

Type 5 17 β -hydroxysteroid dehydrogenase: its role in the formation of androgens in women

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Abstract

Type 5 17 β -HSD, one of the seven types of 17 β -hydroxysteroid dehydrogenase (17 β -HSD) so far characterized in humans, catalyzes the transformation of 4-androstenedione (4-dione) into testosterone (T). This reaction is also catalyzed by type 3 17 β -HSD which is responsible for pseudohermaphroditism in deficient man but is asymptomatic in deficient women. Since type 3 17 β -HSD is not found in the ovary, whereas type 5 is, it is suggested that the latter is involved in the conversion of 4-androstenedione to testosterone in the ovary. The comparison of type 5 17 β -HSD of different species shows that the human enzyme shares 95 and 78% identity with the monkey and mouse enzymes respectively. In addition, the human and monkey enzymes are labile and are destroyed upon homogenization of the transfected cells, whereas the mouse enzyme is not. Human type 5 17 β -HSD also possesses a high 20 α -HSD activity that inactivates progesterone, whereas the monkey and mouse enzymes do not have such high 20 α -HSD activity. Since the endocrine ovary is composed of two types of cells, one producing androgens (theca cells) and the other producing progesterone and estrogens (granulosa cells), it is tempting to suggest that the role of the high 20 α -HSD activity of type 5 17 β -HSD is to protect the theca cells against the progesterone produced by the granulosa cells. The double activity of type 5 17 β -HSD in the female reproductive tissues is probably necessary to the control of the optimal level of progesterone and sex steroids. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Seventeen beta-hydroxysteroid dehydrogenases are enzymes that catalyze the reduction of 17-keto- and/or the oxidation of 17 β -hydroxysteroids and thus play an important role in the control of active sex steroid levels, namely testosterone (T), dihydrotestosterone (DHT), 5-androstenediol (5-diol) and estradiol (E2), in target tissues. The molecular cloning of enzymes that exert 17 β -HSD activity has allowed the identification to up to nine types of 17 β -HSDs (Adamski et al., 1995; Ando et al., 1996; Biswas and Russell, 1997; Deyashiki et al., 1995; Dufort et al., 1999; Fornitcheva et al., 1998; Geissler et al., 1994; Krazeisen et al., 1999; Luu-The et al., 1989a; Mustonen et al., 1997; Nokelainen et al., 1998, 1996; Normand et al., 1995; Peltoketo et al., 1988, 1999; Rheault et al., 1999; Sha et al., 1997; Su et

al., 1999; Wu et al., 1993), named in the chronological order of their discovery.

The human counterparts of types 6, 8 and 9 17 β -HSD, however, have not yet been described. It is noteworthy that these enzymes only have ~20% homology, and thus possess totally different protein structures, although they catalyze the transformation of similar substrates. The 17 β -HSDs are almost members of the short chain alcohol dehydrogenases family, except type 5 which belongs to the aldo-keto reductase family (Deyashiki et al., 1995; Dufort et al., 1999; Rheault et al., 1999). Like almost all other dehydrogenases, 17 β -hydroxysteroid dehydrogenases are considered to be reversible enzymes, able to catalyze the interconversion of substrates and products. It is now well recognized that 17 β -HSDs catalyze their reaction mainly in an unidirectional manner (Dufort et al., 1999; Labrie et al., 1997; Luu-The et al., 1995): types 1 (Dumont et al., 1992), 4 3 (Geissler et al., 1994), 5 (Dufort et al., 1999; Rheault et al., 1999), and 7

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(Krazeisen et al., 1999; Nokelainen et al., 1998) catalyze the reduction of 17-ketosteroids, while types 2 (Wu et al., 1993), 4 (Adamski et al., 1995), 6 (Biswas and Russell, 1997) and 8 (Fornitcheva et al., 1998) catalyze the oxidation of 17 β -hydroxysteroids. Since sex steroids are active in the 17 β -hydroxy configuration and inactive in the 17-keto configuration, types 1, 3, 5, and 7 are activating enzymes, while types 2, 4, 6, and 8 are inactivating enzymes. These enzymes show high substrate selectivity. Types 1 and 7 17 β -HSD catalyze the formation of active estrogens while types 3 and 5 catalyze the formation of androgens.

The most widely known 17 β -HSD is the testicular enzyme which catalyzes the transformation of 4-dione into T. This testicular enzyme is ascribed to type 3 17 β -HSD (Geissler et al., 1994). Its impairment leads to male pseudohermaphroditism. However, in contrast to man, its deficiency in women is asymptomatic (Mendonca et al., 1999).

It is interesting to note that types 3 and 5 17 β -HSD possess similar substrate specificity and that both catalyze the transformation of 4-dione into T, which is a crucial step in the biosynthesis of sex steroids. Since type 3 17 β -HSD is not expressed in the ovary (Zhang et al., 1996), the source of androgens in women, it is likely that this role is filled by type 5 17 β -HSD Qin and Rosenfield, 2000). In this report, we compare the characteristics of human, monkey and mouse type 5 17 β -HSD and describe the localization of this enzyme in the ovary.

2. Materials and methods

2.1. Stable expression of human, mouse and monkey type 5 17 β -HSD in transformed human embryonic kidney (HEK-293) cells

Cells were cultured in 6-well falcon flasks to approximately 3×10^5 cells/well in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% (vol./vol.) calf fetal serum (Hyclone, Logan, UT) at 37°C under a 95% air, 5% CO₂ humidified atmosphere. A total of 5 μ g of pCMVneo-m17 β -HSD5 or pCMVneo-m17 β HSD5 (Dufort et al., 1999) or pCVMneo-mk17 β -HSD5 (Luu-The et al., unpublished results) plasmids were transfected using a lipofectin transfection kit (Life Technologies, Burlington, ON, Canada), as described (Dufort et al., 1999). After 6h incubation at 37°C, the transfection medium was removed and 2 ml of DMEM were added. Cells were further cultured for 48 h and then transferred into 10-cm Petri dishes and cultured in DMEM containing 700 μ g/ml of G-418 in order to inhibit the growth of non-transfected cells.

The medium containing G-418 was changed every two days until resistant colonies were observed.

2.2. Assay of enzymatic activity

Determination of the activities was performed in intact cells as previously described (Luu-The et al., 1995). Briefly, 0.1 μ M of the [¹⁴C]-labeled steroid (Dupont, Mississauga, Ontario) was added to freshly changed culture medium in a 24-well culture plate. When cell homogenates or purified enzyme was used, the reaction was performed in a final volume of 1 ml of 50 mM sodium phosphate buffer (pH 7.5), 20% glycerol, 1 mM EDTA and 0.1 μ M of [¹⁴C]-labeled steroid and 1 mM of the corresponding cofactor (NADPH for reduction and NADP⁺ for oxidation). After incubation, the steroids were extracted twice with 1 ml of ether. The organic phases were pooled and evaporated to dryness. The steroids were solubilized in 50 μ l of 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, revealed by autoradiography and quantified using the Phosphorimager System (Molecular Dynamics, Sunnyval, CA).

2.3. Immunocytochemistry

Antibodies against human type 5 17 β -HSD were prepared as described (El-Alfy et al., 1999; Pelletier et al., 1999) and was used to incubate with sections of human ovarian tissues which were serially cut at 7 μ m, mounted on glass slides, deparaffinized, and hydrated. The tissues were obtained by surgery within 15 min after their removal, and fixed in 4% paraformaldehyde in a 0.2 M phosphate buffer (pH 7.4), then dehydrated and embedded in paraffin as described (El-Alfy et al., 1999). The incubation was performed overnight at 4°C, in Tris-saline buffer pH 7.6 with antiserum diluted 1:1000. The sections were then washed and incubated at room temperature for 4 h with peroxidase-labeled goat anti-rabbit γ -globulin (Hyclone; Logan, UT) diluted as previously described (El-Alfy et al., 1999). Endogenous peroxidase activity was eliminated by preincubation with 3% H₂O₂ for 30 min. Peroxidase was then revealed during incubation with 10 mg of 3,3'-dian-dnobenzidine in 100 ml of Tris-saline buffer containing 0.03% H₂O₂. The intensity of staining was controlled under the microscope. The sections were then counterstained with hematoxylin. Control experiments were performed on adjacent sections by substituting nonimmune rabbit serum (1:1000).

3. Results

3.1. Lability of human and monkey type 5 17 β -HSD

Previously, we have shown that human type 5 17 β -HSD is labile and is destroyed upon homogenization of the stable transfected cells, while mouse type 5 17 β -HSD remains stable after homogenization (Dufort et al., 1999). Following the recent cloning of a monkey type 5 17 β -HSD cDNA clone (Luu-The, unpublished results), we have established HEK-293 cells stably transfected with this clone, and determined whether the monkey enzyme is labile as the human's or remains stable as the mouse enzyme. As shown in Fig. 1, the lability of the monkey type 5 17-HSD is similar to that of the human enzyme: 90% of the activity that catalyzes the transformation of 4-dione into T is destroyed upon homogenization.

3.2. 20 α -HSD activity of human type 5 17 β -HSD

Previously, we have shown that, in addition to the 17 β -HSD activity that catalyzes the transformation of 4-dione into T, human type 5 17 β -HSD also shows a high 20 α -HSD activity (Dufort et al., 1999) that catalyzes the inactivation of progesterone to 20 α -hydroxyprogesterone, and could thus play a role in the protection of androgen producing cells against a female hormone. To determine whether this double activity also exists in the mouse and monkey type 5 17 β -HSD, we have added [¹⁴C]-progesterone and 4-dione to the culture medium of cells stably expressing human, monkey and mouse type 5 17 β -HSD and assessed their ability to convert these substrates. As illustrated in Fig.

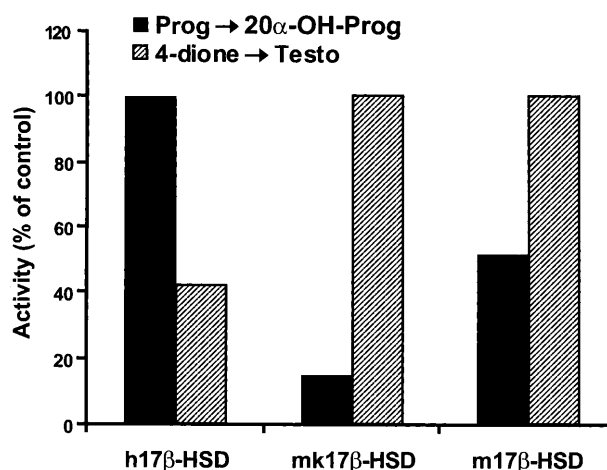


Fig. 2. Determination of 20 α -HSD and 17 β -HSD activities of human, monkey and mouse type 5 17 β -HSD. Cells stably expressing the human (h), monkey (mk) and mouse (m) type 5 17 β -HSD were cultured in 24-well plates to $\sim 10^5$ cells/well. [¹⁴C]-labeled 4-dione, or progesterone (0.1 μ M) were added to a freshly changed culture medium. After 2 h of incubation, the media were collected and extracted. Extraction, separation on TLC and quantification of steroids were performed as described under Section 2.

2, only the human enzyme shows a high 20 α -HSD activity whereas the monkey and mouse type 5 17 β -HSD show much lower activity.

3.3. Immunocytochemical localization of type 5 17 β -HSD

Antibodies raised against the synthetic peptide covering amino acids 297–320 of type 5 17 β -HSD, which have been previously shown (El-Alfy et al., 1999; Pelletier et al., 1999) to specifically recognize type 5 17 β -HSD by western blot analysis, were used to perform immunocytochemical localization in the human ovary. As can be seen in Fig. 3, theca cells, which are respon-

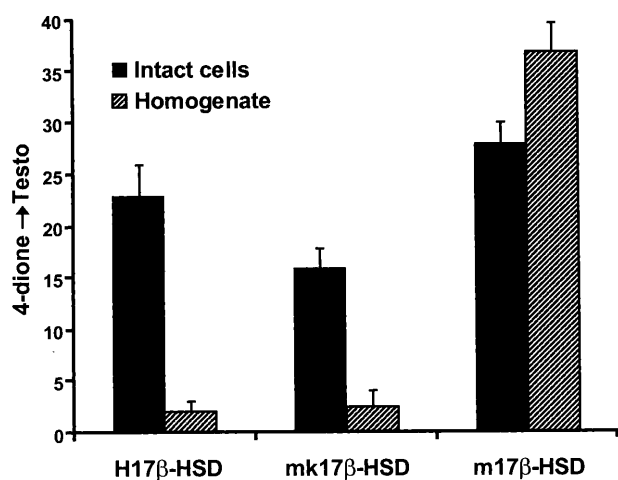


Fig. 1. Comparison of the activities of type 5 17 β -HSD in intact transfected cells and in cell homogenates. Enzymatic activity of expressed cDNAs encoding human (H), monkey (mk) and mouse (m) type 5 17 β -HSD in intact transfected HEK-293 cells (black bar) and their homogenates (striped bar).

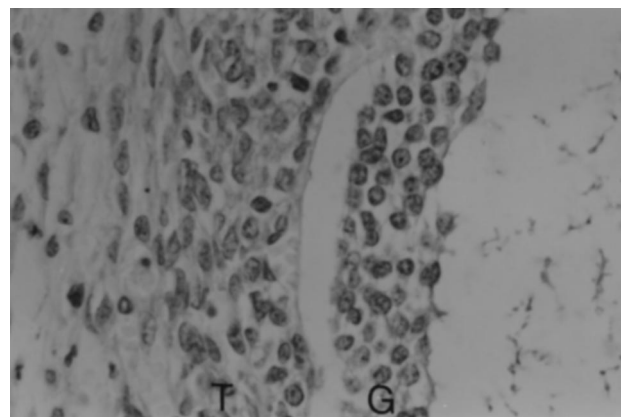


Fig. 3. Immunocytochemical localisation of human type 5 17 β -HSD in the ovary. View of a portion of an antral follicle. Immunostaining can be seen in the theca cell layer (T). No staining can be detected in granulosa cell layer (G).

sible for the production of androgens in women, are more heavily labeled, while the granulosa cells, which produce estrogens and progesterone, are not. These results confirm the hypothesis on the role of type 5 17β -HSD in the production of androgens in women.

4. Discussion

It is generally thought that the same type of steroidogenic enzymes will be found in the gonads of men and women. However, data from the literature show that the type 3 17β -HSD which catalyzes the formation of testosterone and is responsible for pseudo-hermaphroditism in deficient men, is found only in the testis, not in the ovary (Qin and Rosenfield, 2000; Zhang et al., 1996). On the other hand, type 5 17β -HSD which also catalyzes the formation of testosterone (Dufort et al., 1999) is found in the theca cell layer of the ovary the cell layer that produces androgens (Pelletier et al., 1999), (Fig. 3). The data thus strongly suggest that distinct types of 17β -HSD are responsible for the production of testosterone in men and women gonads. Type 5 17β -HSD that catalyzes for the formation of androgens in women, is also found in peripheral tissues of men such as the prostate (El-Alfy et al., 1999) as well as in the prostatic carcinoma cell lines, DU-145 and LnCaP; and in the osteosarcoma cells MG-63 (Dufort et al., 1999). It is likely that this enzyme is responsible for the virilisation of type 3 17β -HSD deficient man in adulthood (Andersson et al., 1996).

Although both human and mouse type 5 17β -HSD efficiently catalyze the transformation of 4-dione into T, the human enzyme differs by its lability and its high 20α -HSD activity. Comparison with monkey type 5 17β -HSD, which has recently been cloned in our laboratory (Luu-The et al., unpublished result) and has 95 and 78% amino acid identity, respectively, with the human and mouse enzymes indicates that the monkey type 5 17β -HSD is as labile as the human enzyme, but it does not possess high 20α -HSD activity. Previously, we have detected the expression of type 5 17β -HSD in the prostate and suggested that this high 20α -HSD activity might protect this male organ against a female hormone progesterone (Dufort et al., 1999). The present result gives strength to this hypothesis and suggests that this high 20α -HSD activity in human could be used to protect androgen-producing cells against female hormones. In the ovary, this role could be even more important since the neighboring cells produce large amounts of female hormones that could have antagonistic effects on androgen producing cells. On the other hand, the differential localization of a testosterone producing enzyme (type 5 17β -HSD) and aromatase in theca and granulosa cells, respectively, strongly suggests that the pathway leading to the pro-

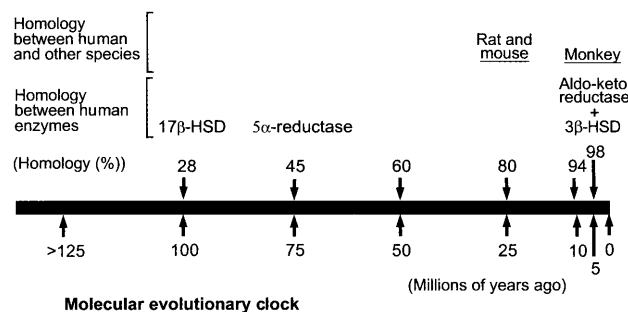


Fig. 4. Hypothetical scheme showing on a molecular evolutionary clock the divergence periods of enzyme families and species, based on homology.

duction of estradiol in the ovary consists of an aromatization of 4-dione to estrone followed by a subsequent conversion of estrone to estradiol by type 1 and/or type 7 17β -HSD in the granulosa cells. It has been suggested that type 1 17β -HSD is the main enzyme in the human whereas type 7 is more important in the mouse (Noelkainen et al., 1998). This pathway is somehow contradictory to the general belief that estradiol is produced directly by aromatisation of testosterone. However, it is in agreement with the higher affinity of aromatase for 4-androstenedione than testosterone.

Type 5 17β -HSD belongs to both 17β -HSD and aldo-keto reductase families. These two families show opposite structure/ activity relationships. Members of the 17β -HSD family share very low homology ($\sim 20\%$) but catalyze similar activities: they catalyze oxidation and/or reduction of the oxygen at the position 17 of the steroid nucleus. On the other hand, members of the aldo-keto reductase family possess high percentage identity ($> 80\%$) but catalyze different activities, namely 3α -HSD, 17β -HSD, 20α -HSD and 5α -reductase activities. Although 5β -reductase activity does not involve the aldo and keto groups, this enzyme has been classified in the aldo-keto reductase family by homology (Jez et al., 1997). It has also been shown that, in the rat 3α -HSD, a single mutation of His 117 to Glu confers 5β -reductase activity to this enzyme (Jez and Penning, 1998). This thus confirms the versatility of activities of this family. To have a better understanding of the differences between these two family, we fitted them on a molecular clock scale, according to their amino acid identity and the model described by Ayala (1986) (Fig. 4). Taking into account that the average homology between human and rodent enzymes, the most widely used laboratory animals is 70–80%, our data suggests that members of the 17β -HSD family diverged before the separation between rodents and primates, and thus that these enzymes could have been conserved while retaining similar activity in humans.

On the other hand, members of the aldo-keto reductase family must have diverged more recently, after the

Table 1
Relationship between cofactor specificity and oxidative or reductive activity catalyzed by some dehydrogenases^a

	Reaction			
	Reduction		Oxidation	
	NADPH	NADH	NADP ⁺	NAD ⁺
17β-HSD				
Type 1	+			
Type 2				+
Type 3	+			
Type 4				+
Type 5	+			
Type 6				+
Type 7	+			
Type 8				+
Type 9				+
3β-HSD				
Type 1				+
Type 2				+
5α-Reductase				
Type 1	+			
Type 2	+			
11β-HSD				
Type 1	+			
Type 2				+

^a +, enzymatic activity.

separation from rodents, and it is thus possible that some enzymes do not have orthologous counterparts. We can take hydroxy steroid dehydrogenase as an example: human types 1 (Luu-The et al., 1989b) and 2 3β-HSD (Rhéaume et al., 1991) share 93.8% identity, and were likely separated after the separation from rodents. It could thus explain that, although six types of 3β-HSD have been found in the mouse and rat, none of these shows the characteristics of human type 2 3β-HSD.

Like almost all other dehydrogenases, 17β-hydroxysteroid dehydrogenases are considered to be reversible enzymes that catalyze the interconversion of substrates and products, mainly because they were first characterized using tissue homogenates or sub-fractions or purified proteins with added oxidized (NAD⁺/NADP⁺) or reduced (NADH/NADPH) cofactors. These exogenously added cofactors drive the reaction in the oxidative or reductive direction depending on their oxidized or reduced state, respectively. However, using a more biologically relevant method of enzymatic activity analysis, namely transfection of expression vectors in mammalian cells, we were able to analyze the enzymatic activity in cultured intact cells without addition of exogenous cofactors. It is noteworthy that all the enzymes that catalyze the reductive reaction prefer a phosphorylated cofactor (NADPH/NADP⁺) while the

enzymes that catalyze the oxidative reaction prefer NAD⁺/NADH. It is likely that this phenomenon is linked to the intracellular ratio of NADPH/NADP⁺ and NADH/NAD⁺. Indeed, it is well recognized that the most abundant intracellular concentrations of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate cofactors are NAD⁺ and NADPH, respectively. It is thus logical to conclude that the preference of an enzyme for a phosphorylated or non-phosphorylated cofactor will cause a dehydrogenase to catalyze an oxidative or a reductive reaction respectively (Table 1). Since the 17β-OH group has to be present in active sex steroids, these enzymes play a major role in the regulation of the intracellular level of biologically active sex hormones.

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