Characterization of a human 20 α -hydroxysteroid dehydrogenase

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ABSTRACT

It has been suggested that 20 α -hydroxysteroid dehydrogenase (20 α -HSD) is a T-cell differentiation marker in mice. In the human, this enzyme has generally been associated with types 1 and 2 17 β -HSDs, which belong to the short-chain alcohol dehydrogenase family, whereas the rat, rabbit, pig and bovine 20 α -HSDs are members of the aldoketo reductase superfamily, which also includes the 3 α -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 20 α -HSD activity but negligible 3 α - and 17 β -hydroxysteroid dehydrogenase activities. A comparison of the amino acid sequence of the human 20 α -HSD with those of other related 20 α - and 3 α -HSDs indicates that the human 20 α -HSD shares 79.9, 68.7 and 52.3% identity with rabbit, rat and bovine 20 α -HSDs, whereas it shows 97, 84 and 65% identity with human type 3, type 1 and rat 3 α -HSDs. In

contrast, the enzyme shares only 15.2 and 15.0% identity with type 1 and type 2 human 17 β -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 20 α -HSD preferentially catalyzes the reduction of progesterone to 20 α -hydroxyprogesterone with a K_m value of 0.6 μ M, 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 20 α -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 20 α -HSD belongs to the aldoketo reductase family, like the 20 α -HSDs from other species.

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INTRODUCTION

The enzyme 20 α -hydroxysteroid dehydrogenase (20 α -HSD) (EC 1.1.1.149) catalyzes the conversion of progesterone (P) into its inactive form, 20 α -hydroxyprogesterone (20 α -OHP). 20 α -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, 20 α -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 20 α -HSD has been shown to play a major role in the termination of pregnancy in the rat and rabbit, the role of 20 α -HSD in the human is still ill-defined. In the placenta, where 17 α -hydroxylase and 5 α -reductase are absent, 20 α -HSD is almost the only enzyme able to catalyze the catabolic transformation of P. It has been observed that a treatment with prostaglandin F2 α increased 20 α -HSD activity with a concomitant decrease in the P concentration (Bussmann & Deis 1979). The activity of 20 α -HSD is suppressed by prolactin during the luteal regression and is increased at the end of pseudo-pregnancy and pregnancy (Kuhn & Briley 1970). 20 α -HSD activity is known to be induced by cytokines such as interleukin-3 and a granulocyte

FIGURE 1. Substrate specificity of the transfected human 20 α -HSD. pCMV-20 α -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-20 α -HSD-transfected cells respectively. The concentration of substrate was 0.1 μ M. Experimental conditions were as described in Materials and Methods. Data are expressed as means of duplicate determinations.

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

MATERIALS AND METHODS

Cloning of 20 α -HSD cDNA

A human skin fibroblast cDNA library constructed in λ gt11 (Clontech Laboratories Inc., Palo Alto,

CA, USA) was screened with a human 3 α -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-20 α -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-20 α -HSD plasmid per well were transfected into 293 cells by the calcium phosphate procedure (Kingston *et al.* 1991). Cells

FIGURE 2. Cofactor specificity for human 20 α -HSD. pCMV-20 α -HSD-transfected HEK-293 cells were examined for their ability to use NAD⁺, NADH, NADP⁺ and NADPH to transform P to 20 α -OHP (A). Lineweaver-Burk plot analysis (B) for the $V_{\max}$ and K_m determinations for NADH (●—●) and NADPH (○—○). The concentration of substrate was 0.1 μM . 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 $\mu\text{g}/\text{ml}$.

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 (Phospho 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 20 α -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 20 α -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

FIGURE 3. 20 α -HSD RNA expression. Poly(A)+ mRNA for human adrenal, brain, liver, prostate and testis was obtained from Clontech Laboratories Inc. and was used to perform RT-PCR amplification. 20 α -HSD- and actin-amplified products stained with ethidium bromide are shown in the upper and middle panels, whereas hybridization of the 20 α -HSD-amplified products with 32 P-labeled oligonucleotide specific for human 20 α -HSD is illustrated in the lower panel. The amplification procedure was as described in Materials and Methods.

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

FIGURE 4. Expression of 20 α -HSD mRNA in HaCaT cells and female tissues. Preparation of HaCaT Poly(A)+ mRNA and RT-PCR amplifications of 20 α -HSD were performed as described in Materials and Methods. Poly(A)+ mRNA for human uterus and mammary gland were obtained from Clontech Laboratories Inc. The amplified product was separated on agarose gel and stained with ethidium bromide. The DNA size marker (L) is indicated on the left.

manufacturers. Amplification of the 20 α -HSD mRNA was performed as described above.

RESULTS

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

We have obtained, by screening a human skin fibroblast cDNA library, a 1.3 kb cDNA clone that expresses 20 α -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 3 α -HSD, dihydrodiol 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-20 α -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), estradiol (E2), P,

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h20 $\alpha$ HSD  MDSKYQCCKLNDGHFMPVLGFGTYAPAEVPSKALEATKLAIEAGFRHIDSAHLYNNEEQVGLAIRSKIADGSVKREDIFYTSKLWCNSHRPELVRPALERSLKNLQLDY 110
rb20 $\alpha$ HSD --P-F-R-A-S---I-----E-----M---I-D-----YF-K--KE-----T-----TF-----S-D-----
r20 $\alpha$ HSD  -N--I-KME-----SI-----TE-NLRK-SM-S--I-DV-----CS--Q--EI-Q--V--E--T-----S--N--R--N--
b20 $\alpha$ HSD  . . . . . -AK--I-L- . . . . . WKS-PG-VT--V-V--DLGY--C--V-Q--NE--LQA-LQEKV--L-IV--TY-DKD--KG-CQKT--SD-K--
h3 $\alpha$ HSD1  --P--R-E-----P--RNR-V-V-----Y-----T-----TFFQ-QM-Q--S--K--
h3 $\alpha$ HSD3  -----V-----H-----V-----S-----
r3 $\alpha$ HSD   --ISLR-A--N-I-----TV-EK-A-DEVIK--I--DN-----F--Y--EV--E--Q-----E--T-----STF-----TC--KT--ST-Q--
h17 $\beta$ HSD5 --Q-----P--R--V-----T-----STF-----N--KA--
m17 $\beta$ HSD5 --T-R-S--I-I-----Q--K--T-AV-I--D--H-----M-Q--KE-----T-----V-----VC--Q--QL--

h20 $\alpha$ HSD  VDLYLIHFVPVSKPGEEVVPKDENGKILFDITVLCATWEAVEKCKDAGLAKSIGVSNFNRRLQLEMILNKPGKYKPCVNQVECHPYFNQRKLLDFCKSKDIDLVAISALG 220
rb20 $\alpha$ HSD ---I---TAL---V-I--T--H-AI---I---M-----L--G--E--G-----
r20 $\alpha$ HSD  --L---D-LL-Q--H-NLIL-----D--M-----K-----HR-----L-L-S--AY--MN-----G--
b20 $\alpha$ HSD  L---L-W-TGF--KDFF-L-D-NVIPSEK-FVD--TVM-ELV-E-V-A--HL-V-K-----AV-I--LT-E-IQY-N-G-VT--P--
h3 $\alpha$ HSD1  ---L---MAL---TPL-----VI---S--VM-----C-----L--S-----H--
h3 $\alpha$ HSD3  -----M-----H-L-----
r3 $\alpha$ HSD   ---I---MALQ--DIFF-R--H--L--E--I-D--M-----C--R-----L-L-S-M--Y-----I--S-CT--
h17 $\beta$ HSD5 ---S-M-L--LS-T--VI--I--T--M-----RS-----
m17 $\beta$ HSD5 ---F--AM---NYL-K--L-Y-A--I-D--M-----H-L-K--K-----L-QG-----R--

h20 $\alpha$ HSD  SHREEPVDPNPVLLEDPLCALAKKHKRTPALIALRYQLQGVVVLAKSYNEQRIQNVQVFELTSEEMKAIDGLNRNVRYLTLDIFAGPPNYPFSDEY 323
rb20 $\alpha$ HSD ---PE---QSA---LIG---QQ-----I-----FT-K--KE-I--P--D--V--S--F--V-A-FAI-H-----
r20 $\alpha$ HSD  TQ-YKYCINEDT--D--I--TM--Y-----E--I-T-V-F--E--E-L--D--A-DD-EIL-N-D-L--FPANM-KAH--F--
b20 $\alpha$ HSD  . PDR--AK-ED-SI---RIK-I-D-YNK-T-QVLI-FPI--NLI-IP--VTPE--AE-F--D-E-DK-D-NT-LSY--DW-ACA-VSCASHRD--HE-F
h3 $\alpha$ HSD1  TQ-HKL-----E-I-----D--VL-----Y--VVM-FLMDH-D-----
h3 $\alpha$ HSD3  -----
r3 $\alpha$ HSD   -S-DKT--QK-----D-----I--Y-Q--V-----P-IR-FKPK--KEPT-----A--D--L--F--NNAKY-DDH--H--T--
h17 $\beta$ HSD5 -Q-DKR-----A-D-----D--LH-FNS-S--SH--Y-----
m17 $\beta$ HSD5 -H-E-Q--QS-----DN--GSM--YN-----FS-K--KE-M-----S--VL-D-NK-IR-ISGS--KD--DF-FW--

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FIGURE 5. Compared sequences of the human 20 α -HSD and related sequences. The deduced amino acid sequence of human 20 α -HSD was aligned with amino acid sequences of rabbit (rb), rat (r) and bovine (b) 20 α -HSDs as well as human (h) and rat 3 α -HSDs. Amino acid sequences are given in the conventional single-letter code and are numbered on the right. Dashes and dots represent identical and missing amino acid residues respectively.

4-androstenedione (4-dione), testosterone (T) and dihydrotestosterone (DHT). As illustrated in Fig. 1, the enzyme possesses a high level of reductive 20 α -HSD activity that transforms P into the inactive 20 α -OHP, whereas other activities such as those of 17 β - and 3 α -HSD (which catalyze the transformation of other substrates) are negligible.

Cofactor specificity

As illustrated in Fig. 2, the 20 α -HSD activity in a cell-homogenate preparation efficiently catalyzes the reduction of P to 20 α -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 $V_{\max}$ and K_m values respectively (Fig. 2). The reverse reaction is negligible. The K_m value obtained in this experiment (6.6 μ M), which used cell homogenate, was much higher than the K_m obtained using intact cells (0.6 μ M). Indeed, 20 α -HSD is moderately labile and the activity decreases rapidly upon homogenization.

Expression of 20 α -HSD mRNA in various tissues

Using specific oligonucleotide primers for 20 α -HSD and amplification by PCR, we have deter-

mined the expression levels of 20 α -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 α -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 α -HSD with that of rabbit, rat and bovine 20 α -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 α -HSD amino acid sequences (Fig. 5).

Relative 20 α -HSD, 3 α -HSD and 17 β -HSD activities catalyzed by human 20 α -HSD, type 3 3 α -HSD and type 5 17 β -HSD

As mentioned above, 20 α -HSD, type 3 3 α -HSD and type 5 17 β -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

FIGURE 6. Relative 20 α -HSD, 3 α -HSD and 17 β -HSD activities catalyzed by human 20 α -HSD, type 3 3 α -HSD and type 5 17 β -HSD. Cells stably expressing human 20 α -HSD, type 3 3 α -HSD and type 5 17 β -HSD were cultured in 6-well plates to approximately 5×10^5 cells/well. 14 C-labeled P, DHT or 4-dione (0.1 μ M) was added to freshly changed culture medium. After 2 h incubation, the medium was collected and subjected to extraction. Extraction, separation on TLC and quantification of steroids were performed as described in Materials and Methods. 100% activity corresponds to 1.70, 0.38 and 1.37 nmol/h/ 10^6 cells for h3 α -HSD3, h17 β -HSD5 and h20 α -HSD respectively.

are illustrated in Fig. 6. 20 α -HSD, 3 α -HSD and 17 β -HSD activities were assessed by the reactions catalyzing the transformation of P into 20 α -OHP, of DHT into 5 α -androstane-3 α ,17 β -diol (3 α -diol) and of 4-dione into T respectively. 20 α -HSD activity is found in all three enzymes. In 20 α -HSD, this activity is rather unique, whereas in type 5 17 β -HSD and in type 3 3 α -HSD a dual 20 α -HSD and 17 β -HSD or 3 α -HSD activity has been observed respectively.

DISCUSSION

This report describes the cloning and the characterization of a human 20 α -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) 20 α -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 3 α -HSD. Proteins that possess the ability to bind bile acids also include type 1 3 α -HSD, type 3 3 α -HSD, type 5 17 β -HSD and rat 3 α -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 3 α -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.

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

TABLE 1. Enzymatic characteristics of 20 α -HSD and related enzymes

Enzyme	Reaction	K _m (μ M)	Cofactor	Direction of reaction
20 α -HSD	P $\rightarrow$ 20 α -OHP	0.6	NADPH	Reduction
	DHT $\rightarrow$ 3 α -diol	ND		
Type 3 3 α -HSD	P $\rightarrow$ 20 α -OHP	3.8	NADPH	Reduction
	DHT $\rightarrow$ 3 α -diol	1.4		
Type 1 17 β -HSD	E1 $\rightarrow$ E2	8.1	NADPH	Reduction
	P $\rightarrow$ 20 α -OHP	200		
Type 2 17 β -HSD	E2 $\rightarrow$ E1	0.21	NAD	Oxidation
	T $\rightarrow$ 4-dione	0.39		
	20 α -OHP $\rightarrow$ P	0.71		
Type 5 17 β -HSD	4-dione $\rightarrow$ T	2.3	NADPH	Reduction
	P $\rightarrow$ 20 α -OHP	0.5		

ND, K_m value could not be determined.

17 β -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 20 α -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 20 α -HSD belongs to the aldoketo reductase superfamily, which also includes 3 α -HSD, as well as aldose and aldehyde reductases. However, the role of the 20 α -HSD activity exerted by type 1 and type 2 17 β -HSDs, which belong to the short-chain alcohol dehydrogenases, is still unclear. After transfection into mammalian cells, the expressed 20 α -HSD catalyzes the reductive conversion of P to 20 α -OHP, while the conversion of DHT to 3 α -diol (3 α -HSD activity) is negligible, although the enzyme possesses 97 and 84% amino acid identity with the human types 3 and 1 3 α -HSD respectively. These 3 α -HSDs catalyze the transformation of DHT to 3 α -diol and 5 α -androstane-3,17-dione to ADT more actively than the transformation of P to 20 α -OHP (Dufort *et al.* 1996).

In the rat ovary, it has been shown that prolactin strongly inhibits the expression of 20 α -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 20 α -HSD in the brain (Fig. 3) suggests that it could play a role in the regulation of myelin formation. 20 α -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 20 α -HSD cDNA offers an opportunity for further study of the role of this enzyme in thymocytes, placenta, gonads and the skin.

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