Pergamon

0960-0760(95)00028-3

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 111–118, 1995

0960-0760/95 $9.50 + 0.00

The Murine 3β-Hydroxysteroid Dehydrogenase Multigene Family: Structure, Function and Tissue-specific Expression

Anita H. Payne,[1–4]* Trent R. Clarke[2,3] and Paul A. Bain[3,4]†

[1]Department of Obstetrics and Gynecology and [2]Department of Biological Chemistry, [3]The Reproductive Sciences Program and [4]The Graduate Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI 48109-0278, U.S.A.

The classical form of the enzyme 5-ene-3β-hydroxysteroid dehydrogenase/isomerase (3βHSD), expressed in adrenal glands and gonads, catalyzes the conversion of 5-ene-3β-hydroxysteroids to 4-ene-3-ketosteroids, an essential step in the biosynthesis of all active steroid hormones. To date, four distinct mouse 3βHSD cDNAs have been isolated and characterized. These cDNAs are expressed in a tissue-specific manner and encode proteins of two functional classes. Mouse 3βHSD I and III function as 3β-hydroxysteroid dehydrogenases and 5-en→4-en isomerases using NAD^+ as a cofactor. The enzymatic function of 3βHSD II has not been completely characterized. Mouse 3βHSD IV functions only as a 3-ketosteroid reductase using NADPH as a cofactor. The predicted amino acid sequences of the four isoforms exhibit a high degree of identity. Forms II and III are 85 and 83% homologous to form I. Form IV is most distant from the other three with 77 and 73% sequence identity to I and III, respectively. 3βHSD I is expressed in the gonads and adrenal glands of the adult mouse. 3βHSD II and III are expressed in the kidney and liver with the expression of form II greater in kidney and form III greater in liver. Form IV is expressed exclusively in the kidney. Although the amino acid composition of forms I, III and IV predicts proteins of the same molecular weight, the proteins have different mobilities on SDS–polyacrylamide gel electrophoresis. This characteristic allows for differential identification of the expressed proteins. The four structural genes encoding the different isoforms are closely linked within a segment of mouse chromosome 3 that is conserved on human chromosome 1.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1–6, pp. 111–118, 1995

INTRODUCTION

The enzyme 5-ene-3β-hydroxysteroid dehydrogenase/isomerase (3βHSD) catalyzes the conversion of 5-ene-3β-hydroxysteroids to 4-ene-3-ketosteroids, an essential step in the biosynthesis of all biologically active steroid hormones, including the adrenal steroid hormones, cortisol (or corticosterone in the rodent) and aldosterone; and the gonadal steroid hormones, progesterone and estradiol in the ovary and testosterone in the testis (Fig. 1). Earlier reports suggested the presence of 3βHSD activity in nonsteroidogenic tissues. Milewich *et al.* [1] reported 3βHSD activity in human epidermal keratinocytes, and Devine *et al.* [2] in guinea pig kidneys. The presence of 3βHSD activity in brain has been suggested by reports from Weidenfeld *et al.* [3], Jung-Testas *et al.* [4] and Bauer and Bauer [5]. There are numerous clinical reports of patients with 3βHSD deficiency whose symptoms suggest that distinct 3βHSD structural genes may encode the activity in peripheral tissues [6–10]. These reports include male and female patients with adrenal hyperplasia, with the male patient exhibiting various degrees of male pseudohermaphroditism who also show signs of virilization during puberty. The defect in 3βHSD activity in these patients is consistent with deficiency of adrenal and gonadal 3βHSD and the expression of one or more peripheral 3βHSD genes. More recently, Rhéaume *et al.* [11] reported the isolation of a 3βHSD cDNA

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
*Correspondence to A. H. Payne at: L1221 Women's Hospital, University of Michigan, Ann Arbor, MI 48109-0278, U.S.A.
†Present address: Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, U.S.A.

from a human adrenal cDNA library that is distinct from the 3βHSD isolated from a human placental cDNA library [12, 13]. The former, referred to as 3βHSD II, is expressed almost exclusively in gonads and adrenal glands, while the latter, referred to as 3βHSD I, is expressed in placenta, skin and mammary gland tissue [12].

Studies in the rat have identified four distinct 3βHSD cDNAs which are expressed in a tissue-specific manner [14]. Rat 3βHSD I and II are both expressed in gonads and adrenal glands and to a lesser extent in the placenta, rat 3βHSD III is expressed exclusively in the male liver and 3βHSD IV is expressed in ovary, placenta and skin.

Our laboratory has isolated and characterized four distinct cDNAs in the mouse. This paper will review the chromosomal location of the four mouse genes, their tissue-specific expression and the functional characteristics of the expressed proteins. The functional significance of the multiple forms of 3βHSD also will be discussed.

CHROMOSOMAL LOCATION

The chromosomal location of the four genes encoding the mouse isoforms has been determined by linkage analysis using gene-specific probes derived from the 3′-untranslated regions of the 3βHSD cDNA clones. The four 3βHSD structural genes (*Hsd3b-1*, *Hsd3b-2*, *Hsd3b-3* and *Hsd3b-4*) were found to be closely linked within a segment of mouse chromosome 3 between *Tshb* and *Gba* [15]. The order of markers on chromosome 3 surrounding the *Hsd3b* locus is: centromere-*Gba*-(4.4 ± 2.2)-*Hsd3b*-(3.3 ± 1.9)-*Tshb*-(6.7 ± 2.7)-*Amy 1*. This segment of mouse chromosome 3 shows conservation of gene order and physical distance with the centromeric region of human chromosome 1. Human 3βHSD has been mapped to 1p13 by *in situ* hybridization [16, 17]. Our results suggested that all of the human 3βHSD genes would be found on the short arm of human chromosome 1, proximal to TSHB. Recently Russell *et al.* reported close genetic linkage between the human genes for 3βHSD type I and II [18]. However, the physical distance of the human *Hsd3b* genes to other human markers has not been established.

In preliminary studies to determine the physical distance separating the mouse *Hsd3b* genes, using pulsed-field gel electrophoresis of large fragments of genomic DNA, we demonstrated that the four genes are all present on a single 400 kb fragment (unpublished data). The close linkage of the four genes demonstrates that the mouse 3βHSD gene family exists as a cluster of related genes which probably arose through duplication and divergence of a single ancestral gene.

CHARACTERIZATION OF MOUSE 3βHSD cDNAs AND THEIR TISSUE-SPECIFIC EXPRESSION

Four distinct mouse cDNA clones have been isolated and characterized [19–21]. They are referred to as 3βHSD I, II, III and IV based on the chronological

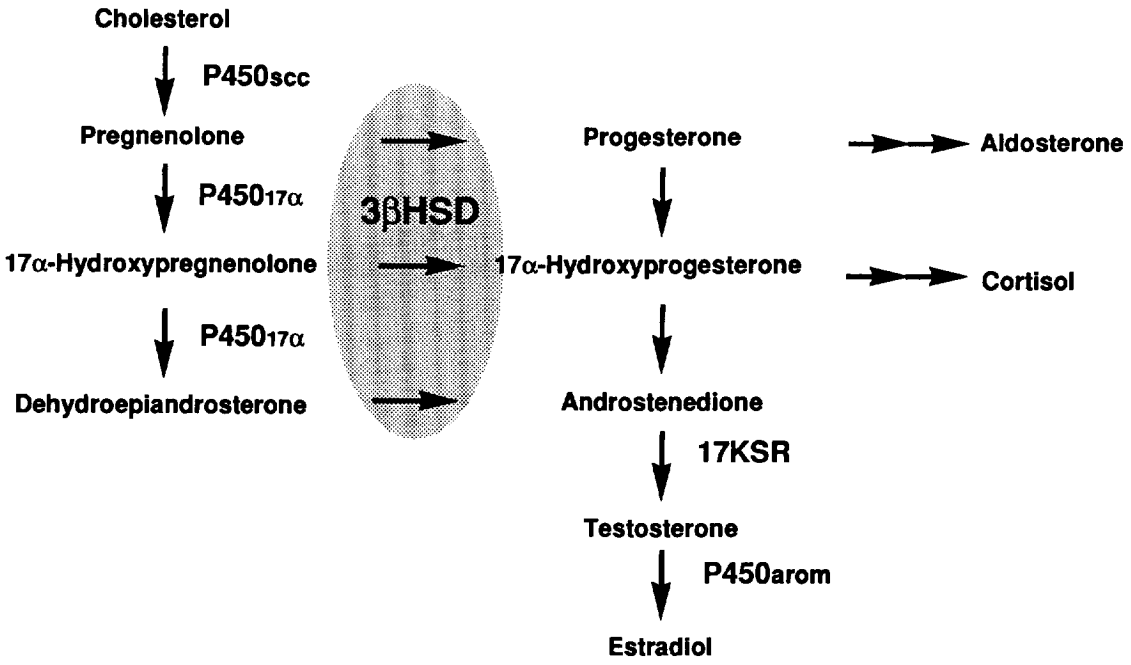

Fig. 1. Steroid biosynthetic pathway in gonads and adrenal glands. *P*450scc, cytochrome *P*450 cholesterol side-chain cleavage; *P*45017α, cytochrome *P*45017α-hydroxylase/C17-20 lyase; 3βHSD, 5-ene-3β-hydroxysteroid dehydrogenase/isomerase; 17 KSR, 17-ketosteroid reductase, *P*450arom, cytochrome *P*450 aromatase.

```
I:    MAGWSCLVTGAGGFVGQRIIKMLVQEKELQEVRALDKVFRPETKEEFSKLQTKTKVTVLEGDILDAQCLR
II:   ************************************************************************
III:  -P------------L-----QL-----D-E-I-V-----K---R-Q-FN-G-SI----------T-Y--
IV:   -P------------L----VR-----E----I---FRT-GRKQE--L----------K---------K

I:    RACQGISVVIHTAAVIDVTGVIPRQTILDVNLKGTQNLLEACVQASVPAFIFCSSVDVAGPNSYKKIVLN
II:   ************************************----I---------S-----------E----
III:  --------------I-------------------------I---------S-----------D----
IV:   -----M-A------A--PL-AAS-------------L--D---E-N--T--YS---L--------E-I--

I:    GHEEQNHESTWSDPYPYSKKMAEKAVLAANGSMLKNGGTLNTCALRPMYIYGERSPFIFNAIIRALKNKG
II:   ----EC-----------------------------------Q-------C-------L-S-I--M---H--
III:  ---DEHR---------------------------------Q-------C------Q-LS-T--K-----F
IV:   A---EH------N-------------------I-------H-----LSF----ECQVTSTTVKT----NS

I:    ILCVTGKFSIANPVYVENVAWAHILAARGLRDPKKSTSIQGQFYYISDDTPHQSYDDLNYTLSKEWGLRP
II:   --RSF---NT------G-----------------PN---E-----------F--IS--------FCL
III:  --RGG----T------G-------------N----PN---E-------------------------FCL
IV:   -IKKNAT---------G-A---------S-Q-----P---------T----------KC---------L

I:    NASWSLPLPLLYWLAFLLETVSFLLRPVYRYRPLFNRHLITLSNSTFTFSYKKAQRDLGYEPLVNWEEAK
II:   DS-----V-----------------S-I---I-P-----V---G-------------------S-----
III:  -SR-Y--V-I---------------S-I---I-P-----V--TA-------------------S-----
IV:   DT--------------------------N---P---L---VL--V-----------------S-----

I:    QKTSEWIGTIVEQHREILDTKCQ
II:   ---------L------T----S-
III:  ---------L------T----S-
IV:   ---------L-M-----GNK-S-
```

Fig. 2. Comparison of the predicted amino acid sequence of mouse 3βHSD I, II, III and IV. Asterisks indicate the region of 3βHSD II that has not been isolated. Identical amino acids are indicated by (-).

order of their isolation. 3βHSD I was isolated from a mouse Leydig cell cDNA library, 3βHSD II and III were isolated from mouse liver libraries and 3βHSD IV was isolated from a mouse kidney library. These four forms exhibit a high degree of sequence identity, with 3βHSD IV being more distantly related to I, II and III than these three forms are to each other. The predicted amino acid sequences of the four isoforms are shown in Fig. 2. As can be seen in Fig. 2, differences in amino acids are scattered throughout the sequences indicating that the different isoforms are products of distinct genes rather than alternative spliced products of a single gene.

Tissue-specific expression of each of the mouse 3βHSD isoforms was analyzed by RNAse protection analysis [19, 20]. 3βHSD I is expressed exclusively in the classical steroidogenic tissues, the gonads and adrenal glands of the adult mouse; 3βHSD II is expressed in the kidney and to a much lesser extent in the liver; 3βHSD III is expressed mostly in the liver and only to a very low extent in the kidney [20]; 3βHSD IV is only expressed in the kidneys of both

Table 1. Characteristics of mouse 3βHSD isoforms

Form	AA Homology to 3βHSD I (%)	Substrates	Preferred cofactor	Substrate K_m (μM)	Tissue-specific expression
I	100	Pregnenolone	NAD^+	0.076	Gonads and
		Dehydroepiandrosterone	NAD^+	0.14	adrenal glands
		Androstanediol	NAD^+	0.16	
		DHT	NADH	5.5	
II	85	—	—	—	Kidney > liver
III	83	Pregnenolone	NAD^+	1.03	Liver > kidney
		Dehydroepiandrosterone	NAD^+	0.49	
		Androstanediol	NAD^+	0.46	
		DHT	NADH	6.8	
IV	77	DHT	NADPH	2.2	Kidney

Clarke *et al.* [20].

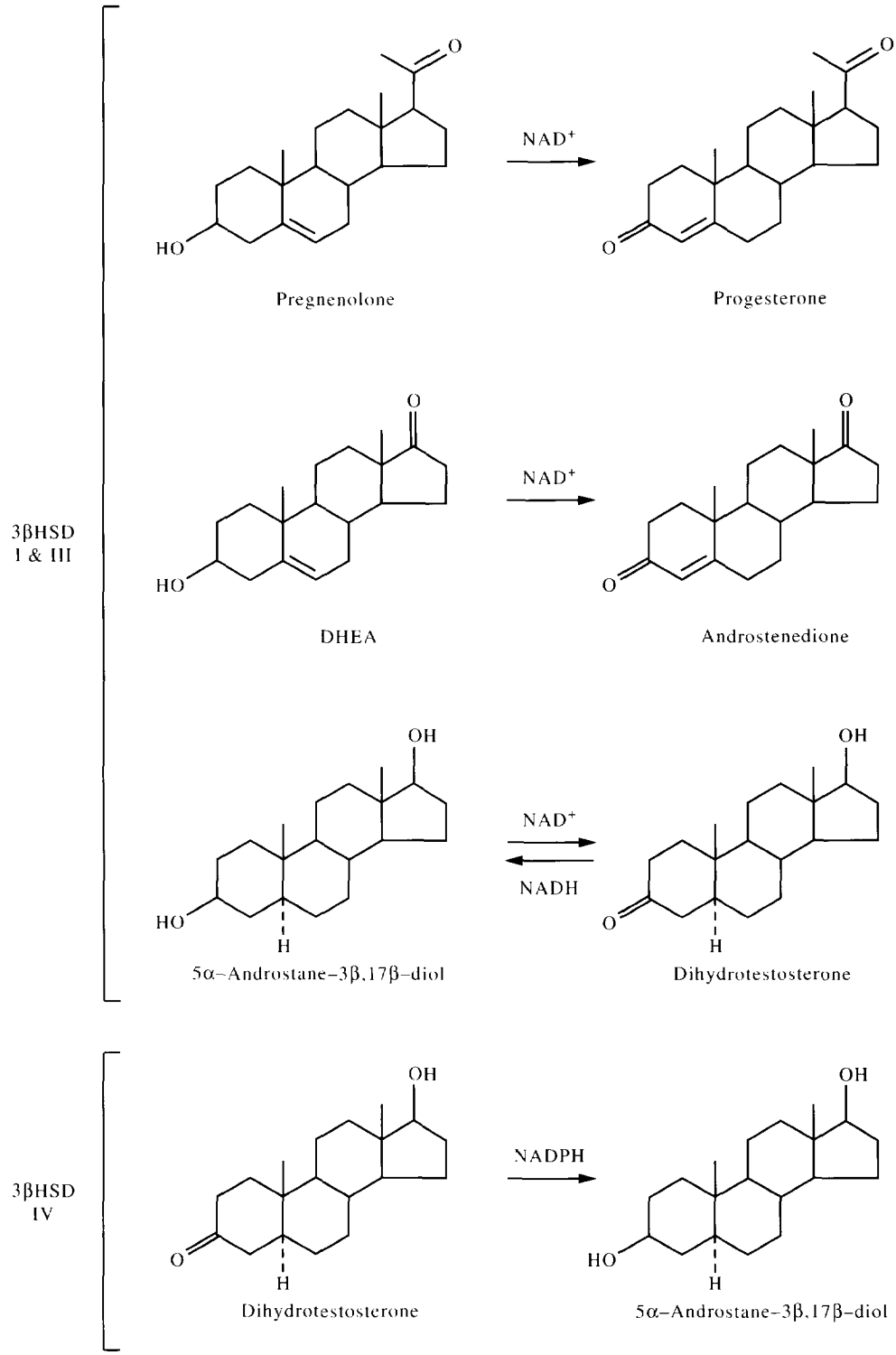

Fig. 3. Enzymatic reactions catalyzed by mouse 3βHSD isoforms. DHEA, dehydroepiandrosterone.

male and female mice [20]. *In situ* hybridization analyses of adult mouse kidney sections with a specific ^{35}S-labeled RNA antisense probe complementary to 3βHSD IV mRNA detected expression only in the cortex with the highest expression in the proximal convoluted tubules in the inner part of the kidney cortex and lower expression in the distal tubules [20]. Expression of 3βHSD IV was not detected in the medulla of the kidney.

CHARACTERIZATION OF THE PROTEINS ENCODED BY THE 3βHSD cDNAs

To characterize the proteins encoded by 3βHSD I, III and IV cDNAs, COS-1 cells were transiently transfected with pCMV5 expression vectors containing the complete coding sequence of the different cDNAs. The complete coding sequence for 3βHSD II has not been isolated. The enzymatic characteristics of the

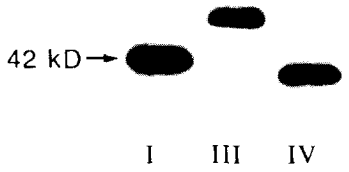

Fig. 4. Immunoblot analysis of 3βHSD proteins in COS-1 transfected cells. Lysates of COS-1 transfected cells were subjected to polyacrylamide gel electrophoresis and Western-blot analysis with the immunoglobulin G fraction of a rabbit antiserum raised against the human placental 3βHSD [19]. I, pCMV5-3βHSD I-transfected cells; III, pCMV5-3βHSD III-transfected cells; IV, pCMV5 IV-transfected cells.

expressed proteins was examined by assaying homogenates of COS-1 cells that had been transfected with one of the 3βHSD cDNAs. Cell homogenates were incubated with the ^{3}H-labeled 5-ene-3β-hydroxysteroids, pregnenolone or dehydroepiandrosterone (DHEA) or the 5α-reduced steroids, 5α-androstane-3β, 17β-diol (Adiol) or dihydrotestosterone (DHT). NAD^+ or $NADP^+$ was used as a cofactor with pregnenolone, DHEA and Adiol; NADH or NADPH was used as a cofactor with DHT.

It was shown that both 3βHSD I and III have the capacity to convert 5-ene-3β-hydroxysteroids to 4-ene-3-ketosteroids as well as to dehydrogenate the 3β-hydroxyl group of Adiol to yield DHT. In all cases NAD^+ was the preferred cofactor (Table 1 and Fig. 3). The K_m values of 3βHSD I for these substrates were below 0.2 μM. K_m values of 3βHSD III were higher for all three substrates with the greatest difference (>10×) observed for pregnenolone (Table 1). Both 3βHSD I and 3βHSD III can catalyze the reduction of DHT to Adiol in the presence of the cofactor NADH, but with considerably higher K_m values. These data indicate that 3βHSD I and III function predominantly as 5-ene-3β-hydroxysteroid dehydrogenase/isomerase rather than as 3-ketosteroid reductase. In contrast, 3βHSD IV cannot convert 5-ene-3β-hydroxysteroids to 4-ene-3-ketosteroids nor does it have the capacity to convert Adiol to DHT in the presence of either NAD^+ or $NADP^+$. 3βHSD IV functions as a 3-ketosteroid reductase catalyzing the reduction of DHT to Adiol, and requires NADPH rather than NADH as the cofactor (Table 1 and Fig. 3). Thus, although, the predicted amino acid sequence of 3βHSD IV is 77 and 73% identical to 3βHSD I and III, these three isoforms of mouse 3βHSD fall into two categories of functionally distinct enzymes. Four distinct 3βHSD isoforms have been described in the rat [14, 22, 23]. Three of these, rat 3βHSD I, II and IV, function as 5-ene-3β-hydroxysteroid dehydrogenase/isomerases while rat 3βHSD III functions as a 3-ketosteroid reductase [23]. Rat 3βHSD III is expressed only in the male liver, while mouse 3βHSD IV, a 3-ketosteroid reductase, is expressed only in kidneys of both sexes. Preliminary studies from our laboratory have identified a new mouse isoform, 3βHSD V, which is expressed only in livers of adult male mice (Abbaszade and Payne, unpublished data). The amino acid sequence of this new mouse isoform shows a high degree of identity (93%) to mouse 3βHSD IV and it can be predicted from the amino acid sequence that 3βHSD V will function as a 3-ketosteroid reductase. Mouse 3βHSD V appears to be the homolog of rat 3βHSD III.

In addition to their difference in function, the expressed proteins representing 3βHSD I, III and IV have distinct mobilities as determined by Western-blot analysis (Fig. 4). Although the amino acid sequences predict proteins of the same molecular weight, 42 kDa, the mobility of the proteins indicate an apparent M_r of 44 kDa for 3βHSD III, 42 kDa for 3βHSD I and 41 kDa for 3βHSD IV, similar to the respective proteins expressed in the liver [21], testis and adrenal

Table 2. Percent amino acid sequence identities among all characterized forms of 3βHSD

	Mouse			Rat				Bovine	Macaque	Human	
	II*	III	IV	I	II	III	IV	I	I	I	II
Mouse I	85	83	77	88	86	77	86	73	72	72	71
Mouse II	—	91	75	84	84	78	88	75	73	72	72
Mouse III	—	—	73	82	82	75	89	74	71	71	70
Mouse IV	—	—	—	78	77	83	77	67	68	68	68
Rat I	—	—	—	—	94	80	91	74	73	72	72
Rat II	—	—	—	—	—	80	88	74	72	72	71
Rat III	—	—	—	—	—	—	79	69	68	68	67
Rat IV	—	—	—	—	—	—	—	76	75	73	73
Bovine I	—	—	—	—	—	—	—	—	79	79	78
Macaque I	—	—	—	—	—	—	—	—	—	94	96
Human I	—	—	—	—	—	—	—	—	—	—	94

Modified from Clarke *et al.* [20].

*As mouse II is a partial cDNA, missing amino acids 1–109, comparisons with mouse II include only amino acids 110–373. For comparison of all other forms, amino acids 1–373 were included.

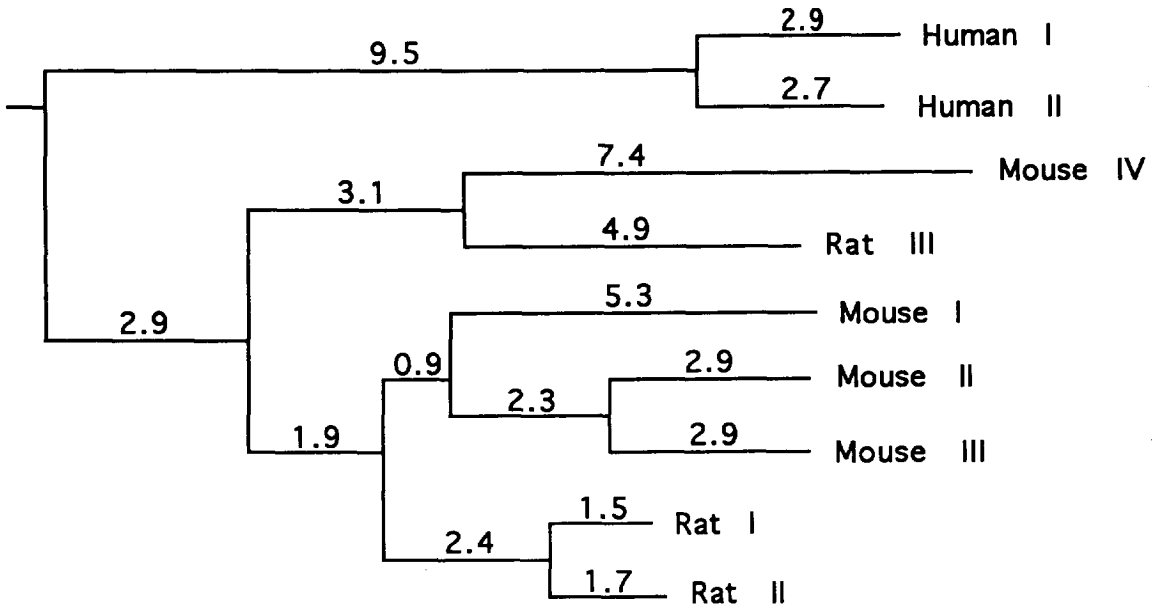

Fig. 5. Proposed evolutionary tree of rodent and human 3βHSDs. The tree was constructed with the neighbor-joining method using the nucleotide sequence from the coding region between nucleotide 328 (counting from the A of the ATG as 1) and 1122. This portion of the coding region was used because mouse II is a partial cDNA that does not contain the 5′-portion of the coding region. The numbers on the branches are the uncorrected percentages of 795 sites substituted. This evolutionary tree was constructed prior to the isolation of rat IV [Clarke *et al.* Ref. 20].

glands [20, 21] and kidney, respectively [20]. The differences in the apparent molecular weights are probably not due to differences in posttranslational modification, but more likely reflect differences in the amino acid composition of the three proteins [21].

DISCUSSION

To date, 3βHSD cDNAs have been isolated from five species. The isoforms of each species have been numbered in the chronological order of their isolation [11–13, 19, 24–26]; the numbers do not imply a correlation of function or tissue of expression. With the exception of mouse 3βHSD IV (and probably 3βHSD V) and rat 3βHSD III, all other cDNAs whose proteins have been examined encode proteins with 3β-hydroxysteroid dehydrogenase and 5-en→4-en-isomerase activities [3, 11, 13, 14, 21, 25–28]. Different forms within a given species exhibit different K_m values for the different 3β-hydroxysteroids and different patterns of tissue-specific expression. Amino acid sequence identities among different 3βHSD isoforms are shown in Table 2. In accord with their similar enzymatic activities, mouse IV and rat III isoforms show a greater degree of amino acid homology to each other than they do to other isoforms (Table 2). Thus, the degree of amino acid homology between different 3βHSD isoforms correlates more closely to enzymatic function than to tissue-specific expression.

From a comparison of the nucleotide sequences of the coding regions of the mouse, rat, and human isoforms, a predicted evolutionary tree of the rodent and human 3βHSDs has been constructed using the neighbor-joining method [20] (Fig. 5). The analysis suggests that there were at least two separate 3βHSD genes in existence before the mouse and rat evolved into separate species. The most ancient form of rodent 3βHSD is predicted to have had an enzyme activity similar to that of the modern rodent gonadal and adrenal isoforms (mouse I and rat I, II and IV), because these activities are essential for reproduction and the maintenance of life in mammals. The differentiation of mouse IV and rat III from this ancestral 3βHSD isoform antedates the speciation of mouse and rat. It appears that the origin of the form from which mouse II and III arise occurred after the origin of the form that became mouse IV and rat III and possibly before the differentiation of the mouse and rat. Mouse forms II and III are derived from the classical ancestral form of 3βHSD, rather than from the one that became mouse IV and rat III.

The physiological significance of the different forms of 3βHSD remains to be determined. The peripheral 5-ene-3β-hydroxysteroid dehydrogenase/isomerases, mouse 3βHSD III or human 3βHSD I, could play an important role in steroid hormone biosynthesis in cases of a genetic deficiency in the expression of the gonadal and adrenal isoform (human 3βHSD II [29] and mouse 3βHSD I [19]). In cases of gonadal and adrenal deficiencies, high amounts of 5-ene-3β-hydroxysteroids are secreted by the adrenal glands and the gonads. These 5-ene-3β-hydroxysteroids could then be

converted to 4-ene-3-ketosteroids by the peripheral 5-ene-3β-hydroxysteroid dehydrogenase/isomerase and further metabolized to active steroid hormones either by the adrenal glands and gonads and/or by peripherally expressed 17β-hydroxysteroid dehydrogenase and aromatase. The mouse can serve as an excellent model for studies to test the importance of the peripheral 5-ene-3β-hydroxysteroid dehydrogenase/isomerase by targeted mutation of 3βHSD I in mouse embryonic stem cells via homologous recombination. In the resulting mouse lines which will lack functional 3βHSD I it will be of interest to determine whether steroid hormone production is accomplished by other isoforms.

The 3-ketosteroid reductases may function in the inactivation of active steroid hormones such as DHT by conversion to 5α-androstanediol thus playing a role in regulating androgen action in androgen sensitive tissues. The physiological importance of the 3βHSD isoforms that function as 3-ketosteroid reductases remains to be established.

Acknowledgements—Supported by NIH Grant HD-17916. T. R. Clarke and P. A. Bain were supported in part by NIH Training Grant HD-07048. The authors are especially grateful for the continued interest and advice of Dr Miriam H. Meisler in the genetic studies of the 3βHSD multigene family. The authors thank C-H. Jason Park for the Western-blot analysis and assisting with the illustrations and Rita Lemorie for typing the manuscript.

REFERENCES

1. Milewich L., Shaw C. B. and Sontheimer R. D.: Steroid metabolism by epidermal keratinocytes. *Ann. N.Y. Acad. Sci.* **548** (1988) 66–89.
2. Devine P. L., Kelly N. S. and Adams J. B.: 3β-Hydroxysteroid isomerase dehydrogenase in guinea-pig kidney: possible involvement in 11-deoxycorticosterone formation *in situ*. *J. Steroid Biochem.* **25** (1986) 265–270.
3. Weidenfeld J., Siegel R. A. and Chowers I.: *In vitro* conversion of pregnenolone to progesterone by discrete brain areas of the male rat. *J. Steroid Biochem.* **13** (1980) 961–963.
4. Jung-Testas I., Hu Z. T., Baulieu E. E. and Robel P.: Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* **125** (1989) 2083–2091.
5. Bauer H. C. and Bauer H.: Micromethod for the determination of 3-β-HSD activity in cultured cells. *J. Steroid Biochem.* **33** (1989) 643–646.
6. Bongiovanni A. M.: Unusual steroid pattern in congenital adrenal hyperplasia: deficiency of 3β-hydroxy dehydrogenase. *J. Clin. Endocr. Metab.* **21** (1961) 860–862.
7. Bongiovanni A. M.: The adrenogenital syndrome with deficiency of 3β-hydroxysteroid dehydrogenase. *Clin. Invest.* **41** (1962) 2086–2092.
8. Parks G. A., Bermudez J. A., Anast C. S., Bongiovanni A. M. and New M. I.: Pubertal boy with the 3β-hydroxysteroid dehydrogenase defect. *J. Clin. Endocr. Metab.* **33** (1971) 269–278.
9. Rosenfield R. I., Rich B. H., Wolfsdorf J., Cassorla F. S., Parks J. S., Bongiovanni A. M., Wu C. H. and Shackelton C. H. L.: Pubertal presentation of congenital Δ^5-3β-hydroxysteroid dehydrogenase deficiency. *J. Clin. Endocr. Metab.* **51** (1980) 345–353.
10. Pang S., Levine L. S., Stoner E., Opitz J. M., Pollack M. S., Dupont B. and New M. I.: Nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency with normal glomerulosa function. *J. Clin. Endocr. Metab.* **56** (1983) 808–818.
11. Rhéaume E., Lachance Y., Zhao H-F., Brenton N., Dumont M., de Launoit Y., Trudel C., Luu-The V., Simard J. and Labrie F.: Structure and expression of a new complementary DNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in human adrenals and gonads. *Molec. Endocr.* **5** (1991) 1147–1157.
12. Luu-The V., Lachance Y., Labrie C., Leblanc G., Thomas J. L., Strickler R. C. and Labrie F.: Full length cDNA structure and deduced amino acid sequence of human 3β-hydroxy-5-ene steroid dehydrogenase. *Molec. Endocr.* **3** (1989) 1310–1312.
13. Lorence M. C., Murry B. A., Trant J. M. and Mason J. I.: Human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase from placenta: expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C_{21} and C_{19} steroids. *Endocrinology* **126** (1990) 2493–2498.
14. Simard J., Couet J., Durocher F., Labrie Y., Sanchez R., Breton N., Turgeon C. and Labrie F.: Structure and tissue-specific expression of a novel member of the rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) family. *J. Biol. Chem.* **268** (1993) 19,659–19,668.
15. Bain P. A., Meisler M. H., Taylor B. A. and Payne A. H.: The genes encoding gonadal and nongonadal forms of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase are closely linked on mouse chromosome 3. *Genomics* **16** (1993) 219–223.
16. Bérubé D., Luu-The V., Lachance Y., Gagné R. and Labrie F.: Assignment of the human 3β-hydroxysteroid dehydrogenase gene (HSDB3) to the p13 band of chromosome 1. *Cytogenet. Cell Genet.* **52** (1989) 199–200.
17. Morrison N., Nickson D. A., McBride M. W., Mueller U. W., Boyd E. and Sutcliffe R. G.: Regional chromosomal assignment of human 3-beta-hydroxy-5-ene steroid dehydrogenase to 1p13.1 by non-isotopic *in situ* hybridization. *Hum. Genet.* **87** (1991) 223–225.
18. Russell A. J., Wallace A. M., Forest M. G., Donaldson M. D. C., Edwards C. R. W. and Sutcliffe R. G.: Mutation in the human gene for 3β-hydroxysteroid dehydrogenase type II leading to male pseudohermaphroditism without salt loss. *J. Molec. Endocr.* **12** (1994) 225–237.
19. Bain P. A., Yoo M., Clarke T., Hammond S. H. and Payne A. H.: Multiple forms of mouse 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase and differential expression in gonads, adrenal glands, liver and kidneys of both sexes. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 8870–8874.
20. Clarke T. R., Bain P. A., Greco T. L. and Payne A. H.: A novel mouse kidney 3β-hydroxysteroid dehydrogenase complementary DNA encodes a 3-ketosteroid reductase instead of a 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase. *Molec. Endocr.* **7** (1993) 1569–1578.
21. Clarke T. R., Bain P. A., Sha L. and Payne A. H.: Enzyme characteristics of two distinct forms of mouse 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase complementary deoxyribonucleic acids expressed in COS-1 cells. *Endocrinology* **132** (1993) 1971–1976.
22. Zhao H-F., Labrie C., Simard J., de Launoit Y., Trudel C., Martel C., Rhéaume E., Dupont E., Luu-The V., Pelletier G. and Labrie F.: Characterization of rat 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. *J. Biol. Chem.* **266** (1991) 583–593.
23. de Launoit Y., Zhao H-F., Belanger A., Labrie F. and Simard J.: Expression of liver-specific member of the 3β-hydrogenase family, an isoform possessing an almost exclusive 3-ketosteroid reductase activity. *J. Biol. Chem.* **267** (1992) 4513–4517.
24. Zhao H-F., Rhéaume E., Trudel C., Couët J., Labrie F. and Simard J.: Structure and dimorphic expression of a liver-specific rat 3β-hydroxysteroid dehydrogenase/isomerase. *Endocrinology.* **127** (1990) 3237–3239.
25. Zhao H-F., Simard J., Labrie C., Breton N., Rhéaume E., Luu-The V. and Labrie F.: Molecular cloning, cDNA structure and pre dicted amino acid sequence of bovine 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase. *FEBS Lett.* **259** (1989) 153–157.
26. Simard J., Melner M. H., Breton N., Low K. G., Zhao H-F., Periman L. M. and Labrie F.: Characterization of macaque 3β-hydroxy-5-ene steroid dehydrogenase/Δ^5-Δ^4 isomerase: structure and expression in steroidogenic and peripheral tissues in primate. *Molec. Cell. Endocr.* **75** (1991) 101–110.
27. Lachance Y., Luu-The V., Labrie C., Simard C., Dumont M., de Launoit Y., Guérin S., Leblanc G. and Labrie F.:

Characterization of human 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene and its expression in mammalian cells. *J. Biol. Chem.* **265** (1990) 20,469–20,475.

28. Simard C., de Launoit Y. and Labrie F.: Characterization of the structure-activity relationships of rat types I and II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase by site-directed mutagenesis and expression in HeLa cells. *J. Biol. Chem.* **256** (1991) 14,842–14,845.
29. Rhéaume E., Simard J., Morel Y., Mebarki F., Zachmann M., Forest M. G., New M. I. and Labrie F.: Congenital adrenal hyperplasia due to point mutations in the type II 3β-hydroxysteroid dehydrogenase gene. *Nature Genet.* **1** (1992) 239–245.