 17β-EDH harbour the oestradiol dehydrogenase activity) show similarities to members of the short-chain alcohol dehydrogenase family. The central region (residues 343–607) resembles enzymes that catalyse peroxisomal β-oxidation of fatty acids: the trifunctional hydratase–dehydrogenase–epimerase of *C. tropicalis* [28] and the FOX2 protein of *S. cerevisiae* [27]. It remains to be clarified whether 17β-HSD IV

Figure 2. Nucleotide and predicted amino acid sequences of human 17β-HSD IV

Numbering of nucleotides and amino acids is given on the right. The nucleotides corresponding to the open reading frame and the 5′- and 3′-untranslated regions are in capital and small letters respectively. The differences in amino acids between human 17β-HSD IV and porcine 17β-EDH [20], and the insertion at amino acid 306 of the porcine enzyme are given below the deduced sequence. The polyadenylation consensus sites are double underlined [25], and asterisks denote potential glycosylation sites. The region corresponding to the porcine probe used in the screening of the libraries is underlined with a single line.

Table 1 Enzymic characterization of human 17β-HSD IV

Hek 293 cells were transfected with pRep10 or pRep10-h17β-HSD IV plasmid by the calcium phosphate co-precipitation method as described in the Experimental section. Enzyme assays were performed on cell extract as previously described using 17β-[^{3}H]oestradiol (E_2), [^{3}H]oestrone (E_1), Δ^5-[^{3}H]androstene-3β-diol (Δ^5-diol), dehydro[^{3}H]epiandrosterone (DHEA), [^{3}H]testosterone (T), 5α-[^{3}H]androstane-3α,17β-diol (3α-diol), dihydro5α-[^{3}H]testosterone (DHT), dihydro20α-[^{3}H]progesterone (20DHP) or [^{3}H]androsterone (A). ND, Not detectable.

Substrate	K_m (μM)	Conversion (pmol/min per ml)
E_2	0.81	0.71
E_1	ND	ND
Δ^5-diol	0.90	0.65
DHEA	ND	ND
3α-diol	ND	ND
T	ND	ND
DHT	ND	ND
A	ND	ND
20DHP	ND	ND

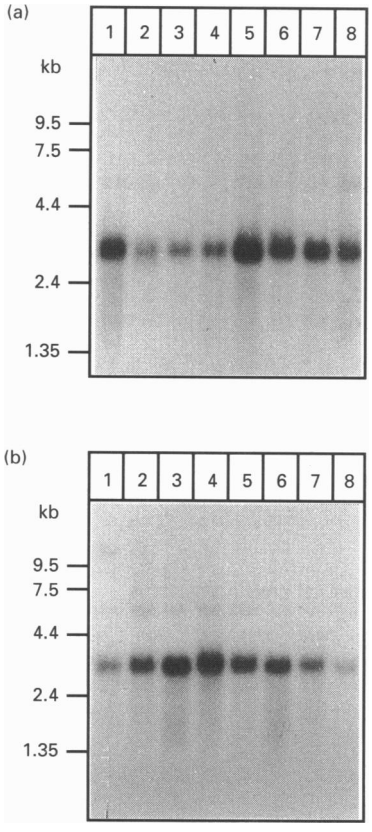

Figure 5. Northern-blot analysis of 17β-HSD IV in human tissues

Samples of polyadenylated mRNA (2 μg per lane) from different human tissues were applied. The *Eco*RI-digested fragment from nucleotides 302 to 1753 of Li-17βIV plasmid was used for hybridization. RNA blot analysis was performed as previously described [22]. Sizes (kb) are indicated on the left. (**a**) mRNA from human heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) and pancreas (lane 8); (**b**) mRNA from human spleen (lane 1), thymus (lane 2), prostate (lane 3), testis (lane 4), ovary (lane 5), small intestine (lane 6), colon (lane 7) and peripheral blood leucocytes (lane 8).

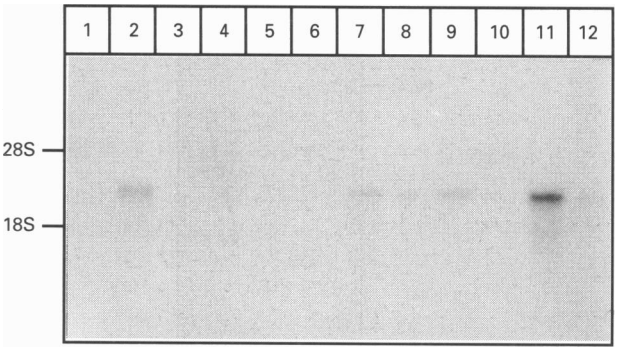

Figure 6. Northern-blot analysis of 17β-HSD in human cancer cells

Total RNA (10 μg) from each cell line was extracted and applied to 1% denaturing agarose gel. After transfer to nylon membranes, blots were hybridized, washed and autoradiographed as described in the legend to Figure 5. The positions of 28S and 18S RNA are indicated on the left. The blot contains RNA from human cancer cell lines of the following origins: mammary adenocarcinoma [MCF-7 (lane 1), T-47D (lane 2), BT-20 (lane 3), MDA-MB-453 (lane 4) and MDA-MB-231 (lane 5)], cervix carcinoma [HeLa (lane 6)], embryo teratocarcinoma [Tera-1 (lane 7)], melanoma [SK-MEL (lane 8)], hepatocarcinoma [HepG2 (lane 9)], erythroleukaemia [MOLT-4 (lane 10)], megakaryocytic leukaemia [Dami (lane 11)] and colon adenocarcinoma [HCT-15 (lane 12)].

is able to catalyse corresponding reactions. Human 17β-HSD IV, porcine 17β-EDH, hydratase–dehydrogenase–epimerase and FOX2 must have diverged some 2 billion years ago. Their common ancestor could have been related to the present form of *Escherichia coli* 3-oxoacyl carrier protein reductase (M. E. Baker, personal communication). The C-termini of 17β-HSD IV and porcine 17β-EDH are similar to the sterol carrier protein 2 including its peroxisomal targeting signal. Immunogold electron-microscopy studies on porcine uterus epithelium have localized 17β-EDH to 120–200 nm vesicles with moderately electron-dense matrix bounded by a single membrane [35]. The morphology of these specialized organelles and their density in sucrose gradients (1.18 g/ml) are indeed close to those of peroxisomes. The final assignment awaits further immunogold electron-microscopy studies on colocalization of typical peroxisomal markers with the dehydrogenase.

The marked resemblance between the amino acid sequences of human 17β-HSD IV and porcine 17β-EDH suggests that their catalytic properties are likely to be similar. They are different from those of 17β-HSD I, II and III. The oxidoreductase 17β-HSD I [10,11] preferentially catalyses the interconversion of oestradiol and oestrone. The oxidoreductase 17β-HSD II [8] and the reductase 17β-HSD III [12] metabolize oestrogens and androgens. Porcine 17β-EDH oxidizes oestradiol and Δ^5-androstene-3β,17β-diol and is virtually devoid of reductase activity [19]. Moreover, 17β-HSD III uses NADP(H) specifically as a cofactor, whereas 17β-HSD I and II preferentially employ NAD(H) for oxidation and reduction. Porcine 17β-EDH prefers NAD^+ for oxidation, but NADPH is required for the little reductase activity that is carried out.

The data on 17β-HSD IV mRNA expression indicate a wide distribution. Besides high levels in liver, heart, prostate and testes, substantial amounts of 17β-HSD IV mRNA were detected in ovary, kidney, pancreas, muscle and small intestine. However,

Figure 7. Multidomain structure of 17β-HSD IV

Regions of similarity are indicated. SCAD, short-chain alcohol dehydrogenase; HDE hydratase–dehydrogenase–epimerase of *C. tropicalis*; SCP2, sterol carrier protein 2.

little mRNA was found in the placenta. This is in contrast with the predominant expression of 17β-HSD I and II in the placenta and 17β-HSD III in testes [2,8,12,36]. Primary meningioma cells were initially found to express 17β-HSD II solely [36]. Several cancer cell lines arising from prostate [37], choriocarcinoma [38] and breast [37,39,40] have been reported to express 17β-HSD I. This enzyme is expressed in oestrogen-receptor-positive as well as oestrogen-receptor-negative mammary cancer cell lines (D. Monté and Y. de Launoit, personal communication). Although the highest expression of 17β-HSD IV was observed in oestrogen-receptor-positive T-47D cells, no correlation between the presence of the oestrogen receptor and the expression of this enzyme could be obtained. A comparison of the expression of the four 17β-HSDs in a wide series of mammary cancer cell lines should therefore be made. Most surprising is the presence of 17β-HSD IV in a non-steroidogenic tissue such as human leukaemia cells. However, as reported by Martel et al. [2], almost all human tissues possess detectable 17β-HSD activity, suggesting that 17β-HSD IV could be responsible for this wide tissue distribution. Moreover, as 17β-HSD IV activity favours oxidation of 17β-oestradiol, the most potent oestrogen, it could have an important house-keeping function, inactivating oestrogens in all tissues.

Several lines of evidence (expression, substrate/cofactor specificity, subcellular localization) indicate a diversity of 17β-HSD enzymes. Cloning of human 17β-HSD IV has only added to the complexity of steroid synthesis and metabolism. The multitude of enzymes that act at C-17 might be necessary to provide precise control of hormone levels.

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