REVIEW

Unique multifunctional HSD17B4 gene product: 17 β -hydroxysteroid dehydrogenase 4 and D-3-hydroxyacyl-coenzyme A dehydrogenase/hydratase involved in Zellweger syndrome

Y de Launoit¹ and J Adamski²

¹Virology Unit, Faculty of Medicine, CP 614, Free University Brussels, 808 route de Lennik, 1070 Brussels, Belgium

²GSF National Research Center for Environment and Health, Institute of Mammalian Genetics, Genome Analysis Center, 85764 Neuherberg, Germany

(Requests for offprints should be addressed to Y de Launoit)

ABSTRACT

Six types of human 17 β -hydroxysteroid dehydrogenases catalyzing the conversion of estrogens and androgens at position C17 have been identified so far. The peroxisomal 17 β -hydroxysteroid dehydrogenase type 4 (17 β -HSD 4, gene name HSD17B4) catalyzes the oxidation of estradiol with high preference over the reduction of estrone. The highest levels of 17 β -HSD 4 mRNA transcription and specific activity are found in liver and kidney followed by ovary and testes. A 3 kb mRNA codes for an 80 kDa (737 amino acids) protein featuring domains which are not present in the other 17 β -HSDs. The N-terminal domain of 17 β -HSD 4 reveals only 25% amino acid similarity with the other types of 17 β -HSDs. The 80 kDa protein is N-terminally cleaved to a 32 kDa enzymatically active fragment. Both the 80 kDa and the N-terminal 32 kDa (amino acids 1–323) protein are

able to perform the dehydrogenase reaction not only with steroids at the C17 position but also with D-3-hydroxyacyl-coenzyme A (CoA). The enzyme is not active with L-stereoisomers. The central part of the 80 kDa protein (amino acids 324–596) catalyzes the 2-enoyl-acyl-CoA hydratase reaction with high efficiency. The C-terminal part of the 80 kDa protein (amino acids 597–737) facilitates the transfer of 7-dehydrocholesterol and phosphatidylcholine between membranes *in vitro*. The HSD17B4 gene is stimulated by progesterone, and ligands of PPAR α (peroxisomal proliferator activated receptor alpha) such as clofibrate, and is down-regulated by phorbol esters. Mutations in the HSD17B4 lead to a fatal form of Zellweger syndrome.

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INTRODUCTION

The genetics and biochemistry of steroid conversion have recently been developing very fast. There is a growing body of reports showing the involvement of steroid converting enzymes in several human disorders. In this review we will focus on the most unique protein among the 17 β -hydroxysteroid dehydrogenases, the type 4 enzyme. For the sake of clarity we will use the abbreviation 17 β -HSD 4, although, as will be discussed later, alternative

names are used frequently. The complexity of related enzymes was reviewed recently elsewhere (Reed 1991, Andersson 1995, Poutanen *et al.* 1995, Penning 1997).

MULTIPLE DEHYDROGENASES CONVERT STEROIDS AT POSITION C17

Biological potency of estrogens and androgens is regulated by conversions at position C17 by

TABLE 1. Human 17β-hydroxysteroid dehydrogenases

Enzyme	Tissue	Subcellular localization	Mass (kDa)	Best substrate	K _m (μM)	nmol/mg protein	Catalysis <i>in vivo</i>
17β-HSD 1 (Peltoketo <i>et al.</i> 1988)	Placenta	Soluble	34	Estrone	8.6	0.02	Reduction
17β-HSD 2 (Wu <i>et al.</i> 1993)	Ovary			Estradiol	5.9		
17β-HSD 3 (Geissler <i>et al.</i> 1994)	Placenta	Microsomal	43	Testosterone	0.4	0.22	Oxidation
17β-HSD 4 (Adamski <i>et al.</i> 1995)	Liver			Estradiol	0.2		
17β-HSD 5 (Lin <i>et al.</i> 1997; Zhang <i>et al.</i> 1995)	Testis	Microsomal	35	Androstenedione	0.5	0.09	Reduction
				Estrone	0.5		
	Liver	Peroxisomal	80	Estradiol	0.2	0.14	Oxidation
	Kidney			Δ5-Androstenediol	0.4		
	Liver	Microsomal	34	5α-Dihydrotestosterone	19.0	2.45	Reduction
	Testis			3α-Androstanediol	17.1	4.83	Oxidation

17β-hydroxysteroid dehydrogenases (17β-HSDs). Several enzymes with close substrate specificity participate in that process. The identification and characterization of individual human 17β-HSDs was limited by the minute amounts of tissue available for purification. However, analyses performed with homogenates or with subcellular fractions allowed the kinetical differentiation of several enzymes such as the soluble 17β-hydroxysteroid oxidoreductase of placenta or the structure-associated 17β-estradiol dehydrogenase of uterus epithelium (Engel & Groman 1974, Tseng & Gursipide 1974, Tseng & Mazella 1981). Before molecular biology techniques became widespread, the readily available human placenta allowed the purification of the first human 34 kDa 17β-hydroxysteroid dehydrogenase (17β-HSD 1), which became a model for studies of steroid converting enzymes. After cloning and elucidation of the gene structure (Peltoketo *et al.* 1988, Luu-The *et al.* 1989), it represents the best characterized human steroid dehydrogenase. *In vivo* this enzyme participates in the synthesis of steroids via a reductive pathway. However, detailed kinetical studies (Blomquist *et al.* 1985) have shown that the placenta expresses additional HSDs and one of them, namely 17β-HSD 2, was cloned (Wu *et al.* 1993) (Table 1). This enzyme is a 43 kDa microsomal dehydrogenase revealing a twofold higher rate of oxidation than reduction for both estrogens and androgens. A further enzyme, 17β-HSD 3, is most abundant in testes and represents a transmembrane microsomal 35 kDa protein with a strong preference for the reduction of androgens (Geissler *et al.* 1994). Mouse and human 17β-HSD type 5 has not yet been fully characterized (Deyashiki *et al.* 1995, Zhang *et al.* 1995). However, this enzyme has wide tissue distribution and has also been identified as 3α-HSD type 2 (Lin *et al.* 1997) (for a review see Penning

et al. 1997). Later, we will discuss the issue of multifunctionality in more detail.

The oxidative 17β-HSD activity found in human uterus endometrium could not be unequivocally ascribed to the known enzymes (Tseng & Mazella 1981). Attempts to isolate the endometrial 17β-HSD from the particulate fraction of homogenates (Pollow *et al.* 1976) resulted in a 40-fold enrichment. However, because of difficulties in collecting and the paucity of starting material, the enriched fractions were not applied to amino acid sequencing and antibody production. Entenmann *et al.* (1980) discovered oxidative activity for 17β-estradiol in porcine endometrium. This microsomal activity revealed comparable kinetical parameters (NAD⁺-dependency, K_m less than 1 μM). The parameters suggested a role in the inactivation of hormones.

PURIFICATION OF PORCINE 17β-ESTRADIOL DEHYDROGENASE 4

To accomplish identification of a novel 17β-HSD we have used a pig (*Sus scrofa*) model. The epithelial layer of the porcine uterus could easily be collected at a preparative scale by curettage (Sierralta *et al.* 1978). The 17β-estradiol dehydrogenase activity had to be solubilized from the particulate fraction (Adamski *et al.* 1992a). Extracts were applied to DEAE-Sepharose, depleted of free detergent on Amberlite XAD-2 and further purified by affinity chromatography on blue Sepharose (Adamski *et al.* 1992b). Further purification on butyl Sepharose resulted in two products rich in 17β-estradiol dehydrogenase activity: a major moderately hydrophobic fraction (EDH) and a minor very hydrophobic fraction (VHF). They were processed in parallel by gel filtration and ion-exchange chromatography on Mono S. The EDH fraction was purified to homogeneity and revealed a

TABLE 2. Kinetic parameters of purified porcine 17 β -estradiol dehydrogenase 4

Parameter	$E_2 \rightarrow E_1$	$E_2 \leftarrow E_1$
Optimal pH	7.8	6.6
K_m for steroid	0.22 μ M	1.10 mM
Best cofactor	NAD ⁺	NADPH
K_m for cofactor	44 mM	21 mM

E_2 , 17 β -estradiol; E_1 , estrone.

single band at 32 kDa in the denaturing SDS-PAGE. The VHF was a mixture of proteins of 32, 45 and 80 kDa (Adamski *et al.* 1992b).

CHARACTERIZATION OF PURIFIED PORCINE 17 β -HSD 4

Both purification products, the EDH and the VHF, show the same K_m for steroids and cofactors as those measured with two-substrate kinetics in the particulate fraction of homogenates of porcine uterus epithelium (Table 2). They reveal an ordered mechanism of reaction with the cofactor binding first (Adamski *et al.* 1992b, Marks 1992). EDH and VHF also share the same substrate specificity, which is highest for 17 β -estradiol and, unexpectedly, comparably high for 5-androstene-3 β ,17 β -diol ($K_m=0.2 \mu$ M). Other androgens or progestagens are not converted.

The molecular mass was estimated in denaturing SDS-PAGE and under native conditions by gel filtration and density gradient centrifugation (Adamski *et al.* 1992b). The 32 kDa protein has a capability of forming dimers with an apparent molecular weight of 75 kDa (Carstensen *et al.* 1996). The interactions in the VHF are more complex. Both gel filtration analyses and density gradient centrifugation revealed the presence of a heterogeneous complex with at least two molecular mass forms of 170 kDa and 240 kDa.

CLONING OF THE PORCINE, HUMAN AND MOUSE 17 β -ESTRADIOL DEHYDROGENASE TYPE 4

With degenerated PCR-primers, designed according to partial amino acid (aa) sequence of the 32 kDa protein, a fragment of 405 base pairs (bp) was amplified from porcine endometrium cDNA. It had a single open reading frame coding for an amino acid sequence which was identical to the 32 kDa protein as confirmed by Edman degradation. Using

this fragment as a probe, a 3 kb cDNA was isolated from a porcine λ ZAP kidney cDNA library. The sequence was later confirmed in porcine uterus (Leenders *et al.* 1994a). The cDNA coded for a protein of 80 kDa consisting of 737 aa and was not similar to any known steroid dehydrogenases. About 70% of its amino acid sequence was already known from peptides of the 32 and 80 kDa proteins. Screening of human and mouse cDNA λ gt11 libraries of liver was performed with porcine enzyme cDNA. Novel 3 kb cDNAs were identified which coded for proteins of 735 and 736 aa representing the human and mouse counterparts respectively of the porcine enzyme (Adamski *et al.* 1995, Normand *et al.* 1995).

PARALLEL WAYS OF HSD17B4 IDENTIFICATION

The product of the HSD17B4 gene, an 80 kDa protein, was detected almost in parallel by other groups. During studies on peroxisomal β -oxidation of pristanic acid and bile acid intermediates in rat and man, 80 kDa D-specific hydroxyacyl-coenzyme A (CoA) dehydrogenase/hydratase (also called multifunctional protein 2-MFP2) were purified (Novikov *et al.* 1994, Qin *et al.* 1997b), characterized and cloned (Dieuaide-Noubhani *et al.* 1996a, 1997a,b, Jiang *et al.* 1996, 1997, Novikov *et al.* 1997). The amino acid sequence of the human enzyme was identical to that of 17 β -HSD 4 (Adamski *et al.* 1995).

Guinea pig enzyme was cloned from a cDNA library while using rat 17 β -HSD 4 as a probe (Caira *et al.* 1998).

Another study pursued the identification of cDNAs up-regulated in rat by peroxisomal proliferators such as WY14,643 (Corton *et al.* 1996, 1997). One of the affected proteins turned out to be the rat ortholog of previously known porcine, mouse and human 17 β -HSD 4.

Unusual was the way that chicken protein was identified (Kobayashi *et al.* 1997). A monoclonal antibody 3b5 was prepared against isolated retinal pigment epithelium cells. The antibody recognized a 75 kDa protein on western blots of retinal pigment epithelium. This antibody was then used to screen a lambda expression library. This approach resulted in the identification of a full length cDNA clone coding for the chicken 17 β -HSD 4.

Amino acid identities between 17 β -HSD 4 from different species are very high, around 80% (Fig. 1). Several hydroxysteroid dehydrogenases could be traced to a common ancestor close to 3-ketoacyl-acyl carrier protein reductase of *Eschericia coli*

FIGURE 1. Alignment of amino acid sequences of 17β-HSD 4 from different species. Sequences were ordered to obtain maximum similarity with ClustalX software. Identical amino acids are grey shaded, similar amino acids are boxed. Accession numbers for protein sequences are: mouse, P51660; chicken, U77911; rat, U37486; human, P51659; porcine, X78201; guinea pig, Y13623.

(Baker 1996). Phylogenetic studies suggest a common ancestor for the 17β-HSD 1 and 2, whereas the 17β-HSD 3 and 4 are examples of convergence (Baker 1994, 1996). In particular 17β-HSD 4 appears to be a very ancient protein whose functionality and amino acid sequence are strongly conserved. Similarities to proteins of primitive organisms will be discussed in the gene structure section.

NAMES USED

Due to multifunctionality of the encoded protein, the HSD17B4 gene product was discovered by many groups using different strategies. Because of that there are several names being used. Table 3 gives a summary of them. The references given should be understood as a source for the representative description. Quite often different synonyms are used in the same reports, depending on the functionality analyzed.

GENE STRUCTURE

The chromosomal assignment and structure of the human HSD17B4 gene were determined recently (Leenders *et al.* 1996a, Novikov *et al.* 1997, Leenders *et al.* 1999). The HSD17B4 gene localizes to chromosome 5q2 and was found to be more than 100 kbp in length (Fig. 2). The size of the exons ranges from 21 bp (exon 5) to 286 bp (exon 13). The smallest intron with 100 bp was found between exons 5 and 6.

The N-terminal domain of 17β-HSD 4 reveals functional similarity and amino acid homology (54%) to the family of short chain alcohol dehydrogenases (SCAD) (Persson *et al.* 1991, Leenders *et al.* 1994b, Jörnvald *et al.* 1995) and the central domain to several fatty acid hydratases (HDE), especially to that of *Candida tropicalis* (40%) (Baker 1996). However, neither sizes of the corresponding genes nor the exon/intron structures are analogous. As discussed later, the 17β-HSD 4 is an exception in the group of 17β-HSDs, since it

TABLE 3. Names used for the *HSD17B4* gene product

Name	Reference
17 β -hydroxysteroid dehydrogenase type 4 or 17 β -HSD 4	Leenders <i>et al.</i> (1994)
Multifunctional protein 2 or MFP-2	Dieuaide-Noubhani <i>et al.</i> (1996)
Peroxisomal multifunctional enzyme 2 or perMFE2	Qin <i>et al.</i> (1997)
D-specific multifunctional protein 2	Caira <i>et al.</i> (1998)
D-3-hydroxyacyl-CoA dehydrogenase	Dieuaide-Noubhani <i>et al.</i> (1997); Novikov <i>et al.</i> (1994)
2- <i>trans</i> -enoyl-CoA hydratase	Dieuaide-Noubhani <i>et al.</i> (1997)
D-bifunctional protein	Jiang <i>et al.</i> (1997)
D-3-hydroxyacyl-CoA dehydratase/ D-3-hydroxyacyl dehydrogenase bifunctional protein	Jiang <i>et al.</i> (1996)

FIGURE 2. Structure of 17 β -HSD 4 protein and HSD17B4 gene. A schematic structure of the human 17 β -HSD IV protein is given above that of the gene. Arrows point to the position of the G16S mutation, cleavage site and C-terminal peroxisomal targeting signal. The black boxes representing exons are drawn to scale according to the indicated 10 kb marker. The sizes of introns are given above: introns smaller than 7 kb are drawn to scale, larger introns are represented by broken lines. The numbers of the exons are below the boxes. Exons 1 and 24 consist of translated (black box) and untranslated (grey box) regions. Accession numbers for the nucleotide sequence are AF057720-AF057740.

consists of 3 domains. Only the N-terminal domain of 320 amino acids participates in steroid metabolism and is coded by 12 exons spanning about 40 kbp. In comparison to other 17 β -hydroxysteroid dehydrogenases, the SCAD domain of the HSD17B4 gene is much bigger than the 17 β -HSD 1 (6 exons on 3.3 kbp) (Luu-The *et al.* 1990), close to the 17 β -HSD 2 (7 exons on 40 kbp) (Labrie *et al.* 1995) and smaller than the 17 β -HSD 3 (11 exons on 60 kbp) (Geissler *et al.* 1994).

The amino acid sequence C-terminal domain of 17 β -HSD 4 reveals 40% identity and the same

functionality as the C-terminal domain (sterol carrier protein (SCP) 2) of the SCPX protein (Ohba *et al.* 1994, Leenders *et al.* 1996b). Interestingly, the gene structure of the last 3 exons is also similar. This observation supports the hypothesis that the HSD17B4 gene is the result of a gene fusion.

PROCESSING OF 80 kDa PROTEIN

Some peroxisomal proteins (SCP2, 3-ketoacyl-CoA thiolase) are cleaved from larger precursors in the

TABLE 4. Distribution of 17β-estradiol dehydrogenase activity in porcine tissues

Tissue	E ₂ →E ₁ (μU/mg)	E ₂ ←E ₁ (μU/mg)	Immunocytochemistry
Liver	687.5	5.6	Hepatocytes
Kidney	336.4	0.7	Epithelium of proximal tubuli
Ovary	293.9	1.5	Granulosa cells
Lung	185.4	0.8	Bronchial epithelium
Testes	69.2	1.8	Leydig cells
Uterus	30.1	1.2	Luminal and glandular epithelium
Skeletal muscle	10.9	ND	Myocytes
Prostate	0.8	0.1	Epithelium
Blood erythrocytes	ND	ND	ND

E₂, 17β-estradiol; E₁, estrone; ND, not detected.

course of translocation (Swinkels *et al.* 1991). A protease present in peroxisomes has been proposed to recognize the sequence Ala-[AlaVal]-Pro (Mori *et al.* 1991). The 80 kDa full length HSD17B4 gene product is N-terminally cleaved, probably after the sequence Ala³²⁰-Ala-Pro-Ser³²⁴, to a 32 kDa fragment representing a SCAD domain (Leenders *et al.* 1994b). Such processing has also been observed in mice and rats (Novikov *et al.* 1994, Normand *et al.* 1995, Dieuaide-Noubhani *et al.* 1997b) although the protease recognition motif was not conserved. The meaning of this cleavage for 17β-HSD 4 is not known. Interestingly, the extent of processing of 80 kDa into 32 kDa varies among porcine tissues (Adamski *et al.* 1997). Western blot analyses of porcine organs revealed that target tissues (uterus and mammary glands) show high processing. In these tissues the 32 kDa form dominates. On the other hand, non-target tissues participating in β-oxidation of fatty acids (such as liver or kidney) showed low processing. Nevertheless, in either tissue both proteins are present. The differential processing raised the question whether the release of the 32 kDa fragment from the 80 kDa protein is an activation step for 17β-hydroxysteroid dehydrogenase. However, both 80 and 32 kDa proteins had comparable kinetical parameters after transient expression in HEK 293 cells or purification of recombinant proteins from *E. coli* (Leenders *et al.* 1996b, Adamski *et al.* 1997). Also the preferred reaction direction, i.e. oxidation, remained unchanged for purified, recombinant and transiently expressed proteins.

TISSUE DISTRIBUTION

In the porcine tissues studied, the oxidation of 17β-estradiol predominates over the reduction.

Highest activities are found in liver and kidney followed by uterus, lung, ovary and testes (Table 4).

Immunohistochemical analysis of tissues showing low 17β-HSD 4 mRNA expression, such as brain, lung and uterus, reveals that the enzyme is present in specific cells within these organs. In the rat and mouse cerebellum 17β-HSD 4 is confined to Purkinje cells, and the expression is also seen in the anterior pituitary (Normand *et al.* 1995). There is high expression of 17β-HSD 4 in chick retinal pigment epithelium but not in other parts of the eye (Kobayashi *et al.* 1997). In the lung the bronchial epithelium expresses high levels of 17β-HSD 4 (Möller *et al.* 1999) and in the uterus the protein is present in luminal and glandular epithelium and not in stromal cells (Husen *et al.* 1994).

A slightly different expression pattern was seen in human tissues. The highest mRNA level of human 17β-HSD 4 was observed in liver, followed by heart, prostate and testis. Moderate expression occurred in lung, skeletal muscle, kidney, pancreas, thymus, ovary, intestine and term placenta. Weak signals were observed in brain, spleen, colon and lymphocytes. In all cases only a single band at 3 kb was detectable (Adamski *et al.* 1995). The wide distribution of 17β-HSD 4 may in part explain oxidative activities measured in human tissues (Martel *et al.* 1992). The expression of 17β-HSD 4 is in contrast with that of 17β-HSD 1 and 2 which are predominantly seen in the placenta.

Several human cancer cell lines also express 17β-HSD 4. The estrogen receptor positive mammary cell line, T47D, expresses more 17β-HSD 4 mRNA transcript than BT-20, MDA-MB-453 and MDA-MB-231 cell lines which are estrogen receptor negative. The megakaryotic cell line, DAMI, reveals a very high level of 17β-HSD 4 mRNA while less is present in hepatocellular carcinoma HEP-G2 and early embryonic Tera-1

cell lines (Adamski *et al.* 1995). The prostate cancer cell lines DU145, LNCaP and PC3 express 17 β -HSD 4 but not 17 β -HSD 1 or 3 (Carruba *et al.* 1997, Castagnetta *et al.* 1997). Interestingly, 17 β -HSD 2, which appears to be the principal isozyme expressed in the prostate, is present only in PC3 cells (Delos *et al.* 1995, 1998, Elo *et al.* 1996, Castagnetta *et al.* 1997). Most probably, 17 β -HSD 2 is the main protective dehydrogenase in the prostate.

REGULATION OF HSD17B4

Porcine 17 β -hydroxysteroid dehydrogenase 4 is the first peroxisomal enzyme known to be stimulated by progesterone as checked by mRNA expression and immunohistochemistry (Kaufmann *et al.* 1995). In the early 1970s the hormone was observed to increase estradiol oxidation in human tissues (Tseng & Gurpide 1975, 1979). Later it was shown to up-regulate 17 β -HSD 1 in human breast cancer cells (Poutanen *et al.* 1990) and to increase mRNA expression of 17 β -HSD 2 in human endometrium (Casey *et al.* 1994).

Although 17 β -HSD 1 is supposed to participate in the synthesis and 17 β -HSD 4 in the inactivation of steroids there is a simultaneous expression of both enzymes in gonads (Luu-The *et al.* 1990, Carstensen *et al.* 1996). However, the corresponding regulatory pathways of protein kinase C are not controlled in the same way. In vitamin D-differentiated human leukemia THP 1 cells, 17 β -HSD 4 mRNA was stimulated twofold by dexamethasone but it was completely down-regulated by phorbol esters (Jakob *et al.* 1995, 1997). This is in contrast to the dose- and time-dependent increase in gene transcripts of 17 β -HSD 1 under similar treatment (Tremblay & Beaudoin 1993).

The recently purified and cloned rat 80 kDa homolog of human 17 β -HSD 4 is up-regulated by peroxisomal proliferators such as clofibrate and WY 14,643 (Novikov *et al.* 1994, Corton *et al.* 1995). In the PPAR α (peroxisomal-proliferator activated receptor α) knock-out mice the expression of 17 β -HSD 4 is low and does not change after treatment with WY 14,643 (Aoyama *et al.* 1998). The 80 kDa protein seems to be controlled by modulators of both steroid and fatty acid metabolism. Another example of PPAR α -mediated regulation is the activation of steroid metabolizing enzyme, NADP⁺-dependent 3 α -HSD in human liver by derivatives of clofibrate (Matsuura *et al.* 1996). In contrast, the guinea pig ortholog is so far the only species in which the same treatment causes

a down-regulation (instead of up-regulation) of the expression of 17 β -HSD 4 (Caira *et al.* 1998).

SUBCELLULAR DISTRIBUTION OF PORCINE 17 β -HSD 4

Immunocytochemical and immunofluorescence studies in porcine uterus restricted porcine 17 β -HSD 4 to luminal and glandular epithelium (Husen *et al.* 1994) similar to analyses of human endometrium (Scublinsky *et al.* 1976, Mäentausta *et al.* 1991). However, the staining in the cytoplasm was not diffuse but showed a punctuate appearance. The intensity of the monoclonal antibody F1-peroxidase staining followed the changes in porcine 17 β -hydroxysteroid dehydrogenase activity. It was raised fourfold after day 5 of the ovarian cycle and rapidly decreased after day 17 in a manner similar to the levels of progesterone. On day 4 faint spots of fluorescence appeared in the cytoplasm of the glandular epithelium. The spots accumulated at the cell bases between days 11 and 17 (luteal phase) and disappeared within one day. The pattern of immunofluorescence staining suggested that porcine 17 β -HSD 4 is localized in vesicles. The latter have been isolated from porcine uterus epithelium homogenates by sequential density gradients of isopycnic 30% Percoll and linear 0.3–2 M sucrose in vertical rotors (Adamski *et al.* 1987, Adamski 1991, Adamski *et al.* 1993). The vesicles harboring the 17 β -HSD activity equilibrated at a density of 1.18 g/ml, were 120–200 nm in diameter, revealed a moderate electron-dense matrix bounded by a single membrane and were morphologically and enzymatically distinct from mitochondria, lysosomes, fragments of plasma membrane, endoplasmic reticulum and the Golgi apparatus. In immunogold electron microscopy the labeling with monoclonal antibody F1 (recognizing the 32 kDa and the 80 kDa protein) and W1 (reacting with 32 kDa only) confirmed that all forms of the enzyme are present in the same vesicles, both in tissue and in the isolated fraction (Adamski *et al.* 1993).

Several clues pointed to the identity of the 17 β -HSD 4 containing vesicles as peroxisomes: (1) the morphology and density is similar to that of peroxisomes, (2) the 80 kDa primary transcript features the peroxisomal targeting signal Ala-Lys-Ile and a putative recognition sequence (Ala-Ala-Pro) for a protease processing peroxisomal protein (Mori *et al.* 1991) and (3) the 80 kDa protein is similar to enzymes participating in peroxisomal β -oxidation of fatty acids. Indeed, typical peroxisomal markers such as catalase and acyl-CoA oxidase co-localized with porcine 17 β -HSD 4 in

immunogold labeling studies in uterus, kidney and liver (Markus *et al.* 1995a,b).

In other species, such as the rat, 17β-HSD 4 was indeed purified from isolated peroxisomes (Novikov *et al.* 1994). However, at that time the amino acid sequence of the rat enzyme was not known.

Peroxisomal localization of 17β-HSD 4 extends our understanding on how steroid, sterol and bile acid metabolism are interlinked. Different sub-cellular distribution of various 17β-HSDs allows for local control of their activities. Peroxisomes were initially believed to play only a minor role in mammalian metabolism. However, they play an indispensable role in many metabolic pathways, like synthesis of cholesterol, β-oxidation of fatty acids, biosynthesis of ether lipids and bile acids, α-oxidation of phytanic acid and, as depicted in this review, oxidation of steroids (Wanders *et al.* 1995, Krisans 1996, Magalhaes & Magalhaes 1997, Verhoeven *et al.* 1997, Seedorf *et al.* 1998).

MULTIFUNCTIONALITY OF 17β-HSD 4

In order to check for the presence of activities predicted by amino acid similarities of the 80 kDa protein (Fig. 2) its three domains were expressed separately (Leenders *et al.* 1996b). The N-terminal domain (aa 1–323) catalyzed both the 17β-hydroxysteroid and the 3-hydroxyacyl-CoA dehydrogenase reactions (Table 5). Kinetic parameters (K_m , V_{max}) of the expressed full length 80 kDa protein were close to those observed for the single expressed domains or for the native purified enzyme (EDH or VHF) (Leenders *et al.* 1996b).

This was the first observation of an enzyme performing dehydrogenase activity not only with steroids but also with 3-hydroxyacyl-CoA derivatives of fatty acids. Furthermore, the central domain (aa 324–596) was responsible for the 2-enoyl-acyl-CoA hydratase activity. Essentially the same data concerning conversion of 3-hydroxyacyl-CoAs and 2-enoyl-acyl-CoAs were obtained simultaneously with expressed or purified proteins of the rat (Novikov *et al.* 1994, Qin *et al.* 1997a,b). Estimated K_m values for the hydratase and fatty acid dehydrogenase are similar to those of other enzymes of β-oxidation of fatty acids (Steinman & Hill 1975, Palosaari & Hiltunen 1990). Both K_m for steroids and fatty acids are in the physiological range.

Further studies on peroxisomal β-oxidation of fatty acids and precursors of bile acids demonstrated that the 17β-HSD 4 specifically forms and dehydrogenates D-3-hydroxyacyl-CoAs and not their L-stereoisomers (Dieuaide-Noubhani *et al.* 1997a, Jiang *et al.* 1997). This differentiates the

TABLE 5. Kinetic parameters of domains of porcine 17β-HSD 4

Sample	17β-Hydroxysteroid dehydrogenase		Fatty acid-CoA hydratase		Fatty acid-CoA dehydrogenase	
	K_m (μM)	V_{max} (nmol × l ⁻¹ × min ⁻¹ × mg prot. ⁻¹)	K_m (μM)	V_{max} (μmol × l ⁻¹ × min ⁻¹ × mg prot. ⁻¹)	K_m (μM)	V_{max} (μmol × l ⁻¹ × min ⁻¹ × mg prot. ⁻¹)
Purified 32 kDa	0.2	0.15	ND	ND	ND	ND
Recombinant 32 kDa	0.3	0.14	ND	ND	ND	ND
VHF	0.3	0.19	34.0	4.44	34.8	3.31
Recombinant 80 kDa	0.4	0.11	37.1	3.92	35.3	2.91
Recombinant central domain	ND	ND	34.7	1.36	21.9	1.25

Kinetic parameters of 17β-HSD 4 were assayed with 17β-estradiol, fatty acid-CoA hydratase with crotonyl-CoA and fatty acid-CoA dehydrogenase with acetoacetyl-CoA (Leenders *et al.* 1996). Recombinant 32 kDa and 80 kDa proteins were assayed in homogenates of transfected cells and were corrected for background conversion (Carstensen *et al.* 1996). The recombinant central domain was characterized after purification on glutathione-agarose and thrombin cleavage. ND, activity not detected.

enzyme from the multifunctional enzyme 1 (MFP1) (Osumi *et al.* 1985b) which is L-specific.

The velocity of 17 β -estradiol oxidation by porcine, mouse and human 17 β -HSD 4 is several fold lower than that of fatty acyl-CoA (Leenders *et al.* 1994a, 1996b, Adamski *et al.* 1995, 1997, Normand *et al.* 1995). The same observation was made in rat (Dieuaide-Noubhani *et al.* 1996b, Qin *et al.* 1997b). However, all other known 17 β -HSDs have the same conversion rates for steroids within the range 0.1 to 2.45 nmol/min/mg protein (Table 1). Because the enzymatic parameters ($V_{\max}$, K_m) of the 17 β -HSD 4 for both fatty acyl-CoA and steroids are close to those known for other enzymes of the SCAD gene family it remains to be settled which substrates are physiological.

In comparison to other 17 β -HSDs the type 4 enzyme accepts the most structurally diverse substrates. Human 17 β -HSD 1–3 and mouse and rat 17 β -HSD 7 (Nokelainen *et al.* 1998) are practically only active with steroids at position 17. However, 17 β -HSD 5 and the recently cloned rat 17 β -HSD 6 (Biswas & Russell 1997) must be considered multifunctional because of their 3 α -hydroxysteroid dehydrogenase activity. Actually, 17 β -HSD 5 was first identified in a row of different 3 α -HSDs and assigned type 2 among them (for reviews see Lin *et al.* 1997, Penning 1997). One 3 α -HSD was even first identified as a bile acid binding protein (Nanjo *et al.* 1995).

Interesting is the novel 17 β -hydroxysteroid dehydrogenase previously known as Ke6 protein in mouse and human (Ando *et al.* 1996, Formicheva *et al.* 1998). This enzyme which might be termed 17 β -HSD 8 has a $V_{\max}$ of 0.27 nmol/min/mg protein with 17 β -estradiol/ NAD^+ and reveals the highest (37%) amino acid sequence identity among the 17 β -HSDs to 17 β -HSD 4. It remains to be verified if 17 β -HSD 8 is able to metabolize bile acids or fatty acids.

ROLE OF SCP2 DOMAIN

The most C-terminal part of the 80 kDa protein (amino acids 597–737) has 39% similarity to rat SCP2 (Leenders *et al.* 1994b). This non-specific lipid transfer protein is highly conserved, even in evolutionarily distant species such as chicken and human (Yamamoto *et al.* 1991, Pfeifer *et al.* 1993). This product of an SCPX gene, which actually encodes two proteins, SCP2 and SCPX, is a fusion between SCP2 and thiolase (Ohba *et al.* 1994, Seedorf *et al.* 1994). SCP2 is a 13 kDa basic protein believed to participate in the intracellular movement of cholesterol and lipids (Wirtz 1997).

The role of SCP2 in steroidogenesis and arteriosclerosis has been extensively studied (Seedorf *et al.* 1993, Krisans 1996, Magalhaes & Magalhaes 1997, Wanders *et al.* 1997). A synopsis of this functionality is beyond the scope and limits of this review.

The role of the SCP2 domain for the functionality of 17 β -HSD 4 is not clear. The expressed porcine SCP2 domain facilitates the transfer of 7-dehydrocholesterol and phosphatidylcholine between membranes *in vitro*. The activities of the N-terminal domain towards steroids or fatty acyl-CoA are not changed if the SCP2 domain is deleted (Leenders *et al.* 1996b).

Recently, gene targeting in mice was used to study the unknown function of SCP2 (Seedorf *et al.* 1998). In the *Scp2*(-/-) mice with complete deficiency of SCP2 and SCPX, marked alterations in gene expression, peroxisome proliferation, hypolipidemia, impaired body weight control, and neuropathy were observed. Knock-out mice showed impaired catabolism of methyl-branched fatty acyl-CoAs, especially of the tetramethyl-branched fatty acid, phytanic acid. The gene disruption led to inefficient import of phytanoyl-CoA into peroxisomes and to defective thiolytic cleavage of 3-ketopristanoyl-CoA.

MUTATIONS IN THE HSD17B4 GENE

Recent research on peroxisomal disorders revealed that 17 β -HSD 4 is deficient in Zellweger syndrome (Novikov *et al.* 1997, Suzuki *et al.* 1997, van Grunsven *et al.* 1998). Peroxisomal organelle deficiency results in disorders of lipid, fatty acid and sterol metabolism such as Zellweger syndrome, adrenoleukodystrophy, infantile refsum disease and hyperpipecolic acidemia (Lazarow & Moser 1995, Wanders *et al.* 1995). Patients with peroxisomal deficiency reveal high plasma concentrations of long chain fatty acids, bile acids and deficient synthesis of plasmalogens. This exerts pleiotropic influence of renal functions impairment and neuronal development (neuronal migration defects and degeneration).

One example of the molecular basis of the recently identified 17 β -HSD 4 deficiency is the mutation G16S (van Grunsven *et al.* 1998). This mutation is localized in the first exon (Fig. 2) and disturbs the conformation of the Rossmann fold required for cofactor (NAD^+) binding. The mutant is inactive with both steroids and bile acids (van Grunsven *et al.* 1998, Möller *et al.* 1999). The mutation is lethal, most probably because the D-specific pathways of pristanic acid and

di/tri-hydroxycholestanoic acid metabolism are disrupted. As mentioned above, the multifunctional protein 1 (Osumi *et al.* 1985a) is L-specific and cannot substitute the deficient D-pathway (Dieuaide-Noubhani *et al.* 1997a). Because 17 β -HSD 4 has a ubiquitous distribution, any mutation would affect the whole organism. In addition, developmental studies have shown that this enzyme is present as early as at least day 7 post coitus of embryonic development (Mustonen *et al.* 1997). The lack of observation of any steroid hormone related phenotype might be due to compensation by other 17 β -hydroxysteroid dehydrogenases.

PHYSIOLOGICAL SIGNIFICANCE IN STEROID METABOLISM

Table 1 compares human 17 β -HSD 1–5 and depicts differences in catalytic parameters, posttranslational processing and subcellular localization. The catalytic property of 17 β -HSD 4, revealing the virtually unidirectional oxidative activity, clearly defines it as a steroid inactivating enzyme (Gurpide & Marks 1981), since it produces estrone which shows little affinity to the estradiol receptor. The conversion of 17 β -estradiol to estrone might be complemented by hydroxylations in positions 6 α or 7 α (Maschler *et al.* 1983) producing steroids devoid of estradiol receptor affinity and permitting fast release from cells after formation. The $V_{\max}$ and K_m values for EDH are similar to those for estrone hydroxylases (Adamski *et al.* 1994) and allows the metabolic conversion 17 β -estradiol $\rightarrow$ estrone $\rightarrow$ 6 α /7 α -hydroxy-estrone without rate-limiting steps.

Our discovery of 17 β -HSD 4 in peroxisomes stimulated discussions about the possible role of peroxisomes in steroid metabolism (Markus *et al.* 1995b). The enzyme has an as yet unseen ability to be stimulated by both (1) progestins (Kaufmann *et al.* 1995), which is a common feature of other 17 β -HSDs (Tseng & Gurpide 1975, Poutanen *et al.* 1990), and (2) peroxisomal activators such as WY14,643 (Corton *et al.* 1996, 1997) or clofibrate (M Markus & J Adamski, unpublished results). The stimulation produces a tissue-specific response: progestins induce about a tenfold increase in 17 β -HSD 4 mRNA and protein synthesis in the uterus epithelium but not in liver whereas clofibrate treatment has no effects on uterus but results in a threefold induction in the liver. It is not known whether the differences are caused by local concentrations of receptors, modulation of receptor action by spatially restricted factors or alternated promoter usage similar to that observed for the estrogen receptor in osteoblasts and breast tissue

(Grandien *et al.* 1995). 17 β -HSD 4 inactivates the conversion of Δ^5 -androstene-3 β , 17 β -diol to dehydroepiandrosterone (DHEA), a known peroxisomal proliferator (Prough *et al.* 1994). Although both DHEA and clofibrate induce peroxisomes they have opposite effects on the concentrations of triglycerides and cholesterol in blood. DHEA increases the levels of lipids while clofibrate acts as a hyperlipidemic drug. Decreased expression of enzymes which inactivate estradiol, including Cyp2C11, and the reported increased expression of aromatase (converting testosterone to estradiol) may explain why male rats exposed to diverse peroxisomal proliferators have higher serum estradiol levels. These higher estradiol levels in male rats have been thought to be mechanistically linked to Leydig cell hyperplasia and adenomas. Increased conversion of estradiol to the less active estrone by 17 β -HSD 4 induction may explain how exposure to the di-(2-ethylhexyl)-phthalate leads to decreases in serum estradiol levels and suppression of ovulation in female rats (Srivastava & Srivastava 1991, Corton *et al.* 1997).

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NOTE ADDED IN PROOF

Radiation hybrid mapping by Genethon assigned HSD17B4 gene to interval D5S471–D5S393 with a physical position of 484.22 cR₃₀₀₀ on chromosome 5.