

# Human Types 1 and 3 3 $\alpha$ -Hydroxysteroid Dehydrogenases: Differential Lability and Tissue Distribution\*

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## ABSTRACT

3 $\alpha$ -Hydroxysteroid dehydrogenases (3 $\alpha$ -HSDs) catalyze the conversion of 3-ketosteroids to 3 $\alpha$ -hydroxy compounds. The best known 3 $\alpha$ -HSD activity is the transformation of the most potent natural androgen, dihydrotestosterone, into 5 $\alpha$ -androstan-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ -diol), a compound having much lower activity. Previous reports show that 3 $\alpha$ -HSDs are involved in the metabolism of glucocorticoids, progestins, prostaglandins, bile acid precursors, and xenobiotics. 3 $\alpha$ -HSDs could, thus, play a crucial role in the control of a series of active steroid levels in target tissues. In the human, type 1 3 $\alpha$ -HSD was first identified as human chordecone reductase. Recently, we have isolated and characterized type 3 3 $\alpha$ -HSD that shares 81.7% identity with human type 1 3 $\alpha$ -HSD. The transfection of vectors expressing types 1 and 3 3 $\alpha$ -HSD in transformed human embryonic kidney (HEK-293) cells indicates that both enzymes efficiently catalyze the transformation of dihydrotestosterone into 3 $\alpha$ -diol in intact cells.

However, when the cells are broken, the activity of type 3 3 $\alpha$ -HSD is rapidly lost, whereas the type 1 3 $\alpha$ -HSD activity remains stable. We have previously found that human type 5 17 $\beta$ -HSD which possesses 84% and 86% identity with types 1 and 3 3 $\alpha$ -HSD, respectively, is also labile, whereas rodent enzymes such as mouse type 5 17 $\beta$ -HSD and rat 3 $\alpha$ -HSD are stable after homogenization of the cells. The variable stability of different enzymatic activities in broken cell preparations renders the comparison of different enzymes difficult. RNA expression analysis indicates that human type 1 3 $\alpha$ -HSD is expressed exclusively in the liver, whereas type 3 is more widely expressed and is found in the liver, adrenal, testis, brain, prostate, and HaCaT keratinocytes. Based on enzymatic characteristics and sequence homology, it is suggested that type 1 3 $\alpha$ -HSD is an ortholog of rat 3 $\alpha$ -HSD while type 3 3 $\alpha$ -HSD, which must have diverged recently, seems unique to human and is probably more involved in intracrine activity. (*J Clin Endocrinol Metab* 86: 841–846, 2001)

3 $\alpha$ -HYDROXYSTEROID DEHYDROGENASES (3 $\alpha$ -HSDs) are members of the aldo-keto reductase (AKR) family and catalyze the conversion of 3-ketosteroids to the corresponding 3 $\alpha$ -hydroxy compounds (1) using NADPH as the cofactor. The best known activities of 3 $\alpha$ -HSDs are the transformation of the most potent natural androgen dihydrotestosterone (DHT) into its much less active form, 5 $\alpha$ -androstan-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ -diol), and the transformation of 5 $\alpha$ -pregnane-3,20-one into 5 $\alpha$ -pregnane-3 $\alpha$ -ol-20-one or allopregnanolone, a naturally occurring neuroactive steroid. It has been reported (2) that this neurosteroid can act as a modulator of reproductive functions by suppressing the release of the hypothalamic GnRH in female rats. This compound can also alter GABA<sub>A</sub> receptor function (3–5). 3 $\alpha$ -HSDs could also be involved in the metabolism of various 3-keto steroids including progestins, glucocorticoids, and bile acid precursors (6, 7).

The enzymatic activity has been found in various mammalian tissues including the liver (8), prostate (9), brain (10), and epididymis (11). In the human, two types of 3 $\alpha$ -HSD have been isolated (12–14), chronologically named type 1 and

type 3 3 $\alpha$ -HSD (13, 14). Type 2 3 $\alpha$ -HSD (13, 15) is now recognized as type 5 17 $\beta$ -HSD (16) because its ability to transform 4-androsten-3,17-dione (4-dione) into testosterone (Testo) in intact transfected cells in culture is much higher than the transformation of DHT into 3 $\alpha$ -diol. According to the new nomenclature for the AKR family (17), human types 1 and 3 3 $\alpha$ -HSD, 20 $\alpha$ -HSD, and type 5 17 $\beta$ -HSD were named AKR1C4, AKR1C2, AKR1C1, and AKR1C3, respectively. On the other hand, it is also well recognized that type 5 17 $\beta$ -HSD is responsible for the formation of androgens in women (18, 19) because of the absence of type 3 17 $\beta$ -HSD in the ovary (19, 20) and the presence of type 5 17 $\beta$ -HSD in the theca cell layer (21).

It is noteworthy that, although types 1 and 3 3 $\alpha$ -HSD share very high homology with type 5 17 $\beta$ -HSD (84% and 86% amino acids identity, respectively) and 20 $\alpha$ -HSD (84% and 97.8%), these enzymes possess very different substrate selectivity. Of particular interest, type 3 3 $\alpha$ -HSD and 20 $\alpha$ -HSD differ only by seven amino acids but they exert their activities at diametrically opposite positions on the steroid nucleus and their respective substrates possess completely different hormonal actions. Indeed, type 3 3 $\alpha$ -HSD inactivates DHT, a male hormone, whereas 20 $\alpha$ -HSD inactivates progesterone, a female hormone.

Numerous members of the AKR family are dihydrodiol dehydrogenases (22, 23) and have a tim-barrel structure (24, 25). In fact, the most studied members and the first to be crystallized are aldose reductases (24) that catalyze the reduction of polyol sugars. It was, thus, expected that types 1 and 3 3 $\alpha$ -HSD, type 5 17 $\beta$ -HSD, and 20 $\alpha$ -HSD possess di-

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hydrodiol dehydrogenase (DD) activity, which led to their labeling as DD4, DD2, DDX, and DD1 (17), respectively. Indeed, these enzymes are able to catalyze the transformation of exogenous diol compounds such as benzenediol (26) and polycyclic aromatic hydrocarbons (27) and show high affinity for bile acids (7). It is, thus, suggested that these multifunctional proteins could be involved in the detoxification of chemical carcinogens and xenobiotics and in the transportation of bile acids in the liver. However, they also efficiently catalyze the transformation of endogenous compounds such as prostaglandins (28) and steroids (13–16, 29) and are involved in the metabolism of these compounds in the target tissues.

The recent cloning and characterization of the distinct members of this highly homologous family (6, 7, 12–14, 16, 29) have shown that these enzymes are very selective. In the rat, only one type of 3 $\alpha$ -HSD has been cloned, overexpressed, and crystallized, whereas, in the human, two types have been identified. Here, we describe the differential characteristics of these two human enzymes.

### Materials and Methods

#### Construction of human types 1 and 3 3 $\alpha$ -HSD expression vectors

The human type 3 3 $\alpha$ -HSD complementary DNA (cDNA) was obtained as described previously (14), whereas type 1 3 $\alpha$ -HSD cDNA was amplified in a human liver cDNA library (CLONTECH Laboratories, Inc. Palo Alto, CA) using PCR and the oligoprimers pair 5'-GGA-ATTCGT-GAC-AGG-GAA-TGG-ATT-CCA-AAC-AG-3' and 5'-GGA-ATTCCTT-TCT-GGA-CCA-TGG-ATA-TC-3', derived from the sequence described by Khanna *et al.* (13). The resulting cDNA fragments were subcloned into a pCMVneo expression vector to generate stably transfected HEK-293 cells, as described below. Plasmid DNA was prepared using the QIAGEN Mega Kit (QIAGEN, Chatsworth, CA). Double-stranded DNA was sequenced according to the dideoxy chain termination method (30).

#### Stable expression in HEK-293 cells

The isolation of stable transfectant HEK-293 cells was performed as described previously (16). Briefly, cells were cultured in 6-well falcon flasks to  $\sim 3 \times 10^5$  cells/well in DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% (vol/vol) FCS (HyClone Laboratories, Inc., Logan, UT) at 37 C under a 95% air-5% CO<sub>2</sub> humidified atmosphere. Five micrograms of pCMVneo-h3 $\alpha$ -HSD1 or pCMVneo-h3 $\alpha$ -HSD3 plasmid was transfected using a lipofectin transfection kit (Life Technologies, Inc., Burlington, Ontario, Canada). After a 6-h incubation at 37 C, the transfection medium was removed and 2 mL DMEM were added. Cells were further cultured for 48 h and then transferred into 10-cm Petri dishes and cultured in DMEM containing 700  $\mu$ g/mL of G-418 to inhibit the growth of nontransfected cells. The medium containing G-418 was changed every 2 days until resistant colonies were observed.

#### Assay of enzymatic activity

The determination of the activities was performed in intact cells as described previously (31). Briefly, 0.1  $\mu$ M of the [<sup>14</sup>C]-labeled steroid (Dupont Inc., Mississauga, Ontario, Canada) was added to freshly changed culture medium in a 24-well culture plate. After incubation, the steroids were extracted twice with 1 mL ether. The organic phases were pooled and evaporated to dryness. The steroids were then solubilized in 50  $\mu$ L dichloromethane, applied to Silica gel 60 thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany), before separation by migration in the toluene-acetone (4:1) 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). The enzymatic activity in broken cells was determined using the same amount of cells as described above after three freezing-thawing cycles. The cells were incubated in a final volume of 1 mL containing 50 mM sodium phosphate (pH 7.4), 20% glycerol, 1 mM EDTA, 1 mM NADPH, and 0.1  $\mu$ M [<sup>14</sup>C]-labeled steroid (Dupont Inc.). The incubation, extraction, separation, and measurement of the products and substrates were carried out as described above.

#### Types 1 and 3 3 $\alpha$ -HSD messenger RNA (mRNA) expression analysis

Poly(A)<sup>+</sup> RNA (0.1  $\mu$ g) from human prostate, brain, testis, liver, and adrenals (Stratagene, La Jolla, CA) were reversed transcribed using a poly (T) primer. The cDNA products were amplified using pairs of specific oligoprimers for type 1 3 $\alpha$ -HSD (5'-GTGGCAAGCAATG-GATCCCAAATAT-3' and 5'-TTTCTGGACCATGGATATC-3') and type 3 3 $\alpha$ -HSD (5'-GTGAACAGAAATGGATTCGAAATAC-3' and 5'-GATGGCTTAGCTGTAGCTT-3') and 30 cycles of PCR. The mRNA expression levels for HaCaT cells were determined as described above, except for the specific pair of oligoprimers for type 1 3 $\alpha$ -HSD (5'-TTTCTGGACCATGGATATCTAG-3' and 5'-CCCAAACCTCCCACTTCTTTGG-3') and type 3 3 $\alpha$ -HSD (5'-GATGGCTTAGCTGTAGCTT-3' and 5'-CCGAACTCCCGGTGCTCTTGG-3'). The amplified DNA products were separated on a 1% agarose gel and stained with ethidium bromide.

### Results

#### Differential expression of types 1 and 3 3 $\alpha$ -HSD

As mentioned above, types 1 and 3 3 $\alpha$ -HSD are highly homologous enzymes and share 81.7% amino acid sequence identity. These enzymes belong to the AKR family and are highly homologous to rat 3 $\alpha$ -HSD (6, 32), human (13, 15, 16) and mouse (33) type 5 17 $\beta$ -HSD, as well as to human (29), rat (34–36), rabbit (37), and bovine (38) 20 $\alpha$ -HSD (Fig. 1). Because of the high level of identity, Northern blot analysis could not distinguish these different mRNAs. Using RT-PCR and oligoprimers specific to types 1 and 3 3 $\alpha$ -HSD, we were able to specifically detect the mRNA expression level of these two enzymes. As shown in Fig. 2, type 1 3 $\alpha$ -HSD is exclusively expressed in the liver, whereas type 3 3 $\alpha$ -HSD is found in several tissues including liver, prostate, adrenal, brain, testis, and keratinocyte cell line HaCaT.

#### Lability of human types 1 and 3 3 $\alpha$ -HSD

To further characterize types 1 and 3 3 $\alpha$ -HSD, we compared the activity of the expressed enzymes in intact transfected cells in culture, with that measured in cell lysates. As shown in Fig. 3, although human type 1 3 $\alpha$ -HSD remains stable after breaking the cells, the human type 3 3 $\alpha$ -HSD is labile. This phenomenon has been previously observed for other members of this family, namely the mouse and human type 5 17 $\beta$ -HSDs (16). In fact, we had a 40% loss of activity of type 3 3 $\alpha$ -HSD during the breaking of the cells, and the 2-h incubation period compares to the level observed in the intact cells assay. In contrast, the activity of the type 1 3 $\alpha$ -HSD in the same treatment shows a tendency for higher levels than that found using intact cells. This is probably due to a higher concentration of the exogenous NADPH cofactor used in the reaction compared with the intact cell situation.

#### Efficiency of human types 1 and 3 3 $\alpha$ -HSD

Because type 3 3 $\alpha$ -HSD is more labile than type 1 on breaking the cells, we used intact HEK-293 cells stably trans-



FIG. 1. Alignment of amino acid sequence of 3 $\alpha$ -HSDs and other HSD members of the AKR family. The deduced amino acid sequence of human (h) type 3 3 $\alpha$ -HSD was aligned with amino acid sequences of human type 1 3 $\alpha$ -HSD, human and mouse (m) type 5 17 $\beta$ -HSD as well as rat (r) 3 $\alpha$ -HSD and human, rabbit (rb), rat, and bovine (b) 20 $\alpha$ -HSD. Amino acid sequences are presented in the conventional single letter code and numbered on the right. Dashes and dots represent identical and missing amino acid residues, respectively.

fectured with the human types 1 and 3 3 $\alpha$ -HSDs to assess the efficiency of their enzymatic activity. As shown in Fig. 4, types 1 and 3 efficiently catalyze the reduction of DHT to 3 $\alpha$ -diol. However, type 1 3 $\alpha$ -HSD shows a higher rate.

This result is confirmed by the determination of the affinity constant values of the two enzymes for DHT. As shown in Table 1, the  $V_{max}/K_m$  value of type 1 3 $\alpha$ -HSD is 8-fold higher and is, thus, a more efficient enzyme than the type 3 enzyme to transform DHT into 3 $\alpha$ -diol.

*Substrate specificity of types 1 and 3 3 $\alpha$ -HSD*

As seen in Fig. 5, types 1 and 3 3 $\alpha$ -HSD show similar substrate specificity profiles. Both enzymes catalyze most efficiently the transformation of DHT into 3 $\alpha$ -diol. Both enzymes also possess a 20 $\alpha$ -HSD activity that catalyzes the transformation of progesterone into 20 $\alpha$ -hydroxyprogesterone. For both enzymes, this activity represents approximately two thirds of the 3 $\alpha$ -HSD activity, whereas the 17 $\beta$ -HSD activity represents about 7% of the 3 $\alpha$ -HSD activity. The transformation of estrogens, on the other hand, is negligible.

**Discussion**

The present study shows that, although types 1 and 3 3 $\alpha$ -HSDs possess the same substrate specificity and 81.7% amino acid sequence identity, these two enzymes possess several different characteristics. In fact, type 1 3 $\alpha$ -HSD is a stable enzyme, its activity remaining high in cell lysates, whereas the activity of type 3 3 $\alpha$ -HSD rapidly decreases. The lability of type 3 3 $\alpha$ -HSD is, however, much less pronounced than that of type 5 17 $\beta$ -HSD (16), another enzyme of the AKR family, which shares 84% and 86% identity with types 1 and 3 3 $\alpha$ -HSD. Indeed, type 5 17 $\beta$ -HSD readily loses more than 90% of its activity on homogenization of the cells (16).

The reason why some members of this enzyme family are

labile, such as observed for human type 5 17 $\beta$ -HSD (16), 20 $\alpha$ -HSD (Luu-The, V., unpublished results), human type 3 3 $\alpha$ -HSD (this study), and monkey type 5 17 $\beta$ -HSD (18), whereas others are stable [for example, mouse type 5 17 $\beta$ -HSD (16, 39), rat 3 $\alpha$ -HSD, and human type 1 3 $\alpha$ -HSD (this study)], is still unknown. It could be related to the fact that members of this gene family have been duplicated relatively recently and are in the process of fixation or elimination. Indeed, members of this family possess a high percentage of identity (>80%) but different substrate specificity patterns. Members of this enzyme family have been named 3 $\alpha$ -HSD (10, 14), 17 $\beta$ -HSD (7, 16), 20 $\alpha$ -HSD (14, 29), and 5 $\beta$ -reductase (17), according to the main activity that has been characterized for each of them. Although 5 $\beta$ -reductase activity does not involve the aldo and keto groups, 5 $\beta$ -reductase has been classified in the AKR family by homology (17). Furthermore, it has been shown that, in the rat 3 $\alpha$ -HSD, a single mutation of His 117 to Glu confers 5 $\beta$ -reductase activity to this enzyme (40). This confirms the high potential versatility of activities of this family.

It is noteworthy that, in the human, it was found that types 1 and 3 3 $\alpha$ -HSD as well as type 5 17 $\beta$ -HSD possess nonnegligible 20 $\alpha$ -HSD activity. It has been suggested that the high 20 $\alpha$ -HSD activity found in type 5 17 $\beta$ -HSD protects male organs (16) or androgen-producing cells (18) against female hormones. Similarly, the 20 $\alpha$ -HSD activity in types 1 and 3 3 $\alpha$ -HSD could serve to inactivate progesterone in addition to the 3 $\alpha$ -HSD activity that inactivates DHT. This could also represent an additional way to inactivate glucocorticoids and mineralocorticoids. Another striking example of diversity is the fact that type 3 3 $\alpha$ -HSD and 20 $\alpha$ -HSD possess 97.8% amino acid identity but show different substrate selectivity (14). Such a high identity percentage suggests that type 3 3 $\alpha$ -HSD and 20 $\alpha$ -HSD were separated, in evolutionary

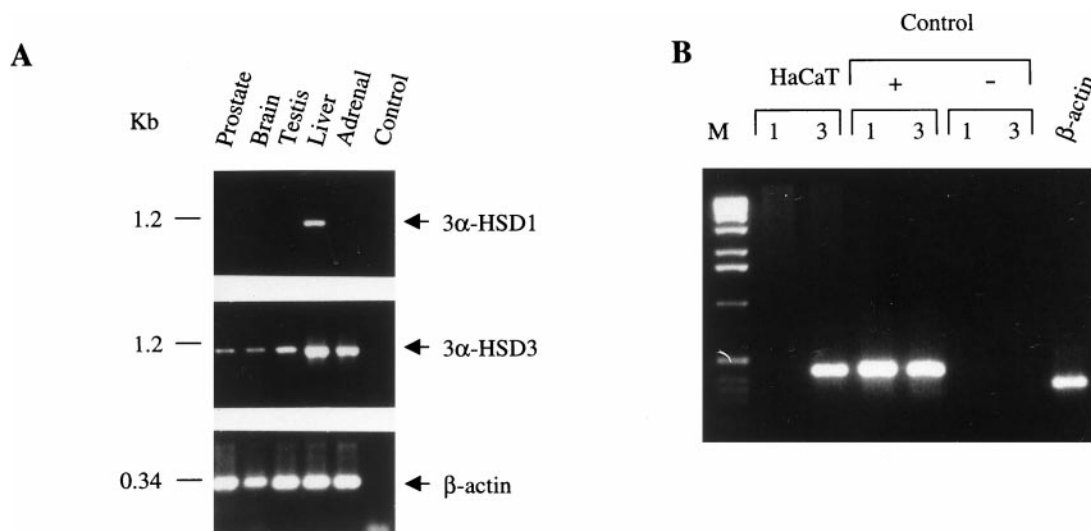


FIG. 2. Tissue distribution of human types 1 and 3  $3\alpha$ -HSD mRNA. Specific oligonucleotide primers for types 1 and 3  $3\alpha$ -HSD were used to perform RT-PCR with commercial poly(A)<sup>+</sup> RNA from human liver, prostate, adrenal, brain, and testis (CLONTECH Laboratories, Inc.; A) and poly(A)<sup>+</sup> RNA from the HaCaT keratinocytes (B). Experimental procedures were performed as described in *Materials and Methods*.

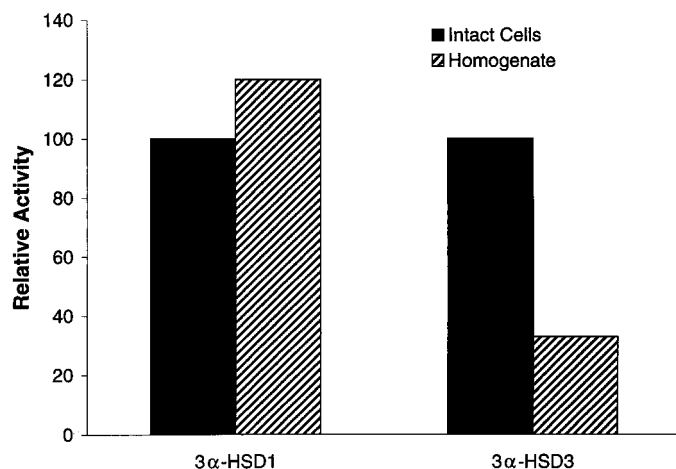


FIG. 3. Activity of human types 1 and 3  $3\alpha$ -HSD in intact and broken transfected cells. Enzymatic activity of expressed cDNAs encoding human types 1 and 3  $3\alpha$ -HSD in intact transfected HEK-293 cells in culture (■) and in broken cells (▨) was compared using 0.1  $\mu$ M [<sup>14</sup>C]-DHT as substrate. Incubation, extraction, separation on TLC, and quantification of steroids were performed as described in *Materials and Methods*.

terms, very recently and that it is likely that one of them does not have an ortholog in other species.

To obtain a better illustration of this hypothesis (Fig. 6), we localized the divergence period of these human AKR genes on a molecular clock scale according to their amino acid sequence identity and the evolution model described by Ayala (41). Taking into account that the average homology between human and rodents, the most widely used laboratory animals, is 70–80% whereas it is about 90–95% between the human and monkey, we suggest that type 1  $3\alpha$ -HSD and type 5  $17\beta$ -HSD diverged at the period of separation of primates from rodents and could have ortholog genes in these species. On the other hand, type 3  $3\alpha$ -HSD is likely to have appeared very recently and is probably unique to the human. A similar example is the  $3\beta$ -HSD family: human

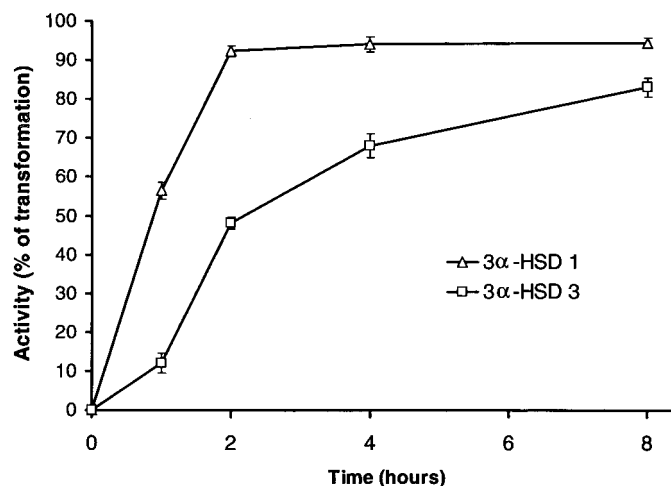


FIG. 4. Comparison of types 1 and 3  $3\alpha$ -HSD activity. Types 1 ( $\Delta$ ) and 3 ( $\square$ )  $3\alpha$ -HSD activity was determined in intact transfected cells in culture using 0.1  $\mu$ M [<sup>14</sup>C]-labeled DHT as substrate. The error bar indicates mean of duplicate. Similar results have been obtained in three different experiments. Incubation, extraction, separation on TLC, and quantification of steroids were performed as described in *Materials and Methods*.

TABLE 1. Kinetic parameters of human types 1 and 3  $3\alpha$ -HSD

Enzyme	$K_m$ ( $\mu$ M)	$V_{max}$ (nmol product/h/ 10 <sup>6</sup> cells)	$V_{max}/K_m$ (ml/h/10 <sup>6</sup> cells)
Type 1 $3\alpha$ -HSD	0.8 $\pm$ 0.2	32.4 $\pm$ 1.8	40.5
Type 3 $3\alpha$ -HSD	1.4 $\pm$ 0.3	7.2 $\pm$ 1.2	5.1

Kinetic constants were determined using HEK-293 cells stably expressing human types 1 and 3  $3\alpha$ -HSD and [<sup>14</sup>C]-DHT as substrate.

types 1 (42) and  $23\beta$ -HSD (43) share 93.8% identity and likely diverged from one another after the separation from rodents. This could be the reason why (18), although six types of  $3\beta$ -HSD have been found in the mouse and rat, none of these shows the characteristics of human type 2  $3\beta$ -HSD.

Types 1 and 3  $3\alpha$ -HSD also show very different tissue

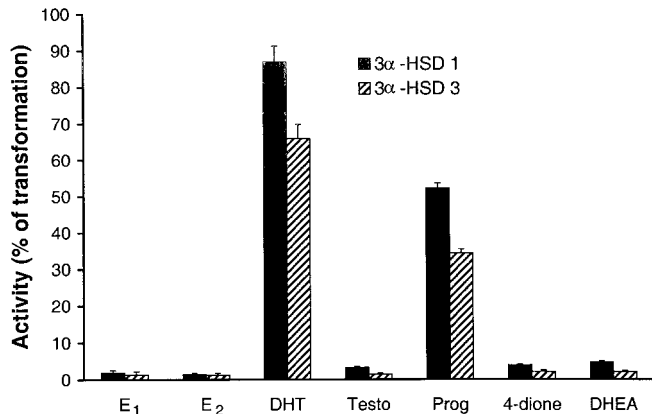


FIG. 5. Substrate specificity of types 1 and 3 3 $\alpha$ -HSD. Activities catalyzed by types 1 (■) and 3 (▨) 3 $\alpha$ -HSD were determined in intact transfected cells in culture using 0.1  $\mu$ M [<sup>14</sup>C]-labeled substrates. E<sub>1</sub>, Conversion of estrone to estradiol; E<sub>2</sub>, conversion of estradiol to estrone; DHT, conversion of DHT to 3 $\alpha$ -diol; Testo, conversion of Testo to 4-dione; Prog, conversion of progesterone to 20 $\alpha$ -hydroxyprogesterone; 4-dione, conversion of 4-dione to Testo and DHEA; DHEA, conversion of DHEA to 5-androstenediol. The error bar indicates mean of duplicate. Similar results have been obtained in three different experiments. Incubation, extraction, separation on TLC, and quantification of steroids were performed as described in *Materials and Methods*.

distribution patterns. Type 1 3 $\alpha$ -HSD (AKR1C4), also known as chordecone reductase, is only found in the liver where it probably plays an important role in the detoxification of exogenous compounds containing a keto-group, such as xenobiotics, environment pollutants, and drugs. Type 1 3 $\alpha$ -HSD reduces a keto group into a hydroxy group that can be conjugated by sulfotransferase or glucuronosyl transferase to facilitate the secretion or elimination of the compound. On the other hand, because it is only found in the liver, type 1 3 $\alpha$ -HSD could play a role in maintaining the homeostasis of steroid hormones by converting 3-keto steroids to more polar 3 $\alpha$ -hydroxy compounds. Human type 1 3 $\alpha$ -HSD likely is an ortholog of the rat 3 $\alpha$ -HSD.

Type 3 3 $\alpha$ -HSD is more widely expressed than type 1 3 $\alpha$ -HSD. This enzyme is found in many peripheral tissues that produce active steroids, such as the prostate, the skin, the adrenals, and the brain. It is, thus, likely that type 3 3 $\alpha$ -HSD plays a role in the intracrine control of active hormone levels in these tissues, especially DHT in the prostate, allopregnanolone in the brain, and glucocorticoids and mineralocorticoids in the adrenals. In the liver, type 3 3 $\alpha$ -HSD could play a role in the metabolism of polycyclic aromatic hydrocarbons (44) and in bile acid transport (45).

The human body produces high levels of circulating DHEA that increase during the adrenarche in children between the ages of 6 and 8 yr. Elevated values of circulating DHEA-sulfate are maintained throughout adulthood, thus providing the high concentration of substrate required for the formation of potent androgens and estrogens in peripheral tissues, depending on the predominance of androgen- or estrogen-synthesizing enzymes in those tissues (46). On the other hand, the adrenals of laboratory animals, except primates, do not produce significant amounts of DHEA. Most sex steroids in these animals are produced by the gonads, a

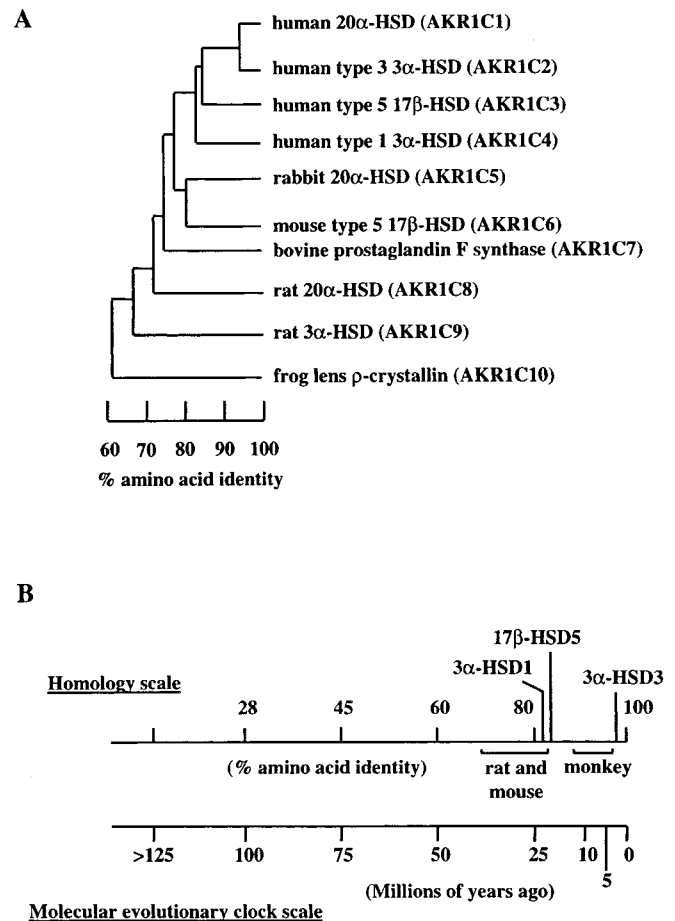


FIG. 6. A, Dendrogram representing a cluster analysis of steroid-metabolizing members of the AKR family. B, Hypothetical scheme based on homology, showing on a molecular evolutionary clock based on the divergence periods of members of the human AKR family that metabolize steroids (*top*) and according to various species (*bottom*).

situation similar to the gonads of young children before adrenarche. This hypothesis is in agreement with the hypothesis being presented here, that type 3 3 $\alpha$ -HSD is unique to human and that its role is to control the intracellular level of active steroids in peripheral target tissues. Furthermore, substrate specificities and cellular expression allow each cell type to control intracellular androgen and/or estrogen concentrations according to local needs, a process called intracrinology (46).

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### References

- Talalay P. 1963 Hydroxysteroid dehydrogenases. In: Boyer PD, Lardy H, Myrback K, eds. *The enzymes*, ed 2. New York: Academic Press; 177–202.
- Genazzani AR, Palumbo MA, de Micheroux AA, et al. 1995 Evidence for a role for the neurosteroid allopregnanolone in the modulation of reproductive function in female rats. *Eur J Endocrinol*. 133:375–380.
- Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. 1986 Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science*. 232:1004–1007.
- Majewska MD. 1992 Neurosteroids: endogenous bimodal modulators of the

- GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol.* 38:379–395.
5. Lambert JJ, Belelli D, Hill-Venning C, Peters JA. 1995 Neurosteroids and GABAA receptor function. *Trends Pharmacol Sci.* 16:295–303.
  6. Pawlowski JE, Huizinga M, Penning TM. 1991 Cloning and sequencing of the cDNA for rat liver 3  $\alpha$ -hydroxysteroid/dihydrodiol dehydrogenase. *J Biol Chem.* 266:8820–8825.
  7. Deyashiki Y, Ogasawara A, Nakayama T, et al. 1994 Molecular cloning of two human liver 3  $\alpha$ -hydroxysteroid/dihydrodiol dehydrogenase isoenzymes that are identical with chlordecone reductase and bile-acid binder. *Biochem J.* 299:545–552.
  8. Penning TM, Mukharji I, Barrows S, Talalay P. 1984 Purification and properties of a 3  $\alpha$ -hydroxysteroid dehydrogenase of rat liver cytosol and its inhibition by anti-inflammatory drugs. *Biochem J.* 222:601–611.
  9. Taurog JD, Moore RJ, Wilson JD. 1975 Partial characterization of the cytosol 3  $\alpha$ -hydroxysteroid:NAD(P)<sup>+</sup> oxidoreductase of rat ventral prostate. *Biochemistry.* 14:810–817.
  10. Penning TM, Sharp RB, Krieger NR. 1985 Purification and properties of 3  $\alpha$ -hydroxysteroid dehydrogenase from rat brain cytosol. Inhibition by nonsteroidal anti-inflammatory drugs and progestins. *J Biol Chem.* 260:15266–15272.
  11. Hastings CD, Hansson V. 1979 Physico-chemical characterization of the NADPH dependent soluble 3 $\alpha$ -hydroxysteroid oxidoreductase in the rat epididymis. *Int J Androl.* 2:263–274.
  12. Winters CJ, Molowa DT, Guzelian PS. 1990 Isolation and characterization of cloned cDNAs encoding human liver chlordecone reductase. *Biochemistry.* 29:1080–1087.
  13. Khanna M, Qin KN, Wang RW, Cheng KC. 1995 Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3  $\alpha$ -hydroxysteroid dehydrogenases. *J Biol Chem.* 270:20162–20168.
  14. Dufort I, Soucy P, Labrie F, Luu-The V. 1996 Molecular cloning of human type 3 3  $\alpha$ -hydroxysteroid dehydrogenase that differs from 20  $\alpha$ -hydroxysteroid dehydrogenase by seven amino acids. *Biochem Biophys Res Commun.* 228:474–479.
  15. Lin HK, Jez JM, Schlegel BP, Peehl DM, Pachter JA, Penning TM. 1997 Expression and characterization of recombinant type 2 3  $\alpha$ -hydroxysteroid dehydrogenase (HSD) from human prostate: demonstration of bifunctional 3  $\alpha$ /17  $\beta$ -HSD activity and cellular distribution [published erratum appears in *Mol Endocrinol* 12:1763, 1999]. *Mol Endocrinol.* 11:1971–1984.
  16. Dufort I, Rheault P, Huang XF, Soucy P, Luu-The V. 1999 Characteristics of a highly labile human type 5 17 $\beta$ -hydroxysteroid dehydrogenase. *Endocrinology.* 140:568–574.
  17. Jez JM, Flynn TG, Penning TM. 1997 A new nomenclature for the aldo-keto reductase superfamily. *Biochem Pharmacol.* 54:639–647.
  18. Luu-The V, Dufort I, Pelletier G, Labrie F. Type 5 17 $\beta$ -hydroxysteroid dehydrogenase: its role in the formation of androgens in women. *Mol Cell Endocrinol.* In press.
  19. Qin KN, Rosenfield RL. 2000 Expression of 17  $\beta$ -hydroxysteroid dehydrogenase type 5 in human ovary: a pilot study. *J Soc Gynecol Investig.* 7:61–64.
  20. Zhang Y, Word RA, Fesmire S, Carr BR, Rainey WE. 1996 Human ovarian expression of 17  $\beta$ -hydroxysteroid dehydrogenase types 1, 2, and 3. *J Clin Endocrinol Metab.* 81:3594–3598.
  21. Pelletier G, Luu-The V, Tetu B, Labrie F. 1999 Immunocytochemical localization of type 5 17 $\beta$ -hydroxysteroid dehydrogenase in human reproductive tissues. *J Histochem Cytochem.* 47:731–738.
  22. Worner W, Oesch F. 1984 Identity of dihydrodiol dehydrogenase and 3  $\alpha$ -hydroxysteroid dehydrogenase in rat but not in rabbit liver cytosol. *FEBS Lett.* 170:263–267.
  23. Takikawa H, Stolz A, Sugiyama Y, Yoshida H, Yamanaka M, Kaplowitz N. 1990 Relationship between the newly identified bile acid binder and bile acid oxidoreductases in human liver. *J Biol Chem.* 265:2132–2136.
  24. Wilson DK, Bohren KM, Gabbay KH, Quiocho FA. 1992 An unlikely sugar substrate site in the 1.65 Å structure of the human aldose reductase holoenzyme implicated in diabetic complications. *Science.* 257:81–84.
  25. Hoog SS, Pawlowski JE, Alzari PM, Penning TM, Lewis M. 1994 Three-dimensional structure of rat liver 3  $\alpha$ -hydroxysteroid/dihydrodiol dehydrogenase: a member of the aldo-keto reductase superfamily. *Proc Natl Acad Sci USA.* 91:2517–2521.
  26. Hara A, Taniguchi H, Nakayama T, Sawada H. 1990 Purification and properties of multiple forms of dihydrodiol dehydrogenase from human liver. *J Biochem (Tokyo).* 108:250–254.
  27. Glatt HR, Vogel K, Bentley P, Oesch F. 1979 Reduction of benzo(a)pyrene mutagenicity by dihydrodiol dehydrogenase. *Nature.* 277:319–320.
  28. Ohara H, Nakayama T, Deyashiki Y, Hara A, Miyabe Y, Tsukada F. 1994 Reduction of prostaglandin D2 to 9  $\alpha$ ,11  $\beta$ -prostaglandin F2 by a human liver 3  $\alpha$ -hydroxysteroid/dihydrodiol dehydrogenase isozyme. *Biochim Biophys Acta.* 1215:59–65.
  29. Zhang Y, Dufort I, Rheault P, Luu-The V. 2000 Characterization of a human 20 $\alpha$ -hydroxysteroid dehydrogenase. *J Mol Endocrinol.* 25:221–228.
  30. Sanger F, Nicklen S, Coulson AR. 1977 DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA.* 74:5463–5467.
  31. Luu-The V, Zhang Y, Poirier D, Labrie F. 1995 Characteristics of human types 1, 2 and 3 17  $\beta$ -hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. *J Steroid Biochem Mol Biol.* 55:581–587.
  32. Cheng KC, White PC, Qin KN. 1991 Molecular cloning and expression of rat liver 3  $\alpha$ -hydroxysteroid dehydrogenase. *Mol Endocrinol.* 5:823–828.
  33. Deyashiki Y, Ohshima K, Nakanishi M, Sato K, Matsuura K, Hara A. 1995 Molecular cloning and characterization of mouse estradiol 17  $\beta$ -dehydrogenase (A-specific), a member of the aldo-ketoreductase family. *J Biol Chem.* 270:10461–10467.
  34. Miura R, Shiota K, Noda K, Yagi S, Ogawa T, Takahashi M. 1994 Molecular cloning of cDNA for rat ovarian 20  $\alpha$ -hydroxysteroid dehydrogenase (HSD1). *Biochem J.* 299:561–567.
  35. Mao J, Duan WR, Albarracin CT, Parmer TG, Gibori G. 1994 Isolation and characterization of a rat luteal cDNA encoding 20  $\alpha$ -hydroxysteroid dehydrogenase. *Biochem Biophys Res Commun.* 201:1289–1295.
  36. Zhong L, Ou J, Barkai U, Mao JF, Frasor J, Gibori G. 1998 Molecular cloning and characterization of the rat ovarian 20  $\alpha$ -hydroxysteroid dehydrogenase gene. *Biochem Biophys Res Commun.* 249:797–803.
  37. Lacy WR, Washenick KJ, Cook RG, Dunbar BS. 1993 Molecular cloning and expression of an abundant rabbit ovarian protein with 20  $\alpha$ -hydroxysteroid dehydrogenase activity [published erratum appears in *Mol Endocrinol* 7:1239, 1993]. *Mol Endocrinol.* 7:58–66.
  38. Warren JC, Murdock GL, Ma Y, Goodman SR, Zimmer WE. 1993 Molecular cloning of testicular 20  $\alpha$ -hydroxysteroid dehydrogenase: identity with aldose reductase. *Biochemistry.* 32:1401–1406.
  39. Rheault P, Charbonneau A, Luu-The V. 1999 Structure and activity of the murine type 5 17 $\beta$ -hydroxysteroid dehydrogenase gene(1). *Biochim Biophys Acta.* 1447:17–24.
  40. Jez JM, Penning TM. 1998 Engineering steroid 5  $\beta$ -reductase activity into rat liver 3  $\alpha$ -hydroxysteroid dehydrogenase. *Biochemistry.* 37:9695–9703.
  41. Ayala FJ. 1986 The theory of evolution: the case for randomness in the evolution of DNA and proteins. 'The neutral theory of molecular evolution.' By Motoo Kimura. *Publ Stn Zool Napoli [II].* 8:129–138.
  42. Luu-The V, Lachance Y, Labrie C, et al. 1989 Full length cDNA structure and deduced amino acid sequence of human 3  $\beta$ -hydroxy-5-ene steroid dehydrogenase. *Mol Endocrinol.* 3:1310–1312.
  43. Rheaume E, Lachance Y, Zhao HF, et al. 1991 Structure and expression of a new complementary DNA encoding the almost exclusive 3  $\beta$ -hydroxysteroid dehydrogenase/delta 5- $\delta$  4-isomerase in human adrenals and gonads. *Mol Endocrinol.* 5:1147–1157.
  44. Burczynski ME, Lin HK, Penning TM. 1999 Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. *Cancer Res.* 59:607–614.
  45. Stolz A, Hammond L, Lou H, Takikawa H, Ronk M, Shively JE. 1993 cDNA cloning and expression of the human hepatic bile acid-binding protein. A member of the monomeric reductase gene family. *J Biol Chem.* 268:10448–10457.
  46. Labrie F. 1991 Intracrinology. *Mol Cell Endocrinol.* 78:C113–C118.