0888-8809/06/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 20(2):437–443
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doi: 10.1210/me.2005-0058

Characterization of Type 12 17β-Hydroxysteroid Dehydrogenase, an Isoform of Type 3 17β-Hydroxysteroid Dehydrogenase Responsible for Estradiol Formation in Women

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A novel 17β-hydroxysteroid dehydrogenase (17β-HSD) chronologically named type 12 17β-HSD (17β-HSD12), that transforms estrone (E1) into estradiol (E2) was identified by sequence similarity with type 3 17β-HSD (17β-HSD3) that catalyzes the formation of testosterone from androstenedione in the testis. Both are encoded by large genes spanning 11 exons, most of them showing identical size. Using human embryonic kidney-293 cells stably expressing 17β-HSD12, we have found that the enzyme catalyzes selectively and efficiently the transformation of E1 into E2, thus identifying its role in estrogen formation, in contrast with 17β-HSD3, the enzyme involved in the biosynthesis of the androgen testosterone in the testis. Using real-time PCR to quantify mRNA in a series of human tissues, the expression levels of 17β-HSD12 as well as two other enzymes that perform the same transformation of E1 into E2, namely type 1 17β-HSD and type 7 17β-HSD, it was found that 17β-HSD12 mRNA is the most highly expressed in the ovary and mammary gland. To obtain a better understanding of the structural basis of the difference in substrate specificity between 17β-HSD3 and 17β-HSD12, we have performed tridimensional structure modelization using the coordinates of type 1 17β-HSD and site-directed mutagenesis. The results show the potential role of bulky amino acid F234 in 17β-HSD12 that blocks the entrance of androstenedione. Overall, our results strongly suggest that 17β-HSD12 is the major estrogenic 17β-HSD responsible for the conversion of E1 to E2 in women, especially in the ovary, the predominant source of estrogens before menopause. (*Molecular Endocrinology* 20: 437–443, 2006)

THE 17β-HYDROXYSTEROID dehydrogenases (17β-HSDs) are the key enzymes responsible for the formation and inactivation of sex steroids (1–5). The hydrogenation catalyzed at position 17β of the steroid backbone by reductive 17β-HSDs leads to active androgens and estrogens whereas removal of the hydrogen by oxidative 17β-HSD inactivates the steroids. A particular property of members of this family is that they possess very different primary structures (an average of only ~20% amino acid identity) despite being highly specific for substrates having closely related structures. Additional regulation of 17β-HSD activity is also achieved by the specificity of tissue distribution of three 17β-HSDs, thus permitting each tissue to control intracellular steroid levels according to local needs. Such local intracellular formation of steroids in peripheral target tissues from the adrenal precursor dehydroepiandrosterone has been called intracrinology (6, 7). To date, 12 types of 17β-HSDs have been described. The first and best-characterized 17β-HSD is type 1 (8, 9), which catalyzes the transformation of estrone (E1) into estradiol (E2). This enzyme is expressed at high levels in the placenta where it acts as a partner of aromatase, which catalyzes the transformation of androstenedione (4-dione) into E1, the substrate of type 1 17β-HSD (17β-HSD1). 17β-HSD1 is also the first mammalian steroidogenic enzyme to have been crystallized (10).

Type 7 17β-HSD (17β-HSD7) was also identified as an enzyme catalyzing the transformation of E1 into E2 (11, 12). This enzyme also possesses a 3β-ketosteroid reductase activity catalyzing the transformation of dihydrotestosterone into 5α-androstane-3β,17β-diol (2, 13–15). A form 2 of 17β-HSD7 has also been characterized (16). More recently, 17β-HSD7 was also described as a 3β-ketosteroid reductase (12) involved in cholesterol metabolism. The high expression level of this enzyme in the liver is in agreement with this role.

Recently, based only upon sequence similarity without any information about substrate specificity, we have identified a thus far uncharacterized but potential 17β-HSD that we named chronologically type 12 17β-

First Published Online September 15, 2005

Abbreviations: CoA, Coenzyme A; 4-dione, androstenedione; E1, estrone; E2, estradiol; ER, estrogen receptor; HEK, human embryonic kidney; HSD, hydroxysteroid dehydrogenase; 17β-HSD1, -3, -7, and -12, types 1, 3, 7, and 12 17β-HSD, respectively; testo, testosterone.

***Molecular Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

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HSD (17β-HSD12) (GenBank accession nos. AF078850 and NM_016142). Independently, Moon and Horton (17) have shown that the enzyme possesses ketoacyl-coenzyme A (CoA) reductase activity and is involved in fatty acid metabolism. In the present report, using human embryonic kidney (HEK)-293 cells stably expressing 17β-HSD12, we show that this enzyme catalyzes selectively the transformation of E1 into E2. Comparison of the expression levels and tissue distribution of 17β-HSD12 with the expression of types 1 and 7 17β-HSDs strongly suggests that this enzyme is the major estrogen-producing 17β-HSD in the ovary as well as in estrogen-sensitive tissues such as the mammary gland. Furthermore, structure-function relationship analysis using sequence alignment, modelization, and site-directed mutagenesis indicates that this enzyme is a homolog of type 3 17β-HSD (17β-HSD3), the enzyme responsible for testosterone (testo) biosynthesis from 4-dione in the testis (18).

RESULTS

Sequence Homology between 17β-HSD12 and 17β-HSD3

Using the sequences available in GenBank, we first compared the genomic structures of types 3 and 12 17β-HSDs. As illustrated in Fig. 1, these two genes show very similar genomic structures: 11 exons spanning large chromosomal regions (240 kb for 17β-HSD12 and 103 kb for 17β-HSD3). Whereas exons 2, 3, 4, 7, 8, 9, and 10 possess the same number of nucleotides, exons 5 and 6 of 17β-HSD12 contain three additional and nine missing nucleotides, respectively. In addition, 17β-HSD12 shows longer 5′- and 3′-untranslated regions. Indeed, the first and last exons of 17β-HSD12 possess additional 0.2- and 1.2-kb sequences.

Amino acid sequence alignment (Fig. 2) shows that 17β-HSD3 and 17β-HSD12 share 41% identity with a conserved active site (YXXXK) and a slightly modified cofactor-binding motif. Both genes show GXXXGXL instead of GXXXGXG as described for 17β-HSD (10). Such data strongly suggest that 17β-HSD3 and 17β-HSD12 are duplicated genes located in different chromosomes, 17β-HSD3 being found in chromosome band 9q22 whereas 17β-HSD12 is located at chromosome band 11q11.

Substrate Specificity of Human 17β-HSD12

We have used 17β-HSD12 stably expressed in HEK-293 cells to characterize the substrate specificity of the enzyme in intact cells. As illustrated in Fig. 3, 17β-HSD12 catalyzes mainly the transformation of E1 into E2 (37% transformation), whereas the transformation of 4-dione to testo is very low (5%) under similar conditions. Under the same experimental conditions, 17β-HSD3 transforms 63% of 4-dione into testo and only 5% of E1 into E2 (data not shown). Comparison of 17β-HSD12 activity with that of types 1 and 7 17β-HSD (Table 1) shows that 17β-HSD12 possesses similar Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) values with 17β-HSD7, whereas they are much higher and lower, respectively, than those of

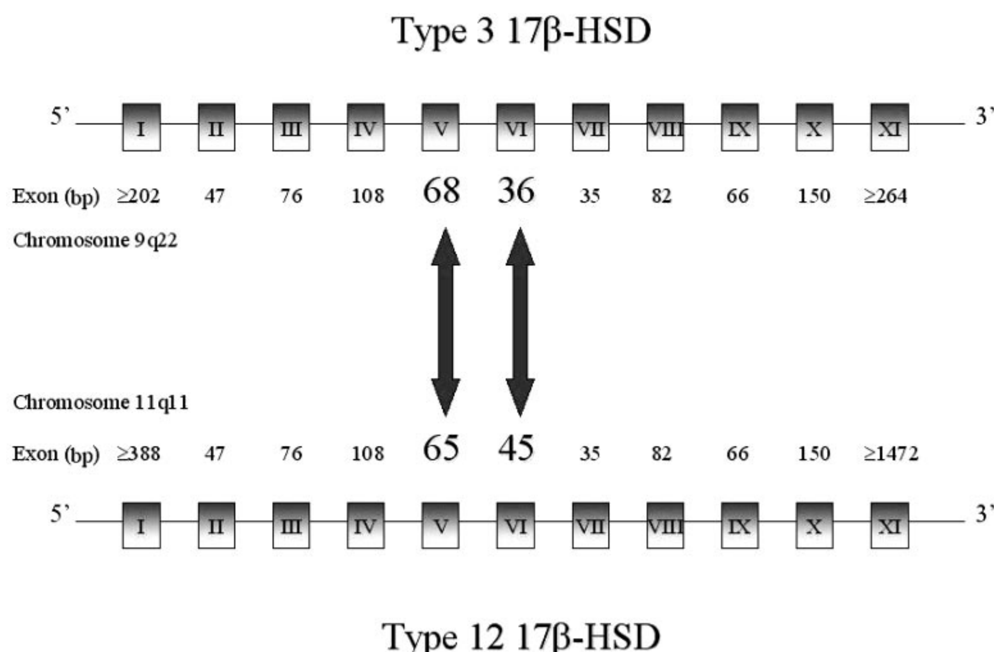

Fig. 1. Schematic Representation of the Genomic Structure of 17β-HSD3 and 17β-HSD12

The exons are *boxed* and numbered I–XI. Exon sizes are indicated *below* (17β-HSD3) and *above* (17β-HSD12) the *boxes*. Chromosomal localizations are also indicated.

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h17β-HSD12      MESALPAAGFLYWVGAGTVAYLALRISYSLFTALRVWGVGNEAGVGPGLG 50
h17β-HSD3       -GDV-EQF*-ILTGLLVCL-C--KCVRF-RCVL-NY-K-LPKSFLRS*M- 48

h17β-HSD12      EWAVVTGSTDGIGKSYAEELAKHGMKVVLISRSKDKLDQVSSEIKEKFKV 100
h17β-HSD3       Q---I--AG-----A-SF----R-LN------TLE--EAIAT--ERTTGR 98

h17β-HSD12      ETRTIAVDFASEDIYDKIKTGLAGLEIGILVNNVGMS*YEYPEYFLDVPD 149
h17β-HSD3       SVKI-QA--TKD---EH--EK---------------LPNLL-SH--NA-- 147

h17β-HSD12      LDNVIKKMININILSVCKMTQLVLPGMVERSKGAILNISSGSGMLPVPLL 199
h17β-HSD3       ***E-QSL-HC--T--V-----I-KH-ES-Q--L-------IALF-W--Y 195

h17β-HSD12      TIYSATKTFVDFFSQCLHEEYRSKGVFVQSVLPYFVATKLAKIRKPTLDK 249
h17β-HSD3       SM---S-A--CA--KA-Q---KA-E-II-VLT--A-S-AMT-YLNTNVIT 245

h17β-HSD12      PSPETFVKSAIKTVGLQSRTNGYLIHALMGSIISNLPSWIYLKIVMN*MN 298
h17β-HSD3       KTADE---ESLNY-TIGGE-C-C-A-EILAGFL-LI-A-AFYSGAFQRLL 295

h17β-HSD12      KSTRAHYLKKTKKN* 312
h17β-HSD3       LTHYVA---LNT-VR 310
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Fig. 2. Comparison of the Amino Acid Sequences of 17β-HSD3 and 17β-HSD12

Amino acids are presented in conventional *single-letter* code and numbered on the *right*. *Dashes* and *stars* represent identical and missing amino acids, respectively. The consensus sequences for cofactor binding and active sites are *underlined.*

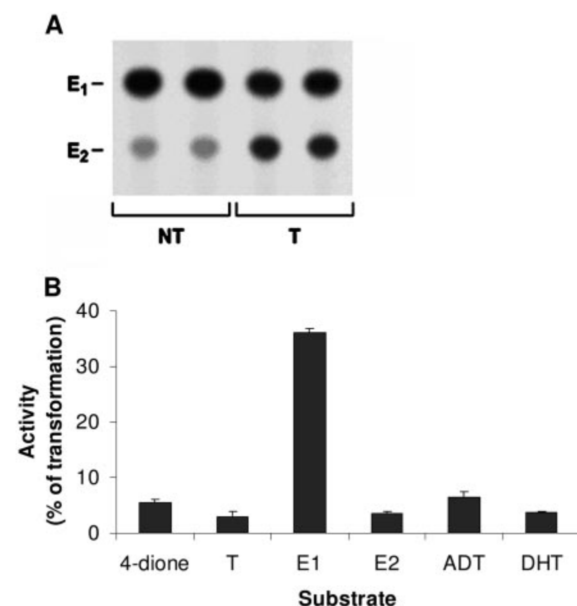

Fig. 3. Substrate Specificity of HEK-293 Cells Stably Transfected with 17β-HSD12

The experiments were performed in intact transfected cells in culture using 0.1 μM of the indicated ^{14}C- or ^{3}H-labeled steroid substrate. A, Thin-layer chromatography showing the conversion of E1 to E2 by nontransfected (NT) and stably transfected (T) cells. B, 4-dione, conversion of 4-dione to testo; T, conversion of testo to 4-dione; E1, conversion of E1 to E2; E2, conversion of E2 to E1; ADT, conversion of androsterone (ADT) to 5α-androstane-3α,17β-diol (3α-diol); DHT, conversion of dihydrotestosterone (DHT) to 3α-diol. The incubation of control HEK-293 cells with the same substrates serves as control and the values obtained are subtracted in the present figure from those obtained with transfected cells. The data are expressed as means ± SEM of triplicate measurements.

17β-HSD1. This could be related to the fact that types 7 and 12 17β-HSD are microsomal enzymes, whereas 17β-HSD1 is cytoplasmic.

F234, a Potential Amino Acid Critically Involved in Substrate Specificity

To obtain a better understanding on the structural basis of the difference in substrate specificity between 17β-HSD12 and 17β-HSD3, we have performed modelization of the tridimensional structures of 17β-HSD12 and 17β-HSD3 using the coordinates of type 1 17β-HSD (1fds) and the Insight II software (Accelrys, San Diego, CA) (Fig. 4). We have thus identified valine at position 196 and alanine at position 234, both located in the substrate-recognition domain of the enzyme, as potential amino acids responsible for the different substrate specificities of 17β-HSD12 compared with 17β-HSD3. The corresponding amino acids in 17β-HSD3 are W192 and A230. The latter has a much smaller size and could thus allow the entrance of C19-steroids. Moreover, the large size of F234 could prevent the entrance of C19-steroids while allowing the passage of C18-steroids. To verify this hypothesis,

Table 1. Kinetic parameters of human types 1, 7, and 12 17β-HSD

Enzyme	K_m (μM)	V_{max} (nmol product/h/10^6 cells)
17β-HSD1	0.2 ± 0.05	110 ± 15
17β-HSD7	4.0 ± 0.3	83 ± 9
17β-HSD12	3.5 ± 0.5	56 ± 7

Kinetic constants were determined using HEK-293 cells stably expressing human types 1, 7 and 12 17β-HSD and [^{14}C]E1 as substrate.

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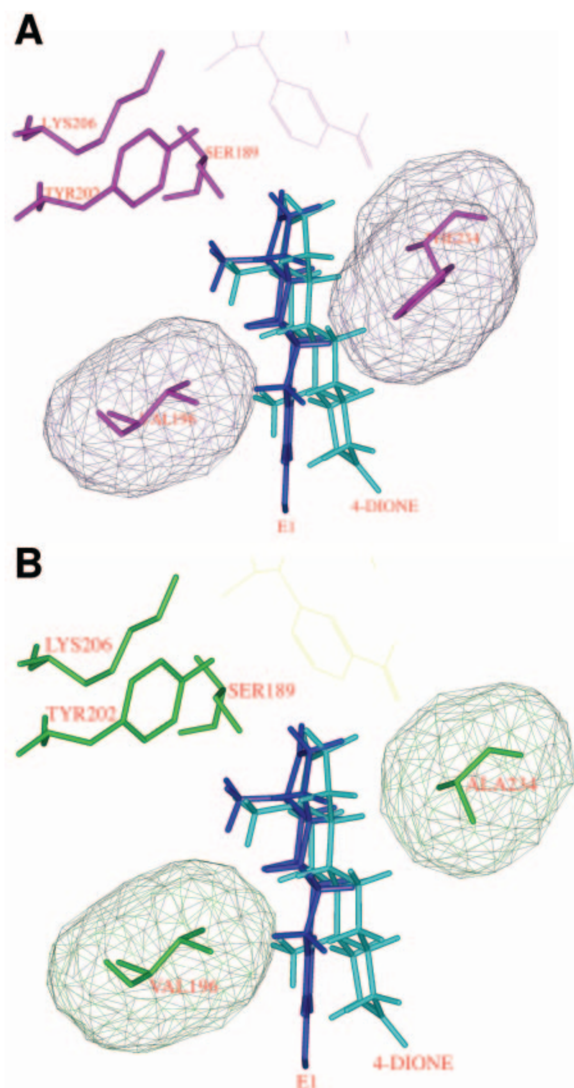

Fig. 4. Schematic Representation of the Possible Steric Hindrance due to a Bulky F234 that Prevents the Transformation of 4-dione by 17β-HSD12

17β-HSD12 was modeled using the coordinates of 17β-HSD1 and the Insight II program. A, The two amino acids V196 and F234 potentially involved in substrate specificity are shown with electronic density, whereas the structures of E1 and 4-dione lay over the entrance of the active site. B, Model showing the substitution of F234 in 17β-HSD12 by a less bulky A234, which tolerates the entrance of 4-dione.

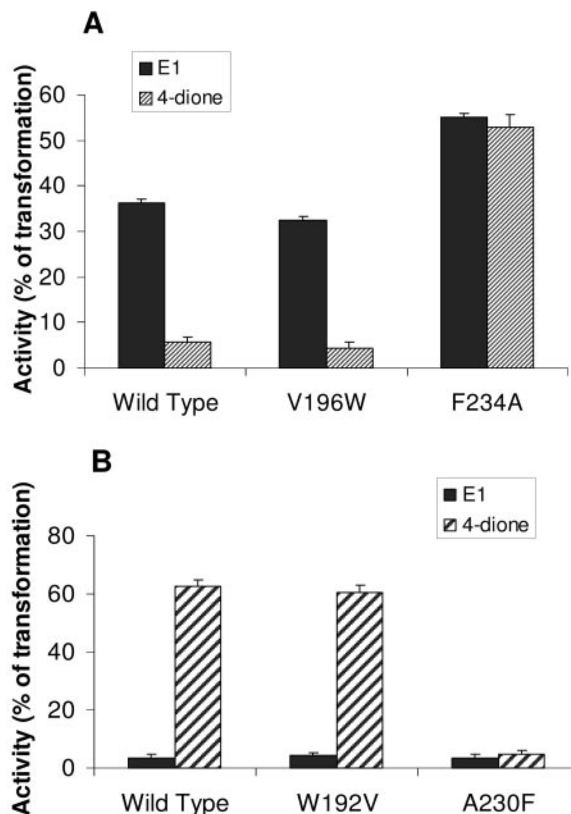

Fig. 5. Effects of V196W and F234A Amino Acid Substitutions in 17β-HSD12 Activity and the Corresponding W192V andA230F Amino Acid Substitutions in 17β-HSD3

A, HEK-293 cells were transiently transfected with expression vectors encoding 17β-HSD12 (Wild Type) and mutants with a substitution of Val for Trp at amino acid position 196 (V196W) and a substitution of Phe for Ala at amino acid position 234 (F234A). B, Similarly, HEK-293 cells were also transiently transfected with expression vectors encoding 17β-HSD3 (Wild Type) and mutants with a substitution of Trp for Val at amino acid position 192 (W192V). The ability of transfected cells to convert E1 to E2 and 4-dione to T (testo) were determined as described in *Materials and Methods.*

we have performed site-directed mutagenesis to substitute F234 in 17β-HSD12 and A230 in 17β-HSD3. As illustrated in Fig. 5, the F234A change in 17β-HSD12 allowed the enzyme to catalyze the transformation of 4-dione to testo at an increased efficiency comparable to the transformation of E1 to E2, whereas the conversion of 4-dione to testo was almost completely abolished in the 17β-HSD3 A230F mutant. On the other hand, the substitutions W192V and V196W in 17β-HSD12 and in 17β-HSD3 do not alter the activity of the mutated enzymes.

Tissue Distribution of 17β-HSD12

Using quantitative real-time PCR, we have determined the tissue distribution of 17β-HSD12 mRNA in some estrogen-sensitive tissues, namely the mammary gland, ovary, uterus, vagina, cervix, and placenta. As illustrated in Fig. 6, 17β-HSD12 mRNA is highly and selectively expressed in the mammary gland and ovary. It can also be seen in this figure that 17β-HSD1, the enzyme thus far thought to be the one responsible of E2 formation in the ovary and peripheral tissues, is usually expressed at a much lower level in all tissues except the placenta. 17β-HSD7, on the other hand, the other enzyme that transforms E1 into E2, is expressed at moderate but usually higher levels than 17β-HSD1 in the tissues studied, with the exception of the placenta.

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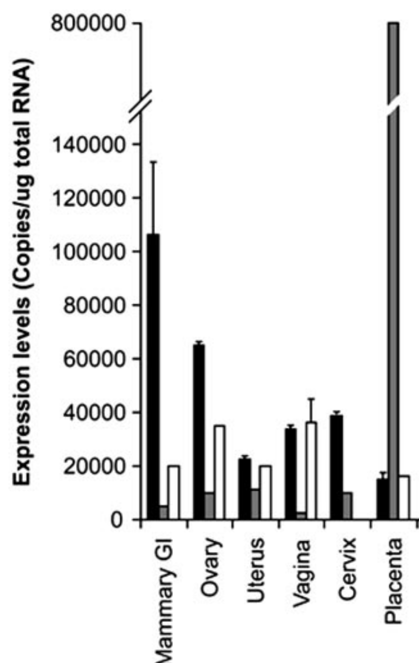

Fig. 6. Comparison of mRNA Expression Levels of 17β-HSD1, 17β-HSD7, and 17β-HSD12 Using Quantitative Real-Time PCR

Total RNA of the indicated tissues was obtained commercially. Quantitative real-time PCR quantification using SYBR green was performed as described in *Materials and Methods.* The expression level is indicated as the number of copies/μg of total RNA. The data are expressed as means ± SEM of duplicate measurements. *Solid, striped, and open bars* represent 17β-HSD12, 17β-HSD1, and 17β-HSD7, respectively. Gl, Gland.

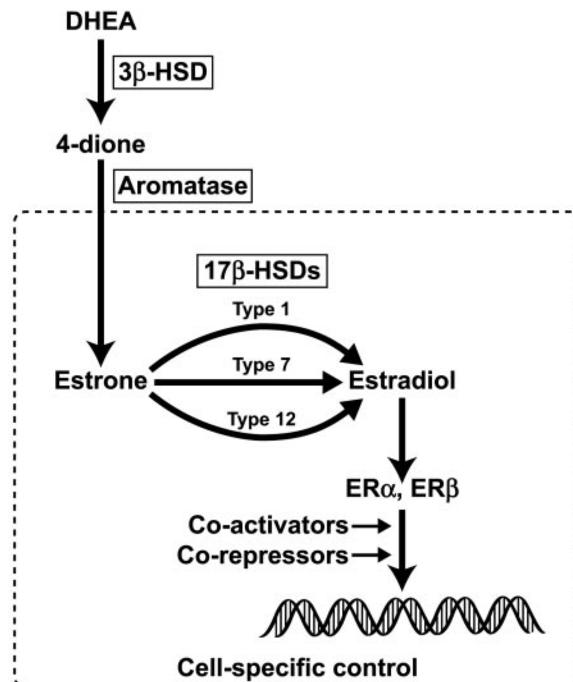

Fig. 7. Schematic Representation of the Pathway Going from Dehydroepiandrosterone (DHEA) to E2

Note the common catalytic activity of types 1, 7, and 12 17β-HSDs, the three enzymes that are responsible for the transformation of E1 into E2. Subsequent to the fine cell-specific tuning of E2 formation by the 17β-HSDs, the estrogen binds to ERα and/or ERβ to recruit cell-specific coactivators and/or corepressors, thus forming specific complexes leading to activation or repression of estrogen-sensitive gene expression.

DISCUSSION

Although being chronologically the third enzyme discovered that catalyzes the transformation of E1 into E2, the present data strongly suggest that 17β-HSD12 is the most important source of E2 in women. In fact, comparison of the mRNA expression levels of 17β-HD12 with types 1 and 7 17β-HSDs in a series of human tissues shows that 17β-HSD12 is expressed more abundantly than types 1 and 7 17β-HSD in the mammary gland, ovary, and uterus, thus suggesting that 17β-HSD12 is the main enzyme responsible for the formation of active estrogen in these important tissues. Moreover, the finding of such a high level of expression of 17β-HSD12 in the human ovary is particularly important because the ovary is the main source of estrogens in women before menopause. After menopause, on the other hand, all the estrogens are made locally in peripheral target tissues, and the specific level of expression of 17β-HSDs in each cell type controls the cell-specific formation of E2 (7, 19). On the other hand, 17β-HSD1 is the major enzyme expressed in the placenta (Fig. 6), whereas 17β-HSD7 is more highly expressed in the liver (11).

As illustrated in Fig. 7, the availability of three estrogenic 17β-HSDs permits the tissue and even the cell-specific fine control of the transformation of E1 into E2. In the main pathway of sex steroid formation, dehydroepiandrosterone is converted into 4-dione by 3β-HSD and then 4-dione is converted into E1 by aromatase, thus providing the required amounts of E1 as substrate for the formation of E2 in a cell-specific fashion according to local physiological needs. Additional fine control of estrogen action is added by the cell-specific expression of estrogen receptor (ERα) and ERβ and the action of a series of coactivators and corepressors of ER action (20).

A recent report from Moon and Horton (17) shows that 17β-HSD12 possesses 3-ketoacyl-CoA reductase activity and is thus involved in fatty acid elongation. This does not contradict our findings because many HSDs are multifunctional enzymes (2, 3), which can accept many substrates and exert different activities in different tissues, their activity and role depending upon the substrate available. Although the 3-ketoacyl-CoA reductase activity observed by Moon and Horton (17) seems to be much weaker than the 17β-HSD activity observed in the present report, it should not be inferred that such fatty acid biosynthetic activity is less important. Indeed, although it is generally accepted that an isoenzyme that possesses higher affinity and/or higher activity will have a more important physiological role, this concept is straightforward for unicellular organisms in which substrates compete for one enzyme, or in which one sub-

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strate is used by multiple enzymes. In the case of differentiated tissues or organs, both the concentration level and affinity of the substrate and activity of the enzyme are key parameters. A high substrate concentration can compensate for a low concentration or activity of the enzyme. As an example, although human type 2 3β-HSD possesses a 10-fold lower affinity and activity than type 1 3β-HSD (21), mutations in the type 2 3β-HSD gene leads to adrenal congenital hyperplasia (22), because it is the only form expressed in the adrenals. Similarly, although 17β-HSD1 catalyzes more efficiently the transformation of E1 to E2 than types 7 and 12 17β-HSD, it is not guaranteed that 17β-HSD1 plays a more important physiological role than types 7 and 12 17β-HSD.

Our finding that 17β-HSD12, a homolog of 17β-HSD3, the enzyme playing a crucial role in the male testis, is an estrogenic 17β-HSD and, therefore, plays a crucial role in the female, points to some parallelism with types 1 and 2 5α-reductases, two homologous genes sharing approximately 45% amino acid identity. In fact, studies using knockout mice (23) have shown that type 1 5α-reductase is important for female physiology, whereas type 2 5α-reductase is more important for the male. In this context, it is noteworthy that inactivating mutations in both 17β-HSD3 and type 2 5α-reductase genes will lead to male pseudohermaphroditism (18, 24). It seems that genes involved in sex steroid metabolism are duplicated and diverge to acquire sex-specific function. Our modelization and site-directed mutagenesis data (Figs. 4 and 5) suggest that during evolution, a change of A to F at position 234 confers the estrogenic specificity to 17β-HSD12 due to the larger size of F234, which prevents the entrance of 4-dione.

In addition to the extremely fine control of E2 formation achieved by the specific expression of types 1, 7, and 12 17β-HSD separately or in tandem in each cell type, such a multiplicity of enzymes performing the same task protects against mutations potentially occurring in one or even two of the 17β-HSDs. Such a high degree of plasticity in the formation of E2 indicates the crucial importance of E2 formation, which is protected against mutations in one and even two of the three genes having the same role in a large series of human tissues. Such a multiplicity of enzymes having similar catalytic activity provides the basis for the high degree of precision that evolution has achieved for an optimal control of peripheral E2 formation and action.

MATERIALS AND METHODS

Isolation of Human 17β-HSD12 and Construction of pCMVneo-HSD17B12 Vector

Using oligonucleotide primers (5′-ggg-aat-tcg-cca-tgg-aga-gcg-ctc-tcc-ccg-ccg-ccg-gct-t-3′ and 5′-ggt-cta-gag-ctt-agt-tct-tct-tgg-ttt-tct-tca-gat-agt-gag-3′) derived from a GenBank sequence having accession no. NM_016142, we have amplified by RT-PCR a cDNA fragment containing the entire coding region of 17β-HSD12. The cDNA fragment was then inserted into a pCMVneo vector downstream from a cytomegalovirus promoter. The resulting pCMVneo-HSD17B12 vector was sequenced to verify its integrity and stably transfected into HEK-293 cells as described elsewhere (25). The positive clones were selected according to their ability to transform E1 into E2.

Site-Directed Mutagenesis

The change from V to W and F to A at amino acid positions 196 and 234 in 17β-HSD12 and a change from W to V and A to F at amino acid positions 192 and 230 in 17β-HSD3 were performed using the oligonucleotide primers, 5′-cat-gct-ccc-ttg-gcc-act-ctt-gac-3′, 5′-gtg-tcc-tgc-cat-acg-ccg-tag-cta-caa-aac t-3′ and 5′-ccc-tgt-ttc-ctg-tgc-ctc-tct-act-c-3′, 5′-gtg-ctg-acc-cca-tat-ttt-gtc-tcg-act-gca-atg-3′, respectively, using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The integrity of the constructs was verified by sequencing of the inserted DNA fragment using the T7 sequencing kit (GE HealthCare, Baie d'Urfé, Quebec, Canada). Plasmid DNA was prepared using the Qiagen Mega Kit (QIAGEN, Chatsworth, CA). Oligonucleotide primers were synthesized with a DNA synthesizer ABI-394 (PerkinElmer-Cetus, Emerville, CA).

Enzymatic Assays

The measurement of enzymatic activities was performed in intact cells as previously described (26). Briefly, 0.1 μM of the ^{14}C-labeled steroid (Dupont, Inc., Mississauga, Ontario, Canada) was added to freshly changed culture medium in a six-well culture plate. To determine the kinetic constants, nonlabeled E1 is added when necessary. After incubation, the steroids were extracted twice with 2 ml of ether. The organic phases were pooled and evaporated to dryness. The steroids were then dissolved in 50 μl of dichloromethane, applied to Silica gel 60 thin layer chromatography plates (Merck, Darmstad, Germany), before separation by migration in the toluene-acetone (4:1, vol/vol) solvent system. Substrates and metabolites were identified by comparison with reference steroids and revealed by autoradiography and quantified using the PhosphorImager System (Molecular Dynamics, Inc., Sunnyvale, CA).

Quantitative Real-Time PCR

Most of human total RNA preparations described in this study were obtained from Ambion, Inc. (Austin, TX), except for total RNA of adrenals and liver that were obtained from CLONTECH Laboratories, Inc. (Palo Alto, CA) and Stratagene (La Jolla, CA), respectively. Quantification of mRNA was performed using 30 pg of the initial total RNA and a fluorescent-based real-time PCR quantification on the LightCycler Real-time PCR apparatus (Hoffman-La Roche, Inc., Nutley, NJ). Reagents were obtained from the same supplier and were used as described by the manufacturer. Specific primer pairs used for amplification were: 17β-HSD1:5′-TTC-TTT-GTC-CCC-TGG-GTC-TGT-GT-3′ and 5′-ATG-GGG-GTC-TCA-CTG-TGT-TGC-T-3′; 17β-HSD7: 5′-TCC-ACC-AAA-AGC-CTG-AAT-CTC-TC-3′ and 5′-GGG-CTC-ACT-ATG-TTT-CTC-AGG-C-3; and 17β-HSD12: 5′-GGC-TGG-TCT-TGA-AAT-CGG-CAT-3′ and 5′-TGC-CAC-TGC-CAG-ATG-AAA-TGT-T-3.

The conditions for the PCRs were: denaturation at 94 C for 15 sec, annealing at 55 C for 10 sec, and elongation at 72 C for 35 sec. The data were normalized using the mRNA expression levels of a housekeeping gene, namely ATP5o (subunit O of ATPase) as internal standard. Atp5o has been shown to have stable expression levels from embryonic life through adulthood in various tissues (27). The mRNA expression levels are expressed as numbers of copies/μg total RNA using a standard curve of Cp *vs*. logarithm of the quantity. The standard curve is established using known cDNA amounts of 0, 10^2, 10^3, 10^4, 10^5, and 10^6 copies of cDNA and

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a LightCycler 3.5 program provided by the manufacturer (Hoffman-La Roche, Inc.).

Acknowledgments

We thank Guy Reimnitz, Nathalie Paquet, and Mélanie Robitaille for their skillful technical assistance.

Received January 24, 2005. Accepted September 8, 2005.

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This work was supported by the Canadian Institute of Health Research (Grant Nos. MOP-53215 and MOP-77698 to V.L.T.).

REFERENCES

1. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, Belanger A 1997 The key role of 17β-HSDs in sex steroid biology. Steroids 62:148–158
2. Luu-The V 2001 Analysis and characteristics of multiple types of human 17β-hydroxysteroid dehydrogenases. J Steroid Biochem Mol Biol 76:143–151
3. Mindnich R, Moller G, Adamski J 2004 The role of 17β-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 218:7–20
4. Labrie F, Luu-The V, Labrie C, Simard J 2001 DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol 22:185–212
5. Labrie F, Luu-The V, Lin SX, Simard J, Labrie C 2000 Role of 17 β-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. Trends Endocrinol Metab 11:421–427
6. Labrie C, Bélanger A, Labrie F 1988 Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate. Endocrinology 123:1412–1417
7. Labrie F 1991 Intracrinology. Mol Cell Endocrinol 78: C113–C118
8. Luu The V, Labrie C, Zhao HF, Couet J, Lachance Y, Simard J, Leblanc G, Cote J, Berube D, Gagne R, Labrie F 1989 Characterization of cDNAs for human estradiol 17β-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 5′-termini in human placenta. Mol Endocrinol 3:1301–1309
9. Peltoketo H, Isomaa V, Maentausta O, Vihko R 1988 Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. FEBS Lett 239:73–77
10. Ghosh D, Pletnev VZ, Zhu DW, Wawrzak Z, Duax WL, Pangborn W, Labrie F, Lin SX 1995 Structure of human estrogenic 17β-hydroxysteroid dehydrogenase at 2.20 A resolution. Structure 3:503–513
11. Krazeisen A, Breitling R, Imai K, Fritz S, Moller G, Adamski J 1999 Determination of cDNA, gene structure and chromosomal localization of the novel human 17β-hydroxysteroid dehydrogenase type 7(1). FEBS Lett 460:373–379
12. Marijanovic Z, Laubner D, Moller G, Gege C, Husen B, Adamski J, Breitling R 2003 Closing the gap: identification of human 3-ketosteroid reductase, the last unknown enzyme of mammalian cholesterol biosynthesis. Mol Endocrinol 17:1715–1725
13. Robert A, Rheault P, Labrie F, Luu-The V, Identification and characterization of an estrogen-activating and androgen-inactivating (EHAL) enzyme. Conférence scientifique nationale. La recherche sur le cancer du sein: raisons d'espérer. Toronto, Ontario, Canada, 1999, p 82
14. Robert A, Luu-The V, Cloning and characterization of a new form of human 3-keto-reductase specific for saturated steroid. CDA/CSEM Professional Conference and Annual Meetings, Ottawa, Ontario, Canada, 1999
15. Torn S, Nokelainen P, Kurkela R, Pulkka A, Menjivar M, Ghosh S, Coca-Prados M, Peltoketo H, Isomaa V, Vihko P 2003 Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. Biochem Biophys Res Commun 305:37–45
16. Liu H, Robert A, Luu-The V 2005 Cloning and characterization of human form 2 type 7 17β-hydroxysteroid dehydrogenase, a primarily 3β-keto reductase and estrogen activating and androgen inactivating enzyme. J Steroid Biochem Mol Biol 94:173–179
17. Moon YA, Horton JD 2003 Identification of two mammalian reductases involved in the two-carbon fatty acyl elongation cascade. J Biol Chem 278:7335–7343
18. Geissler WM, Davis DL, Wu L, Bradshaw KD, Patel S, Mendonca BB, Elliston KO, Wilson JD, Russell DW, Andersson S 1994 Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. Nat Genet 7:34–39
19. Labrie F, El-Alfy M, Berger L, Labrie C, Martel C, Belanger A, Candas B, Pelletier G 2003 The combination of a novel selective estrogen receptor modulator with an estrogen protects the mammary gland and uterus in a rodent model: the future of postmenopausal women's health? Endocrinology 144:4700–4706
20. Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, Gannon F 2003 Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115:751–763
21. Rhéaume E, Lachance Y, Zhao HF, Breton N, Dumont M, de Launoit Y, Trudel C, Luu-The V, Simard J, Labrie F 1991 Structure and expression of a new complementary DNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/D^5-D^4-isomerase in human adrenals and gonads. Mol Endocrinol 5:1147–1157
22. Rheaume E, Simard J, Morel Y, Mebarki F, Zachmann M, Forest MG, New MI, Labrie F 1992 Congenital adrenal hyperplasia due to point mutations in the type II 3 β-hydroxysteroid dehydrogenase gene. Nat Genet 1:239–245
23. Mahendroo MS, Russell DW 1999 Male and female isoenzymes of steroid 5α-reductase. Rev Reprod 4:179–183
24. Andersson S, Berman DM, Jenkins EP, Russell DW 1991 Deletion of steroid 5 α-reductase 2 gene in male pseudohermaphroditism. Nature 354:159–161
25. Huang XF, Luu-The V 2000 Molecular characterization of a first human 3(α→β)-hydroxysteroid epimerase. J Biol Chem 275:29452–29457
26. Luu-The V, Zhang Y, Poirier D, Labrie F 1995 Characteristics of human types 1, 2 and 3 17β-hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. J Steroid Biochem Mol Biol 55:581–587
27. Warrington JA, Nair A, Mahadevappa M, Tsyganskaya M 2000 Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. Physiol Genomics 2:143–147

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