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Characteristics of Human Types 1, 2 and 3 17β-Hydroxysteroid Dehydrogenase Activities: Oxidation/Reduction and Inhibition

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Following transfection of types 1, 2 and 3 17β-hydroxysteroid dehydrogenase (17β-HSD) cDNAs into transformed embryonal kidney (293) cells, we have characterized the selective directional and inhibitory characteristics of these activities. While homogenates of transfected cells could catalyze interconversion of the substrate and product, in agreement with the general belief on the activity of these enzymes, the same activities measured in intact cells, in order to better reflect the physiological conditions, showed an unidirectional reaction. Types 1 and 3 17β-HSD catalyzed the reduction of estrone to estradiol and 4-androstenedione to testosterone, respectively, while type 2 17β-HSD catalyzed the oxidative transformation of both testosterone and 17β-estradiol to 4-androstenedione and estrone, respectively. In addition, types 1, 2 and 3 17β-HSD activities showed different pH optima. While types 1 and 3 showed pH optimum values centered at around 5 and 6, respectively, type 2 17β-HSD activity, which preferentially catalyzes the oxidation reaction, has higher activity at an alkaline pH (8–10). Differences in the optimum incubation temperatures were also observed: type 1 17β-HSD shows a relatively high temperature tolerance (55°C). In contrast, type 2 and 3 functioned best at 37°C. Types 1, 2 and 3 17β-HSD activities could be also differentiated by their sensitivity toward various specific inhibitors: type 1 was potently inhibited by an estradiol derivative containing a bromo/or iodopropyl group at position 16α. On the other hand a derivative of estrone containing a spiro-γ-lactone at position 17 showed a potent inhibitory effect on type 2 17β-HSD, whereas type 3 was strongly inhibited by 1,4-androstadiene-1,6,17-trione.

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INTRODUCTION

The formation of estradiol (E_2) from estrone (E_1), testosterone (T) from 4-androstenedione (Δ4-dione), 5-androstene-3β,17β-diol (Δ5-diol) from dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT) from 5α-androstane-3,17-dione (A-dione) and their respective backward reactions are catalyzed by 17β-HSDs which are widely distributed in human tissues; in fact 17β–HSD activity is not only present in classical steroidogenic tissues, such as the human placenta [1–3], ovary [4], and testis [5, 6], but also in a large series of peripheral intracrine tissues [7], including adipose tissue [8], endometrium [9], ileum [5], liver [10], lung [11], skin, and vaginal mucosa [12], as well as red blood cells [13], breast [14–17], and prostate [18] cancer cells. The presence of 17β-HSD was also demonstrated *in vitro* in several experimental animal tissues, including bovine and rat placenta [19, 20], porcine endometrium [21], porcine and rat testis [4, 22], and rat lung [23].

Recently, four types of human 17β-HSDs were identified and their structure elucidated. Type 1 17β-HSD [24, 25] originally cloned from human placenta libraries was also found in the ovary and mammary gland [4, 7]. Type 2 17β-HSD [26], first isolated from prostatic and placenta cDNA libraries has been found in the placenta, liver, small intestine, endometrium, kidney, pancreas, and colon [27]. Type 3 17β-HSD, on

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the other hand was detected only in the testis [6] and its malfunctioning leads to male pseudohermaphroditism with the absence of the internal male reproductive structures (epididymis, seminal vesicles, and vas deferens) normally formed from Wolffian ducts under the control of testosterone. More recently, human type 4 17β-HSD (Adamski and de Launoit, personal communication), homologous to porcine endometrial 17β-HSD [28], has also been identified. Since there are multiple 17β-HSD genes expressed selectively in various tissues, other types of intracrine dysfunction of 17β-HSD activity in peripheral tissues could also be expected [29]. 17β-HSD activity is indeed an obligatory step in the biosynthesis of all androgens and estrogens, namely, E_2, Δ5-diol, T and DHT. These hormones bind much more strongly to their respective receptors in the 17β-hydroxy configuration than the corresponding 17-keto derivatives.

In this study, we describe the characteristics of the oxidation/reduction activities of types 1, 2 and 3 17β-HSD as well as their inhibition by some selected inhibitors.

MATERIALS AND METHODS

Transformed embryonal kidney (293) cells were obtained from the American Type Culture Collection (Rockville, MD). NAD^+, NADH, $NADP^+$, NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were from Sigma Chemical Co. (St Louis, MO). Restriction enzymes, T4 DNA ligase, and Klenow fragment of *Escherichia coli* DNA polymerase were obtained from Pharmacia Inc. (Canada). Taq DNA polymerase was from Perkin Elmer Inc. (Mississauga, Ontario), and ^{14}C-labeled steroids were from Dupont (Canada) Inc.

Construction of expression vectors

Types 1, 2 and 3 17β-HSD expression vectors were constructed by insertion of the appropriate 17β-HSD cDNA fragments downstream the CMV promoter containing in the pCMV vector (kindly provided by Dr Michael B. Mathews, Cold Spring Harbor Laboratories, NY). Type 1 cDNA was obtained by digestion of the hpE_2DH216 cDNA clone (1) with the endonuclease restriction enzymes *Nco*I and *Eco*RI. Types 2 and 3 17β-HSD cDNAs were obtained by amplification from human placenta and testis cDNA libraries (Clontech Laboratories Inc. (Palo Alto, CA)) using the oligonucleotide primer pairs, respectively (5′-CTG-AAT-TCTT-GAA-GGT-GCA-GCA-AGT-CAC-TG-3′,5′-CCG-AAT-TCT-TTG-AGG-GCT-TCC-ATT-GCC-3′) and (5′-GGA-ATT-CTG-TAT-TCT-ATG-CCT-CTG-TGA-CC-3′, 5′-GGA-ATT-CCA-CGG-CCA-GGG-CTG-AAA-CAG-TC-3′). The *Eco*RI cloning sites are underlined.

Transient expression in 293 cells

Transfection was performed using the calcium phosphate procedure [30] with 10 μg of recombinant plasmid per 10^6 cells. The cells were initially plated at 10^4 cells/cm^2 in Falcon culture flasks and grown in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU penicillin/ml and 100 μg streptomycin sulfate/ml.

Assay of enzymatic activity

Determination of activity in intact cells was performed by adding 0.1 μM of the indicated ^{14}C-labeled substrate to freshly changed culture medium in a 6-well culture plate. After incubation for 1 h, the steroids were extracted and separated by thin layer chromatography (TLC) as previously described [31]. For assays using cell subfraction, cells were sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA and centrifuged at 10,000*g* for 30 min before centrifugation for 100,000*g* for 1 h to separate the mitochondrial and microsomal fractions, respectively. The cytosol fraction (100,000*g* supernatant) was used to determine type 1 activity, while the microsomal fraction (pellet at 100,000*g*) was used for measurement of type 2 and 3 17β-HSD activities. The enzymatic reaction was carried out at 37°C in 0.5 ml 50 mM sodium phosphate buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA, and 0.4 mM cofactors for 1 h in the presence of the indicated concentration of radiolabeled steroid substrates. To determine the pH optimum of enzymatic activities, a Tris–citrate buffer was used. Radioactivity signals were detected and quantitated using a Phosphor Imager (Sunny Vale, CA).

RESULTS

Comparative activity of transfected human types 1, 2 and 3 17β-HSD activities in intact cells

As illustrated in Fig. 1, type 1 17β-HSD measured in intact cells selectively transformed the reductive conversion of E_1 to E_2, while the reverse reaction was negligible. E_1 was thus the most favorable substrate, while DHEA was converted at much smaller extent, and the transformation of Δ4 into T was negligible. In contrast, type 2 17β-HSD was selective for the oxidative reaction. It converts efficiently and almost equally T and E_2 to Δ4-dione and E_1, respectively. Type 3 17β-HSD, on the other hand is the androgenic counterpart of type I 17β-HSD; it specifically converts the reductive transformation of Δ4-dione to T with a low level of transformation of E_1 into E_2.

Comparative activity of transfected human types 1, 2 and 3 17β-HSD activities in cell subfractions

Figure 2 shows that in contrast to the highly predominant unidirectional reaction observed in intact cells, the transfected 17β-HSD activities measured in cytosolic (type 1) or microsomal (types 2 and 3) subfractions catalyze both the oxidative and reductive reactions, the direction of the reaction depending upon the added cofactor. However, the preferred reaction corresponds to the one observed in intact cells.

Optimum temperature for human types 1, 2 and 3 17β-HSD activities

As illustrated in Fig. 3, types 2 and 3 17β-HSD, which are localized in microsomes, function most effectively at physiological temperature; maximal activity being measured at 37°C with a rapid fall in activity at lower or higher temperature. In fact at 55°C, enzymatic activities were almost completely absent. In contrast, type 1 17β-HSD activity possesses a broader temperature tolerance, the optimal temperature being around 45°C. At 55°C, its activity is similar to that found at 37°C and represents approximately two thirds of the activity measured at the optimal temperature of 45°C. In fact, at 65°C only 50% of type 1 17β-HSD activity is lost.

pH optimum for 17β-HSD activities

The pH curves for the forward reaction of types 1, 2 and 3 17β-HSDs are illustrated in Fig. 4. Type 3 17β-HSD possesses the most acidic pH optimum centered around pH 5, while type 1 17β-HSD functions most efficiently at pH 6. In contrast, type 2 17β-HSD activity, which prefers the oxidative reaction, is more efficient at alkaline pH (8–10).

Selective inhibition of types 1, 2 and 3 17β-HSD activities

As illustrated in Fig. 5, 10^{-5} M 16α-bromopropylestradiol selectively inhibits type 1 17β-HSD activity [Fig. 5(A)], whereas 17-(spiro-γ-lactone)-estrone shows a predominant inhibitory effect on type 2 17β-HSD activity [Fig. 5(B)], while 1,4-androstadiene-3,6,17-trione exerts a strong inhibitory effect on type 3 17β-HSD activity [Fig. 5(C)].

DISCUSSION

Although several 17β-HSD cDNAs have been cloned and characterized, their substrate as well as their oxidative/reductive reaction remain poorly-defined. In the present study, using the types 1, 2 and 3 of 17β-HSD activities expressed by transfection of cDNAs into mammalian cells, we characterized the substrate and inhibitor specificity as well as the oxidative/reductive activity of each type of 17β-HSD. The data clearly show that in intact cells, which more closely reflects the physiological conditions, the activity catalyzed by each type of 17β-HSD is almost exclusively unidirectional: types 1 and 3 activities catalyze the reaction in a reductive way, while type 2 17β-HSD activities catalyzes the oxidative reaction. However, in cell homogenates, these 17β-HSDs can drive the

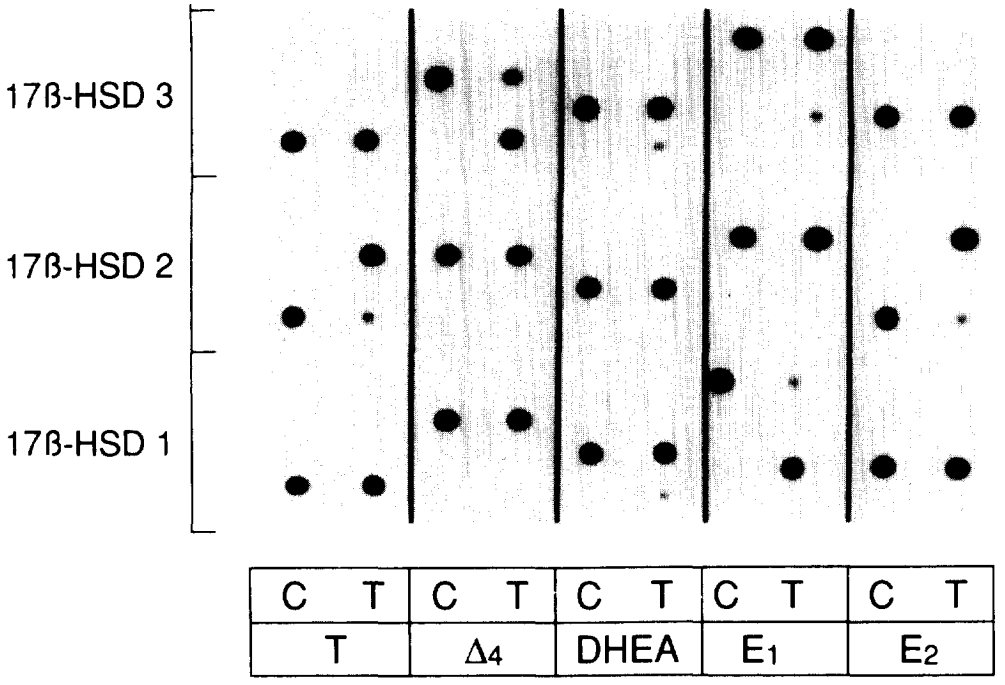

Fig. 1. Enzymatic activities of expressed cDNAs encoding types 1, 2 and 3 17β-HSDs in intact transfected 293 cells. Autoradiograph of TLC of the transformation of 0.1 μM of ^{14}C-labeled-substrate by control mock-transfected cells (C) and cells transfected (T) with the pCMV-17β-HSDs 1, 2 and 3, respectively.

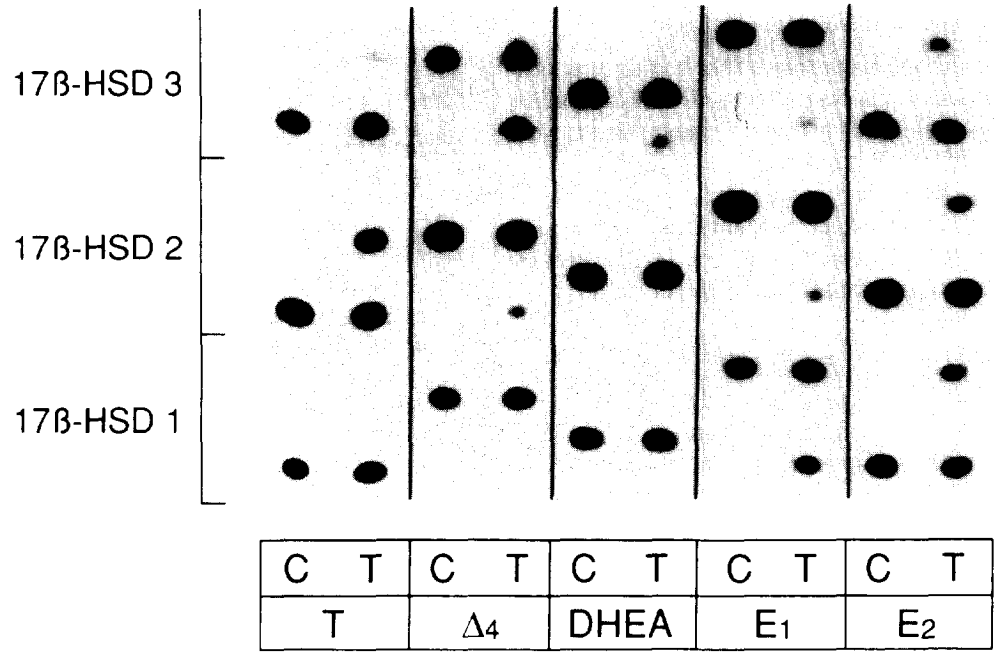

Fig. 2. Enzymatic activities of expressed cDNAs encoding types 1, 2 and 3 17β-HSD in subfraction of transfected 293 cells. Autoradiograph of TLC of the transformation of 0.1 μM of ^{14}C-labeled substrate by control mock-transfected cells (C) and cells transfected (T) with the pCMV-17β-HSDs 1, 2 and 3, respectively. In the reactions using types 1 and 3 17β-HSD, 0.4 mM of $NADP^+$ were added with T and E_2, while NADPH was added with Δ4, DHEA and E_1. In the reaction catalyzed by type 2 17β-HSD, 0.4 mM of NAD^+ was added with T and E_2 and NADH were used with Δ4, DHEA and E_1.

interconversion of the product and substrate, but the direction corresponding to the physiological direction is always favored.

In estrogen-target tissues, such as the placenta, ovary, and breast, the presence of type 1 17β-HSD ensures a high level of estradiol formation. Similarly, in the testis, type 3 17β-HSD drives the conversion of Δ4-dione to T needed for the development and growth of the internal male reproductive structures (epididymis, seminal vesicles, and vas deferens) as well as all secondary sex organs. In fact, impairment of type 3 17β-HSD leads to the well characterized male pseudohermaphroditism [5].

More recently, we have cloned a human placental 20α-hydroxysteroid dehydrogenase that possesses reductive 17β-HSD activities that we named type 5 17β-HSD (Luu-The *et al.*, unpublished data). Thus in peripheral tissues, it is likely that the expression of

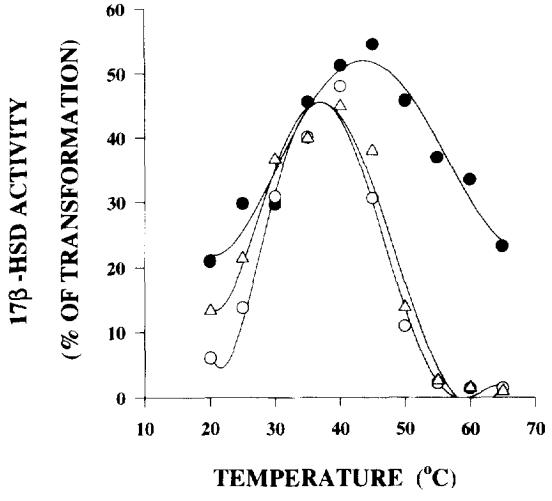

Fig. 3. Effect of temperature on types 1 (●), 2 (○) and 3 (△) 17β-HSD activities. Transfected 293 cells were incubated as described in Materials and Methods, except for the indicated incubation temperature.

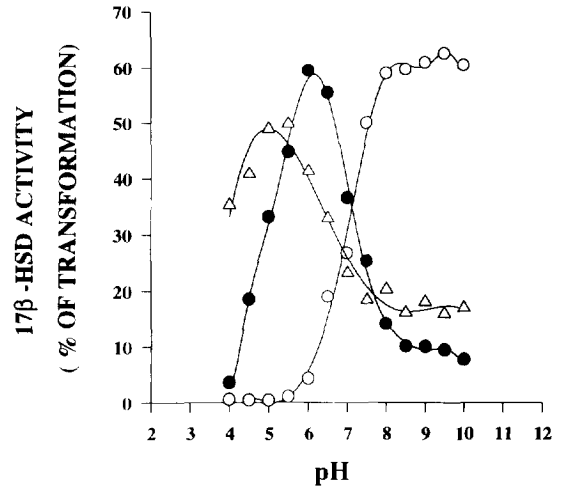

Fig. 4. Effect of pH on types 1 (●), 2 (○) and 3 (△) 17β-HSD activities. Transfected 293 cells were incubated as described in Materials and Methods except for the indicated pH.

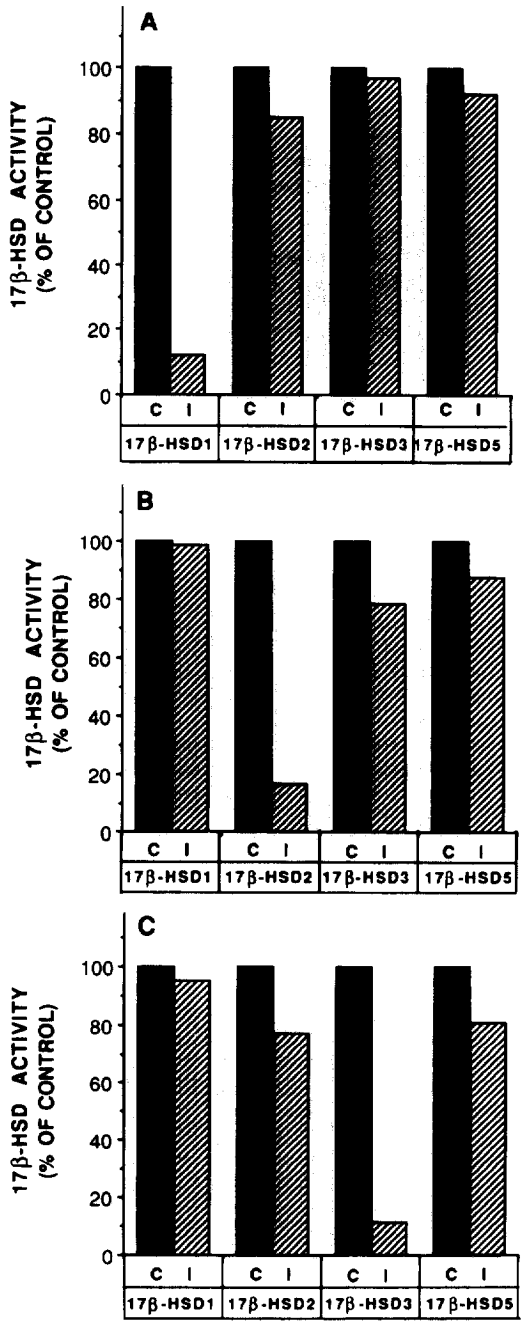

Fig. 5. Inhibitory effect of 16α-bromopropyl-estradiol (A), 17-(spiro-γ-lactone)-estrone (B), and 1,4-androstene-1,6,17-trione (C) on various 17β-HSD activities. The incubation was performed as described in Materials and Methods in the absence (C) or presence (I) of 10^{-5} M inhibitor.

types 1, 2 or 5 17β-HSD activities play an important role in regulating the formation (types 1 and 5) or inactivation (type 2) of active estrogens (E_2 and Δ5-diol) and androgens (T and DHT). Indeed, type 2 17β-HSD is highly expressed in the liver. The contribution of types 1 and 3 17β-HSD activities in peripheral tissues could also be important since, using RT-PCR, we were able to detect type 3 17β-HSD in the prostate (Luu-The *et al.*, unpublished data) while using RNase protection analysis, type 1 17β-HSD was detected in the prostate, adipose tissues, skin, prostate cancer cells (LNCaP), and endometrium [6]. Recently, using immunocytochemistry, we were able to detect type 1 17β-HSD in the rat brain (Pelletier *et al.*, unpublished data).

The isolation of multiple 17β-HSDs strongly favors the hypothesis that intracellular formation and degradation of androgens and estrogens plays an important role in the regulation of cell function and proliferation, an area called intracrinology [29, 32]. This new area of endocrinology describes the biosynthesis of sex steroids in peripheral target cells, these steroids exerting their action in the same cells where synthesis takes place without release in the surrounding space or in the circulation [29]. As multiple 17β-HSD genes are expressed specifically in various tissues, other types of intracrine dysfunction of 17β-HSD activity in peripheral tissues could also be expected in addition to the well-known male pseudohermaphroditism caused by type 3 17β-HSD deficiency [6].

It is interesting to observe the correlation between the oxidation and reduction reactions with the presence of the NAD^+ or NADPH cofactor. Indeed, types 1 and 3 17β-HSDs which preferentially catalyze the reductive reaction, use NADPH as cofactor while type 2 17β-HSD, which preferentially catalyzes the oxidative reaction, uses NAD^+ as a cofactor. Similar results were observed with 11β-hydroxysteroid dehydrogenase (11β-HSD) [33, 34]. Indeed, type 1 11β-HSD which catalyzes the reduction of cortisone to cortisol uses NADPH as cofactor, while the type 2 enzyme which catalyzes the oxidation of cortisol to cortisone uses NAD^+. It is thus tempting to conclude that the enzymes that use nicotinamide adenine dinucleotide phosphate as a cofactor preferentially catalyze the reductive reaction (NADPH) and that the enzymes that use the nicotinamide adenine dinucleotide cofactor preferentially catalyze the oxidative reaction (NAD^+) (Table 1). Such a conclusion has major physiological implications because it is well known that the intracellular concentration of NADPH versus $NADP^+$ is highly in favor of NADPH; in contrast, the intracellular concentration of NAD^+ versus NADH is highly in favor of NAD^+; the most abundant intracellular concentrations of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate cofactors are thus NADPH and NAD^+.

In order to further increase our knowledge about the various types of 17β-HSDs, we synthesized various potential inhibitors of 17β-HSD activities [35, 36]. Among others, the estradiol derivative containing a bromo- or iodopropyl group at position 16α- was found to inhibit selectively and potently type 1 17β-HSD [35], whereas the estrone derivative containing a spiro-γ-lactone group at position 17 inhibits type 2 17β-HSD [36]. Furthermore, a derivative of Δ4-dione, namely 1,4-androstene-3,6,17-trione, strongly inhibits type 3 17β-HSD activity (this study). The availability of

Table 1. Relationship between cofactor specificity and oxidative or reductive activity catalyzed by 17β-HSDs and 11β-HSDs

	Reaction			
	Reduction		Oxidation	
	NADPH	NADH	$NADP^+$	NAD^+
17β-HSD				
Type 1	+			
Type 3	+			
Type 5	+			
Type 2				+
Type 4	+*			+
11β-HSD				
Type 1	+			
Type 2				+

(+) indicates enzymatic activity. *A weak reductive activity using purified enzyme has been observed with NADPH only, using NADH there is no activity, although the preferred cofactor is NAD^+ (J. Adamski, personal communication).

specific inhibitors of 17β-HSD activities could provide most useful tools to better characterize the detailed properties of the 17β-HSD superfamily of steroidogenic enzymes.

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