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Kinetic Analysis of Enzymic Activities: Prediction of Multiple Forms of 17β-Hydroxysteroid Dehydrogenase

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An overview of the application of kinetic methods to the delineation of 17β-hydroxysteroid dehydrogenase (17β-HSD) heterogeneity in mammalian tissues is presented. Early studies of 17β-HSD activity in animal liver and kidney subcellular fractions were suggestive of multiple forms of the enzyme. Subsequently, detailed characterization of activity in cytosol and subcellular membrane fractions of human placenta, with particular emphasis on inhibition kinetics, yielded evidence of two kinetically-differing forms of 17β-HSD in that organ. Gene cloning and transfection experiments have confirmed the identity of these two proteins as products of separate genes. 17β-HSD type 1 is a cytosolic enzyme highly specific for C_{18} steroids such as 17β-estradiol (E_2) and estrone (E_1). 17β-HSD type 2 is a membrane bound enzyme reactive with testosterone (T) and androstenedione (A), as well as E_2 and E_1. Useful parameters for the detection of multiple forms of 17β-HSD appear to be the E_2/T activity ratio, NAD/NADP activity ratios, steroid inhibitor specificity and inhibition patterns over a wide range of putative inhibitor concentrations. Evaluation of these parameters for microsomes from samples of human breast tissue suggests the presence of 17β-HSD type 2. The 17β-HSD enzymology of human testis microsomes appears to differ from placenta. Analysis of human ovary indicates granulosa cells are particularly enriched in the type 1 enzyme with type 2-like activity in stroma/theca. Mouse ovary appears to contain forms of 17β-HSD which differ from 17β-HSD type 1 and type 2 in their kinetic properties.

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INTRODUCTION

There is a large and growing body of evidence that relative levels of biologically active and inactive forms of C_{18} and C_{19} steroid hormones are regulated *in situ* by reversible oxidoreduction of the oxygen function at C-17. These reactions are catalyzed by one or more pyridine nucleotide-dependent enzymes referred to variously as estradiol dehydrogenase, 17β-hydroxysteroid dehydrogenase (17β-HSD), 17-ketosteroid reductase or 17β-hydroxysteroid oxidoreductase.

Because of the reversibility of this reaction, it was postulated early on that 17β-HSD could be an important regulatory enzyme and that the nature of this regulation could depend upon both the level(s) and multiplicity of enzyme forms within a given tissue [1]. This concept was also derived from observations of differences in the subcellular localization and steroid specificity of 17β-HSD activities among various organs and tissues [2]. More recent studies have extended activity measurements to include most human and rat tissues [3, 4], and have supplemented these activity measurements with Northern blot analyses for mRNAs of various 17β-HSD types [4–8], thus confirming the complexity of 17β-HSD enzymology.

Additionally, observations made over 30 years ago that forms of liver alcohol dehydrogenase could recognize steroids and various cyclic secondary alcohols as substrates [9, 10] raised questions about the relationship between alcohol dehydrogenase and hydroxysteroid dehydrogenase activities. The structural bases underlying variations in substrate specificity among these enzyme proteins continue to be of fundamental interest in enzymology [11, 12].

In this paper, work from our laboratory on the application of enzyme kinetics to the analysis of 17β-HSD

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in human term placenta will be reviewed, and an attempt made to delineate useful parameters for analyzing enzyme heterogeneity. Results of our recent application of this approach to human breast, testis and ovary and mouse placenta and ovary are also presented.

ENZYME KINETICS AND MULTIPLE FORMS OF 17β-HSD

17β-HSD of human term placenta

Ryan and Engel were the first to demonstrate the interconversion of 17β-estradiol (E_2) and estrone (E_1) by homogenates of human placenta [1]. With the purification of a soluble form of the enzyme from placental cytosol, attention focused on the characterization of that protein. It was soon apparent that it was highly active with E_2 and E_1 as substrates, had measurable activity in the reductase direction with progesterone, but had little or no activity with testosterone (T) [13, 14]. In the same time period, Lehmann and Breuer [15] and Pollow and coworkers [16] presented evidence of microsomal 17β-HSD activities in term placenta, and Thomas and Veerkamp concluded from a detailed study of the distribution of 17β-HSD in placental subcellular fractions that the majority of activity with T was membrane bound [17].

Those results along with reports that liver alcohol dehydrogenase had activity with steroids under certain conditions [10] prompted us to undertake more detailed studies of soluble 17β-HSD of human placental cytosol and membrane bound forms associated with human placental microsomes and guinea pig liver microsomes.

Because initial velocity measurements only give information about the ability of steroids to act as substrates, we also focused our attention on the ability of non-reacting steroids to act as inhibitors, as an alternate approach to estimating relative affinities. In addition, we examined the inhibitory properties of a variety of non-steroidal alcohols with dimensions comparable to those of the steroid nucleus as a means of probing the dimensions of the steroid binding site and delineating the apparent minimal requirements for tight binding [18–20].

*Table 1. K_I values for a variety of steroids and non-steroidal alcohols as inhibitors of 17β-HSD type 1 purified from human term placenta**

Inhibitor	K_I (comp.)
Non-steroidal compounds	
Cyclopentanol	37 mM
Cyclohexanol	9 mM
1,9-nonanediol	2 mM
1,10-Decanediol	—
C_{18}-steroids	
Estradiol-17β	1.0 μM (K_M)
Estrone	0.4 μM
1,3,5(10)-Estratrien-3-ol	0.04 μM
1,3,5(10),16-Estratetraen-3-ol	0.17 μM
4-Estren-17β-ol	5.2 μM (K_M)
C_{19}-steroids	
Testosterone	>225 μM
Androstenedione	>160 μM
5-Androsten-3β-ol	6.0 μM
3β-Hydroxy-5,16-androstadiene	1.8 μM

*The data have been adapted from Blomquist *et al.* [18].

Data for 17β-HSD purified from term placental cytosol are shown in Table 1. The data suggest there is a minimal structure comparable to that of a C_{18} steroid required for binding and that various C_{19} steroids such as T are not only not substrates, they do not bind appreciably as inhibitors. It is noteworthy for structural considerations that C_{18}-17-desoxysteroids appear to bind with a greater affinity than E_2, and that C_{19}-17-desoxysteroids also have a high affinity, in contrast with T and androstenedione (A). This suggests the presence of an oxygen function at the C-17 position significantly defines the mode of interaction of these steroids with the active site. In contrast, 17β-HSD activity of human placental microsomes and guinea pig liver microsomes have comparable affinities for

*Table 2. Inhibition of 17β-HSD of human placental cytosol ($E_2 \rightarrow E_1$) and microsomes ($T \rightarrow A$) and guinea pig liver microsomes ($T \rightarrow A$) by various steroids**

Steroid	K_I (comp.) Placental cytosol	Placental microsomes	G.P. liver microsomes
Estradiol-17β	1.0 μM (K_M)	0.8 μM	9.0 μM
Estrone	0.4 μM	11.8 μM	15.8 μM
Testosterone	>225 μM	1.3 μM (K_M)	8.7 μM (K_M)
5α-Dihydrotestosterone	>200 μM	1.7 μM	5.8 μM (K_M)
5β-Dihydrotestosterone	>200 μM	1.9 μM	10.1 μM (K_M)
5α-Androstan-3-one	—	—	37 μM
5β-Androstan-3-one	—	—	55 μM
20α-Dihydroprogesterone	>200 μM	1.5 μM	—
Ethinylestradiol	50 μM	0.3 μM	—
Danazol	>15 μM	0.6 μM	—

*The data have been adapted from Refs [18–20].
Where a steroid was not tested is indicated by (—).

both E_2 and T (Table 2), but differ significantly from the cytosolic enzyme in their affinities for C_{19}-17-hydroxysteroids, 20α-dihydroprogesterone and two synthetic steroid derivatives, ethinylestradiol and danazol. These differences are suggestive of differences in amino acid composition of the steroid binding site and mode of steroid recognition between the soluble and membrane bound forms of 17β-HSD. They are suggestive of fundamentally important hydrogen bonding between the C-17 oxygen function and an amino acid in the steroid binding region of the type 1 enzyme. The results of recent inhibition studies of heterocyclic derivatives of estrone [21] and kinetic characterization of 17β-HSD type 1 isoforms modified by site-directed mutagenesis in baculovirus [22] provide further support for this concept.

The observation of apparently fundamental structural differences in the steroid binding region suggested that the cytosolic and microsomal activities of 17β-HSD in human term placenta did not reflect simply the distribution of a single enzyme between the two subcellular fractions, but that there were two distinct forms of the enzyme. This led us to focus our attention on the 17β-HSD of human placental microsomes. Here we could observe a relatively high level of activity with T, although still significantly less than that with E_2.

As an approach to clarifying the relationship between the soluble and membrane bound forms of 17β-HSD, we designed experiments in which the inhibitory properties of E_2 and T were compared. Representative results are shown in Fig. 1. It was confirmed that the soluble enzyme, which we now know to be type 1, was not affected by T or 20α-DHP. It was also apparent that a portion of the microsomal activity with E_2 was inhibited by T or 20α-DHP, but that up to 50% was not, even up to concentrations of T or 20α-DHP of 50–200 μM. In contrast, activity with T was inhibited competitively by E_2.

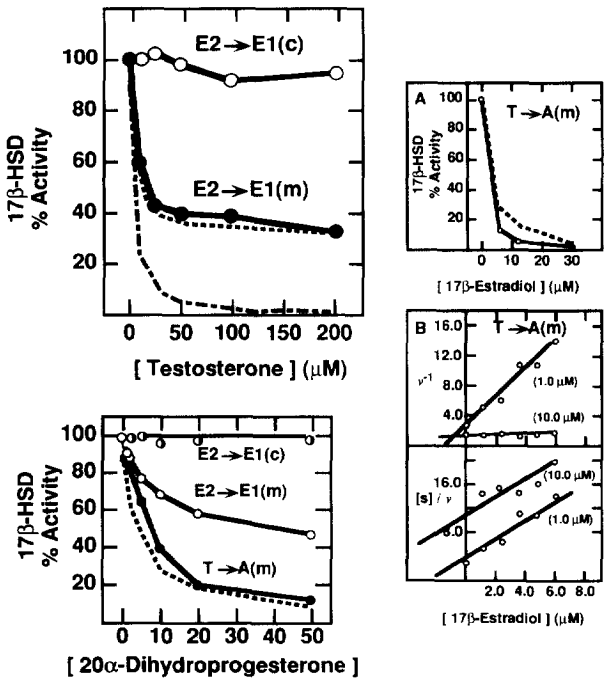

Fig. 1. Inhibition of cytosolic (C) and microsomal (M) 17β-HSD activities of human term placenta by testosterone, 20α-dihydroprogesterone and E_2. The results are adapted from Blomquist *et al.* [23].

Table 3. E_2/T activity ratios for microsomes from human breast tissue

Tissue type	E_2/T activity ratio*
Normal, non-adipose	1.75 ± 1.04 (4)
Adipose tissue	1.93 ± 1.44 (10)
Fibroadenoma	1.19 ± 0.38 (2)

*Reaction mixtures contained 1.0 μM [^{3}H] E_2 or [^{3}H]T, 0.5 μM NAD and microsomal protein in 0.08 M bicine, pH 9.0. The rate of product formations was quantified as described by Blomquist *et al.* [20]. The values used for the ratios were the mean of (n) separate samples assayed in triplicate.

These findings led us to postulate the existence of at least two forms of 17β-HSD, a soluble form highly-specific for E_2 and E_1 and a microsomal form reactive not only with E_2 and T, but with 20α-HSD activity as well [23]. The nature of the microsomal activity with E_2 not inhibited by T or 20α-DHP was problematic, and we speculated it could be type 1 enzyme recovered in the microsomal fraction. Recent data suggest it may be related to a membrane-bound form of 17β-HSD highly specific for E_2, recently purified and cloned from porcine endometrium [24, 25].

17β-HSD of human breast

It is now well-established that the 17β-HSD enzymology of human breast glandular and adipose tissue, as well as breast tumors, is complex [26, 27]. Immunochemical evidence for the occurrence of 17β-HSD type 1 has been presented [28]. Our knowledge of 17β-HSD enzymology of breast tissue microsomes, however, is limited.

On the basis of reports showing that microsomes from normal breast epithelial cells and breast neoplasms have a high microsomal estradiol dehydrogenase specific activity relative to cytosol [29, 30], we felt it to be of interest to examine a series of microsome preparations from breast tissue for 17β-HSD activity with E_2 and T under conditions identical to those used with placental microsomes. As shown in Table 3, the E_2/T activity ratio for three different tissue types is low, characteristic of 17β-HSD type 2 [6]. When inhibition of activity with E_2 or T by 5α-DHT was examined, we detected a pattern similar to that of term placental microsomes in a specimen of breast adipose tissue [Fig. 2(A)]. We also observed inhibition patterns suggestive of the presence of a type 2-like activity in specimens of fibroadenoma [Fig. 2(B)].

These observations suggest breast glandular epithelial cells may be relatively enriched in 17β-HSD

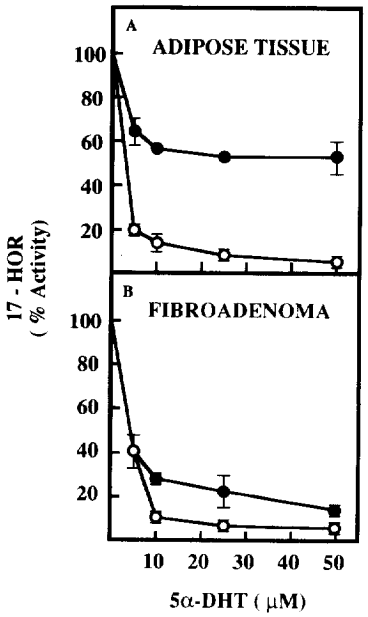

Fig. 2. Inhibition of microsomal 17β-HSD activity with E_2 (●) and T (□) of (A) brest adipose tissue and (B) a sample of fibroadenoma by 5α-DHT. Microsomes were prepared and assays run as described by Blomquist *et al.* [23]. The data are the mean ± SE of duplicate assays.

type 2-like activity, consistent with previous observations [29, 30], whereas breast adipose tissue shows evidence of the E_2-specific, microsomal activity noted with placental microsomes [23].

17β-HSD of human testis

There is evidence from comparisons of 17β-HSD activity of testicular tissue samples from normal males and individuals with testicular feminization of kinetically differing dehydrogenase and reductase activities [31, 32]. There are, however, few data regarding relative activities with E_2 and T. In 1982, Leinonen [33] studied reactivity with E_2 and T in testicular homogenates and observed an E_2/T activity ratio of less than one. 17β-HSD type 3, a reductase, has been cloned from human testicular poly(A)$^+$ mRNA and shown to be highly specific for NADPH [7]. Attempts to detect mRNA for 17β-HSD type 1 [5] and type 2 [8] in human testicular tissue have been unsuccessful. To examine this question further, we assayed testicular microsomes and cytosol, as well as tissue homogenates, for 17β-HSD activity with E_2 and T under optimal conditions for detecting types 1 and 2 in placental homogenates.

Table 4. E_2/T activity ratios for testicular tissue subcellular fractions and homogenates

	17-HSD		
Fraction	E_2	T	E_2/T (n)*
Homogenate	113.8 ± 13.2	63.0 ± 8.2	1.96 ± 0.53 (2)
Cytosol	49.0 ± 26.4	17.7 ± 13.6	3.33 ± 1.25 (3)
Microsomes	95.1 ± 36.9	110.1 ± 55.8	0.99 ± 0.25 (3)

*Reaction mixtures contained 1.0 μM [^{3}H]E_2 or [^{3}H]T, 0.5 μM NAD and tissue fraction in 0.08 M bicine, pH 9.0. The rate of product formation was quantified as described by Blomquist *et al.* [20]. The values ± SE are from (n) separate samples for which activity was assayed in triplicate. Units of activity are pmol/mg protein·h.

When we assayed a series of tissue specimens with NAD as cofactor, the results shown in Table 4 were obtained. The E_2/T activity ratio for testicular cytosol of 3.33 ± 1.25 is markedly less than the ratio of greater than 100, characteristic of placental cytosol [18]. The E_2/T activity ratio for testicular microsomes is characteristic of 17β-HSD type 2, but further study will be required to rationalize the kinetic data with the Northern blot analysis for mRNA [8].

A comparison of specific activities with E_2, T, E_1 and A of a sample of testicular microsomes with those of a sample of placental microsomes is presented in Fig. 3. Quite clearly, relative activities with T and E_2 differ significantly when testicular and placental microsomes are assayed under identical conditions. But with both tissues, optimal dehydrogenase activity assayed at pH 9.0 with NAD as cofactor exceeds reductase activity assayed with NADPH at pH 7.2, consistent with kinetically differing dehydrogenase and reductase activities, as has been suggested [31–33].

17β-HSD of human ovary

The results of Tremblay and collaborators demonstrated the presence of 17β-HSD type 1 mRNA in human ovary and granulosa cells and led to the

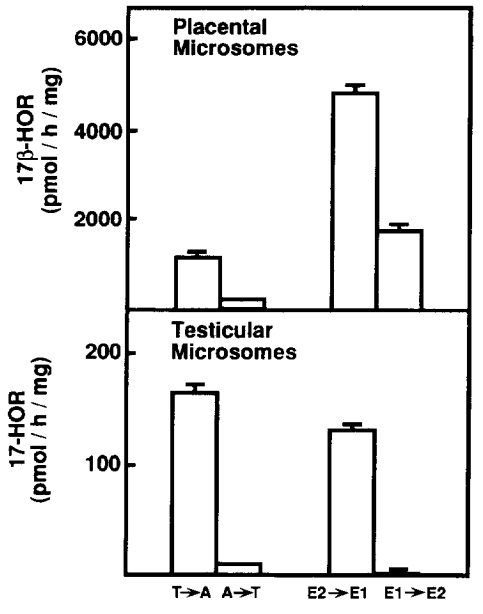

Fig. 3. A comparison of the 17β-HSD activities with T, A, E_2 and E_1 of human term placental and testicular microsomes assayed under identical conditions. Microsomes were prepared and assays run as described by Blomquist *et al.* [23]. Reaction mixtures contained 1.0 μM ^{3}H-labeled E_2 or T and 0.5 mM NAD in 0.08 M bicine, pH 9.0, or 1.0 μM E_1 or A and 0.5 mM NADPH in 0.08 M hepes, pH 7.2.

*Table 5. 17β-HSD activity with E_2 and T and E_2/T activity ratios for cytosol and microsomes from human term placenta, granulosa-luteal (G-L) cells and pre-menopausal ovarian stroma**

Tissue	17β-HSD activity (*n* samples)		
	E_2	T	E_2/T
Placenta			
Cytosol	225 ± 36 (6)	1.63 ± 0.43 (6)	138
Microsomes	70.8 ± 7.2 (6)	20.73 ± 5.58 (6)	3.4
G-L cells			
Cytosol	98.7 ± 17.5 (11)	0.15 ± 0.12 (11)	658
Microsomes	3.5 ± 0.7 (9)	0.13 ± 0.02 (8)	26.9
Ovarian stroma			
Cytosol	0.58 ± 0.07 (8)	0.013 ± 0.006 (8)	44.6
Microsomes	0.12 ± 0.03 (8)	0.04 ± 0.01	3.0

*The data are from Blomquist *et al.* [39]. Units of activity are nmol/mg protein · 30 min.

proposal that a high level of type 1 may be characteristic of E_2-producing tissues [5]. Subsequently, Martel and coworkers [4] presented evidence of a high level of activity with both E_2 and T in homogenates of human ovary. In earlier work, Pittaway *et al.* [34, 35] and Barbieri *et al.* [36] presented evidence of membrane bound activity with T and A, as well as E_1, and suggested ovarian 17β-HSD enzymology may be complex [37]. Additionally, immunohistochemical studies indicate 17β-HSD type 1 is localized to granulosa cells [38]. These observations led us to examine the 17β-HSD activity of human ovarian stroma and granulosa-luteal cells and to compare it with term placental subcellular fractions assayed in parallel [39], with the objectives of evaluating specific activities with E_2 and T and E_2/T activity ratios under conditions which would allow for a comparison of the two organs.

Results from that study are shown in Table 5. The level of 17β-HSD type 1 activity, indicated by cytosolic activity with E_2, is high and comparable for placenta and granulosa-luteal cells. There are some major differences between the two tissues, as well. In particular, the level of microsomal activity with E_2 of placenta is 20-fold greater and that with T over 100-fold greater than that of granulosa-luteal cells, consistent with the high 17β-HSD type 2 mRNA signal on Northern blots of placental mRNA [6] and indicates 17β-HSD type 2-like activity is absent or at a low level in granulosa cells and in stroma/theca, as well. It is also of interest that the E_2/T activity ratio of granulosa-luteal cell microsomes significantly exceeds that of placental microsomes. This suggests that although the type 2-like activity with T is at a very low level, there is an appreciable level of membrane bound activity with E_2 in these cells.

More recently, we have been able to examine tissue samples from a granulosa cell tumor, a luteoma of pregnancy and a corpus luteum. These were particularly useful because of their homogeneity with regard to cell content and because a large amount of material relative to that from samples of granulosa-luteal cells was available for assay. The results in Table 6 indicate an activity pattern similar to that of granulosa-luteal cells with large cytosolic E_2/T activity ratios. The specific activity with E_2 of the granulosa cell tumor, which was very homogeneous with regard to cell content, appears to be significantly lower than that of granulosa-luteal cells. This raises the interesting possibility of differences in the 17β-HSD type 1 content between nonluteinized and luteinized granulosa cells.

Casey and coworkers [8] were unable to detect mRNA for 17β-HSD type 2 in Northern blots of poly A^+ RNA from human ovary. The basis for the discrepancy between the detection of type 2-like activity by direct assay [34–37, 39] and the absence of mRNA by Northern analysis remains to be clarified.

17β-HSD of mouse placenta and ovary

17β-HSD activity is ubiquitous in mouse tissues and variations in the E_2/E_1 activity ratio among tissues are suggestive of multiple forms of the enzyme [40]. In a recent study of mouse placenta during pregnancy, we observed a greater than 100-fold increase in a microsomal 17β-HSD type 2-like activity during the latter half of pregnancy [41]. In contrast, ovarian 17β-HSD activity approximated that of day 9 placenta and was constant from day 9 to day 21, as was the E_2/T activity ratio (Fig. 4). In preliminary experiments, a similar level of activity was observed in non-pregnant ovary.

Because the specific activity of mouse placental 17β-HSD was highest in microsomes, suggestive of a type 2-like activity, we focused initially on the characterization of ovarian microsomes. During the course of those studies, Ghersevich and coworkers [42] reported detecting a type 1-like activity in rat ovary, based on

*Table 6. 17β-HSD activity with E_2 and T and E_2/T activity ratios for a granulosa cell tumour, a luteoma of pregnancy and a corpus luteum**

Tissue	Cytosol			Microsomes		
	E_2	T	E_2/T	E_2	T	E_2/T
Granulosa cell tumor	7.26 ± 1.62	0.006 ± 0.001	1210	2.33 ± 0.15	0.03 ± 0.003	77.7
Luteoma of pregnancy	22.81 ± 0.98	0.14 ± 0.04	163	0.98 ± 0.21	0.19 ± 0.017	5.2
Corpus luteum (18 mm)	26.27 ± 3.36	0.014 ± 0.003	1876	0.62 ± 0.002	0.14 ± 0.01	4.4

*Reaction mixtures containing 1.0 μM [^{3}H]E_2 or [^{3}H]T, 0.5 mM NAD and subcellular fraction of 0.08 M bicine, pH 9.0, were assayed in duplicate as described by Blomquist *et al.* [39].

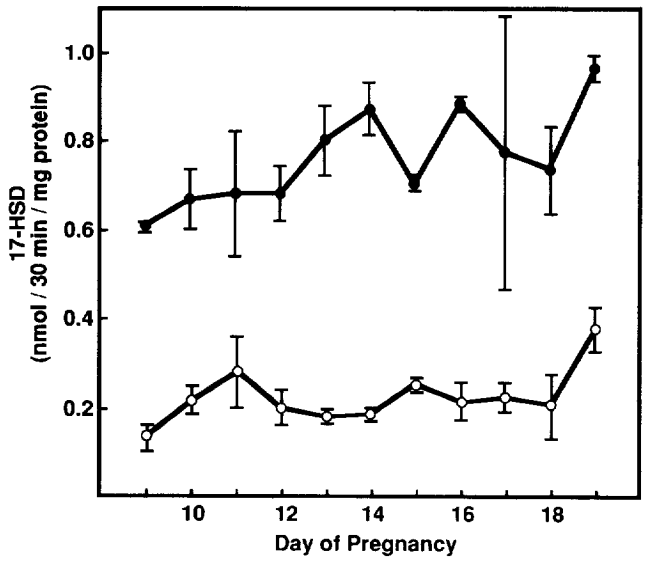

Fig. 4. 17β-HSD activity of homogenates of pregnant CF-1 mouse ovary at days 9–19 of pregnancy. Specific activity was measured with [^{3}H]E_2 or [^{3}H]T at 1.0 μM and with 0.5 mM NAD in 0.08 M bicine, pH 9.0. Homogenates were prepared and activity quantitated as described by Blomquist *et al.* [41].

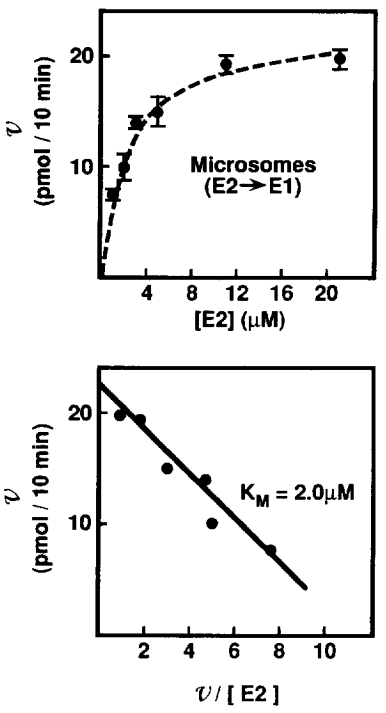

Fig. 5. Estimation of V_{max} and K_M for microsomal 17β-HSD activity of mouse ovary with E_2. Reaction mixtures (20 μl) containing 0.5 mM NAD, 1.0, 2.0, 3.0, 5.0, 11.0 or 21.0 μM [^{3}H]E_2 and 12 μg of microsomal protein in 0.08 M bicine, pH 9.0, were incubated at 37°C for 10 min. Ovaries were from nonpregnant, adult Swiss mice. Microsomes were prepared and reaction velocity quantitated as described by Blomquist *et al.* [41]. The values are the mean ± SE of duplicate assays. The dashed line in the figure is calculated for a K_M for E_2 of 2.0 μM and a V_{max} of 23.4 pmol/10 min.

immunoreactivity with antibody to human placental type 1. They subsequently cloned and sequenced a cDNA with 68% amino acid identity and 80% homology with the type 1 enzyme of human tissues.

These observations prompted us to broaden the scope of our experimentation to include both cytosol and microsomes of non-pregnant mouse ovary and to apply kinetic methods based on our previous work with human placenta and ovary.

Specific activities with E_2 and T of cytosol and microsomes with NAD and NADP as cofactors are shown in Table 7. It is of interest that microsomal activity with E_2 and T approximates that of human granulosa-luteal cells (Table 5). However in marked contrast with human placenta and granulosa-luteal cells, cytosolic 17β-HSD specific activity with E_2 and T approximates or is significantly less than that associated with microsomes. Apparent differences in E_2/T and NAD/NADP activity ratios led us to attempt to further characterize these activities.

An estimate of K_M and V_{max} for microsomal activity with E_2 is shown in Fig. 5. Over a 20-fold range of E_2 concentrations and with NAD as cofactor, the data are consistent with the presence of a single enzyme with a relatively high affinity for E_2 (K_M of 2.0 μM). Similarly, the pattern for microsomal activity with T (Fig. 6) is that expected for a single enzyme with a K_M for T of 19.3 μM (dashed line in Fig. 6). The E_2/T ratio, based on V_{max} values, of 0.4 is similar to that reported

*Table 7. 17β-HSD activity with E_2 and T of cytosol and microsomes from non-pregnant mouse ovary**

	17β-HSD activity			
	E_2		T	
	NAD	NADP	NAD	NADP
Cytosol	0.24 ± 0.01	1.02 ± 0.003	0.15 ± 0.01	0.19 ± 0.004
Microsomes	2.03 ± 0.002	1.35 ± 0.01	1.44 ± 0.01	0.42 ± 0.003

*Six ovaries from three animals were combined, fractionated by centrifugation and fractions assayed in duplicate in reaction mixtures containing 1.0 μM [^{3}H]E_2 or T, 0.5 mM coenzyme and cytosol or microsomes in 0.08 M bicine, pH 9.0. The units of activity ± SE are nmol/mg protein · 30 min. Activity was quantified as described by Blomquist *et al.* [41].

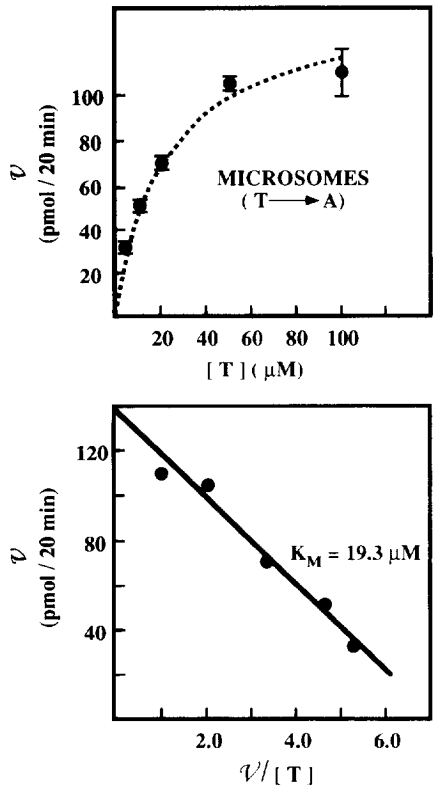

Fig. 6. Estimation of V_{max} and K_M for T with microsomal 17β-HSD of mouse ovary. Reactions were run at 37°C in reaction mixtures (20 μl total volume) containing 0.5 mM NAD, 5.0, 10.0, 20.0, 50.0 or 100 μM [^{3}H]T and 12 μg of microsomal protein in 0.08 M bicine, pH 9.0. Microsomes were prepared from ovaries of adult, random bred Swiss mice, reaction mixtures fractionated and reaction velocity quantitated as described by Blomquist *et. al.* [41]. The data are the mean ± SE of duplicate assays. The dashed line in the figure is calculated for a K_M of 19.3 μM for T with V_{max} equal to 139 pmol/20 min.

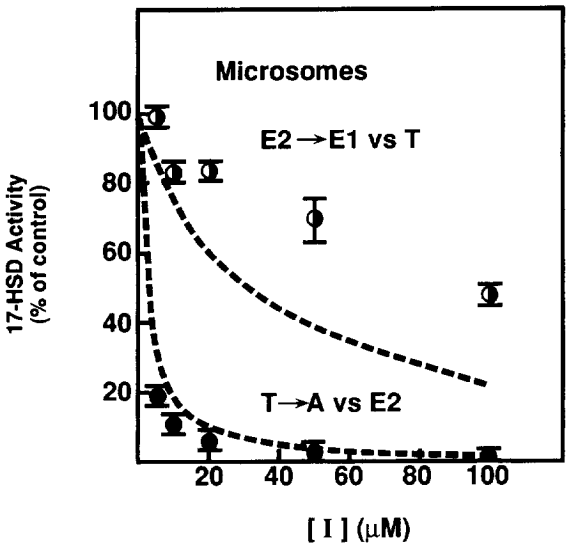

Fig. 7. Inhibition of mouse ovary microsomal 17β-HSD activity with E_2 by T and that with T by E_2. Substrate in each case was at 1.0 μM. Other reaction conditions are described in the legends to Fig. 5 and Fig. 6. The dashed lines in the figure are based on K_I values for E_2 and T (equal to K_M) of 2.0 and 19.3 μM, respectively, and competitive inhibition in both cases.

for 17β-HSD type 2 of human tissues by Wu and colleagues [6].

However when E_2 and T were examined as inhibitors, deviations from the pattern expected for a single enzyme with competitive inhibition between substrates were observed. As shown in Fig. 7, activity with T was inhibited by E_2 and the data fit the calculated line based on competitive inhibition with a K_M for T of 19.3 μM and a K_I for E_2 of 2.0 μM, equal to its apparent K_M value (Fig. 5). In contrast, a significant portion of the activity with E_2 is not inhibited by T, and the results deviate significantly from the pattern predicted for a single enzyme.

Table 8. 17β-HSD activity with E_2 and NADP or NAD of cytosol from mouse ovary, term placenta and G-L cells

Cytosol sample	Activity NADP	Activity NAD	Ratio (NADP/NAD)
Mouse ovary	1.13 ± 0.03	0.55 ± 0.01	2.05
Term placenta	82.42 ± 0.80	155.39 ± 0.82	0.53
G-L cell	40.89 ± 0.41	66.18 ± 2.79	0.62

Units of activity are nmol/mg protein·30 min ± SE for samples assayed in duplicate. Reaction mixtures containing cytosol 1.0 μM E_2 and 0.5 mM NADP or NAD in 0.08 M bicine, pH 9.0 were incubated at 37°C.

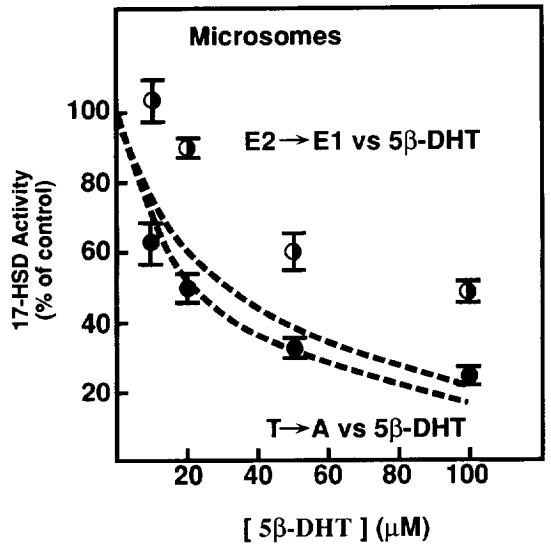

Fig. 8. Inhibition of mouse ovary microsomal 17β-HSD activity with E_2 and T by 5β-DHT. Reaction mixtures (20 μl total volume) containing 1.0 μM [^{3}H]E_2 or [^{3}H]T, 0, 10, 20, 50 or 100 μM 5β-DHT, 0.5 mM NAD and approx. 15 μg of microsomal protein were incubated at 37°C. Reaction velocity was quantitated as described by Blomquist *et al.* [41]. The dashed lines in the figure were calculated for competitive inhibition with K_M values for E_2 and T of 2.0 and 19.3 μM, respectively, and an estimated K_I value for 5β-DHT of 19.0 μM, based on the extent of inhibition observed with T as substrate and 10 μM 5β-DHT.

As an alternate approach, we examined the effect of 5β-DHT as an inhibitor because of its high affinity for 17β-HSD type 2 and extremely low affinity for the type 1 enzyme [20]. As shown in Fig. 8, activity with T was inhibited in a pattern in good agreement with that predicted for a type 2-like activity, but again a significant fraction of the activity with E_2 was unaffected by 5β-DHT. These inhibition patterns are suggestive of the presence of two enzymes, a type 2-like activity reactive with both E_2 and T, and also possibly a microsomal activity relatively specific for E_2 and with a low affinity for T or 5β-DHT, as described by Adamski and colleagues [24, 25].

Because cytosolic specific activity of mouse ovary 17β-HSD with both E_2 and T was highest with NADP as cofactor, we characterized this activity further. Estimates of K_M and V_{max} for E_2 with NADP as cofactor are shown in Fig. 9. The data agree well with the pattern predicted for a single enzyme with a K_M for E_2 of 9.9 μM.

When T was examined as an inhibitor of mouse ovarian cytosolic 17β-HSD activity with E_2, and samples of human term placental cytosol and granulosa-luteal cell sonicate assayed in parallel, the results shown in Fig. 10 were obtained. In marked contrast with the behavior of 17β-HSD type 1, the NADP-dependent 17β-HSD activity of mouse ovarian cytosol

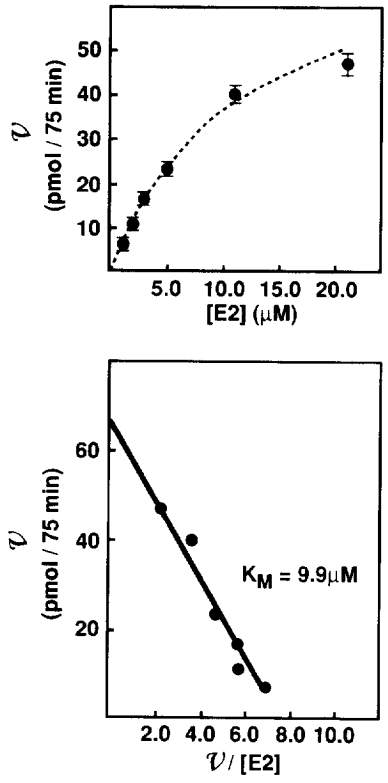

Fig. 9. Estimates of K_M and V_{max} for E_2 with cytosolic 17β-HSD of mouse ovary. Reaction mixtures (20 μl total volume) containing 0.5 mM NADP, 1.0, 2.0, 3.0, 5.0, 11.0 and 21.0 μM [^{3}H]E_2, and 3.5 mg of cytosol protein in 0.08 M bicine, pH 9.0, were incubated at 37°C for 75 min. Mouse ovary cytosol from nonpregnant Swiss mice was prepared and reaction velocity quantitated as described by Blomquist *et al.* [41]. The dashed line in the upper figure is based on an estimated K_M for E_2 of 9.9 μM and a value for V_{max} of 74 pmol/75 min. The data are mean values ± SE of duplicate assays.

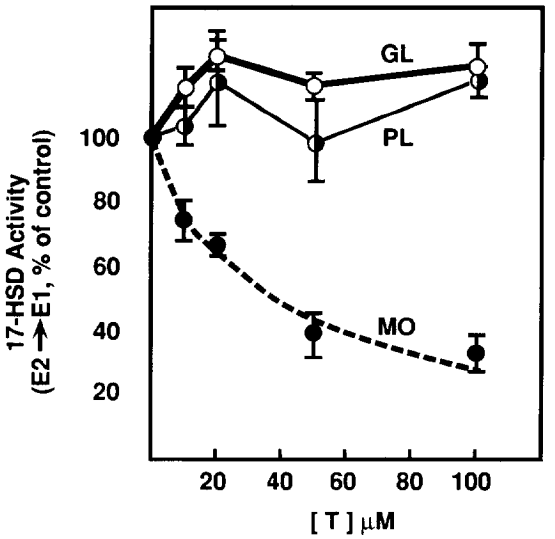

Fig. 10. Effect of T on 17β-HSD activity of mouse ovary cytosol (MO), term placental cytosol (PL) and granulosa-luteal cell (GL) sonicate with E_2. Reaction mixtures contained 1.0 μM [^{3}H]E_2, 0.5 mM NADP, 0, 10, 20, 50 or 100 μM T and cytosol or sonicate protein in 0.08 M bicine, pH 9.0. The values are the mean ± SE of duplicate assays. Reaction rate was quantitated as described by Blomquist *et al.* [41]. Cytosols and sonicate were prepared as described by Blomquist *et al.* [39, 41]. The dashed line in the figure is based on a K_M for E_2 of 9.9 μM and an estimated K_I for T of 31.6 μM, based on the assumption of competitive inhibition and the extent of inhibition observed with T at 10 μM and 20 μM.

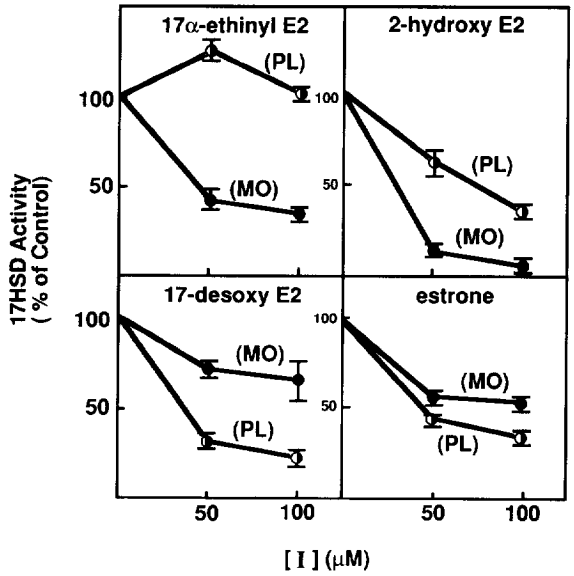

Fig. 11. Effects of 17α-ethinylE_2, 17β-desoxyE_2, 2-hydroxyE_2 and E_1 on cytosolic 17β-HSD activity of mouse ovary (●, MO) and human term placenta (○, PL) with E_2. Reaction mixtures contained 1.0 μM [^{3}H]E_2, 0, 50 or 100 μM inhibitor, 0.5 mM NADP and cytosol in 0.08 M bicine, pH 9.0. The values are the mean ± SE of duplicate assays.

has a significant affinity for T. The data are consistent with a K_I for T of approx. 30 μM (dashed line in Fig. 10).

Further evidence of fundamental differences between 17β-HSDs of mouse ovarian and human placental cytosol was obtained when various C_{18} steroids were compared as inhibitors (Fig. 11). NADP-dependent activity with E_2 of mouse ovary cytosol appears to have a greater affinity for ethinylE_2 and 2-hydroxyE_2 than 17β-HSD type 1, but binds 17β-desoxyE_2 less tightly. The comparable affinities for E_1 are consistent with a significant reductase activity for both enzymes.

These findings suggest the 17β-HSD enzymology of mouse ovary is complex, with multiple enzyme forms distributed between subcellular membrane fractions and the cytosol. A microsomal activity is similar in its substrate specificity to 17β-HSD type 2, but a cytosolic activity reactive with NADP as cofactor appears to have a broader specificity than 17β-HSD type 1 of human placental cytosol and granulosa cells.

That the 17β-HSD enzymology of mouse ovarian cytosol might be complex is also suggested by the results of experiments with NAD and NADP added singly and in combination. As shown in Fig. 12, with E_2 as substrate, the activity in the presence of combined coenzymes is not intermediate to that with NAD or NADP alone, which would fit a competitive inhibition model with a single enzyme. It is also less than that predicted by a model with two separate enzymes, each specific for NAD or NADP. The results are suggestive of the presence of more than one cytosolic form of 17β-HSD. The structural relationship of these to the multiple cytosolic forms of dihydrodiol dehydrogenase purified from mouse liver [43, 44], which are reactive with E_2 and T, remains to be established.

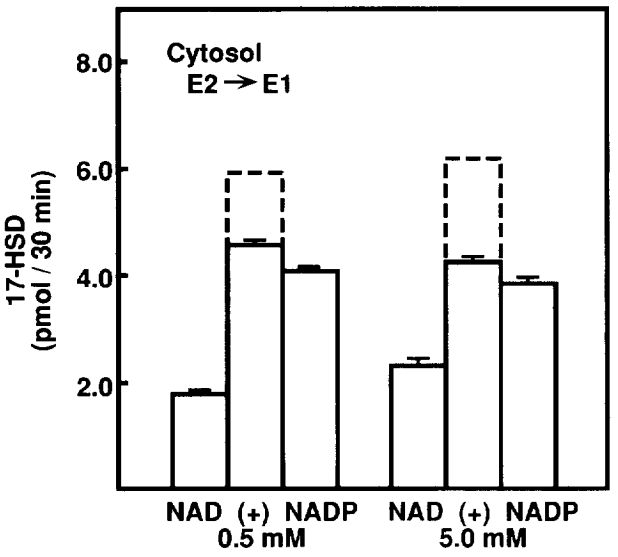

Fig. 12. 17β-HSD activity of mouse ovary cytosol with E_2 (1.0 μM) and NAD or NADP alone or in combination (+), each at 0.5 mM in 0.08 M bicine, pH 9.0. The dashed lines indicate the predicted total activity for two separate enzymes specific for NAD or NADP.

CONCLUDING REMARKS

It has been over 30 years since Aoshima and Kochakian described 17β-HSD activities in subcellular fractions from liver and kidney of a variety of experimental animals [2] and over 40 years since the short paper by Ryan and Engel reporting on the interconversion of E_2 and E_1 by homogenates of human placenta, endometrium and breast [1]. Until recently, progress in delineating the complexity of 17β-HSD enzymology depended on kinetic studies of activity associated with various subcellular fractions and on the purification of soluble or solubilized enzyme proteins. Spectacular progress in cloning and sequencing coupled with the characterization of the properties of 17β-HSD isoforms in transfected cells has allowed for the detailed characterization of four distinct gene products with 17β-HSD activity. The results presented here suggest there are still other forms to be characterized, and that kinetic studies will continue to be a fundamentally useful part of that process.

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REFERENCES

1. Ryan K. J. and Engel L. L.: The interconversion of estrone and estradiol by human tissue slices. *Endocrinology* 52 (1953) 287–291.
2. Aoshima Y. and Kochakian C. D.: Activity, intracellular distribution and some properties of 17β-hydroxy-C_{19}-steroid dehydrogenases in liver and kidney. *Endocrinology* 72 (1963) 106–114.
3. Milewich L., MacDonald P. C. and Carr B. R.: Activity of 17β-hydroxysteroid oxidoreductase in tissues of the human fetus. *J. Endocr.* 123 (1989) 509–518.
4. Martel C., Rheaume E., Takahashi M., Trudel C., Couet J., Luu-The V., Simard J. and Labrie F.: Distribution of 17β-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. *J. Steroid Biochem. Molec. Biol.* 41 (1992) 597–603.
5. Tremblay Y., Ringler G. E., Morel Y., Mohandas T. K., Labrie F., Strauss III J. F. and Miller W. L.: Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen-q25. *J. Biol. Chem.* 264 (1989) 20,458–20,462.
6. Wu L., Einstein M., Geissler W. M., Chan K., Elliston K. O. and Andersson S.: Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. *J. Biol. Chem.* 268 (1993) 12,964–12,969.
7. Geissler W. M., Davis D. L., Wu L., Bradshaw K. D., Patel S., Mendonca B. B., Elliston K. O., Wilson J. D., Russell D. W. and Andersson S.: Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. *Nature Genet.* 7 (1994) 34–39.
8. Casey M. L., MacDonald P. C. and Andersson S.: 17β-Hydroxysteroid dehydrogenase type 2: chromosomal assignment and progestin regulation of gene expression in human endometrium. *J. Clin. Invest.* 94 (1994) 2135–2141.
9. Graves J. M. H., Clark A. and Ringold H.: Sterochemical aspects of the substrate specificity of horse liver alcohol dehydrogenase. *Biochemistry* 4 (1965) 2655–2671.

10. Theorell H., Taniguchi S., Akeson A. and Skursky L.: Crystallization of a separate steroid-active liver alcohol dehydrogenase. *Biochem. Biophys. Res. Commun.* **24** (1966) 603–610.
11. Baker M. E.: Sequence similarity between *Pseudomonas* dihydrodiol dehydrogenase, part of the gene cluster that metabolizes polychlorinated biphenyls, and dehydrogenases involved in metabolism of ribitol and glucitol and the synthesis of antibiotics and 17β-oestradiol, testosterone and corticosterone. *Biochem. J.* **267** (1990) 839–841.
12. Krozowski Z.: The short-chain alcohol dehydrogenase superfamily: variations on a common theme. *J. Steroid Biochem. Molec. Biol.* **51** (1994) 125–130.
13. Engel L. L. and Groman E. V.: Human placental 17β-estradiol dehydrogenase: characterization and structural studies. *Rec. Prog. Hormone Res.* **30** (1974) 139–169.
14. Strickler R. C., Tobias B. and Covey D. F.: Human placental 17β-estradiol and 20α-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **256** (1981) 316–321.
15. Lehmann W. D. and Breuer H.: Characterization and kinetics of a microsomal 17β-hydroxysteroid oxidoreductase of human placenta. *Hoppe-Seyler's Z. Physiol. Chem.* **348** (1967) 1633–1639.
16. Pollow K., Runge W. and Pollow B.: Microsome-associated 17β-hydroxysteroid dehydrogenases of human placenta. II. Kinetic studies and characterization of the solubilized estradiol- and testosterone-sensitive 17-HSD activities. *Z. Naturforsch.* **30c** (1975) 17–24.
17. Thomas C. M. G. and Veerkamp J. H.: The subcellular distribution of 17β-hydroxysteroid dehydrogenase in the human term placenta. *Acta Endocr.* **82** (1976) 150–163.
18. Blomquist C. H., Kotts C. E. and Hakanson E. Y.: Inhibition of human placental 17β-hydroxysteroid dehydrogenase by steroids and nonsteroidal alcohols: Aspects of inhibitor structure and binding specificity. *Arch. Biochem. Biophys.* **186** (1978) 35–41.
19. Blomquist C. H., Kotts C. E. and Hakanson E. Y.: Microsomal 17β-hydroxysteroid dehydrogenase of guinea pig liver: Substrate and inhibitor specificity and effects of pH on steroid-enzyme interactions. *Arch. Biochem. Biophys.* **212** (1981) 63–71.
20. Blomquist C. H., Lindemann N. J. and Hakanson E. Y.: Inhibition of 17β-hydroxysteroid dehydrogenase (17β-HSD) activities of human placenta by steroids and non-steroidal hormone agonists and antagonists. *Steroids* **43** (1984) 571–586.
21. Sweet F., Boyd J., Medina O., Konderski L. and Murdock G. L.: Hydrogen bonding in steroidogenesis: Studies on new heterocyclic analogs of estrone that inhibit human estradiol 17β-dehydrogenase. *Biochem. Biophys. Res. Commun.* **180** (1991) 1057–1063.
22. Puranen T. J., Poutanen M. H., Peltoketo H. E., Vihko P. T. and Vihko R.: Site-directed mutagenesis of the putative active site of human 17β-hydroxysteroid dehydrogenase type 1. *Biochem. J.* **304** (1994) 289–293.
23. Blomquist C. H., Lindemann N. J. and Hakanson E. E.: 17β-Hydroxysteroid and 20α-hydroxysteroid dehydrogenase activities of human placental microsomes: kinetic evidence for two enzymes differing in substrate specificity. *Arch. Biochem. Biophys.* **239** (1985) 206–215.
24. Adamski J., Husen B., Marks F. and Jungblut P. W.: Purification and properties of estradiol 17β-dehydrogenase extracted from cytoplasmic structures of porcine endometrium cells. *Biochem. J.* **288** (1992) 375–381.
25. Leenders F., Adamski J., Husen B., Thole H. H. and Jungblut P. W.: Molecular cloning and amino acid sequence of the porcine 17β-estradiol dehydrogenase. *Eur. J. Biochem.* **222** (1994) 221–227.
26. Tait G. H., Newton C. J., Reed M. J. and James V. H. T.: Multiple forms of 17β-hydroxysteroid oxidoreductase in human breast tissue. *J. Molec. Endocr.* **2** (1989) 71–80.
27. Mann V. Z., Newton C. J. and Tait G. H.: 17β-Hydroxysteroid dehydrogenase in human breast tissues: purification and characterization of soluble enzymes and the distribution of particulate and soluble forms in adipose, non-adipose and tumour tissues. *J. Molec. Endocr.* **7** (1991) 45–55.
28. Poutanen M., Isomaa V., Lehto V.-P. and Vihko R.: Immunological analysis of 17β-hydroxysteroid dehydrogenase in benign and malignant human breast tissue. *Int. J. Cancer* **50** (1992) 386–390.
29. Lubbert H. and Pollow K.: Correlation between the 17β-hydroxysteroid dehydrogenase activity and the estradiol and progesterone receptor concentration of normal and neoplastic mammary tissue. *J. Molec. Med.* **3** (1978) 175–182.
30. Fournier S., Kuttenn F., de Cicco F., Baudot N., Malet C. and Mauvais-Jarvis P.: Estradiol 17β-hydroxysteroid dehydrogenase activity in human breast fibroadenomas. *J. Clin. Endocr. Metab.* **55** (1982) 428–433.
31. Goebelsman U., Hall T. D., Paul W. L. and Stanczyk F.: *In vitro* steroid metabolic studies in testicular 17β-reduction deficiency. *J. Clin. Endocr. Metab.* **41** (1975) 1136–1143.
32. Wilson S. C., Hodgins M. B. and Scott J. S.: Incomplete masculinization due to a deficiency of 17β-hydroxysteroid dehydrogenase: comparison of prepubertal and peripubertal siblings. *Clin. Endocr.* **26** (1987) 459–469.
33. Leinonen P.: 17β-Oxidation of estradiol and testosterone in the human testis. *J. Steroid Biochem.* **16** (1982) 277–281.
34. Pittaway D. E., Andersen R. N. and Giverns J. R.: Characterization of human ovarian oestradiol-17β oxidoreductase activity. *Acta Endocr.* **85** (1977) 624–635.
35. Pittaway D. E., Andersen R. N., Coleman S. A. Jr, Giverns J. R. and Wiser W. L.: Human ovarian 17β-hydroxysteroid oxidoreductase activity: a comparison of normal and polycystic ovarian tissue. *J. Clin. Endocr. Metab.* **56** (1983) 715–719.
36. Barbieri R. L., Osathanondh R. and Ryan K. J.: Danazol inhibition of steroidogenesis in the human corpus luteum. *Obstet. Gynec.* **57** (1981) 722–724.
37. Barbieri R. L.: Human ovarian 17-ketosteroid reductase: Unique characteristics of the granulosa-luteal cell and stromal enzyme. *Am. J. Obstet. Gynec.* **166** (1992) 1117–1126.
38. Sawetawan C., Milewich L., Word R. A., Carr B. R. and Rainey W. E.: Compartmentalization of type I 17β-hydroxysteroid oxidoreductase in the human ovary. *Molec. Cell. Endocr.* **99** (1994) 161–168.
39. Blomquist C. H., Bealka D. G., Hensleigh H. C. and Tagatz G. E.: A comparison of 17β-hydroxysteroid oxidoreductase type 1 and type 2 activity of cytosol and microsomes from human term placenta, ovarian stroma and granulosa-luteal cells. *J. Steroid Biochem. Molec. Biol.* **49** (1994) 183–189.
40. Milewich L., Garcia R. L. and Gerrity L. W.: 17β-Hydroxysteroid oxidoreductase: a ubiquitous enzyme. Interconversion of estrone and estradiol-17β in BALB/c mouse tissues. *Metabolism* **34** (1985) 938–944.
41. Blomquist C. H., Hensleigh H. C., Block D. L. and Feeney L. A.: Placental 17β-hydroxysteroid oxidoreductase, lactate dehydrogenase and malate dehydrogenase during the latter half of pregnancy in the mouse. *J. Steroid Biochem. Molec. Biol.* **46** (1993) 61–67.
42. Ghersevich S., Nokelainen P., Poutanen M., Orava M., Autio-Harmainen H., Rajaniemi H. and Vihko R.: Rat 17β-hydroxysteroid dehydrogenase type 1: primary structure and regulation of enzyme expression in rat ovary by diethylstilbestrol and gonadotropins *in vivo*. *Endocrinology* **135** (1994) 1477–1487.
43. Bolcsak L. E. and Nerland D. E.: Purification of mouse liver benzene dihydrodiol dehydrogenases. *J. Biol. Chem.* **258** (1983) 7252–7255.
44. Sawada H., Hara A., Nakayama T., Nakagawa M., Inoue Y., Hasebe K. and Zhang Y.-P.: Mouse liver dihydrodiol dehydrogenases. *Biochem. Pharmac.* **37** (1988) 453–458.