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Recent advances in 17beta-hydroxysteroid dehydrogenases☆

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ABSTRACT

The metabolism of steroids at position 17 is catalysed by a growing number of 17beta-hydroxysteroid dehydrogenases (17β-HSDs). Several human diseases like breast or prostate cancer, endometriosis, metabolic syndrome and mental diseases were associated with dysfunctions of 17β-HSDs, which consequently became drug targets. This review will focus on identities of 17β-HSDs and recent advances in analyses of their physiological roles in steroid and lipid metabolism. It will also address the potential of metabolomics in drug development.

1. Steroid signaling

Steroid metabolism is observed in most species studied so far including prokaryots, invertebrates and vertebrates. Steroid conversion was reported in bacteria [1,2], fungi [3,4], corals [5], worms [6,7], fish [8,9], reptiles [10,11], birds [12,13], and mammals [14] to name few. With the progression of genome sequencing projects substantial data is provided to verify if the metabolism is associated with signalling or with nutrition. Steroids hydroxylated at position 17, like estradiol or testosterone, have pivotal regulatory functions. They act through membrane sensors like GPR30 [15,16] modulating kinase cascades or the cross-talk between EGFR/HER [17,18], and nuclear receptors [19–21]. The biological potency of certain steroids like androgens and estrogens is controlled by 17β-hydroxysteroid dyhydrogenases (17β-HSDs) requiring cofactors for this reaction (Fig. 1).

2. Identity of known 17β-HSDs

The 17β-HSDs constitute a class of enzymes [14,22] recently attracting considerable attention, due to their ability to specifically modulate activity of hormones, to tightly control cellular responses, and offering unique pharmacological intervention points. The number of 17β-HSDs identified is growing (Table 1). The enzymes belong structurally to a large gene family of short-chain dehydrogenases/reductases (SDRs) [23–25]. One exception is the 17β-HSD type 5 or AKR1C3 which belongs to Aldo-Ketoreductase (AKR) family [26,27]. These enzymes are among targets in the druggable genome [28].

While the present nomenclature reflects the chronology of identification, it is not perfect, because the same enzyme types (highly similar in amino acid sequences) apparently may have different functions in distinct species. In human the numbering goes up to the 14th type of 17β-HSD. Whereas human enzymes type 6, 9 are most probably active in retinoid metabolism, for rodent 17β-HSDs type 6 and 9 steroid activities have been reported [29,30]. The 17β-HSD enzymes are further acting on a large set of substrates like steroids, bile and fatty acids, retinols, and xenobiotics. Their specificity is reached by distinct subcellular localisations, cofactor preferences, spatio-temporal patterns of tissue expression. Although the 17β-HSDs share the same protein fold as demonstrated by crystallisation studies, the differences in non-conserved amino acid sequences result in distinct functionalities [23,31,32]. In addition to position 17 the 17β-HSDs can act on position 3, 7, 15, 20 and 24 of various lipids (Fig. 2).

Because of apparent participation in many pathways the physiological role of 17β-HSDs is controversially discussed for some enzyme types. For example the 17β-HSD type 4 was first identified as the estradiol dehydrogenase from porcine endometrium [33], whereas later experiments [34–36], identification of human mutants [37,38], and gene disruption in the mouse [39] have determined that its main function is in peroxisomal β-oxidation and bile acid metabolism. An open discussion of the same kind is held for the 17β-HSD type 12 [40] reported to be responsible for estradiol

Abbreviations: 17β-HSD, 17beta-hydroxysteroid dehydrogenase; SDR, short-chain dehydrogenases/reductase.

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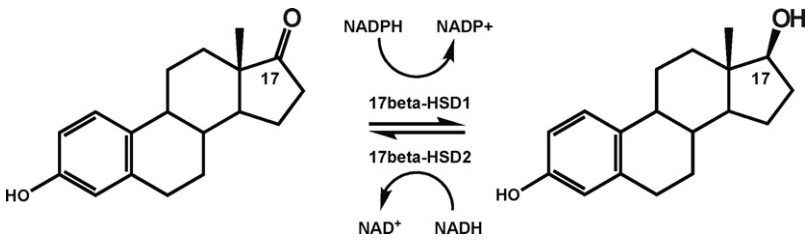

Fig. 1. Conversion of steroids at position 17 modulates the biological potency. Keto-forms (estrone) are less potent than hydroxy-forms (estradiol). Reaction direction is determined by cofactor and substrate presence.

Table 1
Identities of 17beta-hydroxysteroid dehydrogenases.

Type	Gene	Other names	Chr.	Com.	References
1	HSD17B1	E17KSR, EDHB17	17q11	*	[82,83]
2	HSD17B2	E2DH, HSD17	16q24		[53,84]
3	HSD17B3		9q22		[54]
4	HSD17B4	MFP-2, DBP	5q21		[38,85–89]
5	AKR1C3	HSD17B5	10p15		[90–92]
6	HSD17B6	HSE, RODH	12q13	+, R	[93,94]
7	HSD17B7	PRAP	1q23	**	[46,95]
8	HSD17B8		6p21.3		[96]
9	RDH5	HSD17B9	12q13	+, R	[97]
10	HSD17B10	ERAB, HSDH	Xp11.2		[98–102]
11	HSD17B11	retSDR2, Pan1b, DHRS8	4q22.1	#	[103,104]
12	HSD17B12	KAR	11p11.2		[41,105,106]
13	HSD17B13	SCDR9	4q22.1	#, U	[107]
14	HSD17B14	retSDR3, DHRS10	19q13.33		[49]

Chr., chromosome; Com., comments; *, pseudogene present in the same locus; **, pseudogenes present in chr. 1q44 and 10p11; R, probably only retinoid metabolism in human; U, enzymatically not characterised. Gene presence in the same gene clusters is denoted by # and +, respectively. Chromosomal assignments are taken from [95,108,109].

formation in women but known previously as a keto-reductase of fatty acid elongation process [41]. Accumulating evidences in different species including *C. elegans* [42] and human [43,44] point to a side-activity or less critical function of 17β-HSD type 12 towards steroids. The characterisation of mouse models with targeted gene disruption will provide more data to this discussion soon.

3. Searching for new 17β-HSDs

While recalling data on multifunctionality one could rise a question what should be requirements for assigning a new type of 17β-HSD. The guide for that is applicable to many enzyme classes [45]. First, the enzyme gene and the resulting gene product must be known. Solely the observation of activity in a new species or new tissue is not sufficient. Second, metabolic activity should be tested with several substrates and cofactors. Especially distinct classes of substances like steroids, fatty acids, quinones, etc., should be tested. These classes could be inferred from phylogenetic studies or molecular docking. The kinetic parameters found should be then compared to those expected *in vivo*. Third, a comparison to activity known in homologous enzymes should be performed. This should contribute to the knowledge if the observed activity is already

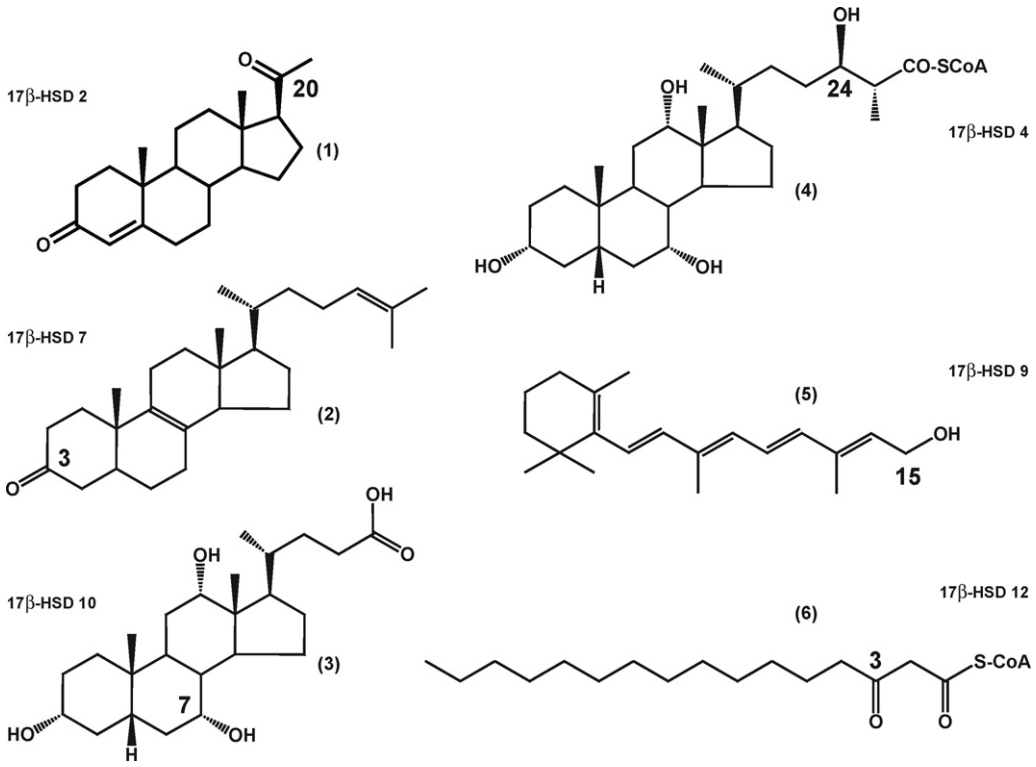

Fig. 2. Examples of different substrates for 17beta-hydroxysteroid dehydrogenases. Enzymes participating are given next to formulas. Conversions can take place at positions indicated. (1) Progesterone, (2) zymosterone (cholesterol precursor), (3) cholic acid (bile acid), (4) tetrahydroxycholestanoic acid (bile acid), (5) retinol, (6) palmitoyl-CoA (long chain fatty acid).

present in the whole phylogenetic clade or is a new evolutionary appearance. Fourth, an inactivation of the candidate enzyme should result in a loss of activity in the assay system. This could be done by inhibitors, siRNA or gene deletion experiments.

The technologies for such verifications and appropriate models are available and were used with success in yeast [46], *C. elegans* [42], human cell lines [44,47] or rodents [48]. Such experiments would definitely consume much time and resources but at the same time they would prevent false-positive identifications. Especially for targeted drug development, this would be of tremendous advantage.

An example for the successful identification of a novel 17b-HSD is the type 14 enzyme [49] known previously as DHRS10 (an uncharacterised SDR enzyme). The protein was crystallised and through modelling approaches some substance classes were suggested and tested for conversion *in vivo* in transfected human cell lines. The shown conversion of estradiol to estrone by the enzyme was independently confirmed in other transfected cell lines and in addition found to be a prognosis factor in breast cancer [43].

An example for an exclusion from the new entries to the 17β-HSDs is the result of analyses of the SDR orphan enzyme HSD-like1 (HSDL1) [50]. In search for functional assignment of human HSDL1, the enzyme was screened for putative substrates suggested by phylogenetics and SDR-substrate spectrum. Surprisingly, human HSDL1 shows an exchange of the amino acid thyrosine in the active center (Y218F) which is considered critical for catalysis. This amino acid exchange in HSDL enzymes is present in many other vertebrate species, including zebrafish. When human HSDL1 was expressed in cells, it did not show enzymatic activity with any of the substrates tested. However, expression of the HSDL1 with the point mutation F218Y resulted in the reconstitution of weak dehydrogenase activity towards steroid and retinoid substrates. The role of this inactivating mutation is uncertain at present. All data gained did not qualify the HSDL1 to be considered as a new 17β-HSD.

4. New approaches for function determination

Presently known human 17β-HSDs were identified after enzymatic profiling of purified [51] or expressed proteins [26,36,52], after expression cloning [53], or by characterisation of genetic effects [38,54]. However, it is an obvious challenge to use the ligand to identify a binding protein. Since this kind of affinity purification procedure turns out to be fairly inefficient for many lipids. For example, neither the estradiol receptor nor any of the 17β-HSDs were purified with estradiol as a ligand. There are many explanations for that, like too many unspecific interactions with the matrix (e.g. sepharose) or steric hindrance of the matrix to ligand–protein interactions. New technologies might overcome these problems. Recent progress in combining highly sensitive detection methods with affinity-purification technologies promise to provide comprehensive lists of ligand or drug binding proteins. An example of such attempts is compound capture mass spectrometry [55]. Capture compounds are trifunctional molecules. They consist of a selectivity feature (e.g. a candidate drug), which reversibly interacts via affinity with proteins. Further, they contain a photo-activable moiety that forms a covalent bond with the captured protein outside the affinity binding site. Finally, they comprise a sorting part (e.g. biotin) that allows the captured protein(s) to be isolated from cellular lysate for mass spectrometric analysis and subsequent characterisation by database queries. Most of the critical interaction and isolation procedures are taking place in solution thus avoiding size exclusion and unspecific effects of matrices.

Another significant contribution to the field is the progress in both crystallisation technologies [56,57] and modeling approaches [58]. Homology modeling nowadays already reaches a quality comparable to that resolved by X-ray diffraction as seen from the publications on the steroid 5β-reductase AKR1D1 crystal structure [59] and its high-resolution homology-build model [60] and allow to explore active site geometries. As the number of protein folds observed in different structures seems to be finite [61], *in silico* approaches might soon be used for molecular docking of candidate drugs to un-crystallised proteins.

5. Introducing metabolomics

Present data on the substrate preferences of various 17β-HSD types illustrate their participations in multiple metabolic pathways. Contemporary characterisation of 17β-HSD roles faces the dimension of metabolomics, i.e. analyses of a multitude of metabolites at the same time. This aspect becomes critical for drug development as there is a need of a much wider validation of inhibitory efficacy than just for steroid conversion. Steroid metabolising enzymes like 17β-HSD type 1 and 3 or 11β-HSD type 1 are drug targets in breast/prostate cancer [62–65] and metabolic syndrome [66–68], respectively. Androgens and estrogens are as well incoming candidates for obesity treatment [69,70] as the imbalance in hormone levels correlates with obesity in human [71] and in mice models [72,73]. There are several mechanisms like modulation of estrogen receptor beta by a negative cross-talk with PPARgamma explaining the associated signaling pathway [74]. At present, most validation studies for the candidate inhibitors of 17β-HSDs were either performed with purified proteins or *ex vivo* using transfected or naturally expressing cell lines. There is a risk of off-side effects by apparently specific inhibitors *in vivo*, e.g. modulation of further lipid pathways.

Metabolomics has been found to be instrumental in analysing responses to animal model treatment [75] and human therapies [76] with rosiglitazone aimed for lipid level normalisation. Search for biomarkers is a tedious and time consuming attempt but pivotal for identifying new characteristic processes of disease or for theranostics. Such *non-targeted* attempts are contributing to our knowledge on unanticipated biochemical processes and their interconnections in common human diseases like diabetes [77]. Another approach is *targeted* metabolomics analysing a defined subset of metabolites (e.g. selected lipids, amino acids and carbohydrates) with the advantage of high-throughput and quantification [78]. Targeted metabolomics has been recently applied to analyse the role of genetic variants on metabolic profiles in a large human population KORA [79]. It showed correlations between genotypes and metabotypes (especially those in lipids) in predisposition to certain diseases, environmental, and nutritional challenge. As most of the contemporary drugs are administered orally, significance of the metabolomics of the gut was recognized recently [80,81]. It is to be expected that metabolomics will further contribute to the research on therapies of steroid-related indications in human disorders. Major advantages to be named are: shorter drug development time, lower associated costs and prevention of unexpected drop-offs in clinical studies because of unanticipated side effects of the drugs.

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