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Biochemical and Biophysical Research Communications 305 (2003) 37–45
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Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7☆

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Received 3 March 2003

Abstract

17β-Hydroxysteroid dehydrogenases (17HSDs) have a central role in the regulation of the biological activity of sex steroid hormones. There is increasing evidence that in addition to their importance in gonads, these hormones also have substantial metabolic roles in a variety of peripheral tissues. In the present study, the cDNA of human 17HSD type 7 was cloned. In silico, the gene corresponding to the cDNA was localized on chromosome 1q23, close to the locus of hereditary prostate cancer 1 (HPC1) (1q24-25) and primary open-angle glaucoma (GLC1A) (1q23-25). Further, a pseudogene was found on chromosome 1q44, close to the locus of predisposing for early-onset prostate cancer (PCaP) (1q42.2-43). Both human (h17HSD7) and mouse 17HSD type 7 (m17HSD7) were for the first time produced as recombinant proteins and purified for functional analyses. Further, kinetic parameters and specific activities were described. h17HSD7 converted estrone (E1) to a more potent estrogen, estradiol (E2), and dihydrotestosterone (DHT), a potent androgen, to an estrogenic metabolite 5α-androstane-3β, 17β-diol (3βA-diol) equally, thereby catalyzing the reduction of the keto group in either 17- or 3-position of the substrate. Minor 3βHSD-like activity towards progesterone (P) and 20-hydroxyprogesterone (20-OH-P), leading to the inactivation of P by h17HSD7, was also detected. m17HSD7 efficiently catalyzed the reaction from E1 to E2 and moderately converted DHT to an inactive metabolite 5α-androstane-3α,17β-diol (3αA-diol) and to an even lesser degree 3βA-diol. The mouse enzyme did not metabolize P or 20-OH-P. The expression of 17HSD type 7 was observed widely in human tissues, most distinctly in adrenal gland, liver, lung, and thymus. Based on the enzymatic characteristics and tissue distribution, we conclude that h17HSD7 might be an intracrine regulator of steroid metabolism, fortifying the estrogenic milieu in peripheral tissues.

Keywords: 17-Ketosteroid reductase; 3β-Ketosteroid reductase; Membrane protein; Steroid metabolism; Chromosomal localization

17β-Hydroxysteroid dehydrogenases (17HSDs) have a central function in controlling the biosynthesis and biological activity of the sex steroid hormones in gonads and peripheral target tissues. They are nicotinamide adenine dinucleotide [NAD(H)]- or its phosphate form [NADP(H)]-dependent enzymes that catalyze the

☆ *Abbreviations:* 17HSD, 17β-hydroxysteroid dehydrogenase; E2, estradiol; E1, estrone; T, testosterone; A-dione, androstenedione; DHT, dihydrotestosterone; 5αA-dione, 5α-androstanedione; PRAP, prolactin receptor-associated protein; RT-PCR, reverse transcription polymerase chain reaction; h17HSD7, human 17β-hydroxysteroid dehydrogenase type 7; m17HSD7, mouse 17β-hydroxysteroid dehydrogenase type 7; 3βA-diol, 5α-androstane-3β,17β-diol; P, progesterone; 20-OH-P, 20α-hydroxyprogesterone; 3αA-diol, 5α-androstane-3α,17β-diol; DHEA, dehydroepiandrosterone.

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doi:10.1016/S0006-291X(03)00694-6

conversions between 17β-hydroxy- and 17-ketosteroids. The reductive reactions catalyzed by 17HSDs are important for the biosynthesis of active sex steroid hormones, e.g., estradiol (E_2) and testosterone (T). Instead, oxidative 17HSD enzymes tend to decrease the activity of sex steroid hormones by converting, for example, E_2 to a less active form of estrogen, estrone (E_1), testosterone (T) to androstenedione (A-dione) or dihydrotestosterone (DHT) to 5α-androstanedione (5αA-dione) ([1,2] and references therein).

17β-Hydroxysteroid dehydrogenase type 7 (17HSD type 7) is a membrane-associated reductive enzyme belonging to the large, phylogenetically related protein family of short-chain dehydrogenases/reductases (SDR) [3,4]. 17HSD type 7 has been reported to catalyze the conversion of E_1 to biologically active estrogen, E_2, in rodents [5]. In this reaction 17HSD type 7 prefers NADPH as a cofactor. 17HSD type 7 was originally identified as a prolactin receptor-associated protein (PRAP) in rat [6]. Later rat PRAP was shown to be rat 17HSD type 7, as Nokelainen et al. [5] cloned mouse 17HSD type 7, which shares 89% identity with rat PRAP and catalyzes the transformation of E_1 to E_2 similar to rat PRAP. Expression of m17HSD7 has been detected by Northern blot and in situ hybridization in mouse ovary (corpora lutea), uterus, and placenta. m17HSD7 mRNA has also been recognized in mouse mammary gland, liver, kidney, and testis and in the HC11 cell line [5,7].

h17HSD7 shares an amino acid identity of 78% with rat and 74% with mouse 17HSD type 7. The human enzyme has been detected with RT-PCR in pregnant uterus and placenta and in the ovaries of non-pregnant women. However, no expression of h17HSD7 was detectable in the ovary samples of pregnant women, which is a difference compared to the rodent enzymes, which are expressed very strongly in the ovaries during pregnancy. h17HSD7 was also present in testis and prostate samples as well as in several neural tissues, including retina, neuronal precursors, infant brain, and glioblastoma [8].

In this study, the cDNA of human 17HSD type 7 was cloned and a pseudogene was found, and both genes were in silico chromosome localized. To clarify the function of the enzyme, human and mouse 17HSD type 7 were for the first time produced as biologically active recombinant proteins in Sf9 (*Spodoptera frugiperda*) insect cells using a recombinant baculovirus expression system and purified by immobilized metal affinity chromatography. Substrate specificities and kinetic parameters were analyzed using the purified recombinant enzymes. The expression of h17HSD7 in different tissues was studied by Northern blotting. A spliced form of h17HSD7 lacking the exon 6 was also produced as recombinant protein and partially purified to characterize its possible enzymatic activities.

Materials and methods

Isolation of RNAs. Total RNA was isolated using TRIzol Reagent (Gibco, Invitrogen, San Diego, CA, USA) and poly(A)-enriched RNA was extracted from total RNA using oligo(dT)-cellulose chromatography [9].

Human 17HSD type 7 cDNA. The first cDNA fragment, h17HSD7.1a (nucleotides 1–585 in human 17HSD type 7 cDNA) was cloned using reverse transcription polymerase chain reaction (RT-PCR) from poly(A)-enriched RNA from granulosa luteal cells of women participating in the IVF program. Primers were based on the human EST clones retrieved from a databank using the sequence of mouse 17HSD type 7 cDNA (see Table 1). The initial RT-step was performed at 42 °C using SuperScriptII (Gibco BRL, Gaithersburg, MD, USA) and an antisense primer, h7-5P1. Following amplification with *Pyrococcus furiosus* (*Pfu*) polymerase (Stratagene, La Jolla, CA, USA) was performed for 30 cycles (94 °C, 1 min; 60 °C, 1 min; and 72 °C, 2 min) using the nested antisense primer, h7-5P2 and the sense primer, h7-RT-3b.

The 3′-RACE technique was applied to clone a 3′-fragment, h17HSD7.1b (nucleotides 517–1141 in human 17HSD type 7 cDNA, Fig. 1) using the *Not*I-$(dT)_{18}$ antisense primer followed by PCR with *Pfu* polymerase using the *Not*I-$(dT)_{18}$ and h7-3P1 primers. In the second round of PCR, the h7-3P1 was replaced by the h7-3P2. The PCR cycles were as mentioned above.

*Bam*HI–*Xho*I digested h17HSD7.1a and *Xho*I–*Not*I digested h17HSD7.1b were ligated to the *Bam*HI–*Not*I digested Bluescript KS+ plasmid to obtain h17HSD7.1c clone.

In addition, the human placental cDNA library (λ-TripIEx-library, HL5020t, BD Biosciences Clontech, Palo Alto, CA, USA) was screened several times with a ^{32}P-labeled cDNA fragment corresponding to the nucleotides 1–722 of human 17HSD type 7 cDNA. One positive plaque was identified, amplified, and purified. A TripIEx plasmid containing human 17HSD type 7 cDNA was released from λ-DNA by homologous recombination in *Escherichia coli* BM25.8. Thereafter, the clone was sequenced and named h17HSD7.2.

Cloning of human 17HSD type 7 genomic clones. The human placental genomic DNA λEMBL library (BD Biosciences Clontech, Palo Alto, CA, USA) was screened by standard methods [10] using a ^{32}P-labeled fragment (1–722) of human 17HSD type 7 cDNA (Fig. 1). Altogether 22 genomic clones were obtained and plaque-purified

Table 1
Primers used in RT-PCR cloning of human 17HSD7 cDNA clones

Name	Sequence (5′–3′)	Nucleotides	EST based on	Restriction site added in 5′-end
h7-5P1	ATATTTGGAAGAGCTGTAGGG	618–598	R92053	—
h7-5P2	GCTGTGCTGGAAGTCCTCG	585–567	R92053	—
h7-RT-3b	GCTTGGAAGTGTGAGTGCG	1–19	T78371	*Bam*HI
h7-3P1	CTGTCACAGTGACAATCCATC	495–515	—	—
h7-3P2	CAGCTCATCTGGACATCATC	517–536	—	—

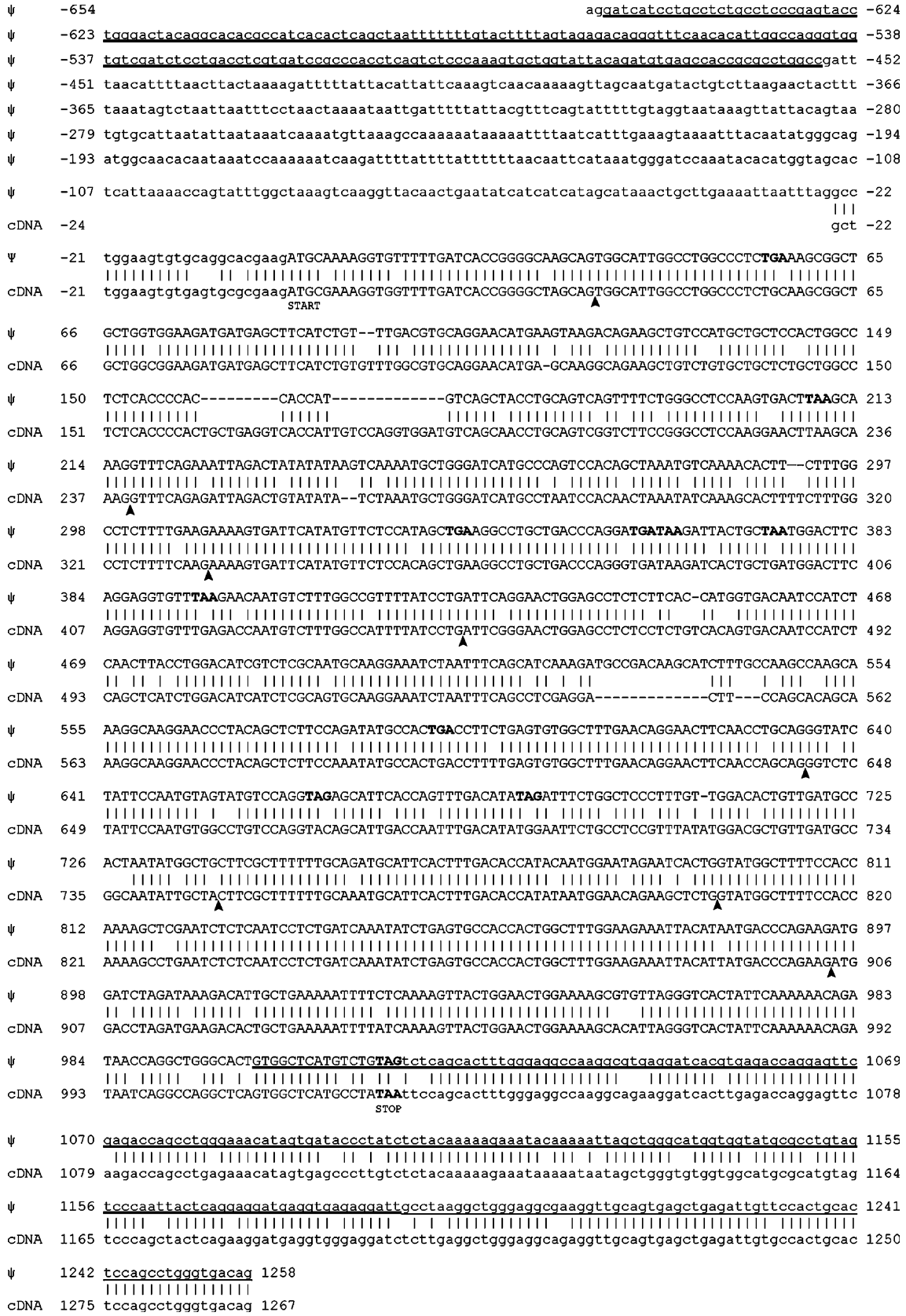

Fig. 1. Nucleotide sequence of human 17HSD type 7 (cDNA) and the genomic region containing a human 17HSD type 7 pseudogene (Ψ). The Alu-like sequence is underlined and the reverse repeat sequence of it is bold-underlined. The stop codons in the pseudogene are bold-faced and the arrowheads below the cDNA sequence represent the intron sites.

through three subsequent rounds of screening, and they were named starting h7-λ1 through h7-λ24. These clones were then amplified and analyzed by Southern blotting and PCR, and some of them were directly sequenced.

In silico chromosomal localization of the human 17HSD type 7 genes. The sequences of the cDNA (AJ249179) and the genes (AJ250550–AJ250558, AJ297815) of human 17HSD type 7 were used to search the database of human genome at National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Sequencing and sequence analysis. Sequencing of cDNA, genomic clones, and all constructs was performed using an automatic DNA sequencer (ABI Prism 377 DNA sequencer, Perkin–Elmer). The sequences were verified by analyses with a GCG package (Genetics Computer Group) on a UNIX computer at Center for Scientific Computing (Espoo, Finland) run through the Internet or using Blast service on the Web site of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Northern blot analysis. A commercial Human RNA Master Blot (BD Biosciences Clontech, Palo Alto, CA, USA) was used for analysis of the tissue distribution of h17HSD7. The filter was hybridized with a ^{32}P-labeled fragment of human 17HSD type 7 (nucleotides 1–722, specific activity 2.9×10^8 cpm/μg) at 68 °C overnight using the hybridization solution provided by the manufacturer of the membrane. After the hybridizations, the membrane was washed 4 × 10 min in 2× SSC containing 0.05% SDS at room temperature and 2 × 20 min in 0.1× SSC containing 0.1% SDS in 50 °C. The membrane was then exposed to Kodak BioMax MR film (Rochester, NY, USA) for the indicated time.

RT-PCR assays for the two forms of human 17HSD type 7. The primers 5′-TGTCTTTGGCCATTTTATCCT-3′ and 5′-ATTTTTCA GCAGTGTCTTCATCTTA-3′ for RT-PCR were designed using the nucleotide sequence of the cDNA deposited in GenBank (Accession No. BC007068) for 17HSD type 7 exon 6. Total RNA was isolated from cultured human eye ciliary epithelial cells using TRIzol reagent (GIBCO, Invitrogen, San Diego, CA, USA), according to the manufacturer's protocol. To eliminate possible genomic DNA contamination in the RNA preparation, the samples were pre-treated with RNase-free DNase (Boehringer Mannheim, Indianapolis, IN, USA) before the RT-PCR reactions were performed using a RT-PCR kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's protocol, 35 cycles (94 °C, 1 min; 54 °C, 1 min; and 72 °C, 1 min). The final polymerization step was extended for an additional 5 min. The amplified DNA products were purified and sequenced.

Preparation of the deletion construct. The deletion of the exon 6 of human 17HSD type 7 (nucleotides 643–747, Fig. 1) was constructed from h17HSD7.1c using ExSite PCR-Based Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) and following the manufacturer's protocol. The primers were 5′-CTTCGCTTTTTTGCAAATGCATTC ACTTTGACACC-3′, 5′ phosphorylated, and 5′-CTGCTGGTTGAA GTTCCTGTTCAAAGCC-3′. The h17HSD7del cDNA coding sequence was amplified by PCR using *Pfu* polymerase. The forward primer contained a *Bgl*II site (underlined) 5′ATAAGATCTCGAAGA TTCGAAGGTGG3′ and the reverse primer contained a nucleotide sequence encoding six histidines and a *Not*I site (underlined) 5′ATA GCGGCCGCTCAATGGTGATGGTGATGATGTAGGCATGAG CCACTGAG3′. The *Bgl*II–*Not*I digested PCR product was subcloned into a pVL1329 vector (Invitrogen, San Diego, CA, USA). The construct was cotransfected in Sf9 cells with linearized baculovirus DNA. This step and the production of the recombinant viruses and protein were performed as described below.

Production of recombinant proteins. To express human and mouse 17HSD type 7 in *S. frugiperda* (Sf9) cells (ATCC, Rockville, MD, USA), fragments containing the whole coding region were amplified using primers corresponding to the nucleotides −5–13 of human 17HSD type 7 cDNA (Fig. 1), the nucleotides 64–81 of mouse 17HSD type 7 cDNA [5] (+*Bgl*II site at 5′-ends), and the nucleotides 996–1023 of human and the nucleotides 1047–1065 of mouse cDNA, both complete with the sequence encoding six histidines just before the stop codon and the *Not*I site for human and the *Eco*RI site for mouse at the 3′-end. The PCR (total 30 cycles) consisted of 5 cycles (94 °C, 1 min; 55 °C, 1 min; and 72 °C, 2 min) and 25 cycles (94 °C, 1 min; 60 °C, 1 min; and 72 °C, 2 min). Thereafter, the human fragment was subcloned into the *Bgl*II and *Not*I sites and the mouse fragment into the *Bgl*II and *Eco*RI sites of the pVL1392 non-fusion transfer vectors. Recombinant *Autographa californica* nuclear polyhedrosis viruses (AcNPV) for human and mouse 17HSD type 7 were produced in Sf9 insect cells using the BaculoGold Transfection system (BD Biosciences Pharmingen, San Diego, CA, USA) according to the manufacturer's instructions. Recombinant h17HSD7 and m17HSD7 were produced in Sf9 insect cells as previously described [11,12].

Purification of recombinant proteins. The purification steps were carried out at room temperature using cold buffers. The purification of m17HSD7 was started by thawing the pellet of cells and diluting it four times with buffer A [50 mM Tris, pH 8.0, 500 mM NaCl, 20% glycerol, and 40 μM NADPH (Roche Molecular Biochemicals, Basel, Switzerland), 0.5 mM PMSF (Sigma Chemical, St. Louis, MO, USA), 5 mM leupeptin (Sigma Chemical, St. Louis, MO, USA), and 0.02% NaN_3] + 10 mM CHAPS (Calbiochem–Novabiochem, San Diego, CA, USA). The suspension was sonicated (Labsonic U, B. Braun Biotech International) on an ethanol-ice bath (four 10-s bursts at 45-s intervals). The remaining cell components were removed by centrifugation (140,000*g*, +4 °C, 1 h). The supernatant was collected and mixed with 1–3 ml of TALON Metal Affinity Resin (BD Biosciences Clontech, Palo Alto, CA, USA) or Ni–NTA agarose (Qiagen, Hilden, Germany), which had previously been equilibrated with buffer A + 10 mM CHAPS, and incubated with rotation at +4 °C for 1 h. The resin was collected by centrifugation, transferred into a 15-ml glass column, and washed first with buffer A + 10 mM CHAPS and then with buffer A + 6 mM CHAPS and 5 mM imidazole. The bound histidine-tagged m17HSD7 was eluted with buffer A + 6 mM CHAPS and 200 mM imidazole.

The purification of h17HSD7 and h17HSD7del was started by thawing the pellet of cells and diluting it four times with buffer A. The suspension was sonicated on an ethanol-ice bath (four 10-s bursts at 45-s intervals) and centrifuged (140,000*g*, +4 °C, 1 h). The pellet was suspended with buffer A + 10 mM CHAPS, homogenized, and sonicated as described above. The suspension was mixed with 1–3 ml of TALON CellThru resin (BD Biosciences Clontech, Palo Alto, CA, USA), which had previously been equilibrated with buffer A + 10 mM CHAPS, and incubated with rotation at +4 °C for 1 h. The resin was collected by centrifugation, transferred into a 15-ml glass column, and washed first with buffer A + 10 mM CHAPS and then with buffer A + 10 mM CHAPS and 5 mM imidazole. The bound histidine-tagged h17HSD7 was eluted with buffer A + 10 mM CHAPS and 200 mM imidazole.

For the analysis of recombinant proteins, SDS–PAGE was performed using 11% polyacrylamide. The proteins in the gel were stained with Coomassie blue. For Western blot analysis, Anti-His (C-term) antibody (Invitrogen, Carlsbad, CA, USA) and alkaline phosphatase-conjugated secondary antibody, Anti-Mouse IgG (Fc specific) (Sigma Chemical, St. Louis, MO, USA) were used.

Measurement of the enzymatic activity and determination of the kinetic parameters of purified recombinant human and mouse 17HSD type 7. Enzymatic activities were measured as previously described [13,14] with minor modifications. Briefly, 150,000 cpm of ^{3}H-labeled substrates DHT (5α-Dihydro[1,2-^{3}H]testosterone, 125 Ci/mmol), E_1 ([2,4,6,7-^{3}H]oestrone, 95 Ci/mmol), P ([1,2,6,7-^{3}H]Progesterone, 86 Ci/mmol), A-dione ([1,2,6,7-^{3}H]androst-4-ene-3,17-dione, 99 Ci/mmol), Cortisol ([1,2,6,7-^{3}H]cortisol, 69 Ci/mmol), Aldosterone ([1,2,6,7-^{3}H]Aldosterone, 61 Ci/mmol) (Amersham Biosciences, Little Chalfont, England), 20-OH-P ([1,2-^{3}H(N)]20α-hydroxyprogesterone, 52 Ci/mmol), or DHEA ([1,2,6,7-^{3}H(N)]Dehydroepiandrosterone, 82 Ci/mmol) (NEN Life Science Products, Boston, MA, USA), and the corresponding unlabeled substrates (Steraloids, Newport, RI, USA), the final concentrations of which were 0.5, 1, 2 or 4 μM, was used.

Table 2
Kinetic parameters of purified recombinant human and mouse 17HSD type 7

Reaction	Human 17HSD type 7			Mouse 17HSD type 7		
	K_m (μM)	K_{cat} (U/mg)[a]	K_{cat}/K_m	K_m (μM)	K_{cat} (U/mg)[a]	K_{cat}/K_m
$E_1 \rightarrow E_2$	3.25 ± 0.38	0.89 ± 0.06	0.27	1.19 ± 0.18	3.61 ± 0.20	3.03
DHT → 3βA-diol	2.60 ± 0.30	0.82 ± 0.05	0.32	ND	ND	ND

Kinetic parameters were determined as described in Materials and methods; ND, not determined.
[a] One nanomole of product formed per minute was defined as one unit of enzyme activity.

For enzyme kinetic studies the amount of substrate formed per minute and per quantity of protein was calculated. The protein concentrations were measured with the method of Lowry et al. [15] using bovine serum albumin as the standard. The kinetic parameters (K_m and K_{cat}) were calculated using the enzyme kinetics template of the GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). The values shown in Table 2 represent averages ± SD of three independent triplicate measurements.

Results and discussion

Human 17HSD type 7 cDNA and genes

Human 17HSD type 7 cDNA sequence was obtained by combining the sequence data from the overlapping cDNA clones h17HSD7.1c and 17HSD7.2, and was deposited into the EMBL Nucleotide Sequence Database (AJ249179). Comparison of the human 17HSD type 7 amino acid sequence to the sequence (AF098786) submitted by Krazeisen et al. [8] revealed 100% identity.

The genomic structure of human 17HSD type 7 was determined by analyzing four overlapping genomic clones (h7-λ1, h7-λ2, h7-λ4, and h7-λ9) screened from the DNA λEMBL genomic library. The sequence of exons and the partial intron sequences were deposited into the EMBL Nucleotide Sequence Database (AJ250550–AJ250558). The exon/intron organization of the human 17HSD type 7 gene was found to be the same as published [8], but in contrast with the published gene location (10p11.2), we in silico localized the gene encoding the cloned cDNA on chromosome 1q23, close to the hereditary prostate cancer 1 (HPC1) locus (1q24-25) [16] and primary open-angle glaucoma (GLC1A) locus (1q23-25) [17]. In addition to the functional human 17HSD type 7 gene, a homologous gene was found on chromosome 10p11.2, but the comparisons revealed several amino acid differences, and a premature STOP codon caused by the change of reading frame in exon 5.

Further, the human genome contained homology (clone h7-λ3, Fig. 1) on chromosome 1q44, close to predisposing to prostate cancer (PCaP) locus (1q42.2-43) [18], having features of a processed pseudogene [19]. Alignment against the human 17HSD type 7 cDNA sequence showed 90% identity. Intervening sequences were precisely spliced. Stop codons and frame shifts due to multiple nucleotide changes, deletions, and insertions in the putative coding region prevent encoding of a functional protein. Lastly, the pseudogene is flanked by invert Alu-sequences. These characteristics indicate that the pseudogene was derived from the retroposited mature 17HSD type 7 mRNA and is a processed pseudogene. The pseudogene sequence was deposited into the EMBL Nucleotide Sequence Database (AJ297815).

Tissue distribution of human 17HSD type 7 cDNA

According to the results obtained by probing the human RNA master blot (Fig. 2) with the human

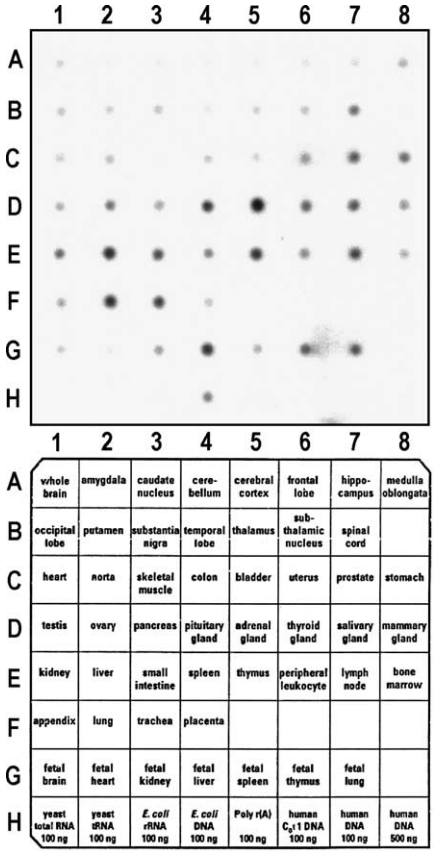

Fig. 2. Tissue distribution of human 17HSD type 7. A membrane containing a ubiquitin-normalized load of 89–514 ng of each poly(A)$^+$ RNA per dot from 50 different human tissues and six different control RNAs and DNAs (see the diagram), Human RNA Master Blot, was hybridized with the human 17HSD type 7 cDNA probe. Exposure time was 4 days.

17HSD type 7 cDNA probe, human 17HSD type 7 mRNA was abundantly expressed in adrenal gland, liver, lung, and thymus. In addition, the enzyme was clearly expressed particularly in pituitary gland, prostate, kidney, lymph node, small intestine, and trachea. A weaker signal was detected in all the other tissues whose RNA was present on the blot. h17HSD7 was weakly, but widely expressed also in tissues not considered steroidogenic, such as lung, kidney, thymus, and lymph node. Therefore, the enzyme might be responsible for the local production of estrogenic metabolites in peripheral tissues.

A spliced form of human 17HSD type 7 with the deletion of the exon 6 (105 bp) has been reported [20]. The two forms were suggested to be expressed differentially, the integral form more abundantly in placenta, adrenals, and testis, whereas the spliced form in brain, testis, and spleen. We found the spliced form in human eye ciliary epithelial cells, neuroendocrine cells, by the RT-PCR. Both the wild type and the spliced form of h17HSD7 were present in the human normal, non-pigmented ciliary epithelial (NPE) cell line (ODM-C4) and the chronic open glaucoma NPE cell line (GCE) (Fig. 3). The spliced form was produced in substantially lesser amounts than the integral form.The sequences of the PCR products confirmed the lack of the entire exon 6 in the spliced form.

Enzymatic activity of human and mouse 17HSD type 7

For the characterization of the enzymatic properties of human and mouse 17HSD type 7, recombinant proteins were produced in Sf9 cells using the baculovirus expression system and single-step purified using immobilized metal affinity chromatography. The purity of the proteins was analyzed by SDS–PAGE and Western blotting. Under reducing conditions, a 35.5 kDa protein band of human and a 36 kDa protein band of mouse 17HSD type 7 were observed (Fig. 4).

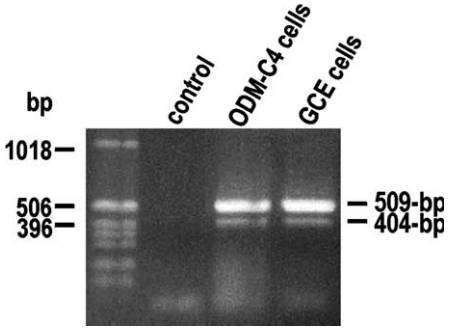

Fig. 3. Wild-type and spliced form of h17HSD7 in human eye ciliary epithelial cells. Lanes: 1, DNA ladder; 2, negative control; 3, human normal non-pigmented ciliary epithelial (NPE) cells (ODM-C4); and 4, chronic open glaucoma NPE cells (GCE).

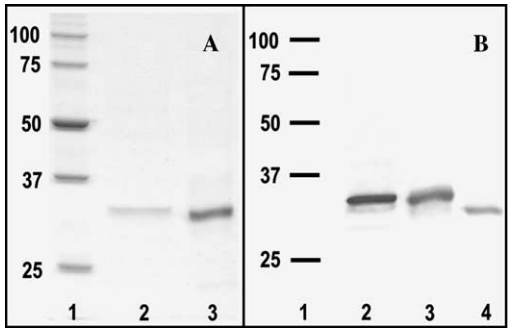

Fig. 4. 17HSD type 7 analyzed by SDS–PAGE (A) and Western blot (B). Lanes: 1, molecular weight marker; 2, purified recombinant mouse 17HSD type 7 (m17HSD7); 3, purified human 17HSD type 7 (h17HSD7); and 4, partially purified spliced form of human 17HSD type 7 (h17HSD7del). Sizes are shown in kDa. Proteins on SDS-gel detected by Coomassie blue, proteins with 6× His-tag on C-terminus detected on Western blot by Anti-HIS antibody.

As shown in Fig. 5, both human and mouse 17HSD type 7 enzymes catalyzed only reductive reactions. Human 17HSD type 7 (Fig. 5A) efficiently catalyzed the conversion of E1 to E2, and when using DHT as a substrate, the human enzyme almost exclusively catalyzed the formation of the estrogenic metabolite, 3βA-diol. h17HSD7 catalyzed the reaction using both estrogenic (E1) and androgenic (DHT) substrates equally effectively. In addition, according to our data, h17HSD7 was able to convert moderately P to 4-pregnen-3β-ol-20-one and 20-OH-P to 4-pregnen-3β,20α-diol and very weakly, DHT to 3αA-diol.

In contrast, mouse 17HSD type 7 (Fig. 5B) efficiently catalyzed only the conversion of E1 to E2. The mouse enzyme was able to convert DHT moderately to both 3α- and 3βA-diol, of which the former inactive metabolite was produced more efficiently than the latter estrogenic metabolite. No conversions were observed when P or 20-OH-P was used as a substrate for the mouse enzyme. The reactions catalyzed by human and mouse 17HSD type 7 reported here are summarized in Fig. 6.

Neither human nor mouse enzyme resulted in enzymatic activity when E2, T, A-dione, DHEA, cortisol, or aldosterone was used as a substrate (data not shown). Because of the pronounced expression of the h17HSD7 in adrenal gland, the gluco- and mineralocorticoids, cortisol and aldosterone, that are produced in the adrenal cortex were presumed to be substrates for the enzyme. The expression of h17HSD7 in both fetal and adult lung also supported these substrate candidates. However, these compounds were not metabolized by h17HSD type 7.

Our data indicate that both human and mouse 17HSD type 7 are able to catalyze the reduction of the keto group at either the 3- or the 17-position of the substrate, thus acting as both 17β- and 3βHSD. Dual activity (3β/17β) has also been observed with human

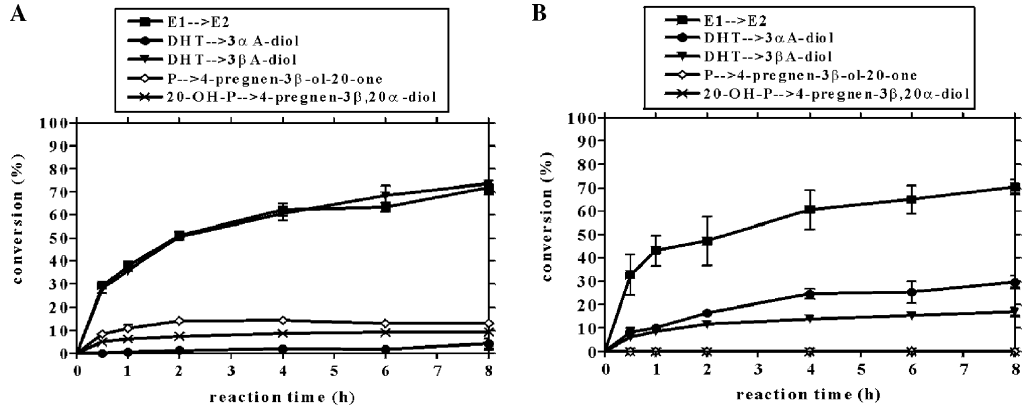

Fig. 5. Characterization of the enzymatic activity of purified recombinant human (A) and mouse (B) 17HSD type 7. The conversions after reaction times of 0.5, 1, 2, 4, 6, and 8 h in 37 °C were measured using estrone (E_1), dihydrotestosterone (DHT), progesterone (P), and 20-hydroxyprogesterone (20-OH-P) as substrates (0.5 μM). The reactions were repeated three times and the curves represent averages with standard deviations.

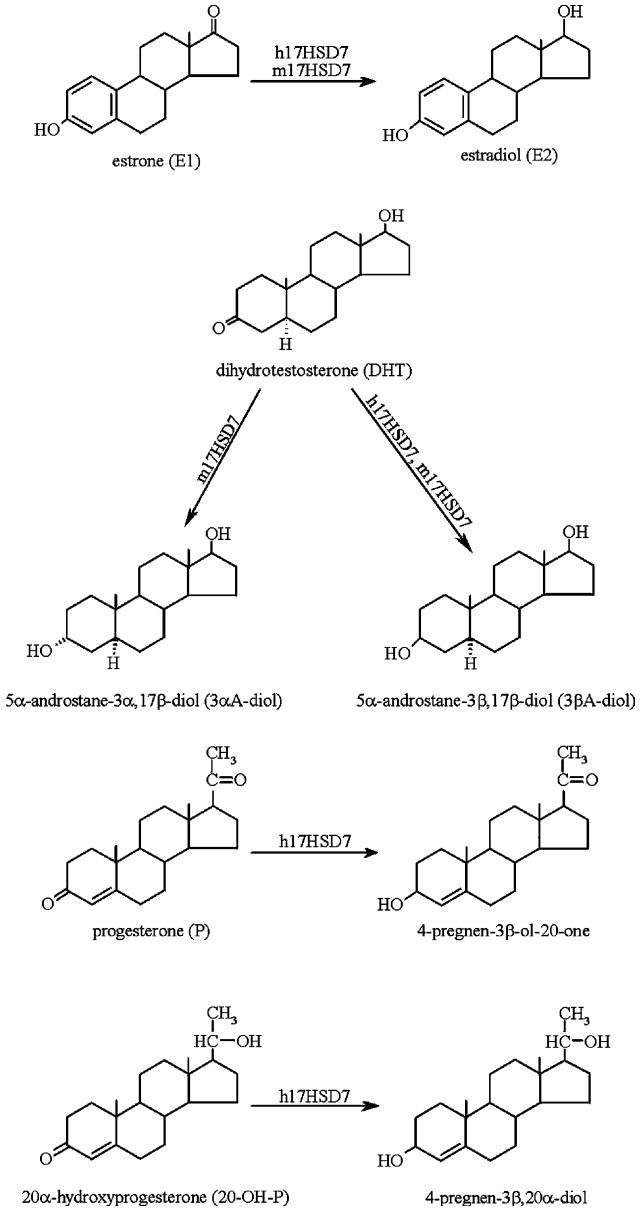

Fig. 6. Reactions in steroid metabolism catalyzed by 17HSD type 7.

17HSD type 2 enzyme [21]. Furthermore, 3αHSD type 2 from human prostate (17HSD type 5) has been reported to act as both 3α- and 17βHSD [22]. However, in these cases the catalytic efficiencies of the reactions in 3-position were distinctly lower than those in 17-position of the steroid.

The kinetic parameters (K_m and K_{cat}) were determined for E1 using both human and mouse 17HSD type 7 and for DHT using the human recombinant enzyme only (Table 2). The kinetic constants for P and 20-OH-P could not be determined as the conversions of these substrates were low. A comparison of the catalytic efficiencies (K_{cat}/K_m) clearly showed that human 17HSD type 7 catalyzes the reactions from E1 to E2 and from DHT to 3βA-diol equally. However, the catalytic efficiency for E1 is 11-fold with the mouse enzyme compared to the human enzyme. Further, the catalytic efficiency of human 17HSD type 7 for E1 is weaker about 10^6-fold compared to that of human 17HSD type 1, which catalyzes the same reaction from E1 to E2 [12] and weaker about 2×10^4-fold for DHT compared to that of human 17HSD type 2, which inactivates DHT to 5αA-dione [23]. However, it is notable that 3βA-diol, the metabolite of DHT by 17HSD7, is a ligand of estrogen receptor β (ERβ) and has been reported to act as an estrogen in prostate [24].

These data and the expression pattern of the enzyme described indicate that human 17HSD type 7 might be an intracrine regulator of steroid metabolism in sex steroid-sensitive peripheral tissues, activating estrogen (E1 → E2) and transforming the potent androgen to the estrogenic metabolite (DHT → 3βA-diol), thereby creating a more estrogenic milieu. A higher catalytic efficiency, such as that of h17HSD1 producing E2 in ovaries for endocrine secretion, might not be required for h17HSD7 for intracrine purposes. Progesterone is able to oppose the action of estrogen by down-regulating the estrogen receptor and acting as an antagonist for the action of estrogen receptor at the molecular level

[25]. Hence, it is logical that progesterone is inactivated (P → 4-pregnen-3β-ol-20-one) by h17HSD7.

We also produced the spliced form of human 17HSD type 7 described above, lacking exon 6 (h17HSD7del), as recombinant protein and partially purified it. The 34-kDa protein was analyzed by SDS–PAGE and detected by Western blot (Fig. 4). h17HSD7del was found to be inactive using both cell homogenate and partially purified enzyme with E1 and DHT as substrates. The purpose of the deleted form is unknown, as the enzyme was inactive with the most adequate known substrates of h17HSD7, E1, and DHT. The exon 6 is located at the putative transmembrane region of the h17HSD7 sequence. The spliced form may originate in chromosome 1q21 [20] or it may be a product of alternative splicing of the functional gene.

Conclusions

Human 17HSD type 7 was expressed widely in human tissues, including peripheral tissues and tissues not considered steroidogenic, and catalyzed the reactions between the active and inactive forms of sex steroid hormones and their metabolites. In conclusion, the present results suggest that the human enzyme might be one of the intracrine regulators of steroid hormone metabolism in peripheral tissues, aiming at the creation of an estrogenic milieu by activating estrogen, transforming a potent androgen into an estrogenic metabolite, and inactivating progesterone. Meanwhile, mouse 17HSD type 7 also activates estrogen but does not convert a potent androgen to an estrogenic metabolite as evidently as the human enzyme does and, further, does not metabolize progesterone. As mouse and human 17HSD type 7 act differently with the substrates, it would be useful to clarify the three-dimensional structure of the enzymes and particularly their active sites.

Acknowledgments

We thank Ms. Minna Eskelinen, Ms. Eeva Holopainen, Ms. Marja-Riitta Hurnasti, Ms. Mirja Mäkeläinen, Ms. Marja-Liisa Norrena, Ms. Pirkko Ruokojärvi, and Ms. Airi Vesala for their skillful technical assistance. This work was supported by the Research Council for Health of the Academy of Finland (Project Nos. 40990, 47630, and 51618), the Finnish Cancer Foundation, and the Sigrid Jusélius Foundation.

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