Pergamon

0960-0760(95)00204-9

J. Steroid Biochem. Molec. Biol. Vol. 55, No. 5/6, pp. 541–548, 1995

0960-0760/95 $9.50 + 0.00

Molecular Characterization of Mouse 17β-Hydroxysteroid Dehydrogenase IV

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17β-hydroxysteroid dehydrogenases (17β-HSD) catalyze the conversion of estrogens and androgens at the C17 position. The 17β-HSD type I, II, III and IV share less than 25% amino acid similarity. The human and porcine 17β-HSD IV reveal a three-domain structure unknown among other dehydrogenases. The N-terminal domains resemble the short chain alcohol dehydrogenase family while the central parts are related to the C-terminal parts of enzymes involved in peroxisomal β-oxidation of fatty acids and the C-terminal domains are similar to sterol carrier protein 2. We describe the cloning of the mouse 17β-HSD IV cDNA and the expression of its mRNA. A probe derived from the human 17β-HSD IV was used to isolate a 2.5 kb mouse cDNA encoding for a protein of 735 amino acids showing 85 and 81% similarity with human and porcine 17β-HSD IV, respectively. The calculated molecular mass of the mouse enzyme amounts to 79,524 Da. The mRNA for 17β-HSD IV is a single species of about 3 kb, present in a multitude of tissues and expressed at high levels in liver and kidney, and at low levels in brain and spleen. The cloning and molecular characterization of murine, human and porcine 17β-HSD IV adds to the complexity of steroid synthesis and metabolism. The multitude of enzymes acting at C17 might be necessary for a precise control of hormone levels.

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INTRODUCTION

The control of growth, differentiation and function of cells by estrogens and androgens is modulated by 17β-hydroxysteroid dehydrogenases (17β-HSD)[1, 2]. Besides the purified and cloned soluble, placental 17β-HSD I consisting of 327 amino acids (aa) a further placental microsomal enzyme 17β-HSD II of 387 aa was cloned [3–8]. The 17β-HSD I is an oxidoreductase with equal affinities for estradiol and estrone [6, 7], whereas 17β-HSD II slightly prefers oxidation of both estrogens and androgens over reduction [8]. 17β-HSD III of 310 aa reducing androgens and estrogens is expressed exclusively in the human testis [9].

Recently, we have purified and cloned a fourth 17β-HSD (17β-HSD IV) in the porcine [10, 11] and the human species [12]. Their respective cDNAs code for proteins of 737 and 736 aa with a calculated molecular mass of about 80 kDa. The enzyme catalyzes NAD^+-dependent oxidation of 17β-estradiol and Δ5-androstene-3β,17β-diol. The respective reverse reactions are about 400-fold less effective [10–12]. After amino-terminal cleavage of the 80 kDa protein a 32 kDa estradiol dehydrogenase is released. It is similar to members of the short chain alcohol dehydrogenase family [12–14]. The amino acid sequence of 17β-HSD IV is markedly different from that of the previously cloned 17β-hydroxysteroid dehydrogenases since it is less than 25% identical with 17β-HSD I, II and III. The first 300 amino-terminal aa of human and porcine 17β-HSD IV are similar to several members of the short chain alcohol dehydrogenase family, such as the N-terminal domains of FOX2 protein from *Saccaromyces cerevisiae* (54% identity) and the multifunctional enzyme of *Candida tropicalis* (50% identity) [12, 13, 15, 16]. The region corresponding to aa 343–607 of the human 17β-HSD IV shares high similarity with the trifunctional C-terminal part of *C. tropicalis* enzyme and the FOX2 protein [12, 13, 15, 16]. The

Proceedings of the Workshop on the Molecular and Cell Biology of Hydroxysteroid Dehydrogenases, Hannover, Germany, 19–22 April 1995.

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carboxy-terminal part of the 17β-HSD IV (aa 595–736) is 39% identical to the human sterol carrier protein 2 (SCP2) which has been reported to participate in the intracellular transport of cholesterol and lipids [12, 13, 17].

A better understanding of the regulation and function of the type IV dehydrogenases would be facilitated by identifying the enzyme in a well-defined animal model such as the mouse. The present work describes the cloning and mRNA expression of the mouse 17β-HSD IV of 735 aa with a predicted molecular mass of 79,524 Da.

MATERIALS AND METHODS

Isolation, subcloning and sequencing of mouse 17β-HSD IV cDNA clones

Molecular cloning of the mouse 17β-HSD IV cDNA was performed with a probe of 2.6 kb corresponding to the partially EcoRI digested full-length Li-17β IV fragment stretching from nucleotide −14 to nucleotide 2548 of human 17β-HSD IV cDNA and with a probe of 352 bp corresponding to the EcoRI digested P1-17β IV fragment stretching from nucleotide −48 to 303 of human 17β-HSD IV cDNA [12]. Approximately 10^6 recombinant phages from an oligo(dT)- + random-primed mouse adult kidney and a random-primed mouse adult skeletal muscle λgt11 cDNA expression libraries were screened with 1×10^6 cpm/ml ^{32}P-labeled probe. The filters were washed in 0.5 × SSC–0.1% SDS at 60°C for 30 min and then autoradiographed 12 h at −80°C. After two purification steps, phages were isolated as described [12] and the inserts were excised by EcoRI digestion. The purified cDNA inserts were subcloned into the polylinker site of pBluescript SK vector (Stratagene, La Jolla, CA). Synthetic oligonucleotides, as well as T7 or T3 vector primers, and modified T7 DNA polymerase were used to sequence both strands of double-stranded plasmid DNA with the dideoxy chain termination method with [^{35}S]ATP (USB, Cleveland, OH). Sequences were confirmed using an Applied Biosystems 370A automatic system with fluorescent dye-labeled cDNA sequence-specific primers and a Taq dye-primer sequencing kit (Applied Biosystem, Foster City, CA).

RNA analysis

A commercial mouse poly(A$^+$) RNA blot was obtained from Clontech (Palo Alto, CA). The membrane was prehybridized in a solution containing 250 μg/ml of denatured salmon sperm DNA, 50% formamide, 5 × Denhardt's, 0.1% SDS, 5 × SSC and 50 mM Na_2HPO_4, for 2 h at 42°C, and hybridization was carried out in the same solution containing 5% dextran sulfate and the ^{32}P-labeled probe for 16 h at 42°C. The [α-^{32}P] EcoRI fragment corresponding to nucleotides 302–1753 of mouse 17β-HSD IV was used as probe. The membrane was washed sequentially in 2 × SSC containing 0.1% SDS at 55°C for 1 h, in 0.5 × SSC–0.1% SDS at 60°C for 30 min and in 0.1 × SSC–0.1% SDS at 65°C for 30 min. Thereafter, the membrane was exposed to X-ray films with an intensifying screen at −80°C for 2 days. Relative 17β-HSD IV signals were quantified using a phosphoimager (Molecular Dynamics).

Western blotting

Kidney cortex was homogenized and a particulate fraction (sedimenting between 2000 g_{av} and 240,000 g_{av} for 2 h) prepared as described [10]. Proteins were separated by SDS–PAGE (10% gel with 20 μg of protein per lane), followed by a transfer to nitrocellulose membranes and incubation with mouse monoclonal antibody F1-peroxidase. The mab F1 recognizes 80 and 32 kDa forms of the porcine 17β-hydroxysteroid dehydrogenase [10].

Immunocytochemistry

Mouse tissues were fixed and processed for paraffin embedding as described [18]. Rehydrated 3 μm sections were incubated with a F1 antibody conjugated with peroxidase and the color developed with diaminobenzidine/H_2O_2 [18].

RESULTS

Isolation of mouse 17β-HSD type IV cDNA

A fragment of human 17β-HSD cDNA [12] corresponding to nucleotides −14 to 2548 was chosen for screening mouse cDNA libraries. Using the ^{32}P-labeled probe, five cDNAs were isolated from adult mouse kidney using 10^6 λgt11 recombinants. The cDNAs were purified and size-characterized. After EcoRI digestion, one clone (mK17β IV) containing two fragments of 0.7 and 1.6 kb was obtained. The fragments were subcloned into pBluescript SK vectors, amplified and the respective 5′ and 3′ regions sequenced. Sequencing revealed that the 1.6 kb fragment contained the stop codon similar to porcine and human 17β-HSD IV [11, 12]. However, the 5′-upstream sequence of the 0.7 kb fragment stopped at a position corresponding to nucleotide 191 of human 17β-HSD IV cDNA [12]. Using the probe of 352 bp corresponding to the EcoRI digested P1-17β IV fragment stretching from nucleotide −48 to 303 of human 17β-HSD IV cDNA [12], one clone (mSM17β IV) was isolated from an adult mouse skeletal muscle λgt11 library. The corresponding subcloned EcoRI fragment contained the first in-frame ATG codon similar to human and porcine 17β-HSD IV and contained 14 nucleotides upstream from the ATG (Fig. 1). The 3′-untranslated region of mK17β IV clone is 248 bp long. One classical polyadenylation consensus AATAAA site [19] was detected 225 nucleotides downstream from the stop codon. However, Northern blot analysis indicates that this clone is truncated in its 5′- or 3′-untranslated

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                                                                                                                caggctgagctc   -1

ATG GCT TCC CCC CTG AGG TTC GAC GGG CGT GTG GTC TTG GTC ACC GGC CCC GGG GGA GGA TTG GGC CGA GCT TAC GCC CTG GCG    84
 M   A   S   P   L   R   F   D   G   R   V   V   L   V   T   G   P   G   G   G   L   G   R   A   Y   A   L   A    28

TTT GCA GAA AGA GGA GCA TTA GTC ATT GTG AAC GAC TTA GGA GGG GAC TTC AAG GGA ATT GGT AAA GGC TCC TCT GCT GCA GAC   168
 F   A   E   R   G   A   L   V   I   V   N   D   L   G   G   D   F   K   G   I   G   K   G   S   S   A   A   D    56

AAG GTT GTG GCA GAG ATA AGA AGG AAA GGC GGA AAA GCA GTG GCC AAT TAC GAT TCA GTT GAA GCA GGC GAG AAG CTT GTG AAG   252
 K   V   V   A   E   I   R   R   K   G   G   K   A   V   A   N   Y   D   S   V   E   A   G   E   K   L   V   K    84

ACG GCA CTG GAC ACA TTT GGC AGA ATA GAC GTT GTG GTC AAC AAT GCT GGA ATC CTG AGG GAC CGT TCC TTC TCC AGG ATA AGT   336
 T   A   L   D   T   F   G   R   I   D   V   V   V   N   N   A   G   I   L   R   D   R   S   F   S   R   I   S   112

GAT GAA GAC TGG GAT ATA ATT CAT AGA GTT CAT TTG CGG GGC TCC TTC CAA GTG ACC CGG GCA GCA TGG GAC CAT ATG AAG AAA   420
 D   E   D   W   D   I   I   H   R   V   H   L   R   G   S   F   Q   V   T   R   A   A   W   D   H   M   K   K   140

CAG AAT TAT GGA AGA ATC CTT ATG ACT TCC TCA GCT TCT GGA ATA TAT GGC AAC TTT GGC CAG GCG AAT TAT AGT GCT GCA AAG   504
 Q   N   Y   G   R   I   L   M   T   S   S   A   S   G   I   Y   G   N   F   G   Q   A   N   Y   S   A   A   K   168

CTG GGC ATT CTG GGT CTC TGC AAT ACT CTC GCC ATT GAA GGC AGG AAG AAC AAC ATT CAT TGC AAC ACC ATT GCC CCC AAC GCT   588
 L   G   I   L   G   L   C   N   T   L   A   I   E   G   R   K   N   N   I   H   C   N   T   I   A   P   N   A   196

GGG TCA CGG ATG ACG GAG ACT GTG TTG CCG GAA GAT CTT GTT GAA GCC CTG AAG CCA GAG TAT GTG GCC CCT CTG GTG CTT TGG   672
 G   S   R   M   T   E   T   V   L   P   E   D   L   V   E   A   L   K   P   E   Y   V   A   P   L   V   L   W   224

CTT TGC CAT GAG AGC TGT GAG GAA AAT GGT GGC CTA TTT GAG GTT GGA GCA GGA TGG ATT GGA AAA TTG CGC TGG GAG AGG ACC   756
 L   C   H   E   S   C   E   E   N   G   G   L   F   E   V   G   A   G   W   I   G   K   L   R   W   E   R   T   252

CTG GGC GCC ATC GTC AGA AAG CGG AAT CAG CCC ATG ACT CCC GAG GCA GTG AGG GAC AAC TGG GAG AAG ATC TGT GAC TTC AGC   840
 L   G   A   I   V   R   K   R   N   Q   P   M   T   P   E   A   V   R   D   N   W   E   K   I   C   D   F   S   280

AAT GCC AGC AAG CCG CAG ACC ATT CAA GAA TCA ACA GGT GGT ATA GTC GAA GTT TTA CAT AAG GTA GAT TCA GAA GGA ATC TCA   924
 N   A   S   K   P   Q   T   I   Q   E   S   T   G   G   I   V   E   V   L   H   K   V   D   S   E   G   I   S   308

CCA AAC CGT ACC AGT CAC GCG GCA CCT GCA GCC ACG TCA GGA TTC GTT GGT GCT GTT GGC CAT AAA CTT CCT TCA TTT TCT TCT  1008
 P   N   R   T   S   H   A   A   P   A   A   T   S   G   F   V   G   A   V   G   H   K   L   P   S   F   S   S   336

TCG TAT ACG GAG CTG CAG AGT ATT ATG TAT GCC CTC GGA GTG GGA GCG TCA GTC AAA AAT CCA AAG GAT TTG AAG TTT GTT TAT  1092
 S   Y   T   E   L   Q   S   I   M   Y   A   L   G   V   G   A   S   V   K   N   P   K   D   L   K   F   V   Y   364

GAA GGC AGT GCT GAC TTC TCC TGT TTG CCC ACC TTC GGA GTC ATT GTC GCT CAG AAG TCC ATG ATG AAT GGA GGG CTG GCA GAG  1176
 E   G   S   A   D   F   S   C   L   P   T   F   G   V   I   V   A   Q   K   S   M   M   N   G   G   L   A   E   392

GTT CCT GGG CTG TCA TTC AAC TTT GCA AAG GCT CTT CAC GGG GAG CAG TAC TTG GAG CTG TAT AAG CCA CTT CTT CGA TCA GGA  1260
 V   P   G   L   S   F   N   F   A   K   A   L   H   G   E   Q   Y   L   E   L   Y   K   P   L   L   R   S   G   420

GAA TTA AAA TGT GAA GCA GTT ATT GCT GAC ATC CTG GAT AAA GGC TCT GGC GTA GTG ATT GTT ATG GAC GTC TAT TCT TAT TCT  1344
 E   L   K   C   E   A   V   I   A   D   I   L   D   K   G   S   G   V   V   I   V   M   D   V   Y   S   Y   S   448

GGG AAG GAA CTT ATA TGC TAT AAT CAG TTC TCT GTC TTT GTT GTT GGC TCT GGG GGC TTT GGT GGA AAA CGG ACA TCA GAA AAA  1428
 G   K   E   L   I   C   Y   N   Q   F   S   V   F   V   V   G   S   G   G   F   G   G   K   R   T   S   E   K   476

CTC AAA GCA GCT GTA GCT GTA CCA AAT CGA CCT CCA GAT GCT GTA CTG AGA GAT GCC ACC TCA CTG AAT CAG GCC GCG CTG TAC  1512
 L   K   A   A   V   A   V   P   N   R   P   P   D   A   V   L   R   D   A   T   S   L   N   Q   A   A   L   Y   504

CGC CTC AGC GGA GAC TGG AAT CCT CTA CAC ATT GAC CCG GAC TTT GCG AGC GTT GCC GGT TTT GAG AAG CCC ATA TTA CAT GGA  1596
 R   L   S   G   D   W   N   P   L   H   I   D   P   D   F   A   S   V   A   G   F   E   K   P   I   L   H   G   532

CTA TGT ACC TTT GGA TTT TCT GCA AGG CAT GTT TTA CAG CAG TTT GCA GAT AAT GAT GTA TCA AGA TTC AAG GCG ATT AAG GTT  1680
 L   C   T   F   G   F   S   A   R   H   V   L   Q   Q   F   A   D   N   D   V   S   R   F   K   A   I   K   V   560

CGT TTT GCC AAA CCA GTG TAT CCA GGA CAG ACT CTA CAA ACT GAG ATG TGG AAG GAA GGA AAC AGA ATT CAT TTT CAA ACC AAG  1764
 R   F   A   K   P   V   Y   P   G   Q   T   L   Q   T   E   M   W   K   E   G   N   R   I   H   F   Q   T   K   588

GTC CAC GAG ACT GGA GAT GTT GTC ATT TCA AAT GCG TAC GTG GAT CTC GTG CCT GCA TCT GGA GTT TCA ACC CAG ACA CCT TCA  1848
 V   H   E   T   G   D   V   V   I   S   N   A   Y   V   D   L   V   P   A   S   G   V   S   T   Q   T   P   S   616

GAG GGT GGA GAG CTC CAG AGT GCT CTT GTG TTT GGG GAG ATA GGC CGC CGC CTC AAG AGT GTT GGC CGT GAG GTG GTA AAG AAA  1932
 E   G   G   E   L   Q   S   A   L   V   F   G   E   I   G   R   R   L   K   S   V   G   R   E   V   V   K   K   644

GCG AAT GCT GTG TTT GAA TGG CAT ATC ACG AAA GGT GGG ACT GTT GCA GCC AAG TGG ACC ATT GAC CTG AAG AGC GGC TCA GGG  2016
 A   N   A   V   F   E   W   H   I   T   K   G   G   T   V   A   A   K   W   T   I   D   L   K   S   G   S   G   672

GAG GTG TAC CAA GGC CCC GCA AAG GGC TCT GCT GAT GTG ACC ATC ATC ATT TCC GAT GAG GAT TTT ATG GAA GTG GTC TTC GGC  2100
 E   V   Y   Q   G   P   A   K   G   S   A   D   V   T   I   I   I   S   D   E   D   F   M   E   V   V   F   G   700

AAG CTT GAC CCA CAG AAG GCC TTC TTC AGT GGC AGG CTG AAG GCC AGA GGG AAC ATC ATG CTG AGC CAG AAA CTA CAG ATG ATT  2184
 K   L   D   P   Q   K   A   F   F   S   G   R   L   K   A   R   G   N   I   M   L   S   Q   K   L   Q   M   I   728

CTT AAA GAC TAT GCC AAG CTC TGA  agggaacccactgtgtgctgttaaaggagtcaataattaaatactgtctacccagctgagccgcagccttctgcgatcc  2287
 L   K   D   Y   A   K   L   *                                                                                    735

acaggagtgtgcaggagaaatcgcttcacatttccagattcagataacttgcatattttcattttctactaatttttcacatatttttacaaggaactgtaatctaggtagc  2399
aaaataatcattctgttcatagatctgtatctt<u>aataaa</u>aaaaatcaaccaaaaacc                                                    2456
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Fig. 1. Nucleotide and predicted amino acid sequences of mouse 17β-HSD IV. Nucleotide and amino acid numbering are given at the right. The nucleotides corresponding to the open reading frame and the 5′- and 3′ untranslated regions are in capital and small letters, respectively. The putative polyadenylation consensus site is underlined.

region and that the corresponding full-length cDNA is about 3.0 kb.

Deduced amino acid sequence and similarity with other 17β-HSDs

The sequence of the first in-frame initiating codon of mouse 17β-HSD IV, GCTCATGG (Fig. 1), contains incomplete consensus sequence required for optimal initiation by eukaryotic ribosomes [20], similar to porcine and human 17β-HSD IV [11, 12]. The 13 nucleotides upstream from this first ATG are not conserved when compared to the 5′-noncoding region of porcine and human 17β-HSD IV. Thirteen in-frame ATG codons are found in the sequence. The second in-frame ATG downstream from the first is not followed by a G at position +4, which is essential for efficient translation [20]. The open reading frame (ORF) starting at the first ATG encodes for a protein of 735 aa of 85 and 81% similarity with human and porcine 17β-HSD IV, respectively (Fig. 2). The

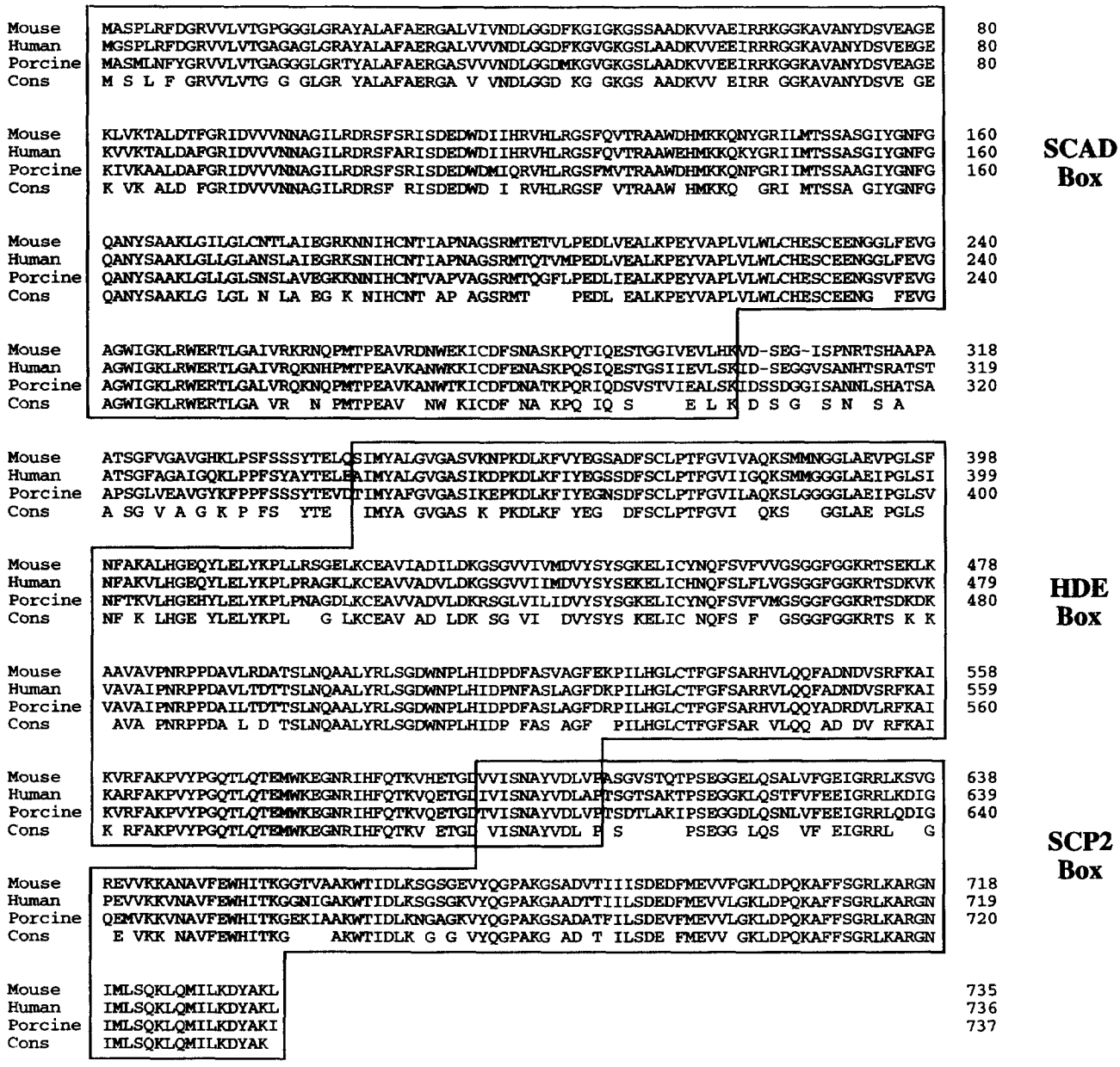

Fig. 2. Alignment of mouse, human and porcine 17β-HSD IV protein sequences. The amino acid (aa) sequences of mouse, human and porcine 17β-HSD IV were aligned by computerized alignment software using the CLUSTAL package [25]. The residues are numbered relative to the first putative amino terminus methionine. Conserved aa identical for all three members are shown at the Cons line. Three regions of similarity have been previously described [12, 13] and are delineated in the SCAD, HDE and SCP2 boxes which corresponds to the first 300 aa similar to short chain alcohol dehydrogenase (SCAD), aa 342–606 similar to the C-terminal part of hydratase-dehydrogenase epimerase (HDE) and aa 595–735 to sterol carrier protein 2 (SCP2), respectively.

calculated molecular mass of the product is 79,524 Da. The translation of the ORF predicts a protein with 9 cysteines, rich in glycine (10.5%), alanine (9.3%), valine (8.8%), leucine (8.3%) and serine (7.5%) residues. An alignment of the amino acid sequences of the 17β-HSD IV cloned in the mouse (this report), human [12] and porcine [11] reveals that all regions are conserved. The lowest similarity (46%) is seen from aa 291 to 337 (Fig. 2). The first 300 aa are similar to members of the short chain alcohol dehydrogenase family (SCAD box). The central region corresponding to aa 343–607 of the human 17β-HSD IV shares high similarity with the C-terminal part of the trifunctional hydratase-dehydrogenase-epimerase (HDE box) [16] and the carboxy-terminal part reveals 39% identity to the human sterol carrier protein 2 (SCP2 box) [12, 13, 17].

Northern blot analysis of 17β-HSD IV in mouse tissues

Expression levels of the mouse 17β-HSD IV were analyzed by Northern blotting. Poly(A^+) mRNA (2 μg/lane) from different mouse tissues were hybridized with the [α-^{32}P] EcoRI fragment corresponding to nucleotides 302–1753 of mouse 17β-HSD IV. Using stringent conditions, an approx. 3.0 kb mRNA transcript was found to be expressed in virtually all mouse tissues tested (Fig. 3). The apparent highest expression was seen in liver (Fig. 3, lane 5) followed by kidney (Fig. 3, lane 7) and skeletal muscle (Fig. 3, lane 6). Moderate expression occurred in the heart (Fig. 3, lane 1), testis (Fig. 3, lane 8) and lung (Fig. 3, lane 4). Faint signals were detected in the brain (Fig. 3, lane 2) and spleen (Fig. 3, lane 3). Quantification with a phosphorimager revealed that the 17β-HSD IV message was 50-fold less abundant in the spleen than in the liver. The message in the kidney is 2-fold less intense than that in the liver.

Reactivity of monoclonal antibody F1 with murine tissues

The affinity of mouse monoclonal antibody F1 prepared against porcine 17β-HSD IV was demonstrated on western blots of two murine species (Fig. 4). Typical 80 and 32 kDa bands, corresponding to the primary translation product and its N-terminal enzymatically active fragment, were seen in western blots of porcine, rat and mouse kidney particulate fractions.

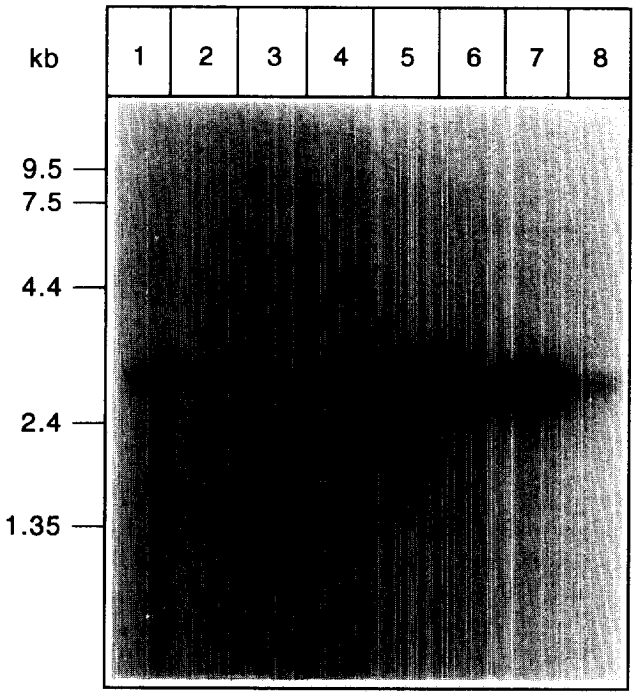

Fig. 3. Northern blot analysis of 17β-HSD IV in mouse tissues. Samples of poly(A^+) mRNA (2 μg per lane) from different mouse tissues were applied. The EcoRI digested fragment from nucleotides 302–1753 of Li-17βIV plasmid was used as probe. RNA blot analysis was performed as previously described [12]. The sizes (kb) are indicated on the left. Blot contains mRNA from mouse heart (lane 1), brain (lane 2), spleen (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) and testis (lane 8).

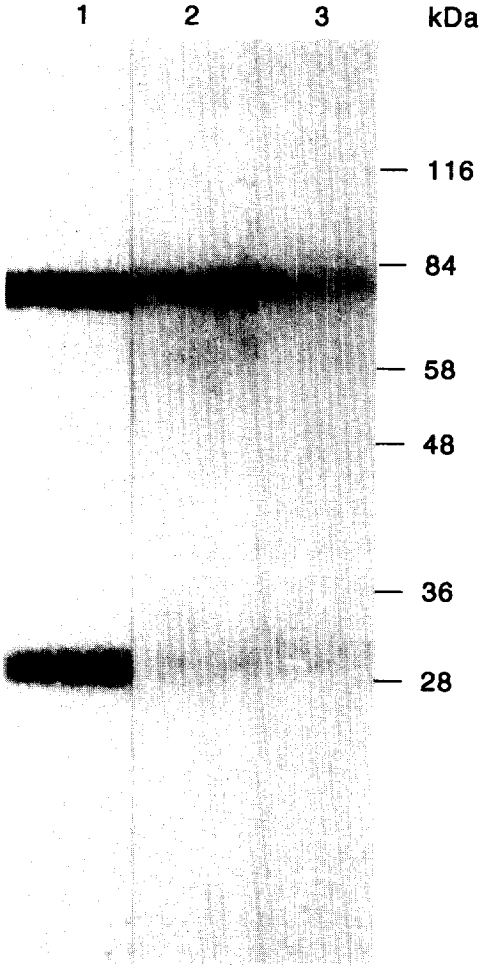

Fig. 4. Reactivity of monoclonal antibody F1. Samples (20 μg) of particulate fractions of kidney homogenates from pig (lane 1), rat (lane 2) and mouse (lane 3) were subjected to SDS-PAGE, blotted to nitrocellulose, incubated with mab F1-peroxidase and visualized by reaction with diaminobenzidine/H_2O_2 [10]. Molecular mass standards are indicated.

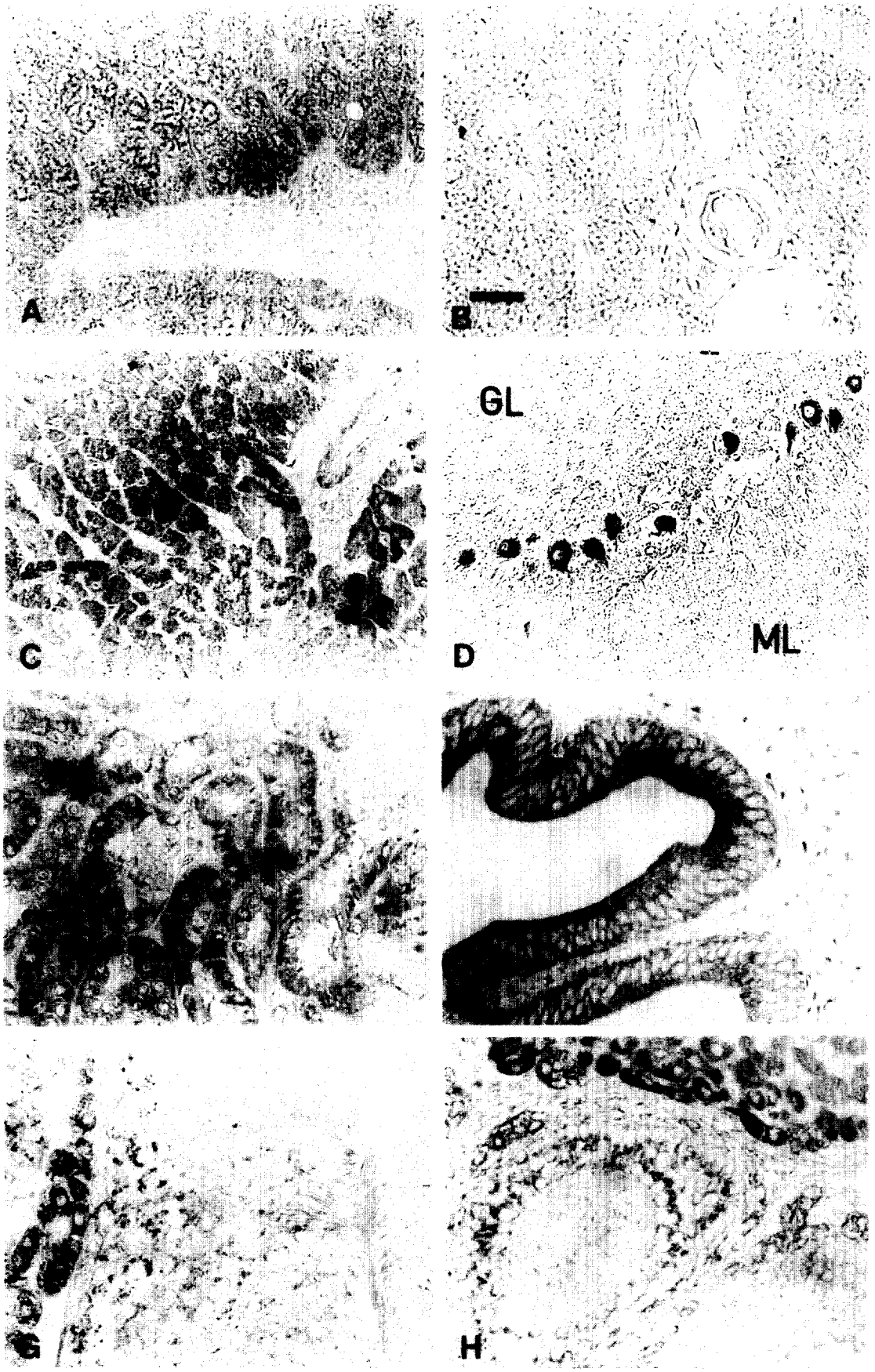

Fig. 5. Immunocytochemical survey of 17β-HSD IV in mouse tissues. Paraffin sections were incubated with mab F1-peroxidase and the color reaction performed as described [18]. Control (B): incubation of liver sections with peroxidase only. Clear labelling was seen in hepatocytes (A), heart myocytes (C), Purkinje cells of cerebellum (ML, molecular layer; GL, granular layer) (D), proximal tubules of kidney (E), uterus epithelium at estrus (F), Leydig cells (G) and granulosa cells (H). Bar 20 μm.

The mab F1 was used to investigate the presence of mouse HSD IV in several tissues. High mRNA levels in liver and heart were paralleled by intense and specific staining of hepatocytes [Fig. 5(A)] and myocytes of the heart [Fig. 5(C)]. Epithelial labelling was seen in luminal and glandular uterine epithelium [Fig. 5(D)] and in the proximal tubules of the kidney [Fig. 5(E)]. The cerebellum showed exclusive staining of Purkinje cells [Fig. 5(F)]. Leydig cells of the testes and the granulosa cells of the ovary and showed clear labelling [Fig. 5(G) and (H), respectively].

DISCUSSION

The cloning of mouse 17β-HSD IV permits an alignment of the amino acid sequences of three currently cloned mammalian 17β-HSD IV. These proteins feature a multidomain structure. The first 300 aa of the consensus 17β-HSD IV sequence (Fig. 2) show similarities to the conserved motifs of the short chain alcohol dehydrogenase family. This domain is followed by a sequence AAP (aa 315–317) which is the suggested processing site for a protease cutting the 32 kDa fragment from the primary translation product [13, 21]. The central part resembles the C-terminal part of enzymes which catalyzes peroxisomal β-oxidation of fatty acids such as the trifunctional hydratase-dehydrogenase-epimerase (HDE) [16]. The carboxy-terminus of the consensus 17β-HSD IV sequence is similar to sterol carrier protein 2 including its peroxisomal targeting signal AKL or AKI [22]. The functionality of the three domains must be examined by a detailed single-domain expression studies. Recent data indicated that the porcine 17β-HSD IV is localized in vesicles of 120–200 nm with moderate electron-dense matrix bounded by a single membrane [23]. The identity of these vesicles with peroxisomes was clarified by immunogold electron microscopy [24].

The data on 17β-HSD IV mRNA expression and immunocytochemistry indicate a wide distribution in different tissues of the mouse. Besides high levels in the liver and kidney, substantial amounts of 17β-HSD IV mRNA were detected in skeletal muscle, testis, lung and heart. Low mRNA levels measured in spleen and brain, as well as in the uterus and intestine (data not shown) might not reflect the expression of 17β-HSD IV in specialized cells such as uterine epithelium or Purkinje cells. These levels could be locally much higher.

Similar observations apply to the widely distributed human 17β-HSD IV [12]. The mouse 17β-HSD IV mRNA is more predominant in liver than its human counterpart. The presence of the immunologically related 80 and 32 kDa proteins in another murine species, the rat, suggests the existence of additional 17β-HSD IV. This enzyme might be responsible for the oxidative activity of 17β-hydroxysteroid dehydrogenases observed in rat tissues [2]. The mouse model allows for studies of regulation and tissue-specific expression of the 17β-HSD IV.

Acknowledgements—We thank Professor P. W. Jungblut for his help and advice during the course of this work. T.N. was grantee of the "Société de Secours des Amis des Sciences", France. The work performed in CNRS Lille was supported by grants awarded in part by the "Centre National de la Recherche Scientifique" (France), by the "Ligue Nationale Contre le Cancer" and by the "Association pour la Recherche contre le Cancer" (France). The novel amino acid and nucleotide sequences published here have been submitted to the EMBL Data Library Accesion No. X89998.

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