Differential androgen and estrogen substrates specificity in the mouse and primates type 12 17β-hydroxysteroid dehydrogenase

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Abstract

Recently, we have shown that human and monkey type 12 17β-hydroxysteroid dehydrogenases (17β-HSD12) are estrogen-specific enzymes catalyzing the transformation of estrone (E_1) into estradiol (E_2). To further characterize this novel steroidogenic enzyme in an animal model, we have isolated a cDNA fragment encoding mouse 17β-HSD12 and characterized its enzymatic activity. Using human embryonic kidney cells (HEK)-293 cells stably expressing mouse 17β-HSD12, we found that in contrast with the human and monkey enzymes, which are specific for the transformation of E_1 to E_2, mouse 17β-HSD12 also catalyzes the transformation of 4-androstenedione into testosterone (T), dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (5-diol), as well as androsterone into 5α-androstane-3α,17β-diol (3α-diol). Previously, we have shown that the specificity of human and monkey 17β-HSD12s for C18-steroid is due to the presence of a bulky phenylalanine (F) at position 234 creating steric hindrance, preventing the entrance of C19-steroids into the active site. To determine whether the smaller size of the corresponding leucine (L) in the mouse sequence is responsible for the entrance of androgenic substrates, we performed site-directed mutagenesis to substitute Leu 234 for Phe in the mouse enzyme. In agreement with our hypothesis, the mutated enzyme has a highly reduced ability to metabolize androgens. mRNA quantification in several mouse tissues using real-time PCR shows that mouse 17β-HSD12 mRNA is highly expressed in the female clitoral gland, male preputial gland, as well as in retroperitoneal fat and adrenal of both sexes. The differential androgenic/estrogenic substrate specificity of type 12 17β-HSD in the mouse and primates seems to agree with the observation that androgen and estrogen in the mouse are provided almost exclusively by gonads, while in primates an important part of these steroid hormones are produced locally from adrenal precursors.

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Introduction

Active sex steroids (testosterone, dihydrotestosterone, and estradiol (E_2)) are characterized by the presence of a hydroxyl group at position 17β on the steroid nucleus. The formation of the 17β-hydroxy group is catalyzed by 17β-hydroxysteroid dehydrogenases (17β-HSDs). To date, 14 types of 17β-HSDs have been identified (Peltoketo *et al.* 1999, Luu-The 2001, Mindnich *et al.* 2004). In the human, a clear substrate specificity pattern for estrogen and androgen substrates has been observed. Types 1, 7, and 12 17β-HSDs (17β-HSD1, 17β-HSD7, and 17β-HSD12) catalyze the transformation of estrone (E_1) into E_2, while 17β-HSD3 and 17β-HSD5 are able to catalyze the transformation of 4-androstenedione (4-dione) into testosterone (T). Others catalyze mostly the transformation of 5α-reduced C19-steroids. In rodents, the enzymatic characteristics of steroidogenic enzymes are somehow different from their human counterparts. For example, rodent 17β-HSD1 catalyzes, in addition to the formation of E_2 from E_1, the transformation of 4-dione into T while human 17β-HSD1 catalyzes selectively the transformation of E_1 into E_2. In the human, the enzyme is abundantly expressed in the placenta where it exerts its function as a partner of aromatase, which is also abundantly expressed to produce E_2. In the rodent, 17β-HSD1 and aromatase are absent from the placenta and thus rodent placenta does not produce E_2 (Gibori *et al.* 1988). The production of E_2 in rodents is provided by granulosa cells (Mustonen *et al.* 1997, Nokelainen *et al.* 1998). Indeed, 17β-HSD1 is expressed in granulosa and cumulus cells in both the human (Sawetawan *et al.* 1994) and rodents (Ghersevich *et al.* 1994*a*,*b*, Nokelainen *et al.* 1998).

It is noteworthy that human type 7 17β-HSD, in addition to its ability to catalyze the transformation of E_1 into E_2, also possesses 3-ketoreductase activity able to transform dihydroxy testosterone (DHT) into 5α-androstane-3β,17β-diol (3β-diol; Torn *et al.* 2003, Liu *et al.* 2005) and zymosterone into zymosterol, an important step in the cholesterol biosynthetic pathway (Marijanovic *et al.* 2003).

Recently, we have characterized primate and human 17β-HSD12 (Luu-The *et al.* 2006, Liu *et al.* 2007) that

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share high homology with 17β-HSD3 and found that both 17β-HSD12s catalyze selectively the transformation of E_1 into E_2, thus suggesting that this enzyme is most probably an important partner of aromatase in the biosynthesis of E_2. Indeed, the two steps that are specific for E_2 biosynthesis are the aromatization of 4-dione precursor into E_1 (a weak estrogen) followed by the transformation of E_1 into E_2, the most potent natural estrogen, by estrogenic 17β-HSD. The isolation of cDNAs encoding enzymes that are specific for the conversion of E_1 into E_2 (human 17β-HSD1, 17β-HSD7, and 17β-HSD12) seems to agree with this model, suggesting strongly that the step of reduction by 17β-HSD follows the step of aromatization (Luu-The *et al.* 2006). It is noteworthy that data of Moon & Horton (2003) also suggest that 17β-HSD12 could be involved in the two-carbon fatty acyl elongation cascade.

In this report, we describe the characterization of mouse 17β-HSD12. We show that, similar to 17β-HSD1, mouse 17β-HSD12 is also able to catalyze the transformation of C19-steroids, which is in contrast with the corresponding human enzymes that are selective for estrogenic substrates. The role of the amino acid at position 234 of 17β-HSD12 in substrate selectivity and the mRNA expression levels in different mouse tissues are also investigated.

Materials and Methods

Tissue collection and RNA preparation

C57BL6 mice at 12–15 weeks of age were obtained from Charles River Inc. (Saint-Constant, Que., Canada) and housed individually in vinyl cages where the photoperiods were 12 h light:12 h darkness. The mice were provided with certified rodent food (Lab Rodent Diet) and tap water available *ad libitum*. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care and performed in accordance with the CCAC Guide for Care and Use of Experimental Animals. During the tissue collection, organs were rapidly trimmed, snap-frozen in liquid nitrogen, and stored at −80 °C. Total RNA was isolated by Trizol (Invitrogen). Using oligo-d(T)24 as primer in a reaction buffer containing 50 mM Tris–HCl (pH 8·3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, and 0·5 mM dNTPs, 20 μg RNA were then converted to cDNA by incubation at 42 °C for 1 h with 400 U SuperScript II reverse transcriptase (Invitrogen).

Isolation of mouse 17β-HSD12 and construction of pCMVneo-HSD17B12 vector

To amplify a cDNA fragment containing the entire coding region of mouse 17β-HSD12 by RT-PCR, we used the oligonucleotide primers (5′-ggg-gaa-ttc-gcc-atg-gag-tgc-gct-ccc-ccg-gcg-3′) and (5′-ggg-tct-aga-tta-gtt-ctt-ctt-cct-ttt-ctt-cag-gta-g-3′) derived from the GenBank sequence having accession number NM_019657. The resulting cDNA fragment was then subcloned into a pCMVneo vector, downstream of a cytomegalo virus (CMV) promoter. To verify the integrity of the new construct, namely pCMVneo-mHSD17B12, we sequenced it before stably transfecting into HEK-293 cells as described (Huang & Luu-The 2000).

Assay of enzymatic activity in intact HEK-293 cells stably expressing mouse 17β-HSD12

Enzymatic activity measurements were performed in intact cells as previously described (Dufort *et al.* 2001). Cells were plated in 6-well plates to $\sim 5 \times 10^5$/well in minimum essential medium (MEM). Briefly, 0·1 μM of the (^{14}C)-labeled steroids (Dupont Inc., Mississauga, Ont., Canada) was added to freshly changed culture medium. After incubation, steroids were extracted with 2 ml ether and evaporated to dryness. The steroids were then dissolved in 50 μl dichloromethane, applied to silica gel 60 thin layer chromatography plates (Merck), before separation by migration in the toluene/acetone (4:1) solvent system. Substrates and metabolites were identified by comparison with reference steroids, revealed by autoradiography, and quantified using the PhosphoImager System (Molecular Dynamics Inc., Sunnyvale, CA, USA).

Site-directed mutagenesis

In order to replace the L at amino acid position 234 in the mouse 17β-HSD12 with an A or F residue, we performed site-directed mutagenesis using the Quick Change Site-directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) as described (Dufort *et al.* 1999) and the following oligonucleotide primers: 5′-agt-gtc-atg-cca-tac-gct-gta-gct-aca-aaa-ctg-3′ and its complementary sequence for L234A substitution, and 5′-agt-gtc-atg-cca-tac-ttt-gta-gct-aca-aaa-ctg-3′ and its complementary sequence for L234F substitution. Those oligonucleotide primers were synthesized by an ABI-394 DNA synthesizer (Perkin–Elmer-Cetus, Emerville, CA, USA). The integrity of the constructs was verified by sequencing the inserted DNA fragment. Plasmid DNA was prepared with the help of the Qiagen Mega Kit (Qiagen).

mRNA expression measurement by quantitative real-time PCR (Q_RTPCR)

A fragment of 225 bp from mouse 17β-HSD12 was amplified with the gene-specific primers 5′-tgg-cac-tga-tgg-aat-tgg-aaa-agc-3′ and 5′-ttc-act-aaa-acg-cca-atc-tca-aga-c-3′. Using cDNA corresponding to 30 ng of the initial total RNA, a fluorescent-based real-time PCR quantification was performed with the LightCycler Realtime PCR apparatus (Hoffman-La Roche Inc., Nutley, NJ, USA) as described

(Luu-The *et al.* 2005*b*). Reagents were obtained from the same supplier and were used as described by the manufacturer. The conditions for the PCRs were: denaturation at 95 °C for 10 s, annealing at 54 °C for 5 s, and elongation at 72 °C for 10 s. To normalize the data, we used the expression levels of the *Mus musculus* housekeeping gene ATP synthase. The mRNA expression levels are expressed as numbers of copies per microgram total RNA using a standard curve of Cp versus logarithm of the quantity as described (Luu-The *et al.* 2005*b*). The standard curve was established using known cDNA amounts of 0, 10^2, 10^3, 10^4, 10^5, and 10^6 copies of cDNA from ATP synthase (primers: 5′-tac-tct-gct-gca-tct-aag-gag-aag-3′ and 5′-gtc-att-tag-gct-ttt-cac-ttt-gac-3′) and a LightCycler 3.5 software provided by the manufacturer. Samples were run in triplicate and the results are expressed as mean ± S.E.M.

Results

Sequence homology between human and mouse 17β-HSD12

Using the sequences available in GenBank, we first compared the amino acid sequence of human and mouse 17β-HSD12. The alignment shows that these proteins share 81% amino acid identity. They also exhibit the conserved signatures seen in short chain dehydrogenase/reductase (SDR) family members, namely the putative YXXXK active center and the GXXXGXG consensus sequence of the cofactor-binding motif of the NADPH-preferring SDR proteins (Mazza *et al.* 1998). Previously, we determined that the amino acid F234 in the human enzyme plays a critical role in substrate specificity as its substitution for a smaller amino acid leads to an enzyme able to convert 4-dione into T. It is noteworthy that the amino acid in the mouse sequence corresponding to this F234 is an L residue (Fig. 1).

Substrate specificity of mouse 17β-HSD12

HEK-293 cells stably expressing 17β-HSD12 were used to characterize the substrate specificity of the enzyme in cultured intact cells without addition of exogenous cofactor. As illustrated in Fig. 2, mouse 17β-HSD12 catalyzes preferentially the transformation of androsterone (ADT) into 3α-diol (38% transformation), while the transformation of 4-dione to T (22%), E_1 to E_2 (25%), and dehydroepiandrosterone (DHEA) to 5-diol (22%) are also at similar level after 24-h incubation. Similar conclusions can be observed during a longer-term (48 h) incubation with the same labeled steroids. Under comparable experimental conditions, human and monkey 17β-HSD12 mainly catalyzes the transformation of E_1 into E_2. The ability to process both androgen and estrogen steroids makes the mouse 17β-HSD12 different from its primate homologs.

L234 role in substrate preference

We have previously shown that amino acid F234 in human 17β-HSD12 is responsible for the selectivity of this enzyme for estrogen (Luu-The *et al.* 2006). Indeed, due to its large size, this amino acid prevents, by steric hindrance, C19-steroids from entering the active site. It is interesting to note that in the homolog 17β-HSD3 that catalyzes specifically the transformation of androgen, the corresponding amino acid is A, the second smallest natural amino acid. Since the corresponding amino acid in the mouse sequence is an L residue that has much smaller size than the F found in human 17β-HSD12, we investigated whether the loss of estrogenic specificity in the mouse enzyme when compared with human is due to this amino acid. We thus performed site-directed mutagenesis to substitute the L residue for A and F found in 17β-HSD3 and 17β-HSD12 respectively. As illustrated in Fig. 3, the ability of the L234F substitution to transform 4-dione is much lower than the one observed with the wild-type enzyme or the L234A substitution.

Tissue distribution of mouse 17β-HSD12

We then examined the tissue distribution of 17β-HSD12 mRNA in 23 mouse tissues, namely the adrenal, liver, colon, kidney, stomach, lung (bronchi), pituitary gland, thymus, spleen, heart, gastrocnemius, preputial gland, epididymis, testis, prostate, seminal vesicle, clitoral gland, fat (retroperitoneal), mammary gland, uterus, vagina, ovary, and oviduct using quantitative real-time PCR. As illustrated in Fig. 4, mouse 17β-HSD12 mRNA is ubiquitously expressed in all those tissues. In the male, the highest level of expression of 17β-HSD12 mRNA is seen in the preputial gland, retroperitoneal fat, and adrenal. In the female, mouse 17β-HSD12 is highly expressed in the clitoral gland, retroperitoneal fat, and mammary gland. Moreover, it is interesting to note that the expression level of this enzyme in the male preputial gland, retroperitoneal fat, and adrenal is much higher than in the female clitoral gland, retroperitoneal fat, and adrenal.

Discussion

In this report, we show that mouse 17β-HSD12 catalyzes efficiently the transformation of C19- as well as C18-steroids, in contrast with the human and primate enzymes that catalyze selectively the transformation of E_1 into E_2. This difference is in part due to the presence of a Leu residue at position 234 in the mouse enzyme. Because of its relatively small size, this amino acid does not exert steric hindrance toward entrance of C19-steroids in the binding site. On the other hand, a bulky Phe residue at this position causes major steric hindrance in both the human and monkey enzymes. Interestingly, human and monkey 17β-HSD3 possess an Ala residue that allows the enzyme to transform efficiently 4-dione into T due to its

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m17β-HSD12    ****MECAPPAAGFLYWVGASTIAYLALRASYSLFRAFQVWCVGNEALVGPRLGEWAVVT  56
h17β-HSD12    ****--S-L-----------G-V------I-----T-LR--G-----G---G--------  56
mk17β-HSD12   ****--S-L-----------G-V------I-----T-LR--G-----G---G--------  56
m17β-HSD3     ****--KLFI---LFVGL*****VC-VKCMRF-QHLFLRFCKALPSSFLRS*M-Q---I-  50
h17β-HSD3     MGDVL-QFFILT-L-VCL*****-C--KCVRF-RCVLLNY-K-LPKSFLRS*M-Q---I-  54
mk17β-HSD3    MGDVL-QFFILT-L-VCL*****-C-VKCMRF-RCILLNY-K-LPKPFLRS*M-Q---I-  54

m17β-HSD12    GGTDGIGKAYAEELAKRGMKIVLISRSQDKLNQVSNNIKEKFNVETRTIAVDFSLDDIYD  116
h17β-HSD12    -S------S-------H---V------K---D---SE-----K----------ASE----  116
mk17β-HSD12   -G------S-----------V------Q---D---SE-----K----------T-E----  116
m17β-HSD3     -AG-------SF---RH-LNV-----TLE--QTIAEE-ERTTGSGVKIVQA--TRE----  110
h17β-HSD3     -AG-------SF------LNV-----TLE--EAIATE-ERTTGRSVKI-QA--TK----E  114
mk17β-HSD3    -AS-------SF---R--LDV-----MLE--QATATE-ERTTGRSVKI-QA--TK----E  114

m17β-HSD12    KIKTGLSGLEIGVLVNNVGMS*YEYPEYFLEIPDLDNTIKKLININVLSVCKVTRLVLPG  175
h17β-HSD12    ------A-----I--------*--------DV-----V---M----I-----M-Q-----  175
mk17β-HSD12   ------A-----I--------*--------DV-----V---M----I-----M-Q-----  175
m17β-HSD3     H--EH-E---N-I-------LPSFF-SH--SSSGE***SQN--HC-IT--V-M-Q---KH  167
h17β-HSD3     H--EK-A-----I-------LPNLL-SH--NA--E***-QS--HC-IT--V-M-Q-I-KH  171
mk17β-HSD3    H--EK-T-----I-------LPNLL-SH--NA--E***-QSV-HC-IT--V-M-Q-I-KH  171

m17β-HSD12    MVERSKGVILNISSASGMLPVPLLTIYSATKAFVDFFSQCLHEEYKSKGIFVQSVMPYLV  235
h17β-HSD12    -------A------G---------------T-------------R---V-----L--F-  235
mk17β-HSD12   -------A------G---F-----------T-------------R---------L--F-  235
m17β-HSD3     -ES-R--L------GAALR-W--YSL---S----YT--KA-SV--RD---II-VLT--SI  227
h17β-HSD3     -ES-Q--L------GIALF-W--YSM---S----CA--KA-Q----A-EVII-VLT--A-  231
mk17β-HSD3    -ES-R--L------GIALF-W--YSM---S----CT--KA-Q---RA-EVII-VLT--A-  231

m17β-HSD12    ATKLAKIQKPTLDKPSAETFVKSAIKTVGLQTRTTGYVIHSLMGSINSIMPRWMYFKIIM  295
h17β-HSD12    -------R--------P--------------S--N--L--A-----I-NL-S-I-L--V-  295
mk17β-HSD12   -------RK------TP--------------S--N--L--V---W-I-NL-S-I-L--A-  295
m17β-HSD3     S-PMT-YLN*NKMTKT-DE---ESL-Y-TIGAESC-CLA-EIIAI-LNRI-SRIFYSSTA  286
h17β-HSD3     S-AMT-YLNTNVITKT-DE---ESLNY-TIGGE-C-CLA-EILAGFL-LI-A-AFYSGAF  291
mk17β-HSD3    S-AMT-YLNTNVITKT-DE--QESLNY-TIGGE-C-CLA-EILAGFL-LI-A-AFYSGAF  291

m17β-HSD12    G*FSKSLRNRYLKKRKKN*  312
h17β-HSD12    N*MN--T-AH----T---*  312
mk17β-HSD12   N*MN-AT-VH----I---*  312
m17β-HSD3     QR-LLTRYSD---RNIS-R  305
h17β-HSD3     QRLLLTHYVA---LNT-VR  310
mk17β-HSD3    QRLLLTHYVA---LNT-VR  310
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Figure 1 Amino acid sequence comparison of the mouse, human, and monkey 17β-HSD12 and 17β-HSD3. The amino acid sequences of the mouse 17β-HSD12 and 17β-HSD3 are aligned with their primate homologs. Amino acid sequences are presented in conventional single letter code and numbered on the right. Dashes represent identical amino acids, while asterisks (*) show the gaps resulting from the alignment. The conserved sequences for the active (YXXXK) and cofactor-binding (GXXXGXG) sites of the NADPH-preferring SDR proteins (Mazza *et al.* 1998) as well as the amino acid number 234 suspected for the substrate specificity in the 17β-HSD12 are underlined.

smaller size. However, the molecular cause of 17β-HSD3 inability to metabolize C18-steroids is still unknown.

The mRNA tissue distribution analysis performed by quantitative real-time PCR indicates that mouse 17β-HSD12 is distributed ubiquitously in numerous sexual tissues and adrenal gland, thus suggesting a central role in androgen and estrogen synthesis in this organism. Adipose tissue is now considered as an important endocrine organ, which is a major site for metabolism of sex steroids (Kershaw & Flier 2004, Blouin *et al.* 2006) and interestingly, the retroperitoneal fat

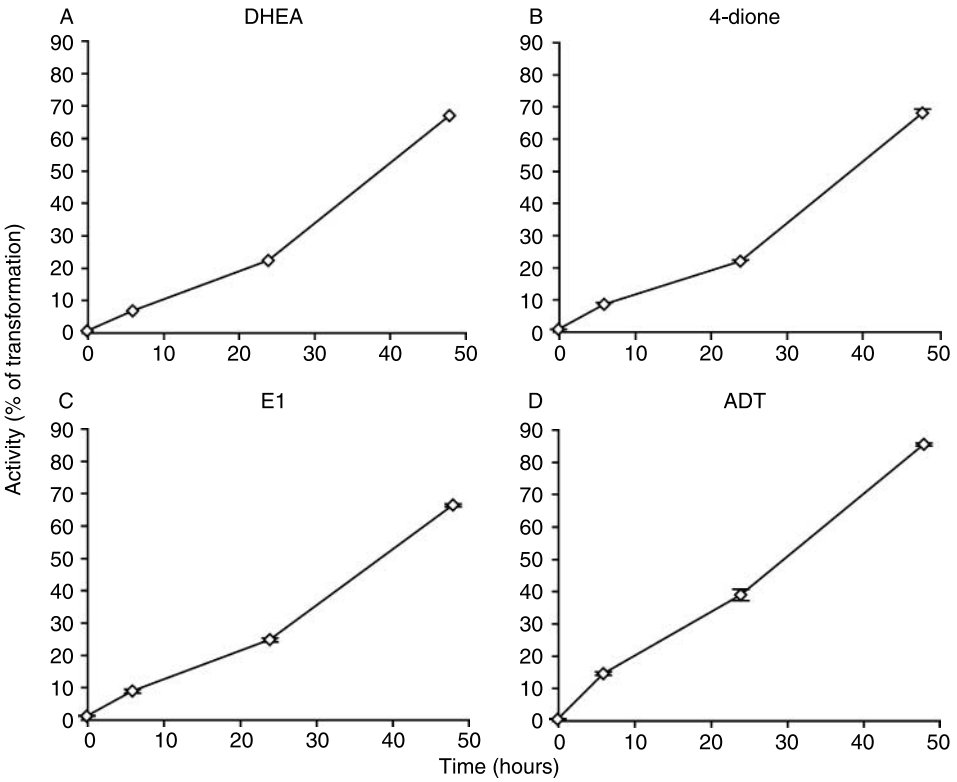

Figure 2 Substrate specificity of HEK-293 cells stably transfected with mouse 17β-HSD12. Different (^{14}C)-labeled steroids (DHEA (A), 4-dione (B), E_1 (C) and ADT (D)) were added to freshly changed culture medium at a concentration of 0·1 μM. After 0, 6, 24, and 48 h of incubation, the media were collected to extract steroids. Metabolites were quantified as described under Materials and Methods. Non-transfected HEK-293 cells were used as controls. The data are expressed as mean ± S.E.M. of triplicate measurements.

exhibits a high level of 17β-HSD12 mRNA. This finding emphasizes the importance of enzyme in numerous steroidogenic organs.

The different characteristics of mouse and human steroidogenic enzymes strongly suggest that sex steroid biosynthesis and their modes of action are very different in these two species. The mouse adrenal does not express P450c17 (Luu-The *et al*. 2005*a*) and thus does not produce androgen adrenal precursors (DHEA and 4-dione) for the biosynthesis of active androgen and estrogen like the human and primate, which produce large amount of adrenal precursors. Consequently, sex steroids in the mouse are produced almost exclusively by the gonads. Hence, upon gonadectomy, the size of androgen- and estrogen-sensitive organs such as the prostate and uterus is reduced drastically. However, administration of an inactive steroid precursor to gonadectomized mice brings back the prostate and uterus to a normal size. The latter observation indicates that despite the fact that local production of active steroids in peripheral tissues is minimally active in rodents, due to the very low level of adrenal precursors, evolution has already put in place an enzymatic structure necessary for their production. This system becomes highly active in the human and primate that possess high circulating levels of these steroid precursors. Indeed, sex steroid formation in peripheral target tissues (intracrinology) accounts for 40–50% of active androgens in the human prostate (Labrie 1991). Many observations clearly demonstrate that there are

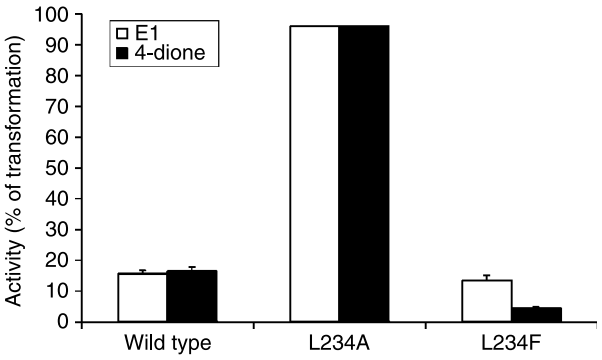

Figure 3 Effects of L234A and L234F amino acid substitutions on mouse 17β-HSD12 activity. HEK-293 cells were transiently transfected with the expression vectors encoding mouse 17β-HSD12 (wild-type) and mutants in which the L at amino acid position 234 has been substituted with either A or F (L234A and L234F respectively). The ability of transfected cells to convert E_1 into E_2 and 4-dione into T was determined as described under Materials and Methods.

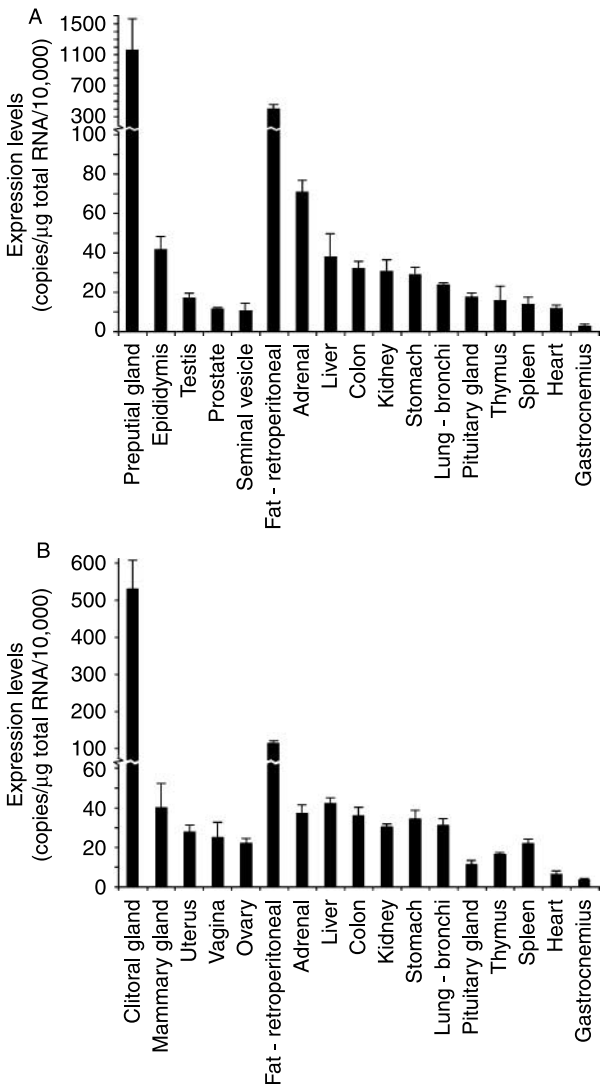

Figure 4 Tissue mRNA expression levels of mouse 17β-HSD12 measured by real-time PCR. The mRNA expression levels were quantified in the indicated (A) male and (B) female tissues using real-time PCR. Real-time PCR was performed as described under Materials and Methods. Results are expressed as mean ± S.E.M. of triplicate measurements.

additional steroid hormone sources other than gonads. For example, in men having their testicles surgically removed or in whom androgen testicular secretion is blocked by treatment with a luteinizing hormone releasing hormone (LHRH) agonist, it is observed that despite a 90–95% decrease of T levels in the blood, intraprostatic DHT concentration is decreased by only 50%. Further evidence lies in the normal development of secondary sexual characteristics in boys deficient in type 2 3β-HSD. In that case, the conversion of DHEA into active steroids via type 1 3β-HSD, expressed in peripheral tissues, is responsible for the apparition of those secondary sexual characteristics. Furthermore, the cloning of multiple genes encoding isoforms of steroidogenic enzymes in the human, namely types 1 and 2 3β-HSD (Luu The *et al.* 1989, Lachance *et al.* 1990, 1991, Rhéaume *et al.* 1991, Rheaume *et al.* 1992), types 1 to 12 17β-HSDs (Peltoketo *et al.* 1988, Luu The *et al.* 1989), as well as types 1 and 2 5α-reductases (Andersson *et al.* 1991, Labrie *et al.* 1992), and 3α-HSDs (Cheng *et al.* 1991, Dufort *et al.* 2001) advocate for the existence of a local biosynthesis in peripheral tissues. During recent years, inhibitors of steroidogenic enzymes have already led to the development of some interesting therapeutic compounds. For example, Proscar (Merck-Frost), an inhibitor of 5α-reductase, has been used to treat successfully androgen-sensitive diseases such as alopecia (Van Neste *et al.* 2000), hirsutism (Fruzzetti *et al.* 1999), and benign prostatic hyperplasia (Ekman 1999). More recently, inhibitors of aromatase have been shown to be highly efficient in breast cancer therapy (Buzdar 2000, Howell *et al.* 2003, Goss & Strasser-Weippl 2004). The gene encoding aromatase is a single gene having many alternative promoters allowing a tissue-specific expression of different transcripts encoding the same protein (Simpson *et al.* 1994, 1997). On the other hand, estrogenic 17β-HSDs (types 1, 7, and 12 17β-HSD) possess very different primary structures, sharing only ~20% amino acids identity. These enzymes could represent more tissue-specific drug targets than aromatase for the treatment of estrogen-sensitive diseases such as breast cancers.

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