Differential expression of 17β-hydroxysteroid dehydrogenases types 2 and 4 in human endometrial epithelial cell lines

B Husen[1], N Psonka[1], M Jacob-Meisel[2], C Keil[3] and **G M Rune[1]**

[1]Institute of Anatomy, Ernst-Moritz-Arndt-University, Greifswald, Germany

[2]Department of Obstetrics and Gynecology, Ernst-Moritz-Arndt-University, Greifswald, Germany

[3]Institute of Biochemistry, Ernst-Moritz-Arndt-University, Greifswald, Germany

(Requests for offprints should be addressed to B Husen, Deutsches Primatenzentrum, Abteilung Reproduktionsbiologie, Kellnerweg 4, D-37077 Göttingen, Germany; Email: bhusen@www.dpz.gwdg.de)

ABSTRACT

In the endometrium two enzymes are known to convert estradiol to its inactive metabolite estrone: microsomal 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) and peroxisomal 17β-HSD4. In order to elucidate the particular function of each of these two different enzymes, the human endometrial epithelial cell lines HEC-1-A and RL95–2 were examined with respect to the expression of 17β-HSD isozymes. They were compared with human endometrium *in vivo*. Non-radioactive *in situ* hybridization revealed both enzymes in glandular epithelial cells of human endometrium. The two cell lines were screened for mRNA expression of 17β-HSD 1–4 by RT-PCR and Northern blot. 17β-HSD2 and 4 could be detected by either method, 17β-HSD1 only by RT-PCR, 17β-HSD3 not at all. Both cell lines were proven to have no receptor for progesterone which is known as a physiological inducer of several 17β-HSD isozymes. To study the regulation of 17β-HSD2 and 17β-HSD4, the concentration of fetal calf serum in the cell culture media was reduced stepwise to 0·3% by dilution with a defined serum replacement. This treatment led to an inhibition of 17β-HSD2 mRNA expression and an increase in the mRNA expression of 17β-HSD4. Concomitantly, distinct morphological changes were observed, such as a decrease in the number and length of microvilli and a decrease in the formation of domes on top of the monolayers.

The endometrial epithelial cell lines HEC-1-A and RL95–2 represent a suitable *in vitro* model for further studies of the differential expression of the major endometrial HSD isozymes, independent of the effect of progesterone.

Journal of Molecular Endocrinology (2000) **24,** 135–144

INTRODUCTION

Inactivation of estradiol by its oxidation to estrone is an important step in the steroid metabolism of endometrial epithelial cells, since estrone has a much lower potency for the activation of the estrogen receptor than estradiol (Tseng & Gurpide 1973). Thus, oxidative 17β-hydroxysteroid dehydrogenase (17β-HSD) enzyme activity can control the occupancy of the estrogen receptor (Gurpide & Marks 1981; for review see Roy 1992, Penning 1997, Peltoketo *et al*. 1999*a*,*b*). This is a prerequisite for the adequate differentiation of the endometrium in the secretory phase of the menstrual cycle. Consequently, during this period the highest levels of oxidative 17β-HSD enzyme activity can be measured (Scublinsky *et al*. 1976, Liu & Tseng 1979, Tseng & Gurpide 1974).

Oxidative 17β-HSD activity in the uterus has been assigned to two isozymes: 17β-HSD2 (in the human: Casey *et al*. 1994, Mustonen *et al*. 1998, Zeitoun *et al*. 1998) or 17β-HSD4 (up to now shown only in porcine uterus: Adamski *et al*. 1992, Husen *et al*. 1994). Both belong to the gene family of short chain alcohol dehydrogenases, but they share only 16·4% amino acid identity (Labrie *et al*.

1997). They are characterized by a similar substrate specificity for estradiol and a rather broad tissue distribution (17β-HSD2: Casey *et al*. 1994, Akinola *et al*. 1996, Mustonen *et al*. 1997; 17β-HSD4: Adamski *et al*. 1995), and they seem to originate from different ancestors by convergent evolution (Baker 1996, Lanisnik-Rizner *et al*. 1999). 17β-HSD2 was first cloned from a human placenta cDNA library (Wu *et al*. 1993). It is a microsomal enzyme that also displays 20α-HSD enzyme activity. 17β-HSD4 was first purified from porcine endometrium (Adamski *et al*. 1992) and later cloned from a human liver cDNA library (Adamski *et al*. 1995). It was the first steroid metabolic enzyme localized in peroxisomes (Markus *et al*. 1995). This isozyme has a multi-domain structure. Two additional domains located C-terminally from the dehydrogenase domain catalyze hydratase activity of peroxisomal β-oxidation of fatty acids and sterol carrier activity (Leenders *et al*. 1996). 17β-HSD4 was also shown to play an important role in bile acid metabolism. In this context, it was termed D-specific multifunctional protein 2 (Dieuaide-Noubhani *et al*. 1996) and some authors even doubt the physiological function of the type 4 isozyme in estradiol metabolism (Dieuaide-Noubhani *et al*. 1996, Mustonen *et al*. 1997, Novikov *et al*. 1997, Qin *et al*. 1997; for review see de Launoit & Adamski 1999).

Up to now, there has been no comparative study of the distribution of oxidative 17β-HSD isozymes in endometrial tissue. Measurement of 17β-HSD enzyme activity does not allow for a proper discrimination between isozymes. The fact that two different enzymes are expressed in the same tissue leads to the assumption that they should have different functions and should be regulated differentially. We investigated this hypothesis using an *in vitro* model. We chose the human endometrial epithelial cell lines HEC-1-A and RL95–2 because they retain a quite well-differentiated phenotype in cell culture. They differ from one another in the extent of their epithelial polarization as judged by organization of the cytoskeleton, presence of microvilli and polarized distribution of adhesion molecules mediating intercellular contacts (Thie *et al*. 1995, 1996). Miettinen *et al*. (1996) detected a pronounced oxidative 17β-HSD enzyme activity in RL95–2 cells and mRNA expression of 17β-HSD1 and 2 in both cell lines. 17β-HSD4 was not considered in earlier studies.

We tested to what degree the steroid metabolic features of the cell lines resemble those of endometrial epithelial cells *in vivo*. The effect of different culture conditions on the expression of 17β-HSD2 and 17β-HSD4 mRNA and on cell morphology was investigated.

MATERIALS AND METHODS

Tissue

Human endometrial tissue was obtained from six 26- to 39-year-old women with regular cycles undergoing hysterectomy for benign conditions at the Department of Obstetrics and Gynecology of the University of Greifswald. Human testis tissue was obtained from patients undergoing orchidectomy because of prostate carcinoma at the Department of Urology of the University of Greifswald. Informed consent was given by all patients. The tissue was frozen in liquid nitrogen and maintained at –70 °C until RNA isolation or cryosectioning. Endometrial dating was performed by measurement of serum levels of estradiol and progesterone and histologically according to the method of Noyes *et al*. (1950).

Cell culture and treatment of cells

HEC-1-A and RL95–2 endometrial epithelial cells and T-47D mammary epithelial cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and stock cultures were maintained according to the supplier's instructions. Serum reduction was performed by stepwise 1:2 dilution with the same basal medium containing 2% TCM defined serum replacement (ICN Biomedicals, Eschwege, Germany), beginning with 5% fetal calf serum (FCS) and ending with 0·31% FCS/1·875% TCM. The cells were cultured in each dilution for at least 2 weeks before part of them was subjected to the next dilution step. The cells did not survive culture with TCM only. Treatment with peroxisome proliferator chemicals was performed using 0·1 mM or 1 mM gemfibrozil or clofibrate (both from Sigma, Deisen-hofen, Germany) dissolved in dimethylsulfoxide (DMSO), for time-periods of 6, 24, 48 and 72 h. For these experiments medium containing 5% FCS or medium containing 0·31% FCS/1·85% TCM was used.

Cell morphology was monitored by phase contrast microscopy. Photographs were taken with a conventional microscope (Axioskop, Zeiss, Germany).

Radioligand assay

Confluent monolayers of cell lines (corresponding to a cell number of 10^7 cells) were washed with phosphate-buffered saline (PBS) and removed from the cell culture flask with a rubber scraper. Cells were homogenized by repeated passage through a syringe with a 20 gauge needle on ice. The homogenate was centrifuged at 20 000 ***g*** for 2 h at 4 °C. The supernatant was used for a radioligand assay according to the standards of the E.O.R.T.C.

Breast Cancer Cooperative Group (1980) with the progesterone analogue ORG 2058 as a ligand. Protein concentrations were determined by a modified Lowry procedure (Markwell *et al*. 1981).

RT-PCR

Total RNAs were isolated using the RNA Mini Kit from QIAGEN (Hilden, Germany) according to the manufacturer's instructions. To avoid contamination with genomic DNA, total RNA preparations were digested with DNAse I (Boehringer Mannheim, Mannheim, Germany) for 1 h at 37 °C prior to cDNA synthesis. The synthesis of cDNA was catalyzed by Superscript II reverse transcriptase (RT) (GIBCO BRL, Karlsruhe, Germany) according to the manufacturer's protocol from 5 μg total RNA. The PCR amplification was performed with Pfu-Polymerase (Stratagene Europe, Amsterdam, The Netherlands) and the following primers: 5′-^{55}GGC CTG CAC TTG GCC GTA CG^{74}-3′ and 5′-^{365}GGC CTG CAG CAT CCG CAC AG^{384}-3′ for 17β-HSD1, 5′-^{112}GGA TCT GCC TGG CTG TCC CC^{131}-3′ and 5′-^{463}CCG AGA GGC GCG GAG AGC TGG^{483}-3′ for 17β-HSD2, 5′-^{119}GCG TGA GAT TCT TCC AGA TGT G^{139}-3′ and 5′-^{459}CAG GAA ATG GCT TGG GAG AAG^{480}-3′ for 17β-HSD3 and 5′-^{110}TGG GCC GAG CCT ATG CCC TG^{129}-3′ and 5′-^{432}CCA TGC TGC CCG TGT CAC TTG^{453}-3′ for 17β-HSD4 respectively.

Preparation of riboprobes

Total RNA was isolated from human endometrial tissue and used for cDNA synthesis. Specific sequences were amplified from this template cDNA by RT-PCR with Pfu-Polymerase and with primers designed according to the known human nucleotide sequences of 17β-HSD1 (Peltoketo *et al*. 1988), 17β-HSD2 (Wu *et al*. 1993) and 17β-HSD4 (Adamski *et al*. 1995). The PCR products obtained were cloned into the vector pPCRScript (Stratagene) and used for *in vivo* transcription with T3- and T7-polymerase (DIG RNA labeling kit; Boehringer Mannheim) to obtain digoxigenin-labeled riboprobes.

The identity of the probes was proven by hybridization to the original PCR products on Southern blots and to total RNA preparations on Northern blots. Automated sequencing of the plasmid preparations (ALF; Amersham Pharmacia Biotech, Freiburg, Germany) was carried out at the Technologietransferstelle Biotechnologie, Greifswald, Germany.

In situ hybridization

In situ hybridization was performed using an established non-radioactive protocol (Trezise *et al*. 1992). Frozen sections of 8 μm thickness were fixed by 20-min paraformaldehyde fixation at 4 °C and permeabilized by 3 min digestion with proteinase K (10 μg/ml) at 37 °C followed by 10-min 0·2% glycin and 5-min postfixation in 4% paraformaldehyde at 4 °C. Slides were washed briefly with 0·1 M triethanolamine and acetylated with 0·25% acetic anhydride in triethanolamine. After two washes in 2 × SSC (20 × SSC = 3 M NaCl, 0·3 M $Na_3C_6H_5O_7$, pH 7·4), sections were dehydrated through graded alcohols and prehybridized with hybridization mix (40% deionized formamide, 10% dextransulfate, 4 × SSC, 0·02% (w/v) ficoll, 0·02% (w/v) polyvinylpyrrolidone, 0·2 mg/ml bovine serum albumin, 2·5 mM dithiothreitol, 25 μg/ml tRNA) for 2 h in a humid chamber at 42 °C. Hybridization was performed under the same conditions overnight with 500 ng/ml of the riboprobes diluted in hybridization mix. Sections were then washed three times with 4 × SSC and digested with RNAse A for 30 min at 37 °C and subsequently washed with a final stringency of 0·1 × SSC at 55 °C for 30 min. The digoxigenin label of the hybridized probes was detected immunologically by antibodies coupled to alkaline phosphatase using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as the substrate for an incubation time of 12–20 h in the dark.

Northern blot analysis

The same riboprobes as for *in situ* hybridization were used for non-radioactive Northern blotting according to standard protocols (Boehringer Mannheim). Densitometric analysis was performed after digitalization of X-ray films with a video imaging system (MWG Biotech, Ebersberg, Germany).

Fluorescence staining

Cells were seeded on Permanox two-chamber slides (Nalge Nunc, Naperville, IL, USA). Confluent monolayers were washed with PBS. Subsequently, they were fixed with 3·7% formaldehyde for 20 min. After two more PBS washes, F-actin was labeled with 0·25 μg/ml phalloidin-fluorescein-isothiocyanate (FITC) for 20 min at room temperature.

Photographs were taken by a digital confocal imaging system (Openlab, Improvision Ltd, Coventry, UK).

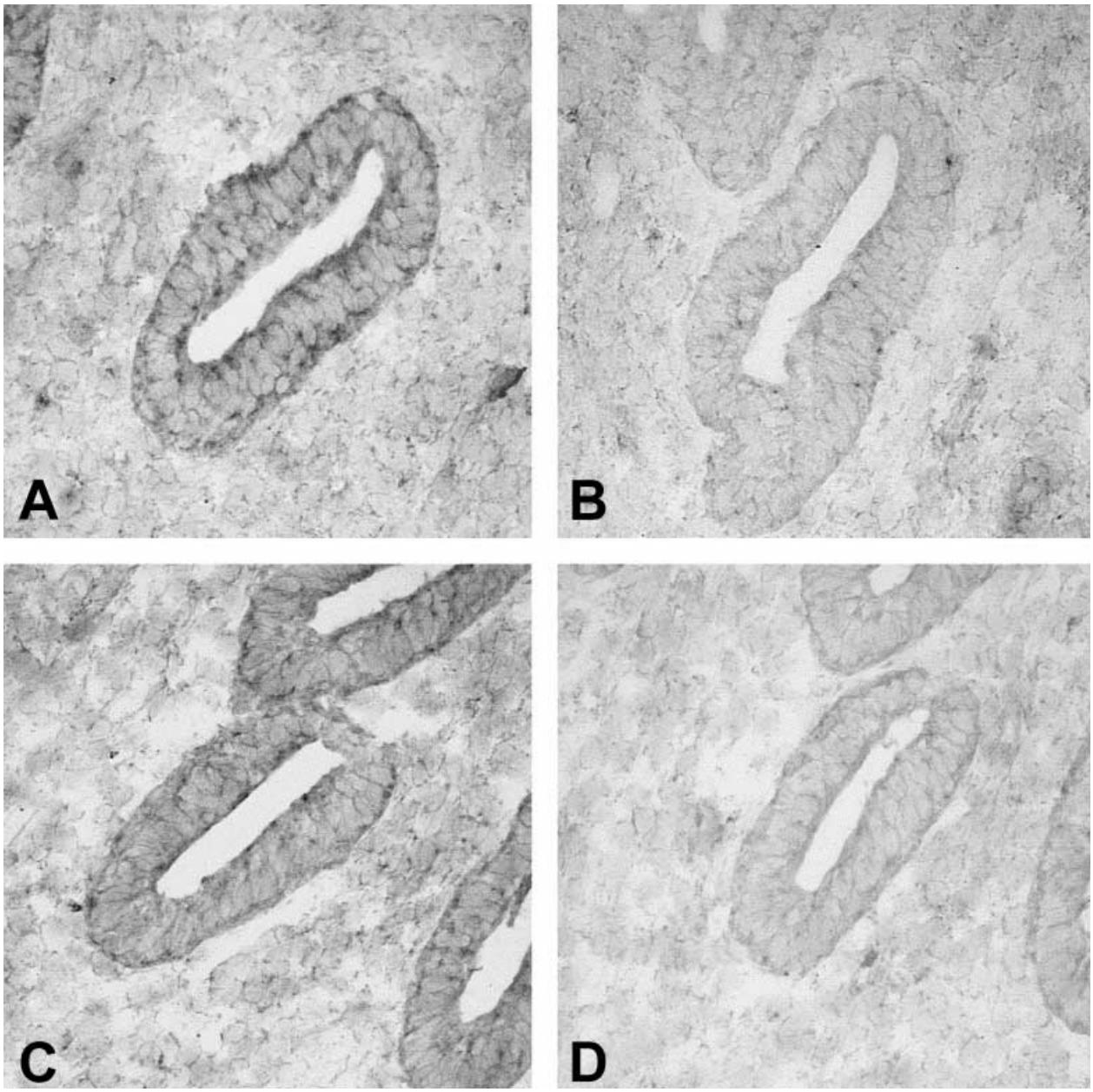

FIGURE 1. *In situ* hybridization of human endometrial tissue obtained in the secretory phase of the menstrual cycle. Frozen sections (8 μm) were hybridized with digoxigenin-labeled riboprobes as described in Materials and Methods. (A)17β-HSD2-anti-sense probe, (B) 17β-HSD2-sense probe as a negative control, (C) 17β-HSD4-anti-sense probe, and (D) 17β-HSD4-sense probe. Magnification: × 370. With both probes, positive hybridization signals are observed in glandular epithelial cells. No mRNA is detected in stromal cells. Negative controls are devoid of any staining.

RESULTS

Expression of 17β-HSD isozymes in human endometrium and in endometrial cell lines

Highest oxidative HSD activity is observed in the secretory phase of the menstrual cycle (Tseng & Gurpide 1974, Scublinsky *et al*. 1976, Liu & Tseng 1979). To verify expression of estradiol-inactivating 17β-HSD isozymes we performed non-radioactive *in situ* hybridization. The simultaneous expression of 17β-HSD2 and 17β-HSD4 in glandular epithelium could be demonstrated on frozen sections of

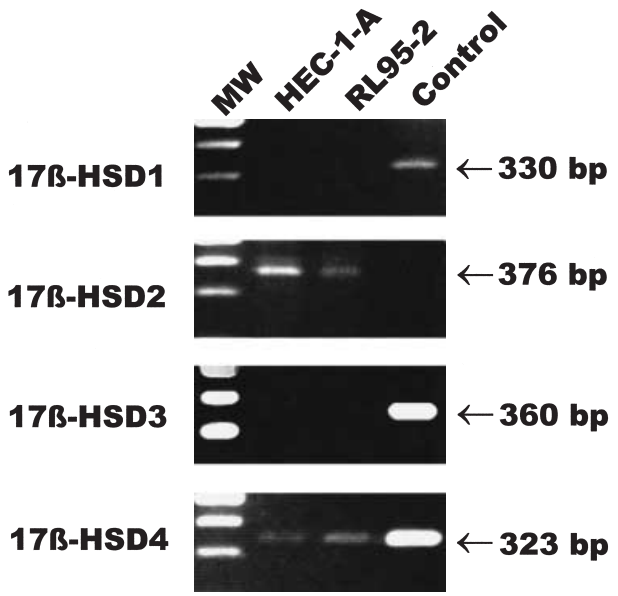

FIGURE 2. RT-PCR analysis of mRNA expression for different 17β-HSD isoforms in the human endometrial epithelial cell lines HEC-1-A and RL95–2. Specific primers for human 17β-HSD1, 2, 3 and 4 were used as described in Materials and Methods. PCR fragments of the expected sizes of the estradiol-inactivating isoforms 17β-HSD2 and 17β-HSD4 were amplified from both endometrial cell lines. The testes-specific 17β-HSD3 was not detected in either cell line. Positive controls were T-47D breast cancer cells for 17β-HSD1, 2 and 4 and human testis for 17β-HSD3. MW=molecular weight marker.

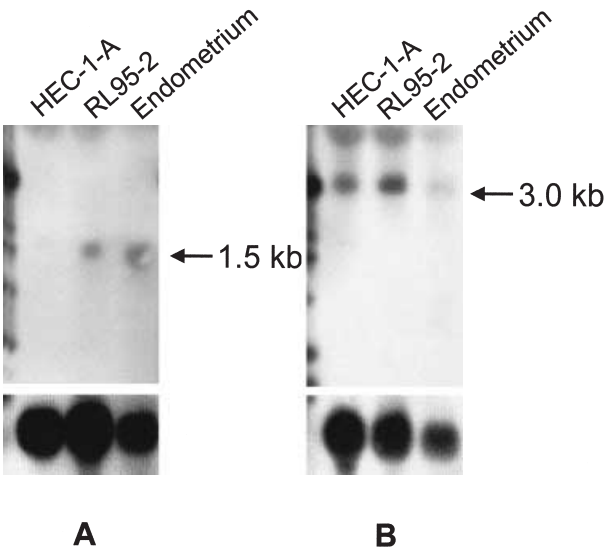

FIGURE 3. Northern blot analysis of human endometrial tissue and cell lines. Total RNA was extracted from human endometrial tissue (secretory phase of the menstrual cycle) and from stock cultures of the endometrial cell lines HEC-1-A and RL95–2 as described in Materials and Methods. Ten micrograms total RNA were loaded per lane. (A) Hybridization with 17β-HSD2 probe and (below) rehybridization with a β-actin probe, and (B) hybridization with 17β-HSD4 probe and (below) rehybridization with a β-actin probe. Both isozymes are expressed in both cell lines as well as in endometrial tissue.

human endometrium from the secretory phase of the menstrual cycle (Fig. 1). Stroma cells, endothelial cells, as well as myometrium, were devoid of specific staining.

We checked further what expression pattern is revealed by the human endometrial epithelial cell lines HEC-1-A and RL95–2. Using primers for the human 17β-HSD isozymes (types 1–4), 17β-HSD2 and 17β-HSD4 could be amplified from cDNA of the cell lines (Fig. 2). Only a faint band of a 17β-HSD1–PCR fragment was evident with cDNA from RL95–2-cells. T-47D mammary epithelial cells were used as a positive control because they are known to express 17β-HSD1 (Poutanen *et al*. 1990, 1992). Testis-specific 17β-HSD3 (Geissler *et al*. 1994) was not present in any of the cell lines.

In Northern blots the expected transcripts of 1·5 kb for 17β-HSD2 and 3·0 kb for 17β-HSD4 were detected in total RNA preparations from HEC-1-A and RL95–2 cells as well as in human endometrial tissue (Fig. 3).

Regulation of 17β-HSD isozymes in HEC-1-A and RL95–2 cells

As progesterone is known to be a physiological inducer of 17β-HSD activity we performed a radioligand assay to determine the progesterone receptor content of the cell lines. In contrast to T-47D cells (243 fM receptor/mg protein) the endometrial cells were devoid of progesterone receptors. Accordingly, with this culture model we could try to reveal a differential regulation of 17β-HSD2 and 17β-HSD4, independent of the effects of progesterone. For this purpose, FCS in the cell culture medium was reduced by stepwise dilution with the defined serum supplement TCM. Maximal serum concentration was 10%, minimal serum concentration was 0·25%. TCM concentration ranged from 1% to 1·85% respectively. The difference in mRNA expression between 10% and 5% serum was negligible. Below 5% serum this treatment caused a stepwise decrease in 17β-HSD2 transcription and concomitantly a stepwise increase in mRNA expression of 17β-HSD4 in HEC-1-A cells (Fig. 4). The two enzymes were each regulated in the opposite way.

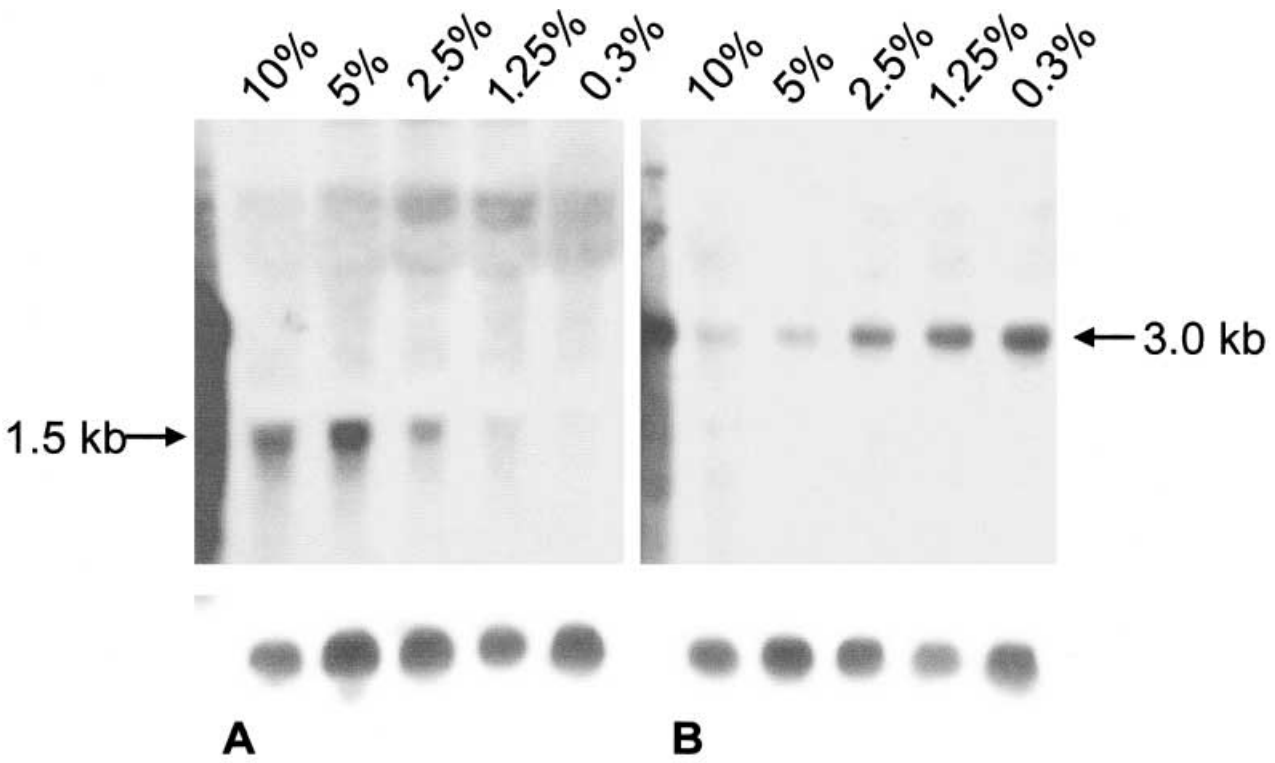

FIGURE 4. Regulation of RNA expression of (A) 17β-HSD2 and (B) 17β-HSD4 in HEC-1-A cells. The Northern blot was loaded with 10 μg total RNA/lane from cells cultured with the FCS concentration indicated at the top of each lane and hybridized with riboprobes for the two isozymes (top). The same blot is shown after rehybridization with a β-actin probe (bottom). The effect of serum replacement becomes evident with FCS concentrations below 5%. There is a down-regulation of 17β-HSD2 and an induction of 17β-HSD4.

The effect of serum replacement on enzyme expression was observed not only in HEC-1-A cells but also in RL95–2 cells (Fig. 5A). It could be corroborated by a densitometric analysis relative to the expression of actin as a house-keeping gene (Fig. 5B). In both cell lines, 17β-HSD2 mRNA dominated at 5% FCS, while the expression of 17β-HSD4 was most pronounced in cultures with 0·3% serum/1·85% TCM.

We tried to identify a specific factor causing this phenomenon and cultured the cells with two peroxisome proliferator chemicals (clofibrate, gemfibrozil) that induce the expression of 17β-HSD4 in liver *in vivo* (Corton *et al*. 1996). Both substances had no effect on the expression of either enzyme in this cell culture model.

Changes in cell morphology were observed by phase contrast microscopy and by fluorescence staining of the F-actin cytoskeleton (Fig. 6). Cells grown with 0·3%/1·82% TCM did not reach the same cell densities as stock cultures with 5% serum. Especially in RL95–2 cells, the size and number of domes forming on top of the monolayer were reduced and, especially in HEC-1-A cells, the presence of microvilli visualized with F-actin staining was less evident with low serum concentration.

DISCUSSION

In this study it was shown for the first time that the estradiol-inactivating enzymes 17β-HSD2 and 17β-HSD4 are expressed simultaneously in human endometrial epithelial cells *in vivo*. Since 17β-HSD4 was also identified as an enzyme involved in peroxisomal β-oxidation and bile acid synthesis, some authors have questioned the physiological function of this protein as a steroid-metabolizing enzyme (Dieuaide-Noubhani *et al*. 1996, Mustonen *et al*. 1998). However, its presence in a classical estradiol target tissue together with 17β-HSD2 and the fact that it has the same substrate specificity for estradiol as 17β-HSD2 (Wu *et al*. 1993, Mustonen *et al*. 1998) strongly indicate that the estradiol dehydrogenase action of 17β-HSD4 is a major function of this enzyme in the endometrium.

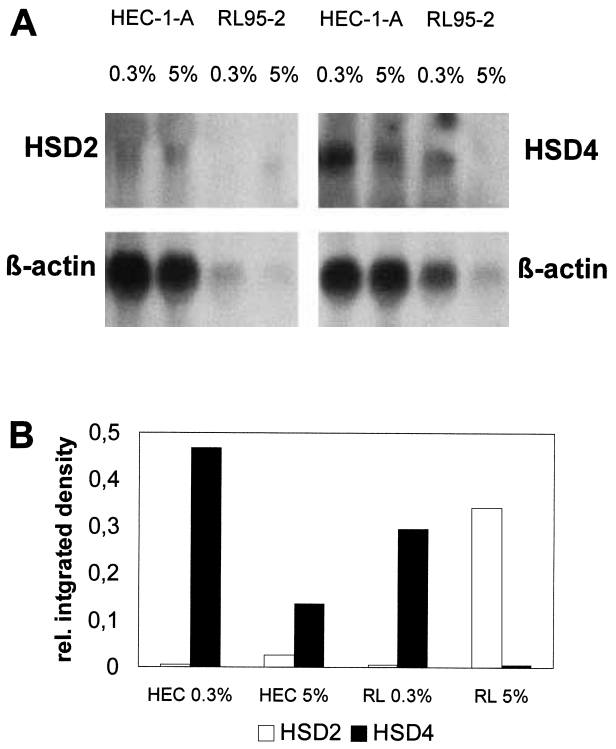

FIGURE 5. Influence of cell culture conditions on mRNA expression in HEC-1-A cells and RL95–2 cells. Cells were adapted to 0·3% FCS/1·82% TCM or adapted to 5% FCS (stock cultures). (A) Northern blot analysis using 10 μg total RNA from HEC-1-A cells and 5 μg total RNA from RL95–2 cells per lane. The two blots at the top of the figure show the hybridization with 17β-HSD probes, the two blots at the bottom show the corresponding rehybridization with a β-actin probe. (B) Densitometric analysis of the blots in (A). In both cell lines, expression levels of 17β-HSD2 are reduced by serum replacement, while expression levels of 17β-HSD4 are elevated by serum replacement. HEC, HEC-1-A cells; RL, RL95–2 cells.

Estradiol levels in the uterus may not only be controlled by oxidative 17β-HSD2 and 17β-HSD4, but also by reductive 17β-HSD1, which catalyzes the biosynthesis of estradiol. The type 1 isozyme was the first 17β-HSD isozyme identified in endometrium (Mäentausta *et al*. 1991). The importance of its function in this tissue is controversial, because it is expressed at very low levels compared with the other isozymes (Milewich *et al*. 1989, Casey *et al*. 1994, Zeitoun *et al*. 1998). This was confirmed in our study, since the expression of both 17β-HSD types 2 and 4 is much higher in human endometrial tissue and in cell lines.

The presence of two estradiol-inactivating isozymes in endometrial epithelial cells offers the possibility of a differential regulation in response to different stimuli. This was tested using a cell culture model. The cell lines HEC-1-A and RL95–2 showed the same isozyme pattern but, in contrast to endometrial epithelial cells *in vivo*, they have no progesterone receptor. They grow independent of progesterone which is known as an important regulatory factor of oxidative 17β-HSD activity *in vivo* (Tseng & Gurpide 1974, 1975, 1979). Progesterone stimulates the expression of 17β-HSD1 (Poutanen *et al*. 1990, 1992, Mäentausta *et al*. 1993), of 17β-HSD2 (Casey *et al*. 1994) as well as that of 17β-HSD4 (Kaufmann *et al*. 1995). With these characteristics of the cell lines in mind, they represent an appropriate model to study the regulation of 17β-HSD isozymes independent of progesterone.

There is an extensive database concerning the regulation of 17β-HSD1 in several mammary carcinoma and choriocarcinoma cell lines and in primary granulosa cell cultures (for review see Peltoketo *et al*. 1996). Little is known about the regulation of other isozymes. We report here, for the first time, that a differential regulation of 17β-HSD2 and 17β-HSD4 can be achieved by different culture conditions *in vitro*. The reduction of FCS and replacement by a supplement that is free of growth factors and steroids has the opposite effect on each of the two isozymes. This may be a general phenomenon because it occurs in two different cell lines. But in spite of their different phenotypes these cell lines are still of the same tissue origin. Further experiments need to be performed with cells derived from other organs. The particular factor causing these changes in enzyme expression in HEC-1-A and RL95–2 cells still has to be identified. One possibility is the action of ligands of the peroxisome proliferator activated receptor α (PPARα), e.g. unsaturated long-chain fatty acids. These were shown to induce 17β-HSD4 expression in rat liver (Corton *et al*. 1996) and may be included in the TCM serum supplement, which is only confirmed to be free of growth factors and steroids by the manufacturer. However, we were not able to achieve this effect in cell culture with the peroxisome proliferator chemicals clofibrate or gemfibrozil. This might be due to a tissue-specific effect of peroxisome proliferator chemicals as reported by Fan *et al*. (1998): 17β-HSD4 is induced only in liver and kidney, but not in uterus and other organs. Although the binding motif for the PPAR on 17β-HSD4 has been identified, its functionality has not yet been characterized (Leenders *et al*. 1998). It was shown recently that the physiological

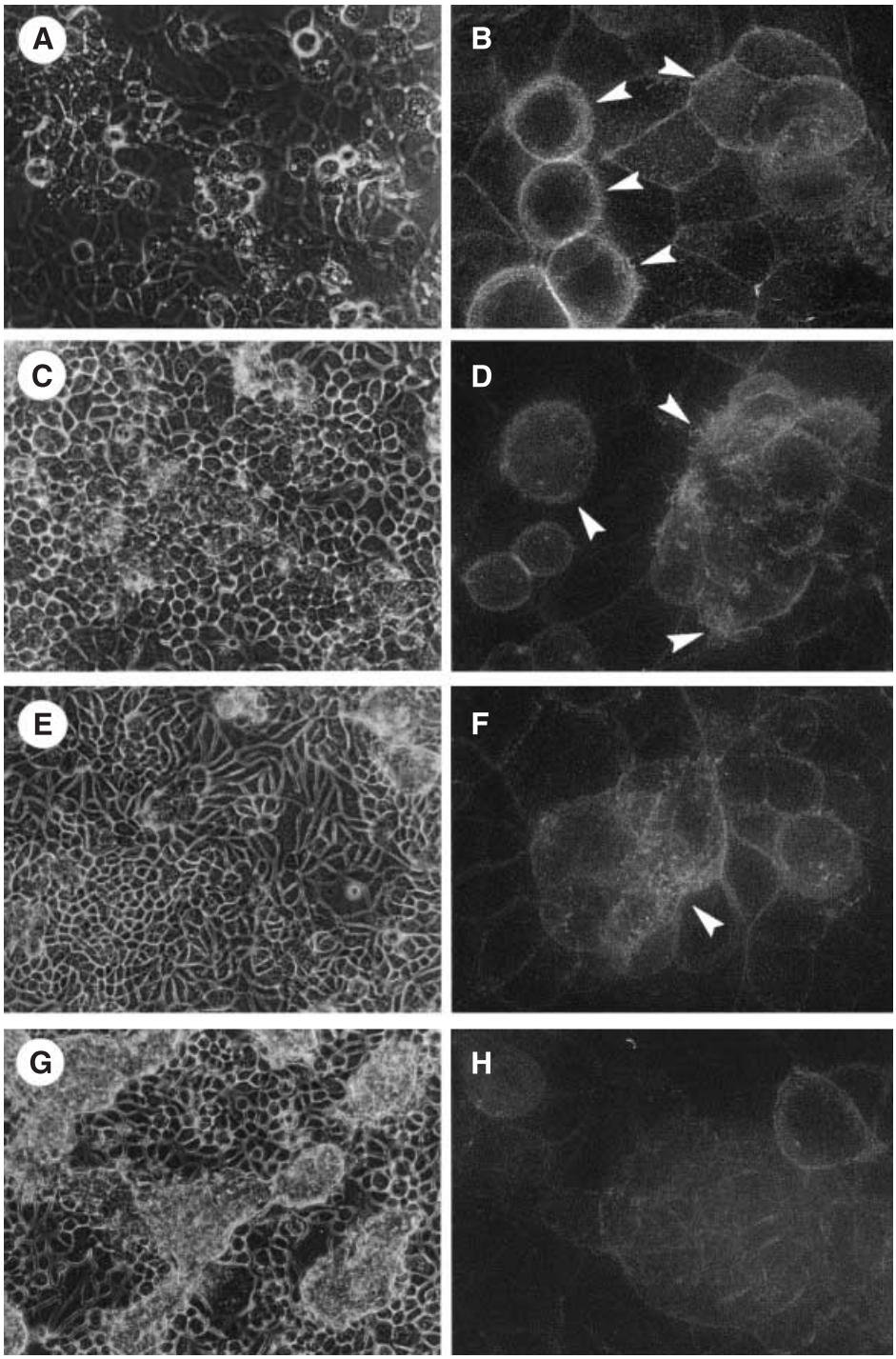

FIGURE 6. Effect of serum replacement on the morphology of HEC-1-A cells (A–D) and RL95–2 cells (E–H) adapted to 0·3% FCS/1·82% TCM (A, B, E, F) or adapted to 5% FCS (C, D, G, H). (A, C, E, G) Phase contrast, × 185; (B, D, F, H) digital confocal image of fluorescent staining of the F-actin cytoskeleton with FITC–phalloidin, × 400. After serum replacement, both cell lines grow with lower cell densities. The formation of microvilli (arrow heads) is impaired and they lose their tendency to form three-dimensional structures on top of the epithelial monolayer. In (H) resolution of the image is lost due to the increased thickness of the specimen by these three-dimensional structures (more than 50 μm, compared with 15–25 μm in B, D and F).

ligand for PPARα in mouse is phytanic acid (Ellinghaus *et al*. 1999) and this should be tested next as an inducer of endometrial 17β-HSD4.

During the adaptation of the cell lines to different culture conditions the changes in steroid metabolic enzyme expression coincided with phenotypic changes. A reorganization of the F-actin cytoskeleton was observed. This finding is especially interesting when considered in relation to earlier results. There are covalent as well as non-covalent interactions between 17β-HSD4 and actin *in vivo* (Adamski *et al*. 1993). The distribution of peroxisomes which harbor 17β-HSD4 changes in glandular endometrial epithelial cells during the estrous cycle of the pig (Husen *et al*. 1994). Thus, the correlation between enzyme expression and cytoskeletal organization may have physiological relevance for the regulation of estradiol metabolism and should be studied in more detail.

The data presented in this work provide new insights into regulatory mechanisms for estradiol inactivation in the uterus proving the differential expression of 17β-HSD isozymes.

ACKNOWLEDGEMENTS

The authors appreciate the skilful technical assistance of Mrs S Hanisch. We are also obliged to Dr Schwesinger from the Institute of Pathology at the University of Greifswald for the endometrial dating. We thank Dr U Zimmermann from the Department of Urology of the University of Greifswald for the supply of testis tissue.

REFERENCES

Adamski J, Husen B, Marks F & Jungblut PW 1992 Purification and properties of oestradiol 17β-dehydrogenase extracted from cytoplasmic vesicles of porcine endometrial cells. *Biochemical Journal* **288** 375–381.

Adamski J, Husen B, Thole HH, Gröschel-Stewart U & Jungblut PW 1993 Linkage of 17β-oestradiol dehydrogenase to actin by ε-(γ-glutamyl)lysine in porcine endometrial cells. *Biochemical Journal* **296** 797–802.

Adamski J, Normand T, Leenders F, Monte D, Begue A, Stehelin D, Jungblut PW & DeLaunoit Y 1995 Molecular cloning of a novel widely expressed human 80 kDa 17β-hydroxysteroid dehydrogenase IV. *Biochemical Journal* **311** 437–443.

Akinola LA, Poutanen M & Vikho R 1996 Cloning of rat 17β-hydroxysteroid dehydrogenase type 2 and characterization of tissue distribution and catalytic activity of rat type 1 and type 2 enzymes. *Endocrinology* **137** 1572–1579.

Baker ME 1996 Unusual evolution of 11β-and 17β-hydroxysteroid and retinol dehydrogenases. *BioEssays* **18** 63–70.

Casey ML, MacDonald PC & Andersson S 1994 17β-Hydroxysteroid dehydrogenase type 2: chromosomal assignment and progestin regulation of gene expression in human endometrium. *Journal of Clinical Investigation* **94** 2135–2141.

Corton JC, Bocos C, Moreno ES, Merritt A, Marsman DS, Sausen PJ, Cattley RC & Gustafsson JA 1996 Rat 17β-hydroxysteroid dehydrogenase type 4 is a novel peroxisome proliferator-inducible gene. *Molecular Pharmacology* **50** 1157–1166.

Dieuaide-Noubhani M, Novikov D, Baumgart E, Vanhooren, JCT, Fransen M, Goethals M, Vanderkerckhove J, Van Veldhoven PP & Mannaerts GP 1996 Further characterization of the peroxisomal 3-hydroxyacyl-CoA dehydrogenases from rat liver. *European Journal of Biochemistry* **240** 660–666.

Ellinghaus P, Wolfrum C, Assmann G, Spener F & Seedorf U 1999 Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2/sterol carrier protein X-deficient mice. *Journal of Biological Chemistry* **274** 2766–2772.

E.O.R.T.C. Breast Cancer Cooperative Group 1980 Revision of the standards for the assessment of hormone receptors in human breast cancers; report on the second E.O.R.T.C. Workshop, held on 16–17 March 1979, in The Netherlands. *European Journal of Cancer* **11** 1513–1515.

Fan LQ, Cattley RC & Corton, JC 1998 Tissue-specific induction of 17β-hydroxysteroid dehydrogenase type 4 by peroxisome proliferator chemicals is dependent on the peroxisome proliferator-activated receptor α. *Journal of Endocrinology* **158** 237–246.

Geissler WM, Davis, DL, Wu L, Bradshaw KD, Patel S, Mendonca BB, Elliston KO, Wilson JD, Russell DW & Andersson S 1994 Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. *Nature Genetics* **7** 34–39.

Gurpide E & Marks C 1981 Influence of endometrial 17β-hydroxysteroid dehydrogenase activity on the binding of estradiol to receptors. *Journal of Clinical Endocrinology and Metabolism* **52** 252–255.

Husen B, Adamski J, Szendro PI & Jungblut PW 1994 Alterations in the subcellular distribution of 17β-estradiol dehydrogenase in porcine endometrial cells over the course of the estrous cycle. *Cell and Tissue Research* **278** 227–233.

Kaufmann M, Carstensen J, Husen B & Adamski J 1995 The tissue distribution of porcine 17β-estradiol dehydrogenase and its induction by progesterone. *Journal of Steroid Biochemistry and Molecular Biology* **55** 535–539.

Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R & Belanger A 1997 The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* **62** 148–158.

Lanisnik-Rizner T, Moeller G, Thole HH, Zakelj-Mavric M & Adamski J 1999 A novel 17β-hydroxysteroid dehydrogenase in the fungus *Cochliobolus lunatus*: new insights into the evolution of steroid-hormone signalling. *Biochemical Journal* **337** 425–431.

de Launoit Y & Adamski J 1999 Unique multifunctional HSD17B gene product: 17β-hydroxysteroid dehydrogenase 4 and D-3-hydroxyacyl-coenzyme A dehydrogenase/hydratase involved in Zellweger syndrome. *Journal of Molecular Endocrinology* **22** 227–240.

Leenders F, Tesdorpf JG, Markus M, Engel T, Seedorf U & Adamski J 1996 Porcine 80-kDa protein reveals intrinsic 17β-hydroxysteroid dehydrogenase, fatty acyl-CoA-hydratase/dehydrogenase, and sterol transfer activities. *Journal of Biological Chemistry* **271** 5438–5442.

Leenders F, Dolez V, Begue A, Möller G, Gloeckner JC, de Launoit Y & Adamski J 1998 Structure of the gene for 17β-hydroxysteroid dehydrogenase type IV. *Mammalian Genome* **9** 1036–1041.

Liu H & Tseng L 1979 Estradiol metabolism in isolated human endometrial epithelial glands and stromal cells. *Endocrinology* **104** 1674–1681.

Mäentausta O, Sormunen R, Isomaa V, Lehto VP, Jouppila P & Vikho R 1991 Immunohistochemical localization of 17β-hydroxysteroid dehydrogenase in the human endometrium during the menstrual cycle. *Laboratory Investigation* **65** 582–587.

Mäentausta O, Svalander P, Gemzell K, Bygdeman M & Vikho R 1993 The effects of an antiprogestin, mifepristone, and an antiestrogen, tamoxifen, on endometrial 17β-hydroxysteroid dehydrogenase and progestin and estrogen receptors during the luteal phase of the menstrual cycle: an immunohistochemical study. *Journal of Clinical Endocrinology and Metabolism* **77** 913–918.

Markus M, Husen B, Leenders F, Jungblut PW, Hall PF & Adamski J 1995 The organelles containing porcine 17β-estradiol dehydrogenase are peroxisomes. *Journal of Cell Biology* **68** 263–267.

Markwell MAK, Haas SM, Tolbeit NE & Bieber LL 1981 Protein determination in membrane and lipoprotein samples: manual and automated procedures. *Methods in Enzymology* **72** 296–299.

Miettinen MM, Mustonen MV, Poutanen MH, Isomaa VV & Vikho RK 1996 Human 17β-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression. *Biochemical Journal* **314** 839–845.

Milewich L, MacDonald PC & Carr BR 1989 Activity of 17β-hydroxysteroid oxidoreductase in tissues of the human fetus. *Journal of Endocrinology* **123** 509–518.

Mustonen MVJ, Poutanen M, Isomaa VV, Vikho PT & Vikho RK 1997 Cloning of mouse 17β-hydroxysteroid dehydrogenase type 2, and analysing expression of the mRNAs for types 1, 2, 3, 4, and 5 in mouse embryos and adult tissues. *Biochemical Journal* **325** 199–205.

Mustonen MVJ, Isomaa VV, Vaskivuo T, Tapanainen J, Poutanen MH, Stenbäck F, Vikho RK & Vikho PT 1998 Human 17β-hydroxysteroid dehydrogenase type 2 messenger ribonucleic acid expression and localisation in term placenta and in endometrium during the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **83** 1319–1324.

Novikov D, Dieuaide-Noubhani M, Vermeesch, JR, Fournier B, Mannaerts GP & VanVeldhoven PP 1997 The human peroxisomal multifunctional protein involved in bile acid synthesis: activity measurement, deficiency in Zellweger syndrome and chromosome mapping. *Biochimica et Biophysica Acta* **1360** 229–240.

Noyes RW, Hertig AT & Rock J 1950 Dating the endometrial biopsy. *Fertility and Sterility* **1** 3–25.

Peltoketo H, Isomaa V, Mäentausta O & Vikho R 1988 Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Letters* **239** 73–77.

Peltoketo H, Isomaa V, Poutanen M & Vikho R 1996 Expression and regulation of 17β-hydroxysteroid dehydrogenase type 1. *Journal of Endocrinology* **150** 21–30.

Peltoketo H, Vikho P & Vikho R 1999*a* Regulation of estrogen action: role of 17β-hydroxysteroid dehydrogenases. *Vitamins and Hormones* **55** 353–398.

Peltoketo H, Luu-The V, Simard J & Adamski J 1999*b* 17β-Hydroxysteroid dehydrogenase/17-ketosteroid reductase family: nomenclature and main characteristics of the 17HSD/KSR enzymes. *Journal of Molecular Endocrinology* **23** 1–11.

Penning TM 1997 Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocrine Reviews* **18** 281–305.

Poutanen M, Isomaa V, Kainulainen K & Vikho R 1990 Progestin induction of 17β-hydroxysteroid dehydrogenase enzyme protein in the T47D human breast cancer cell line. *International Journal of Cancer* **46** 897–901.

Poutanen M, Moncharmont B & Vikho R 1992 17β-Hydroxysteroid dehydrogenase gene expression in human breast cancer cells: regulation of expression by a progestin. *Cancer Research* **52** 290–294.

Qin Y, Poutanen MH, Helander HM, Kvist A, Siivari KM, Schmitz W, Conzelmann E, Hellman U & Hiltunen JK 1997 Peroxisomal multifunctional enzyme of β oxidation metabolizing D-3-hydroxyacyl-CoA esters in rat liver: molecular cloning, expression and characterization. *Biochemical Journal* **321** 21–28.

Roy AK 1992 Regulation of steroid hormone action in target cells by specific hormone-inactivating enzymes. *Proceedings of the Society for Experimental Biology and Medicine* **199** 265–272.

Scublinsky A, Marin C & Gurpide E 1976 Localization of estradiol 17β dehydrogenase in human endometrium. *Journal of Steroid Biochemistry* **7** 745–747.

Thie M, Harrach-Ruprecht B, Sauer H, Fuchs P, Albers A & Denker HW 1995 Cell adhesion to the apical pole of epithelium: a function of cell polarity. *European Journal of Cell Biology* **66** 180–191.

Thie M, Fuchs P, Butz S, Siekmann F, Hoschützky H, Kemler R & Denker HW 1996 Adhesiveness of the apical surface of uterine epithelial cells: the role of junctional complex integrity. *European Journal of Cell Biology* **70** 221–232.

Trezise AE, Romano PR, Gill DR, Hyde SC, Sepulveda FV, Buchwald M & Higgins CF 1992 The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. *EMBO Journal* **11** 4291–4303.

Tseng L & Gurpide E 1973 Effect of estrone and progesterone on the nuclear uptake of estradiol by slices of human endometrium. *Endocrinology* **93** 245–248.

Tseng L & Gurpide E 1974 Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. *Endocrinology* **94** 419–423.

Tseng L & Gurpide E 1975 Induction of human endometrial estradiol dehydrogenase by progestins. *Endocrinology* **97** 825–833.

Tseng L & Gurpide E 1979 Stimulation of various 17β- and 20α-hydroxysteroid dehydrogenase activities by progestins in human endometrium. *Endocrinology* **104** 1745–1748.

Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO & Andersson S 1993 Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. *Journal of Biological Chemistry* **268** 12964–12969.

Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, Meng L, Putman M, Carr B & Bulun SE 1998 Deficient 17β-hydroxysteroid dehydrogenase Type 2 expression in endometriosis: failure to metabolize 17β-estradiol. *Journal of Clinical Endocrinology and Metabolism* **83** 4474–4480.

RECEIVED 14 July 1999