Human 17 β -hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression

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17 β -Hydroxysteroid dehydrogenase (17HSD) isoenzymes catalyse the interconversion between highly active 17 β -hydroxy- and low-activity 17-keto-steroids and thereby regulate the biological activity of sex steroids. The present study was carried out to characterize 17HSD activity and the expression of 17HSD type 1 and 2 isoenzymes in several human cell types and tissues. The data indicate that in cultured cells the direction of 17HSD activity is exclusively determined by the expression of these distinct isoenzymes. The intracellular environment could not modulate the direction of the enzyme activities in any of the cell types analysed. 17HSD type 1 acts as a reductase converting oestrone into oestradiol, whereas 17HSD type 2 possesses oxidative activity inactivating oestradiol by converting it into oestrone. The data, furthermore, suggest that of the two 17HSD

type 1 mRNAs (1.3 and 2.3 kb), expression of the 1.3 kb mRNA is related to enzyme concentration in all the cell types studied. This mRNA is principally expressed in cells of placental and ovarian origin, but is also present in malignant breast epithelial cells. In contrast, 17HSD type 2 is more widely expressed. It is present in several oestradiol-metabolizing tissues as well as in some target cells of sex steroid action. The opposite reaction directions observed in the cultured cells, together with differences in the distribution of the isoenzymes, suggest that type 1 is involved in oestradiol production in females while type 2 plays a role in the inactivation of this sex steroid in peripheral tissues, both in females and in males. However, some examples exist of simultaneous expression of both enzymes in the same cell type or tissue.

INTRODUCTION

17 β -Hydroxysteroid dehydrogenases (17HSDs) catalyse the interconversion of neutral and phenolic 17 β -hydroxy- and 17-keto-steroids, thereby regulating the biological activity of sex steroids. Both androgens and oestrogens have their highest biological activity in their 17 β -hydroxy configuration, and reductive 17HSD activity is essential for the biosynthesis of testosterone and oestradiol in the testis and ovary respectively. 17HSD activity is not limited to steroidogenic tissues only, but is also found in a number of other tissues [1,2], including target tissues for both androgens and oestrogens. It has therefore been suggested that in addition to the hormone concentration in the blood circulation, local interconversion of sex steroids by 17HSDs modulates their biological efficacy [3–5].

The conversion of oestradiol [E2, 1,3,5(10)-oestratriene-3,17 β -diol] into oestrone [E1, 3-hydroxy-1,3,5(10)-oestratriene-17-one] is the predominant 17HSD activity in the human endometrium. The progestin-dependent increase in activity of this enzyme during the luteal phase of the menstrual cycle is thought to be an important factor in decreasing exposure of the endometrium to oestrogens [6–8]. In contrast, other studies have shown that the conversion of E1 into E2 is predominant in human breast tissue [9]. In cultured breast cancer cells, however, both oxidative (E2 into E1) and reductive (E1 into E2) activities have been detected [10–12]. Based on these results it has been suggested that different intracellular environments and cofactor availabilities in different cell types could determine the reaction direction [13,14],

and that opposite 17HSD activities would be expressed, for example, in oestrogen receptor-positive and -negative breast cancer cells [10].

At least three different human 17HSD enzymes (17HSD types 1, 2 and 3) exist [15–17] and the diversity of 17HSD activities in different tissues could be explained by the presence of different isoenzymes. The expression of the enzymes in different cell types and the physiological functions of the isoenzymes are, however, only superficially known. Our current interest is focused on defining the role of each isoenzyme in steroid metabolism, both in steroidogenic tissues and in the target tissues of steroid hormone action. In the present study we have characterized 17HSD activity and the expression of human 17HSD type 1 and type 2 isoenzymes in several human cell types and tissues. In addition, the roles of the two 17HSD type 1 mRNAs (1.3 kb and 2.3 kb) in controlling human 17HSD type 1 protein concentration and its activity were investigated.

EXPERIMENTAL

Materials

[2,4,6,7- 3 H]Oestrone (101 Ci/mmol) and [2,4,6,7- 3 H]oestradiol (98 Ci/mmol) were purchased from Amersham International plc (Amersham, Bucks., U.K.). Unlabelled oestrone and oestradiol were obtained from Steraloids Inc. (Wilton, NH, U.S.A.). Cell culture media and supplements were from Gibco

Abbreviations used: 17HSD, 17 β -hydroxysteroid dehydrogenase; E1, oestrone, 3-hydroxy-1,3,5(10)-oestratriene-17-one; E2, oestradiol, 1,3,5(10)-oestratriene-3,17 β -diol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DOTAP, *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium-methylsulphate.

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(Grand Island, NY, U.S.A.) or the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Guanidine isothiocyanate was from Fluka (Buchs, Switzerland), caesium chloride and oligo(dT) cellulose were from Boehringer Mannheim (Mannheim, Germany), agarose was from FCM BioProducts (Rockland, ME, U.S.A.) and RNA molecular markers (RNA Ladder) were from BRL (Gaithersburg, MD, U.S.A.). Other reagents were either from the Sigma Chemical Co. or Merck A. G. (Darmstadt, Germany) and were of the highest purity grade available. The full-length cDNAs encoding 17HSD type 2 and type 3, cloned into the pCMV6 eukaryotic expression vector, together with the intact pCMV6 vector, were provided by Dr. S. Andersson (Merck Research Laboratories, Rahway, NJ, U.S.A.).

Cell culture

All the carcinoma cell lines used (breast cancer cell lines: BT-20, MCF-7, MDA-MB-231, MDA-MB-361 and T-47D; chorio-carcinoma cell lines: BeWo, JAR and JEG-3; endometrial cancer cell lines: HEC-1-A, HEC-1-B and RL95-2; ovarian cancer cell line: OVCAR-3; prostate cancer cell lines: DU145, LNCaP and PC-3; and monkey kidney cell line: CV-1) were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and were cultured according to the instructions of the supplier. Stock cultures were maintained in almost continuous exponential growth by weekly passage of the appropriate number of cells following treatment with 0.05% trypsin and 0.02% EDTA in PBS without Mg^{2+} and Ca^{2+} .

Tissue specimens

Tissue specimens were obtained surgically from patients with breast lesions or patients undergoing hysterectomy. All tissue specimens were stored in liquid nitrogen until processed.

17HSD activity measurements

Both reductive and oxidative 17HSD activities were measured in cultured cells. Cells were applied in 6-well plates (200 000 cells/well) and were allowed to attach overnight before activity measurements were made. The culture medium was aspirated from the wells and 2 ml of serum-free RPMI medium with 25 mM Hepes, containing oestrone or oestradiol at a concentration of 1 nM, was applied. The metabolism of the steroids was followed by adding the corresponding 3H -labelled substrate (200 000 c.p.m./ml). The cells were then incubated for the indicated times at 37 °C in the cell culture conditions. After incubation the media were removed, frozen in solid CO_2 and kept at -20 °C until the steroids were extracted from a 1 ml fraction into ether. The specimens were then evaporated to dryness, dissolved in acetonitrile/water (45:55 v/v) and further separated on a Sephasil C_{18} reverse-phase chromatography column connected to a SMART® System (Pharmacia LKB Biotechnology, Uppsala, Sweden). Fractions containing oestrone and oestradiol were dissolved in scintillation liquid and the radioactivities were measured in a β -counter. 17HSD activities were calculated as a percentage of the substrate used.

Measurement of 17HSD type 1 concentrations

Immunoreactive 17HSD type 1 concentrations in cell and tissue specimens were measured by time-resolved immunofluorimetric assay using a previously described method [18]. Cultured cells were harvested by washing the plates with PBS, scraping the cells from the plates into PBS and centrifuging at 1000 g for 5 min.

The cell pellets were then suspended in 1 ml of buffer A [10 mM Na_2HPO_4 (pH 7.5)/1 mM EDTA/0.5 mM PMSF/0.02% (w/v) NaN_3 /20% (v/v) glycerol] and sonicated twice for 15 s. The homogenates were then centrifuged at 800 g for 10 min and measurements were carried out in 100 µl aliquots of the supernatants. The tissue specimens were homogenized in buffer A with an Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany). The homogenates were centrifuged at 100 000 g for 1 h and measurements were carried out in 100 µl aliquots from the supernatants.

Northern blot analysis of 17HSD type 1 and type 2

Total RNA in cultured cells was prepared by washing the plates with PBS and lysing the cells in 4 M guanidine thiocyanate with 25 mM sodium acetate. The homogenates were centrifuged (21 h, 179 000 g) through a cushion of 5.7 M CsCl, followed by ethanol precipitation. Total RNA from breast cancer specimens and normal endometrium was prepared similarly, except that the tissues were homogenized in 4 M guanidine thiocyanate with 25 mM sodium acetate with an Ultra-Turrax homogenizer. Poly(A)⁺ RNA was isolated by oligo(dT) cellulose chromatography. For each sample, 10 µg of poly(A)⁺ RNA was resolved by 1% (w/v) agarose gel electrophoresis, transferred to nylon membrane (Hybond; Amersham, Bucks., U.K.) by capillarity and cross-linked by UV radiation. Tissue distribution of the 17HSD type 1 and type 2 enzymes was analysed using commercially available Northern-blots containing 2 µg of poly(A)⁺ RNA from different human tissues (Clontech, Palo Alto, CA, U.S.A.).

The membranes were prehybridized for 2 h at 42 °C in 5 × SSPE [1 × SSPE = 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/0.1 mM EDTA] containing 50% (v/v) formamide, 0.1% (w/v) BSA, 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinyl pyrrolidone, 0.5% (w/v) SDS and 20 µg/ml salmon sperm DNA. The membranes were then hybridized overnight using ^{32}P -labelled 1.0 kb 17HSD type 1 cDNA (*EcoRI*-*SacI* fragment), 1.0 kb 17HSD type 2 cDNA (*SacI* fragment) or 1.2 kb 17HSD type 3 cDNA (full length) as a probe at 42 °C. After hybridization, the membranes were washed twice in 2 × SSPE, containing 0.1% SDS, for 15 min each and once in 1 × SSPE, containing 0.1% SDS, for 30 min. The membranes were then exposed to Kodak XAR films (Rochester, NY, U.S.A.) for 4 days. To quantify the amount of mRNA applied to the gel, the membranes were also hybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or γ -actin cDNA-probes.

Transient expression of 17HSD type 1 and type 2

Both 17HSD type 1 and type 2 were transiently expressed in HEC-1-B, LNCaP and MCF-7 cells under the CMV promoter using pRC/CMV (Invitrogen, San Diego, CA, U.S.A.) and pCMV6 vectors. These plasmids were also used to transiently express 17HSD type 1 in PC-3 cells and 17HSD type 2 in JAR and T-47D cells. Recombinant plasmids together with intact plasmids were amplified in *Escherichia coli* JM109 cells (pRC/CMV-17HSD type 1) and in DH5 α cells (CMV6-17HSD type 2), and thereafter purified using QIAGEN®-columns (Qiagen, Hilden, Germany).

Transient transfections were then carried out using the DOTAP method {N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniummethylsulphate; Boehringer, Mannheim, Germany}}, according to the manufacturer's instructions. Briefly, cells from the stock cultures were plated in 10 cm-diameter dishes at 1.5×10^6 cells/dish and were allowed to attach overnight in the

culture medium. For each dish, 10 μ g of plasmid DNA was incubated for 10 min with 150 μ g/ml DOTAP in 350 μ l of 20 mM Hepes/150 mM NaCl, pH 7.4. After incubation, this reaction mixture was added to 10 ml of the culture medium containing 5% fetal calf serum and the reagent was applied to the cells. Transfection was carried out for 20 h, after which the transfection mixture was replaced with fresh medium and the cells were further cultured for 48 h. Activity measurements in the transfected cells were then carried out in 6-well plates as described above.

RESULTS

We first analysed 17HSD activity in several cultured human breast (BT-20, MCF-7, MDA-MB-231, MDA-MB-361 and T-47D), endometrial (HEC-1-A, HEC-1-B and RL95-2), ovarian (OVCAR-3) and prostate cancer cells (DU145, LNCaP and PC-3), human choriocarcinoma cells (BeWo, JAR and JEG-3), human skin fibroblasts and monkey kidney fibroblast-like tumour cells (CV-1). When 200 000 cells were incubated for 20 h, using 3 pmol of E1 or E2 as substrate, considerable 17HSD activity was found in 9 of the 16 analysed cell lines (more than 10% of substrate converted, Table 1). In T-47D breast cancer cells and in BeWo, JAR and JEG-3 choriocarcinoma cells a predominant reductive activity converting E1 to E2 was found. In contrast, RL95-2 endometrial cancer cells, PC-3 prostate cancer cells and CV-1 monkey kidney cells showed oxidative activity almost exclusively, leading to accumulation of oestrone over oestradiol in the culture medium. In BT-20 and MDA-MB-361 breast cancer cells, both reductive and oxidative activities were found: reductive activity prevailing in BT-20 cells and oxidative activity prevailing in MDA-MB-361 cells. No significant reductive or oxidative 17HSD activity was detected in the other analysed cell lines. After 20 h incubation with OVCAR-3 ovarian cancer cells, both E1 and E2 were effectively metabolized to metabolites other than E2 and E1 respectively. In these cells, only 10% of the steroids were eluted as E1 or E2

when 3 pmol of the steroids was used as substrate for 200 000 cells. The other metabolites formed were not characterized.

To further characterize 17HSD activities, expression of 17HSD type 1 and 2 mRNAs and 17HSD type 1 protein were analysed in all of the cell lines. Using Northern blot analysis of 10 μ g of poly(A)⁺ RNA, two mRNAs, 1.3 and 2.3 kb in size, were detected for 17HSD type 1 (Figure 1). The shorter 1.3 kb mRNA was detected only in BT-20, T-47D and MDA-MB-361 breast cancer cells and in BeWo, JAR and JEG-3 choriocarcinoma cells. In these cell lines the amount of 1.3 kb mRNA was related to 17HSD type 1 protein expression (Figure 1) and to the reductive 17HSD activity in the cultured cells (Table 1, Figure 2). The highest 17HSD type 1 protein concentration was measured in BT-20 cells.

In contrast to 1.3 kb mRNA, the 2.3 kb mRNA was present in all analysed human cell types including cell lines without detectable 17HSD type 1 protein or major reductive 17HSD activity (Table 1). CV-1 monkey kidney fibroblast-like cells were the only cells where no signal for 17HSD type 1 was found. A 1.5 kb signal for 17HSD type 2 mRNA was detected in BT-20 and MDA-MB-361 breast cancer cells, RL95-2 endometrial cancer cells, PC-3 prostate cancer cells and CV-1 monkey kidney cells. Hence, it is evident that 17HSD type 2 expression is related to the oxidation of E2 into E1 measured in the cultured cells (Table 1). In addition, in BeWo choriocarcinoma cells, minor oxidative activity was detected (Table 1). This activity could be related to slight 17HSD type 2 expression, not detected by Northern analysis, or to a non-characterized human 17HSD isoenzyme. Two of the five breast cancer cell lines analysed, namely BT-20 and MDA-MB-361 cells, expressed both 17HSD type 1 and type 2 enzymes and showed both oxidative (E2 into E1) and reductive (E1 into E2) 17HSD activities.

Conversions of E1 and E2 were then followed at different time points (Figure 2) in cells expressing 17HSD type 1 (BeWo, JAR, JEG-3 and T-47D), 17HSD type 2 (CV-1, PC-3 and RL95-2) and in cells expressing both type 1 and type 2 enzymes (BT-20 and MDA-MB-361). The data further confirmed that oestradiol

Table 1 Oxidative and reductive 17HSD activities in cultured cells

Serum-free medium containing 3 pmol of oestrone (E1 $\rightarrow$ E2) or oestradiol (E2 $\rightarrow$ E1) was applied to cells grown on 6-well plates (200 000 cells/well). After 20 h incubation the amounts of E1 and E2 in the media were measured. 17HSD activity was measured in triplicate wells and the results are presented as the percentage of substrate used in 20 h $\pm$ S.D. The whole experiment was repeated, with identical results (not shown).

Cell lines		Substrate converted in 20 h (%)	
		E1 $\rightarrow$ E2	E2 $\rightarrow$ E1
Breast cancer cells	BT-20	70.2 $\pm$ 0.9	26.7 $\pm$ 2.4
	MCF-7	4.5 $\pm$ 1.0	1.7 $\pm$ 0.5
	MDA-MB-231	1.6 $\pm$ 0.1	1.8 $\pm$ 0.1
	MDA-MB-361	23.3 $\pm$ 1.0	73.0 $\pm$ 1.0
	T-47D	89.0 $\pm$ 1.9	7.1 $\pm$ 0.2
Choriocarcinoma cells	BeWo	78.0 $\pm$ 0.6	19.9 $\pm$ 0.6
	JAR	92.2 $\pm$ 0.3	4.9 $\pm$ 0.1
	JEG-3	91.6 $\pm$ 0.8	6.0 $\pm$ 0.1
Endometrial cancer cells	HEC-1-A	4.7 $\pm$ 0.9	3.5 $\pm$ 0.9
	HEC-1-B	3.3 $\pm$ 0.1	2.8 $\pm$ 0.4
	RL95-2	0.8 $\pm$ 0.9	95.0 $\pm$ 0.4
Ovarian cancer cells	OVCAR-3	7.3 $\pm$ 1.5	8.0 $\pm$ 6.3
Prostate cancer cells	DU145	2.4 $\pm$ 1.2	0.7 $\pm$ 0.2
	LNCaP	5.8 $\pm$ 0.5	8.9 $\pm$ 0.9
	PC-3	1.5 $\pm$ 0.2	59.5 $\pm$ 0.2
Human skin fibroblasts		2.8 $\pm$ 0.2	1.2 $\pm$ 0.2
Monkey kidney cells	CV-1	7.7 $\pm$ 0.2	77.2 $\pm$ 1.5

Figure 1 Expression of 17HSD type 1 and type 2 mRNAs and 17HSD type 1 protein in (A) breast, endometrial and ovarian cancer cells, and (B) choriocarcinoma, prostate cancer, fibroblast and CV-1 monkey kidney cells

Poly(A)⁺ RNA (10 µg) from the indicated cells was used to analyse 17HSD type 1 (top) and 17HSD type 2 (middle) mRNA expression by Northern blot analysis. The amount of the RNA loaded was controlled by hybridizing the membranes with GAPDH cDNA (bottom). 17HSD type 1 protein concentrations were measured by time-resolved immunofluorimetric assay.

production is the predominant reaction in cells expressing 17HSD type 1, although minor 17 β -dehydrogenation was also detected in the cells. In BeWo and JEG-3 cells, an equilibrium between E1 and E2 was formed at about 90% E2:10% E1. All the cells expressing 17HSD type 2 showed oxidative activity almost exclusively and no E2 was produced in these cells. In the cells expressing both of the enzymes, the relative conversion rates for oxidation (E2 into E1) and reduction (E1 into E2) were related to the amounts of isoenzymes expressed.

Figure 2 17HSD activities in cell lines expressing either 17HSD type 1 or type 2 and in cells expressing both type 1 and type 2 enzymes

Reductive and oxidative 17HSD activities were followed for 20 h in cultured cells endogenously expressing 17HSD type 1 (BeWo, JAR, JEG-3 and T-47D), in cells expressing 17HSD type 2 (CV-1, PC-3 and RL95-2) and in cells expressing both type 1 and type 2 enzymes (BT-20 and MDA-MB-361). Serum-free medium containing 3 pmol of E1 or E2 was applied to the cells grown on 6-well plates (200 000 cells/well). After incubation (time points 0.5, 1, 2, 4, 10 and 20 h) the amounts of E1 and E2 in the media were measured. 17HSD activity is expressed as a percentage of the substrate used.

We further analysed whether the intracellular environment could modulate enzyme activities in different cell types by transiently expressing both 17HSD type 1 and type 2 in three different cell lines (LNCaP, HEC-1-B and MCF-7, Figure 3A) in which no significant 17HSD activity was endogenously present. The data showed that the enzyme activities of the transiently expressed enzymes were identical with those found in the cells endogenously expressing the enzymes. In all the cell types, 17HSD type 1 showed predominantly reductive activity (E1 into E2), whereas 17HSD type 2 showed oxidative activity (E2 into E1). Thus, it is unlikely that cofactor pools or other intracellular factors play a role in determining the direction of 17 β -oxidoreduction catalysed by the enzymes. Furthermore, we transfected cells endogenously expressing 17HSD type 1 with type 2 cDNA, and vice versa. The results obtained again indicated

Figure 3 Transient expression of 17HSD type 1 and type 2 in different cell types

(A) Three different cell types in which no significant 17HSD activity was present (endometrial cancer cells HEC-1-B, prostate cancer cells LNCaP and breast cancer cells MCF-7) were transiently transfected with 17HSD type 1 or 17HSD type 2. (B) Breast cancer cells T-47D, endogenously expressing 17HSD type 2 cDNA (○), were transfected with 17HSD type 2 cDNA (●), and prostate cancer cells PC-3 expressing 17HSD type 2 (□), were transfected with 17HSD type 1 cDNA (■). Cells (1.5×10^6) grown in 10 cm-diameter dishes were transfected with 10 μ g of plasmid DNA (pRC/CMV, pRC/CMV-17HSD type 1, CMV6 and CMV6-17HSD type 2). Activity measurements were thereafter carried out in 6-well plates (200 000 cells/well) in the presence of 3 pmol of oestrone (E1 $\rightarrow$ E2) or oestradiol (E2 $\rightarrow$ E1). After incubation (time points 0.5, 1, 2, 4, 10 and 20 h), the amounts of E1 and E2 in the media were measured. 17HSD activity is presented as a percentage of the substrate used.

opposite reactions for the enzymes (Figure 3B). Although the reductive activity of 17HSD type 1 in T-47D and JAR cells (results not shown) was not strongly affected by the transfected type 2 enzyme, the type 2 activity of PC-3 cells was effectively reversed by the transfected 17HSD type 1. The results, therefore, suggest that in cultured cells 17HSD type 1 has a higher catalytic efficacy than the type 2 enzyme.

The tissue distribution of 17HSD type 1 mRNA expression was then compared with that of 17HSD type 2. High expression

Figure 4 Tissue distribution of 17HSD type 1 and type 2 mRNAs

Membranes containing 2 μ g of poly(A)⁺ RNA from different human tissues were hybridized with 17HSD type 1 cDNA (top), 17HSD type 2 cDNA (middle) and with γ -actin cDNA (bottom).

Figure 5 Expression of 17HSD type 1 and type 2 mRNAs and 17HSD type 1 protein in breast cancer specimens and endometrial tissue

17HSD type 1 (top) and 17HSD type 2 (middle) mRNA expression was analysed by Northern blot analysis using 50 μ g of total (tot) RNA and 10 μ g of poly(A)⁺ RNA from breast cancer specimens and 10 and 20 μ g of Poly(A)⁺ RNA from endometrium. 17HSD type 1 protein concentrations from the breast cancer specimens were measured by time-resolved immunofluorimetric assay.

of the 1.3 kb mRNA for 17HSD type 1 was detected only in the steroidogenic tissues, placenta and ovary (Figure 4), and in some breast cancer specimens (Figure 5). In addition, low expression of the 1.3 kb mRNA was detected in the endometrium. As in the various cell lines, the amount of the 1.3 kb mRNA in the different breast cancer specimens was related to the enzyme protein concentration, whereas the 2.3 kb mRNA was found in all the breast cancer specimens and in almost all of the other tissues analysed. It was most abundantly expressed in the

endometrium, prostate, heart, muscle and placenta (Figures 4 and 5). The tissue distribution of the 1.5 kb mRNA for 17HSD type 2 expression is known to have a totally different pattern. It is strongly expressed in the endometrium, liver, placenta and small intestine. In addition, low levels of this mRNA are expressed in the colon, kidney, pancreas and prostate. Furthermore, 17HSD type 3 was not detected in any of the cell and tissue specimens, except the testis (results not shown).

DISCUSSION

It is well known that 17HSD activity is present in several mammalian tissues, and the possibility that 17HSD activity could modulate steroid action at the target cell level is interesting. It has been found that in different tissues, 17HSD activity may catalyse opposite reactions [8,9], and the factors governing the predominant reaction direction *in vivo* in different tissues and cell types are not known in detail [13]. Our present data strongly suggest that the types of 17HSD isoenzymes expressed are of key importance in regulating the direction of the reaction in different cell types. In this study, carried out with cultured cells, the balance of E1 and E2 in the culture media was exclusively determined by cell-specific expression of 17HSD type 1 or type 2 isoenzymes. All the cells expressing 17HSD type 1 showed predominantly reductive activity, thereby converting E1 into E2. In contrast, the cells expressing 17HSD type 2 showed exclusively the opposite, oxidative activity. The intracellular environment could not modulate the direction of the enzyme activities in any of the cell types analysed. The predominantly opposite activities found in cultured cells are in line with recent kinetic data observed *in vitro*. Even if both of the reactions catalysed by the two enzymes are reversible, the $V_{\max}/K_m$ value for type 2 enzyme has been shown to be about 20-fold higher for the oxidative direction [16], whereas for type 1 enzyme a 2-fold higher value for the reductive activity has been found [19]. It is evident, however, that compared with cell homogenates, 17HSD type 1 expressed in cultured cells showed a higher 17 β -reduction/oxidation ratio, therefore suggesting that the intracellular environment may promote the reductive activity of the enzyme.

These two enzymes have been shown to have different cofactor and substrate specificities, and subcellular localizations which could contribute to the opposite reactions shown for the enzymes in intact cells. 17HSD type 2 has been shown to prefer NAD⁺/NADH over the corresponding phosphorylated forms of the coenzyme (NADP⁺/NADPH), while 17HSD type 1 has been shown to use both of the coenzymes equally well *in vitro* [16,20]. As there is evidence that NAD⁺ exists mainly in the oxidized form and NADP⁺ mainly in the reduced form in cells, the differential cofactor specificities could partially control the oxidation/reduction direction catalysed by the enzymes ([21] and refs. therein). Furthermore, the data suggest that 17HSD type 2 is a membrane-bound protein, the active site being located in the lumen of the endoplasmic reticulum, while 17HSD type 1 is located in the cytosol. There is evidence implying that the thermodynamic environment in the lumen of the endoplasmic reticulum is more oxidative than that in the cytosol [22], and therefore, the different subcellular localizations of the 17HSD type 1 and type 2 isoenzymes could also contribute to the different reaction directions in intact cells in culture. Both 17HSD type 1 and type 2 enzymes belong to the family of short-chain alcohol dehydrogenases. The primary structures of the two enzymes have the typical conserved regions of the family members, but they share only low overall similarity. The three-dimensional structure of the active site of the type 1 enzyme has

been characterized recently [23], but the reason for the difference in the preferred directions for the hydrogen transfer of 17HSD type 1 and type 2 enzymes still remains to be characterized.

The predominantly reductive activity of 17HSD type 1 in cultured cells, together with its strong expression in the ovaries and placenta, suggest an active role for the enzyme in E2 biosynthesis. This is further supported by the fact that 17HSD type 1 expression in human and rat ovarian granulosa cells is related to the maturation stage of the follicles [24–26]. However, it is evident that the enzyme is also expressed in some oestrogen target tissues such as breast cancer cells [27,28]. Our present findings suggest that 17HSD type 1 may have a central role in the process leading to the accumulation of E2 over E1 in the breast tumours of post-menopausal women [9,29,30]. We found, however, that 17HSD type 2 is also expressed in BT-20 and MDA-MB-361 breast cancer cells. Hence, it is likely that both 17HSD type 1 and type 2 participate in local oestrogen metabolism in breast tissue, and further studies are needed to fully understand their roles in the regulation of oestrogen availability in breast cancer tissue.

17HSD type 1 is expressed in endometrial epithelial cells during the luteal phase of the menstrual cycle [31]. The relatively high expression of 17HSD type 2 in normal endometrium [32] and in RL95-2 endometrial cancer cells is in line with the strong oxidative nature of 17HSD activity found in the endometrium [8]. Both 17HSD type 1 and type 2 are expressed in term placenta, but only the type 1 enzyme was found in chorio-carcinoma cells. The reason for this is not yet known, but it may indicate that the enzymes are expressed in distinct placental cell types.

17HSD type 2 is less oestrogen-specific than the type 1 enzyme, and it also converts testosterone into androstenedione as well as 5 α -dihydrotestosterone into androstanedione. Thus, the finding that 17HSD type 2 is expressed in normal prostate and in PC-3 prostate cancer cells suggests that the enzyme may have a role in the inactivation of androgens in prostate epithelial cells, in a similar manner as suggested for its oestrogenic activity in the endometrium. As 17HSD type 2 is also expressed in the liver, small intestine and kidney, it most likely also contributes to the metabolism of 17 β -hydroxysteroids in these tissues. The present results, together with others [17], suggest that 17HSD type 3 is highly testis-specific and does not contribute significantly to oestrogen metabolism in females, but is essential for testosterone biosynthesis in Leydig cells.

The data shown in this report suggest that the two 17HSD type 1 mRNAs (1.3 kb and 2.3 kb) do not contribute to the same extent to the enzyme protein concentration and to the reductive 17HSD activity in cultured cells. None of the cell lines expressing only the 2.3 kb mRNA showed significant reductive 17HSD activity. Furthermore, in all the cell lines and tissues analysed, up-regulation of the 1.3 kb mRNA for 17HSD type 1 was related to an increased protein concentration and to a significant reductive activity typical of the enzyme. Furthermore, those cell lines (BeWo, BT-20) and tissues (placenta) having the highest enzyme protein concentration predominantly expressed the 1.3 kb mRNA. It is therefore suggested that expression of the 2.3 kb mRNA alone does not result in a significant 17HSD type 1 concentration, and this should be taken into account when the activity of 17HSD type 1 is predicted on the basis of mRNA expression. The structures of the two mRNAs contain an identical translational region, but the 2.3 kb mRNA has an additional 5' non-coding region of about 971 nucleotides [33]. There is growing evidence that translation of a gene can be inhibited by stable secondary structures in mRNAs in the vicinity of the initiator ATG codon [34,35]. The 2.3 kb mRNA, having an unusually

long 5'-untranslated sequence, may contain hairpin structures reducing translation of the mRNA. However, the constitutive expression of the 2.3 kb mRNA in all the cell types analysed, including both epithelial cells and fibroblasts, may contribute to the low 17HSD activity present in several peripheral tissues. The central role of 1.3 kb mRNA in human 17HSD type 1 protein expression is also supported by previous findings showing that in the three different cell types analysed (JAR, JEG-3 and T-47D), only the 1.3 kb mRNA is under hormonal regulation [28,36,37]. Hence, activation of the promoter which is responsible for expression of the 1.3 kb mRNA with a shortened 5'-untranslated sequence seems to be the key regulatory event controlling 17HSD type 1 concentration and the reason for the low translational efficacy of the 2.3 kb mRNA remains to be clarified.

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