Characterization of a human 20α-hydroxysteroid dehydrogenase

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ABSTRACT

It has been suggested that 20α-hydroxysteroid dehydrogenase (20α-HSD) is a T-cell differentiation marker in mice. In the human, this enzyme has generally been associated with types 1 and 2 17β-HSDs, which belong to the short-chain alcohol dehydrogenase family, whereas the rat, rabbit, pig and bovine 20α-HSDs are members of the aldo-keto reductase superfamily, which also includes the 3α-HSD family. In this study, we report the cloning, from a human skin cDNA library, of a cDNA that shows, after transfection into human embryonic kidney (HEK-293) cells, high 20α-HSD activity but negligible 3α- and 17β-hydroxysteroid dehydrogenase activities. A comparison of the amino acid sequence of the human 20α-HSD with those of other related 20α- and 3α-HSDs indicates that the human 20α-HSD shares 79·9, 68·7 and 52·3% identity with rabbit, rat and bovine 20α-HSDs, whereas it shows 97, 84 and 65% identity with human type 3, type 1 and rat 3α-HSDs. In contrast, the enzyme shares only 15·2 and 15·0% identity with type 1 and type 2 human 17β-HSDs. DNA analysis predicts a protein of 323 amino acids, with a calculated molecular weight of 36 767 Da. In intact transfected cells, the human 20α-HSD preferentially catalyzes the reduction of progesterone to 20α-hydroxyprogesterone with a $K_m$ value of 0·6 μM, the reverse reaction (oxidation) being negligible. In a cell cytosolic preparation, the enzyme could use both NADPH and NADH as cofactors, but NADPH, which gave 4-fold lower $K_m$ values, was preferred. We detected the expression of 20α-HSD mRNA in liver, prostate, testis, adrenal, brain, uterus and mammary-gland tissues and in human keratinocyte (HaCaT) cells. The present study clearly indicates that the genuine human 20α-HSD belongs to the aldo-keto reductase family, like the 20α-HSDs from other species.

Journal of Molecular Endocrinology (2000) 25, 221–228

INTRODUCTION

The enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) (EC 1.1.1.149) catalyzes the conversion of progesterone (P) into its inactive form, 20α-hydroxyprogesterone (20α-OHP). 20α-HSD is found in the mouse thymus and in T-dependent areas of other lymphoid organs (Weinstein 1977). It has been suggested that the enzyme is a T-cell differentiation marker (Ihle et al. 1981). In addition to being present in thymic-derived lymphocytes, 20α-HSD activity has been found in many steroidogenic tissues such as the liver, ovary, testis, adrenal and placenta (Wiest 1959, Matthijssen et al. 1964, Armstrong & King 1971, Pineda et al. 1985, Nakajin et al. 1989) and also in haemopoietic cells, erythrocytes and certain micro-organisms (Weinstein 1977, Sharaf & Sweet 1982, Hapel et al. 1985, Rimsay et al. 1988). While 20α-HSD has been shown to play a major role in the termination of pregnancy in the rat and rabbit, the role of 20α-HSD in the human is still ill-defined. In the placenta, where 17α-hydroxylase and 5α-reductase are absent, 20α-HSD is almost the only enzyme able to catalyze the catabolic transformation of P. It has been observed that a treatment with prostaglandin F2α increased 20α-HSD activity with a concomitant decrease in the P concentration (Bussmann & Deis 1979). The activity of 20α-HSD is suppressed by prolactin during the luteal regression and is increased at the end of pseudo-pregnancy and pregnancy (Kuhn & Briley 1970). 20α-HSD activity is known to be induced by cytokines such as interleukin-3 and a granulocyte

Journal of Molecular Endocrinology (2000) 25, 221–228

Online version via http://www.endocrinology.org

0952–5041/00/025–221 © 2000 Society for Endocrinology Printed in Great Britain

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macrophage-colony-stimulating factor (Hapel et al. 1985). Two isoenzymes of 20α-HSD have been purified, respectively, from rat corpora lutea (Seong et al. 1992) and pig adrenal (Nakajin et al. 1989). In the human placenta, 20α-HSD activities have been associated with type I (Strickler et al. 1981) and type II (Wu et al. 1993) 17β-HSDs. Recently, cDNAs encoding rat (Mao et al. 1994, Miura et al. 1994), rabbit (Lacy et al. 1993) and bovine (Warren et al. 1993) 20β-HSD have been cloned. They are highly homologous to the 3β-hydroxysteroid dehydrogenase family, and belong to the aldoketo reductase superfamily. In contrast, type 1 and type 2 17β-HSDs (Peltoketo et al. 1988, Luu-The et al. 1989, Wu et al. 1993) are members of the short-chain alcohol dehydrogenase superfamily.

In this study, we report the isolation and expression of a human skin 20α-HSD cDNA that does not belong to the short-chain alcohol dehydrogenase family but is a member of the aldoketo reductase superfamily – the same family as the 3α-HSD and 20α-HSD of other species.

MATERIALS AND METHODS

Cloning of 20α-HSD cDNA

A human skin fibroblast cDNA library constructed in λgt11 (Clontech Laboratories Inc., Palo Alto, CA, USA) was screened with a human 3α-HSD probe labeled with [α-32P]dCTP by the random primer method (Feinberg & Vogelstein 1983) as previously described (Luu-The et al. 1994). Positive recombinant phage plaques were purified by replication (twice) and phage DNA was isolated by centrifugation for 90 min at 105 000 g followed by phenol extraction. DNA inserts were obtained by digestion with EcoRI and subcloned into a pCMV expression vector (kindly provided by Dr M B Mathews, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA). Recombinant plasmids (pCMV–20α-HSD) were prepared and purified, using the Qiagen Mega kit (Qiagen, Chatsworth, CA, USA).

Sequence determination

Sequencing of double-stranded plasmid DNA was performed according to the dideoxy chain termination method (Sanger et al. 1977), using a T7 sequencing kit (Pharmacia LKB Biotechnology, Baie d’Urfe, Quebec, Canada).

Transient expression in HEK-293 cells

Six micrograms pCMV–20α-HSD plasmid per well were transfected into 293 cells by the calcium phosphate procedure (Kingston et al. 1991). Cells

FIGURE 1. Substrate specificity of the transfected human 20α-HSD.

pCMV–20α-HSD-transfected HEK-293 cells were examined for their ability to transform DHEA, E1, E2, P, 4-dione, T and DHT. Hatched and open bars indicate the activity of mock-transfected and pCMV–20α-HSD-transfected cells respectively. The concentration of substrate was 0·1µM. Experimental conditions were as described in Materials and Methods. Data are expressed as means of duplicate determinations.
were plated initially at $10^4$ cells/cm² in 6-well culture plates and grown in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) calf fetal serum (Hyclone, Logan, UT, USA) at 37°C under a 95% air, 5% CO₂ humidified atmosphere. Gentamicin (Roussel, Montreal, Quebec, Canada) was added to all media to a final concentration of 15 µg/ml.

**Assay of enzymatic activity**

Determination of the activity in intact cells was performed by adding 0·1 µM of the indicated 14C-labeled substrate (Dupont Inc., Mississauga, Canada) to freshly changed culture medium in a 6-well culture plate. Because of the low radio-specific activity of the commercial 14C-steroid (10 mCi/mmol), we used 0·1 µM substrate (without addition of cold steroid) to obtain a sensitivity range that is accurate and convenient for the assay of overexpressed enzymes. We preferred 14C-labeled rather than 3H-labeled steroids (although the latter possess higher radio-specific activity) because of the low background of 14C and the availability and convenience of the detection method (Phosphor Imager; Molecular Dynamics, Sunny Vale, CA, USA). After 1 h incubation, the steroids were extracted and separated by thin-layer chromatography (TLC) as previously described (Luu-The et al. 1994). For assays using a cell homogenate preparation, cells were scraped, frozen and thawed three times in 50 mM sodium phosphate buffer (pH 7·4) containing 20% glycerol and 1 mM EDTA, and then centrifuged at 10 000 g for 15 min. The enzymatic reaction was carried out at 37°C in 1 ml 50 mM sodium phosphate buffer (pH 7·4), containing 20% glycerol, 1 mM EDTA and 0·4 mM cofactors for 1 h in the presence of the indicated concentration of radiolabeled steroid substrates. Radioactivity signals were detected and quantified using a Phosphor Imager.

**Analysis of the 20α-HSD expression level**

Poly(A)+ RNA (0·1 µg) from human prostate, brain, testis, liver and adrenal, uterus and mammary glands (Clontech Laboratories Inc., Palo Alto, CA, USA) was reversed-transcribed using a poly(T) primer. The cDNA products were amplified for 20α-HSD using a pair of specific primers (5′-CTG-AAT-TCT-TGA-AGG-TGC-AGC-AAG-TCA-CTG-3′, 5′-GGA-ATT-CTG-TAT-TCT-ATG-CCT-CTG-TGA-CC-3′) and the PCR. Conditions for Taq-PCR amplification were as described.
previously (Luu-The et al. 1994). The amplified DNA products were separated on a 1% agarose gel and stained with ethidium bromide or transferred to nylon filters and hybridized to an oligonucleotide probe following the Southern procedure (Southern 1975). The oligonucleotide probe was labeled with $^{32}$P-ATP by T4 nucleotide kinase and hybridized to the blots as described (Luu-The et al. 1994). To avoid non-specific hybridization, the selected oligonucleotide probe was chosen from a region different from that of the primers used for amplification.

HaCaT cells were grown in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% (v/v) calf fetal serum (Hyclone) at 37°C under a 95% air, 5% CO2 humidified atmosphere as described above.

The total RNA from HaCaT (human keratinocyte) cells was extracted using a Tri-reagent RNA extraction Kit (Molecular Research Center Inc., Cincinnati, OH, USA) and Poly(A)+ mRNA was isolated using the Oligotex-dT mRNA kit (Qiagen). Experimental procedures were performed as described by the manufacturers. Amplification of the 20α-HSD mRNA was performed as described above.

**RESULTS**

**Isolation and characterization of the expressed activity of a human 20α-HSD cDNA clone**

We have obtained, by screening a human skin fibroblast cDNA library, a 1.3 kb cDNA clone that expresses 20α-HSD activity upon transfection into HEK-293 cells. Sequence comparison with sequences from GenBank indicates that the sequence of our cDNA clone is highly homologous to 3α-HSD, dihydriodiol dehydrogenase and the aldoketo reductase family and is almost identical to the human hepatic bile acid-binding protein (Stolz et al. 1993). We inserted the isolated cDNA fragment into a pCMV expression vector. The resulting pCMV-20α-HSD plasmid was transiently transfected into HEK-293 cells and the activities were analyzed to determine the ability of the expressed enzyme to catalyze the transformation of dehydroepiandrosterone (DHEA), estrone (E1), estriol (E2), P,
4-androstenedione (4-dione), testosterone (T) and dihydrotestosterone (DHT). As illustrated in Fig. 1, the enzyme possesses a high level of reductive 20\(^{-}\)-HSD activity that transforms P into the inactive 20\(^{-}\)-OHP, whereas other activities such as those of 17\(^{-}\}- and 3\(^{-}\)-HSD (which catalyze the transformation of other substrates) are negligible.

### Cofactor specificity

As illustrated in Fig. 2, the 20\(^{-}\)-HSD activity in a cell-homogenate preparation efficiently catalyzes the reduction of P to 20\(^{-}\)-OHP using both NADPH and NADH as cofactors. However, the transformation using NADPH is much more efficient, with 3-6-fold higher and 4-fold lower \(V_{\text{max}}\) and \(K_{\text{m}}\) values respectively (Fig. 2). The reverse reaction is negligible. The \(K_{\text{m}}\) value obtained in this experiment (6.6 \(\mu\)M), which used cell homogenate, was much higher than the \(K_{\text{m}}\) obtained using intact cells (0.6 \(\mu\)M). Indeed, 20\(^{-}\)-HSD is moderately labile and the activity decreases rapidly upon homogenization.

### Expression of 20\(^{-}\)-HSD mRNA in various tissues

Using specific oligonucleotide primers for 20\(^{-}\)-HSD and amplification by PCR, we have determined the expression levels of 20\(^{-}\)-HSD mRNA in the liver, prostate, testis, adrenal and the brain (Fig. 3), as well as in the uterus, mammary gland and the HaCaT cell line (Fig. 4). As can be seen in Figs 3 and 4, 20\(^{-}\)-HSD is highly expressed in the liver, mammary gland and the brain, whereas lower expression was detected in the prostate, testis and uterus, and very low expression was observed in the adrenal.

### Homology

The comparison of the amino acid sequence of the human 20\(^{-}\)-HSD with that of rabbit (rb), rat (r) and bovine (b) 20\(^{-}\)-HSDs indicates that they share 78\(^{-}\)-9, 68\(^{-}\)-7 and 52\(^{-}\)-3% identity respectively. The sequence also shows a high level of homology with the human types 1 (84%) and 3 (97%) and rat (65%) 3\(^{-}\)-HSD amino acid sequences (Fig. 5).

### Relative 20\(^{-}\)-HSD, 3\(^{-}\)-HSD and 17\(^{-}\)-HSD activities catalyzed by human 20\(^{-}\)-HSD, type 3 3\(^{-}\)-HSD and type 5 17\(^{-}\)-HSD

As mentioned above, 20\(^{-}\)-HSD, type 3 3\(^{-}\)-HSD and type 5 17\(^{-}\)-HSD are highly homologous and they all belong to the aldoketo reductase family. These enzymes could thus have some common activities. The relative activities for each enzyme...
illustrated in Fig. 6. 20α-HSD, 3α-HSD and 17β-HSD activities were assessed by the reactions catalyzing the transformation of P into 20α-OHP, of DHT into 5α-androstane-3α,17β-diol (3α-diol) and of 4-dione into T respectively. 20α-HSD activity is found in all three enzymes. In 20α-HSD, this activity is rather unique, whereas in type 5 17β-HSD and in type 3 3α-HSD a dual 20α-HSD and 17β-HSD or 3α-HSD activity has been observed respectively.

**DISCUSSION**

This report describes the cloning and the characterization of a human 20α-HSD that is highly homologous to rabbit (Lacy et al. 1993), rat (Mao et al. 1994, Miura et al. 1994), and bovine (Warren et al. 1993) 20α-HSDs. The clone has been identified previously as a bile acid-binding protein (Stolz et al. 1993). It is noteworthy that this name was also given to type 3 3α-HSD. Proteins that possess the ability to bind bile acids also include type 1 3α-HSD, type 3 3α-HSD, type 5 17β-HSD and rat 3α-HSD. This is probably because bile acids are steroids and thus can bind to this family of enzymes (the active sites of which could accept a broad range of steroids, though their catalytic activity is much more selective). Indeed, it is well known that 3α-HSD follows an ordered mechanism. In the liver and intestine, this enzyme may have a role in the transport of bile acids, but it is unlikely that such activity would be present in the brain, the testis or the adrenal.

20α-HSD activity in the human has previously been associated with type 1 (Strickler et al. 1981) and type 2 (Wu et al. 1993) 17β-HSDs or 3α-HSD (Smirnov 1990). The expressed enzyme reported in the present study shows an almost exclusive 20α-HSD activity after transfection into HEK-293 cells. However, 20α-HSD activity (Table 1) is relatively high in human type 3 3α-HSD and type 5 17β-HSD. Interestingly, in most of the tissues in which 20α-HSD is expressed, type 5 17β-HSD is also found (except for the brain) and type 3 3α-HSD is expressed in all these tissues, albeit at different levels, e.g. 20α-HSD is very low in the adrenal whereas type 3 3α-HSD is much higher (I Dufort, F Labrie & V Luu-The, unpublished observations). Since type 3 3α-HSD and type 5

![Graph showing relative activities of HSD enzymes](image-url)
17β-HSD are involved in the regulation of the intracellular concentration of androgen and are expressed in androgen-sensitive tissues such as the prostate and the testis (El-Alfy et al. 1999, Pelletier et al. 1999), the relatively important 20α-HSD activity associated with these enzymes could help to protect those tissues from the action of P, a female-specific hormone. Our results also indicate that, in agreement with the findings in other species, the human 20α-HSD belongs to the aldo-keto reductase superfamily, which also includes 3α-HSD, as well as aldose and aldehyde reductases. However, the role of the 20α-HSD activity exerted by type 1 and type 2 17β-HSDs, which belong to the short-chain alcohol dehydrogenases, is still unclear. After transfection into mammalian cells, the expressed 20α-HSD catalyzes the reductive conversion of P to 20α-OHP, while the conversion of DHT to 3α-diol (3α-HSD activity) is negligible, although the enzyme possesses 97 and 84% amino acid identity with the human types 3 and 1 3α-HSD respectively. These 3α-HSDs catalyze the transformation of DHT to 3α-diol and 5α-androstane-3,17-dione to ADT more actively than the transformation of P to 20α-OHP (Dufort et al. 1996).

In the rat ovary, it has been shown that prolactin strongly inhibits the expression of 20α-HSD (Albarracin et al. 1994) and could thus repress this activity throughout pregnancy when P is essential for fetal survival. Recently, P has been shown to be synthesized in the brain (Koenig et al. 1995), where it stimulates myelin formation. The presence of 20α-HSD in the brain (Fig. 3) suggests that it could play a role in the regulation of myelin formation. 20α-HSD activity has also been found in mouse macrophages, hemopoietic cells, and cell lines. Its induction by colony-stimulating factors (Hapel et al. 1985) and interleukin-3 (Ihle et al. 1981) is of particular interest.

It is suggested (Ihle et al. 1981) that the enzyme plays a role in protecting thymocytes against the toxic effects of P. The role of this enzyme in the human skin is still ill-defined. Thus the availability of the human skin 20α-HSD cDNA offers an opportunity for further study of the role of this enzyme in thymocytes, placenta, gonads and the skin.

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council (MRC) of Canada, and Endorecherche Inc. We would like to thank Nathalie Paquet, Guy Reimnitz and Mei Wang for their skillful technical assistance.

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